

NEWSLETTER

European Union Reference Laboratory for *Salmonella*

Vol. 30 No. 3
September 2024

ISSN 2211-6877



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

Produced by

European Union Reference Laboratory for *Salmonella*

National Institute of Public Health and the Environment
P.O. Box 1, 3720 BA Bilthoven, The Netherlands

e-mail: EURLSalmonella@rivm.nl

website: www.eurlsalmonella.eu

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
the European Union**

Contents

EDITORIAL NOTE	4
CONTRIBUTION OF THE EURL-<i>SALMONELLA</i>	6
EURL- <i>SALMONELLA</i> ACTIVITIES IN 2024.....	6
TIMETABLE EURL- <i>SALMONELLA</i> COMBINED PT-ILS PRIMARY PRODUCTION STAGE (PPS) AND FOOD 2024	7
TIMETABLE EURL- <i>SALMONELLA</i> PROFICIENCY TEST TYPING 2024.....	8
FROM THE LITERATURE	9

Editorial Note

Bilthoven, 1 October 2024

Dear colleagues,

I hope you had a good and relaxing summer break and gained new energy to come back to daily business again.

At the EURL-*Salmonella* we have been busy with the preparations of the Proficiency Tests (PTs) of this autumn.

Currently the **combined PT for NRLs-*Salmonella* for Food and for primary production stage (PPS)** is organised. This PT will focus on the detection of *Salmonella* in environmental samples from the food and animal production and the samples were sent to the participants on 30 September 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. Because of this combined use of data, it is preferred to start this PT within 2 days after dispatch of the samples. This is different from previous PTs, for which the performance started one week after dispatch of the samples. For your information, the timetable for this combined PT-ILS is (again) included in this Newsletter.

In November, the **2024 PT on typing of *Salmonella*** will be organised. Like in previous 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 (again) included in this Newsletter.

During the summer, we also discussed internally the set-up of the **EURL-*Salmonella* PT Detection to be organised in the first half of 2025**. As you may remember, it is planned that our institute (RIVM) will move to a new building in Utrecht (the Netherlands) during April-July 2025. Currently, we are already very busy with the many preparations needed to move such a large organisation (approx. 2000 employees) with several accredited laboratories in different working fields. It is foreseen, that in the second quarter of 2025 the laboratory activities will be limited to only the 'essential' activities, to make sure that most people have their hands free for the big relocation. For sure this relocation also influences the planning of the EURL-*Salmonella* activities. Still, we are considering to organise a PT early 2025. To do so, we will choose a matrix that was used before in our PTs and has shown to be sufficiently stable to prepare the samples well in advance. Quite likely, the matrix of choice may be flaxseed, meaning that this will be a combined Food-Feed PT. Additionally, the PT will need to be organised before the actual relocation starts, so probably in February/March 2025. More details about this PT Detection of 2025 will be shared in the coming months.

To keep you updated, I want to inform you that we have submitted a proposal for the workplan and estimated budget for the **activities of EURL-*Salmonella* for 2025-2027** in the portal of HaDEA, before 1 September 2024. A reaction from HaDEA to all EURL proposals is expected later this year. If possible, the EURL-*Salmonella* activities foreseen for 2025 will be shared in the next Newsletter. For now, the table with the main activities performed and planned in 2024 is included in this Newsletter again.

Interesting to know is the fact that the 10th edition of the **International Symposium *Salmonella* and Salmonellosis (I3S)** will be organised in Saint-

Malo, France, on 23-25 June 2025. Until 30 November 2024 you can still submit an abstract for an oral presentation. For more information on the I3S symposium, please visit the website of I3S 2025: <https://i3scongress.innozh.fr/>

In September 2024, the following **EURL-*Salmonella* report** was published:

Pol-Hofstad, I.E. and Mooijman, K.A., 2024. EURL-*Salmonella* Proficiency Test Primary Production Stage, 2023. Detection of *Salmonella* in chicken faeces samples. RIVM report 2023-0342. National Institute for Public Health and the Environment, Bilthoven, the Netherlands.

<https://www.rivm.nl/bibliotheek/rapporten/2023-0342.pdf>

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:

Irene Pol-Hofstad

E-mail: Irene.Pol@RIVM.nl

Tel. number: + 31 6 2964 6897

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

P.O. Box 1, 3720 BA Bilthoven, the Netherlands

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

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:

Wilma Jacobs

E-mail: wilma.jacobs@rivm.nl

Mobile number: +31 6 3114 2419

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

From the Literature

Salmonella-related Literature selection from Scopus: July – September 2024

Ma J., Dai J., Cao C., Su L., Cao M., He Y., Li M., Zhang Z., Chen J., Cui S., Yang B.
Prevalence, serotype, antimicrobial susceptibility, contamination factors, and control methods of Salmonella spp. in retail fresh fruits and vegetables: A systematic review and meta-analysis

(2024) *Comprehensive Reviews in Food Science and Food Safety*, 23 (4), art. no. e13407
ABSTRACT: This research presents a comprehensive review of Salmonella presence in retail fresh fruits and vegetables from 2010 to 2023, utilizing data from recognized sources such as PubMed, Scopus, and Web of Science. The study incorporates a meta-analysis of prevalence, serovar distribution, antimicrobial susceptibility, and antimicrobial resistance genes (ARGs). Additionally, it scrutinizes the heterogeneous sources across various food categories and geographical regions. The findings show a pooled prevalence of 2.90% (95% CI: 0.0180–0.0430), with an increase from 4.63% in 2010 to 5.32% in 2022. Dominant serovars include *S. Typhimurium* (29.14%, 95% CI: 0.0202–0.6571) and *S. Enteritidis* (21.06%, 95% CI: 0.0181–0.4872). High resistance rates were noted for antimicrobials like erythromycin (60.70%, 95% CI: 0.0000–1.0000) and amoxicillin (39.92%, 95% CI: 0.0589–0.8020). The most prevalent ARGs were blaTEM (80.23%, 95% CI: 0.5736–0.9692) and parC mutation (66.67%, 95% CI: 0.3213–0.9429). Factors such as pH, water activity, and nutrient content, along with external factors like the quality of irrigation water and prevailing climatic conditions, have significant implications on Salmonella contamination. Nonthermal sterilization technologies, encompassing chlorine dioxide, ozone, and ultraviolet light, are emphasized as efficacious measures to control Salmonella. This review stresses the imperative need to bolster prevention strategies and control measures against Salmonella in retail fresh fruits and vegetables to alleviate related food safety risks. ISSN: 15414337

Parolini F., Ventura G., Rosignoli C., Rota Nodari S., D'Incau M., Marocchi L., Santucci G., Boldini M., Gradassi M.

Detection and Phenotypic Antimicrobial Susceptibility of Salmonella enterica Serotypes in Dairy Cattle Farms in the Po Valley, Northern Italy

(2024) *Animals*, 14 (14), art. no. 2043

ABSTRACT: The presence of Salmonella spp. in dairy cattle farms poses a major risk to animal health and welfare. This study focused on Salmonella detection in dairy farms located in the Cremona and Mantua provinces (northern Italy) in samples collected and submitted to laboratories in 2021–2022. A total of 2710 samples from different sources, including calf carcasses/organs (n = 128), rectal swabs (n = 1937), feces (n = 390), bulk milk (n = 93), and overshoes/swabs (n = 127) for environmental sampling, were analyzed for the presence of Salmonella spp. and were included in the present study. Our results indicate that Salmonella was most commonly firstly identified from calf carcasses and organs (61.67%) and that the serotypes most frequently detected in dairies were *S. Dublin* (38.33%), *S. Typhimurium* (23.33%), and *S. Typhimurium* monophasic variant (14.17%). The most common pathological findings in calf carcasses were enteritis, hepatosplenomegaly, and pneumonia. The antimicrobial resistance pattern analyzed using the MIC assay of 51 Salmonella isolates revealed the presence of multi-resistant strains, which pose a major risk to public and animal health. ISSN: 20762615

Kesby M., Jorgensen F., Willis C., Aird H., Lai S., Sadler-Reeves L., Jenkins C., Chattaway M.

The microbiological quality of flour products in the UK with respect to Salmonella and Shiga-toxin-producing Escherichia coli

(2024) *Journal of Applied Microbiology*, 135 (7), art. no. Ixae183

ABSTRACT: Aim: To investigate the possible contamination of raw flour and raw flour-based products, such as pancake/batter mixes, with Salmonella, generic Escherichia coli, and Shiga-toxin-producing E. coli (STEC). Samples included flours available for sale in the UK over a period of four months (January to April 2020). The Bread and Flour regulations, 1998 state the permitted ingredients in flour and bread but it does not specify the regular monitoring of the microbiological quality of flour and flour-based products. Methods and results: Samples of raw flour were collected by local authority sampling officers in accordance with current guidance on microbiological food sampling then transported to the laboratory for examination. Microbiological testing was performed to detect Salmonella

spp., generic *E. coli*, and STEC characterized for the presence of STEC virulence genes: *stx1*, *stx2*, and subtypes, *eae*, *ipah*, *aggR*, *lt*, *sth*, and *stp*, using molecular methods Polymerase Chain Reaction (PCR). Of the 882 flours sampled, the incidence of *Salmonella* was 0.1% (a single positive sample that contained multiple ingredients such as flour, dried egg, and dried milk, milled in the UK), and 68 samples (7.7%) contained generic *E. coli* at a level of >20 CFU/g. Molecular characterization of flour samples revealed the presence of the Shiga-toxin (*stx*) gene in 10 samples (5 imported and 5 from the UK) (1.1%), from which STEC was isolated from 7 samples (0.8%). *Salmonella* and STEC isolates were sequenced to provide further characterization of genotypes and to compare to sequences of human clinical isolates held in the UKHSA archive. Using our interpretive criteria based on genetic similarity, none of the STEC flour isolates correlated with previously observed human cases, while the singular *Salmonella* serotype Newport isolate from the mixed ingredient product was similar to a human case in 2019, from the UK, of *S. Newport*. Although there have been no reported human cases of STEC matching the isolates from these flour samples, some of the same serotypes and *stx* subtypes detected are known to have caused illness in other contexts. Conclusion: Results indicate that while the incidence was low, there is a potential for the presence of *Salmonella* and STEC in flour, and a genetic link was demonstrated between a *Salmonella* isolate from a flour-based product and a human case of salmonellosis. Impact Statement These results demonstrate the importance of raising awareness amongst food businesses and members of the public regarding the potential risks of consuming raw flour, dough, and batter. ISSN: 13645072

Wang J., Fenster D.A., Vaddu S., Bhumanapalli S., Kataria J., Sidhu G., Leone C., Singh M., Dalloul R.A., Thippareddi H.

Colonization, spread and persistence of Salmonella (Typhimurium, Infantis and Reading) in internal organs of broilers

(2024) *Poultry Science*, 103 (7), art. no. 103806

ABSTRACT: Transfer of *Salmonella* to internal organs of broilers over a 35 d grow-out period was evaluated. A total of 360 one-day old chicks were placed in 18 floor pens of 3 groups with 6 replicate pens each. On d 0, broilers were orally challenged with a cocktail of *Salmonella* (equal population of marked serovars; nalidixic acid-resistant *S. Typhimurium*, rifampicin-resistant *S. Infantis*, and kanamycin-resistant *S. Reading*) to have 3 groups: L (low; ~2 log CFU/bird); M (medium; ~5 log CFU/bird); and H (High; ~8 log CFU/bird). On d 2, 7 and 35, 4 birds/pen were euthanized and ceca, liver, and spleen samples were collected aseptically. Gizzard samples (4/pen) were collected on d 35. The concentration of *Salmonella* in liver and spleen were transformed to binary outcomes (positive and negative) and fitted in glm function of R using cecal *Salmonella* concentrations (log CFU/g) and inoculation doses (L, M, and H) as inputs. On d 2, H group showed greater ($P \leq 0.05$) cecal colonization of all 3 serovars compared to L and M groups. However, M group showed greater ($P \leq 0.05$) colonization of all 3 serovars in the liver and spleen compared to L group. *Salmonella* colonization increased linearly in the ceca and quadratically in the liver and spleen with increasing challenge dose ($P \leq 0.05$). On d 35, L group had greater ($P \leq 0.05$) *S. Infantis* colonization in the ceca and liver compared to M and H groups ($P \leq 0.05$). Moreover, within each group on d 35, the concentration of *S. Reading* was greater than those of *S. Typhimurium* and *S. Infantis* for all 3 doses in the ceca and high dose in the liver and gizzard ($P \leq 0.05$). *Salmonella* colonization diminished in the ceca, liver, and spleen during grow-out from d 0 to d 35 ($P \leq 0.05$). On d 35, birds challenged with different doses of *Salmonella* cocktail showed a similar total *Salmonella* spp. population in the ceca (ca. 3.14 log CFU/g), liver (ca. 0.54 log CFU/g), spleen (ca. 0.31 log CFU/g), and gizzard (ca. 0.42 log CFU/g). Estimates from the fitted logistic model showed that one log CFU/g increase in cecal *Salmonella* concentration will result in an increase in relative risk of liver and spleen being *Salmonella*-positive by 4.02 and 3.40 times ($P \leq 0.01$), respectively. Broilers from H or M group had a lower risk (28 and 23%) of being *Salmonella*-positive in the liver compared to the L group when the cecal *Salmonella* concentration is the same ($P \leq 0.05$). Oral challenge of broilers with *Salmonella* spp. with various doses resulted in linear or quadratic increases in *Salmonella* colonization in the internal organs during early age and these populations decreased during grow-out (d 35). This research can provide guidance on practices to effectively mitigate the risk of *Salmonella* from chicken parts and enhance public health. ISSN: 00325791

Um M.M., Dufour S., Bergeron L., Gauthier M.-L., Paradis M.-È., Roy J.-P., Falcon M., Molgat E., Ravel A.

Development of a decision support tool to compare diagnostic strategies for establishing the herd status for infectious diseases: An example with Salmonella Dublin infection in dairies

(2024) *Preventive Veterinary Medicine*, 228, art. no. 106234

ABSTRACT: The diagnosis of infectious diseases at herd level can be challenging as different stakeholders can have conflicting priorities. The current study proposes a “proof of concept” of an approach that considers a reasonable number of criteria to rank plausible diagnostic strategies using multi-criteria decision analysis (MCDA) methods. The example of *Salmonella* Dublin diagnostic in Québec dairy herds is presented according to two epidemiological contexts: (i) in herds with no history of *S. Dublin* infection and absence of clinical signs, (ii) in herds with a previous history of infection, but absence of clinical signs at the moment of testing. Multiple multiparty exchanges were conducted to determine: 1) stakeholders' groups; 2) the decision problem; 3) solutions to the problem (options) or diagnostic strategies to be ordered; 4) criteria and indicators; 5) criteria weights; 6) the construction of a performance matrix for each option; 7) the multi-criteria analyses using the visual preference ranking organization method for enrichment of evaluations approach; 8) the sensitivity analyses, and 9) the final decision. A total of nine people from four Québec's organizations (the dairy producers provincial association along with the DHI company, the ministry of agriculture, the association of veterinary practitioners, and experts in epidemiology) composed the MCDA team. The decision problem was “What is the optimal diagnostic strategy for establishing the status of a dairy herd for *S. Dublin* infection when there are no clinical signs of infection?”. Fourteen diagnostic strategies composed of the three following parameters were considered: 1) biological samples (bulk tank milk or blood from 10 heifers aged over three months); 2) sampling frequencies (one to three samples collection visits); 3) case definitions to conclude to a positive status using imperfect milk- or blood-ELISA tests. The top-ranking diagnostic strategy was the same in the two contexts: testing the bulk tank milk and the blood samples, all samples collected during one visit and the herd being assigned a *S. Dublin* positive status if one sample is ELISA-positive. The final decision favored the top-ranking option for both contexts. This MCDA approach and its application to *S. Dublin* infection in dairy herds allowed a consensual, rational, and transparent ranking of feasible diagnostic strategies while taking into account the diagnostic tests accuracy, socio-economic, logistic, and perception considerations of the key actors in the dairy industry. This promising tool can be applied to other infectious diseases that lack a well-established diagnostic procedure to define a herd status. ISSN: 01675877

Mahgoub S.A., Qattan S.Y.A., AlMalki F., Kamal M., Alqurashi A.F., Almuraee A.A., Alhassani W.E., Abu-Hiamed H.A., Almarkhan W.D., Alsanei W.A., Alfassam H.E., Rudayni H.A., Allam A.A., Moustafa M., Alshaharni M.O., Taha A.E.

Impact of packaging atmosphere, oregano essential oil, and storage temperature on cold-adapted Salmonella Enteritidis and Salmonella Typhimurium on ready-to-eat smoked turkey

(2024) *Poultry Science*, 103 (7), art. no. 103846

ABSTRACT: The hazard of diseases created by *S. Enteritidis* and *S. Typhimurium* is relatively high in turkey meat products. Combinations of preservation methods are utilized in many strategies, such as mild heat with decreased water activity, a changed atmosphere, refrigerated storage, and decreased heat treatment with some acidification. Within the domain of ready-to-eat food technology, a range of preservation methods are typically utilized to enhance shelf life, such as applying mild heat in tandem with reduced water activity, employing modified atmosphere packaging, utilizing refrigerated storage, and utilizing reduced heat treatment combined with acidification. This investigation aimed to determine how *S. Enteritidis* and *S. Typhimurium* grew when sliced ready-to-eat smoked turkey (RTE-SM) was stored at 0, 5, 10, and 15°C for various periods. The study also examined the effects of modified atmosphere packaging (MAP) (40% CO₂ and 60% N₂) and VP on these growth patterns. Total viable count (TVC), lactic acid bacteria (LAB), pH, and redox potential levels were determined. The control experiment on RTE-SM showed no *Salmonella* growth within 30 d of storage at any temperature. This indicated that the RTE-SM in use did not initially contain *S. Typhimurium* and *S. Enteritidis*. Results indicated that the storage of RTE-SM using a combination of VP, MAP, and MAPEO with storage at 0 and 5°C did not allow for the pathogen to grow throughout storage. In comparison, at 10 and 15°C after one day, which allowed for minor growth (0.17–0.5 log CFU/g)? In contrast, at 0 and 5°C, *Salmonella* survives until the end of storage (173 d). However, the combination of MAPEO with the same storage temperatures achieved the elimination of the pathogen in the meat after 80 d. The combination of both packaging systems with high temperatures (10 or 15°C) allowed for the multiplication and growth of the bacterium through the product's shelf life of more than 1 log CFU/g. Thus, a combination of MAP or MAPEO with low storage temperatures (0 or 5°C) inhibited the growth of the pathogen. ISSN: 00325791

Arrieta-Gisasola A., Martínez-Ballesteros I., Martínez-Malaxetxebarria I., Garrido V., Grilló M.J., Bikandi J., Laorden L.

Pan-Genome-Wide Association Study reveals a key role of the salmochelin receptor IroN in the biofilm formation of Salmonella Typhimurium and its monophasic variant 4,[5],12:i:- (2024) International Journal of Food Microbiology, 419, art. no. 110753

ABSTRACT: *Salmonella enterica* subsp. *enterica* serovar Typhimurium variant 4,[5],12:i:- (so called S. 4,[5],12:i:-) has rapidly become one of the most prevalent serovars in humans in Europe, with clinical cases associated with foodborne from pork products. The mechanisms, genetic basis and biofilms relevance by which S. 4,[5],12:i:- maintains and spreads its presence in pigs remain unclear. In this study, we examined the genetic basis of biofilm production in 78 strains of S. 4,[5],12:i:- (n = 57) and S. Typhimurium (n = 21), from human gastroenteritis, food products and asymptomatic pigs. The former showed a lower Specific Biofilm Formation index (SBF) and distant phylogenetic clades, suggesting that the ability to form biofilms is not a crucial adaptation for the S. 4,[5],12:i:- emerging success in pigs. However, using a pan-Genome-Wide Association Study (pan-GWAS) we identified genetic determinants of biofilm formation, revealing 167 common orthologous groups and genes associated with the SBF. The analysis of annotated sequences highlighted specific genetic deletions in three chromosomal regions of S. 4,[5],12:i:- correlating with SBF values: i) the complete fimbrial operon stbABCDE widely recognized as the most critical factor involved in *Salmonella* adherence; ii) the *hxA*, *hxB*, and *pgiA* genes, which expression in S. Typhimurium is induced in the tonsils during swine infection, and iii) the entire *iroA* locus related to the characteristic deletion of the second-phase flagellar genomic region in S. 4,[5],12:i:-. Consequently, we further investigated the role of the *iro*-genes on biofilm by constructing S. Typhimurium deletion mutants in *iroBCDE* and *iroN*. While *iroBCDE* showed no significant impact, *iroN* clearly contributed to S. Typhimurium biofilm formation. In conclusion, the pan-GWAS approach allowed us to uncover complex interactions between genetic and phenotypic factors influencing biofilm formation in S. 4,[5],12:i:- and S. Typhimurium. ISSN: 01681605

Arista-Regalado A.D., Viera-Segura O., de Oca S.A.-M., Hernández-Hernández L., González-Aguilar D.G., León J.B.

Characterization and efficacy of Salmonella phage cocktail PHA46 in the control of Salmonella Newport and Typhimurium internalized into cherry tomatoes (2024) International Journal of Food Microbiology, 419, art. no. 110745

ABSTRACT: Non-typhoid *Salmonella enterica* causes salmonellosis illness, and this bacterium can contaminate food throughout the production chain, including those that are consumed as raw products. *Salmonella enterica* can adhere to and internalize into fresh produce such as cherry tomatoes. It has been reported that lytic bacteriophages (phages) can be used as a biocontrol agent in the agricultural field, being an alternative for the control of *Salmonella* in red meat, fish, lettuce, and cabbage. The aim of this study was to characterize the two phages present in the PHA46 cocktail to determine their morphology, genome, host range, and resistance to different temperatures and pHs values; and later evaluate their lytic activity to reduce the adherence to and internalization of *Salmonella enterica* serovars Newport and Typhimurium into cherry tomatoes. In addition, in this work, we also explored the effect of the PHA46 cocktail on the virulence of S. Newport-45 and S. Typhimurium SL1344, recovered from the interior of cherry tomatoes, on the lifespan of the animal model *Caenorhabditis elegans*. The nematode *C. elegans*, recently has been used to test the virulence of *Salmonella* and it is easy to maintain and work with in the laboratory. The results revealed that the morphology obtained by Transmission Electron Microscopy of two phages from the PHA46 cocktail correspond to a myovirus, the analyses of their genomes sequences did not report virulence or antimicrobial resistance genes. The PHA46 sample is specific for 33 different serovars from different *Salmonella* strains and shows stability at 7 °C and pH 6. Also, the PHA46 cocktail was effective in reducing the adherence of S. Newport-45 and S. Typhimurium SL1344 to cherry tomatoes, at an average of 0.9 log₁₀, respectively. Regarding internalized bacteria, the reduction was at an average of 1.2 log₁₀, of the serovars mentioned above. The lifespan experiments in *C. elegans* showed by itself, that the PHA46 cocktail was harmless to the nematode, and the virulence from both *Salmonella* strains grown in vitro is diminished in the presence of the PHA46 cocktail. In conclusion, these results showed that the PHA46 cocktail could be a good candidate to be used as a biocontrol agent against *Salmonella enterica*. ISSN: 01681605

García-Díez J., Moura D., Grispoldi L., Cenci-Goga B., Saraiva S., Silva F., Saraiva C., Ausina J.

Salmonella spp. in Domestic Ruminants, Evaluation of Antimicrobial Resistance Based on the One Health Approach—A Systematic Review and Meta-Analysis

(2024) *Veterinary Sciences*, 11 (7), art. no. 315

ABSTRACT: *Salmonella* spp. pose a global threat as a leading cause of foodborne illnesses, particularly prevalent in the European Union (EU), where it remains the second cause of foodborne outbreaks. The emergence of antimicrobial resistance (AMR) in *Salmonella* spp. has become a critical concern, complicating treatment strategies and escalating the risk of severe infections. The study focuses on large and small ruminants, identifying a prevalence of *Salmonella* spp. in slaughterhouses and revealing varied AMR rates across antimicrobial families throughout a meta-analysis. Also, comparison with AMR in human medicine was carried out by a systematic review. The results of the present meta-analysis displayed a prevalence of *Salmonella* spp. in large and small ruminants at slaughterhouses of 8.01% (8.31%, cattle; 7.04%, goats; 6.12%, sheep). According to the AMR of *Salmonella* spp., 20, 14, and 13 out of 62 antimicrobials studied were classified as low (<5%), high (>5% but <10%), and very high (>10%), respectively. *Salmonella* spp. did not display AMR against aztreonam, mezlocillin, ertapenem, meropenem, ceftazidime, levofloxacin, tilmicosin, linezolid, fosfomycin, furazolidone, quinupristin, trimethoprim and spectinomycin. In contrast, a prevalence of 100% of AMR has been described against ofloxacin, lincomycin, and cloxacillin. In the context of the main antibiotics used in the treatment of human salmonellosis, azithromycin was shown to have the highest resistance among *Salmonella* spp. isolates from humans. Regarding cephalosporins, which are also used for the treatment of salmonellosis in humans, the prevalence of *Salmonella* spp. resistance to this class of antibiotics was similar in both human and animal samples. Concerning quinolones, despite a heightened resistance profile in *Salmonella* spp. isolates from ruminant samples, there appears to be no discernible compromise to the efficacy of salmonellosis treatment in humans since lower prevalences of AMR in *Salmonella* spp. isolated from human specimens were observed. Although the resistance of *Salmonella* spp. indicates some degree of concern, most antibiotics are not used in veterinary medicine. Thus, the contribution of cattle, sheep and goats to the rise of antibiotic resistance of *Salmonella* spp. and its potential impact on public health appears to be relatively insignificant, due to their low prevalence in carcasses and organs. Nevertheless, the observed low prevalence of *Salmonella* spp. in ruminants at slaughterhouse and the correspondingly low AMR rates of *Salmonella* spp. to key antibiotics employed in human medicine do not indicate that ruminant livestock poses a substantial public health risk concerning the transmission of AMR. Thus, the results observed in both the meta-analysis and systematic review suggests that AMR is not solely attributed to veterinary antibiotic use but is also influenced by factors such as animal health management (i.e., biosecurity measures, prophylactic schemes) and human medicine. ISSN: 23067381

Musa L., Toppi V., Stefanetti V., Spata N., Rapi M.C., Grilli G., Addis M.F., Di Giacinto G., Franciosini M.P., Casagrande Proietti P.

High Biofilm-Forming Multidrug-Resistant Salmonella Infantis Strains from the Poultry Production Chain

(2024) *Antibiotics*, 13 (7), art. no. 595

ABSTRACT: The ability of *Salmonella* species to adhere to surfaces and form biofilms, leading to persistent environmental reservoirs, might represent a direct link between environmental contamination and food processing contamination. The purpose of this study was to investigate the biofilm-forming ability of 80 multidrug-resistant (MDR) and extended-spectrum beta-lactamase (ESBL) producing *Salmonella enterica* serovar *Infantis* strains isolated from the broiler food chain production through whole genome sequencing (WGS), PCR, and morphotype association assays. Biofilm formation was quantified by testing the strains at two different temperatures, using 96-well polystyrene plates. The rough and dry colony (rdar) morphotype was assessed visually on Congo red agar (CRA) plates. Based on our results, all tested *S. Infantis* strains produced biofilm at 22 °C with an rdar morphotype, while at 37 °C, all the isolates tested negative, except one positive. Most isolates (58.75%) exhibited strong biofilm production, while 36.25% showed moderate production. Only 5 out of 80 (6.25%) were weak biofilm producers. WGS analysis showed the presence of the fim cluster (fimADF) and the csg cluster (csgBAC and csgDEFG), also described in *S. Typhimurium*, which are responsible for fimbriae production. PCR demonstrated the presence of csgD, csgB, and fimA in all 80 *S. Infantis* strains. To our knowledge, this is the first study comparing the effects of two different temperatures on the biofilm formation capacity of ESBL producing *S. Infantis* from the broiler production chain. This study highlights that the initial biofilm components, such as curli and cellulose, are specifically expressed at lower temperatures. It is important to emphasize that within the broiler farm, the environmental temperature ranges between 18–22 °C, which is the optimum temperature for in vitro biofilm formation by *Salmonella* spp. This temperature range facilitates the expression of biofilm-associated genes, contributing to the persistence of *S. Infantis* in the environment. This complicates biosecurity measures and makes

disinfection protocols on the farm and in the production chain more difficult, posing serious public health concerns. ISSN: 20796382

Yildiz M., Demirbilek S.K.

Investigation of prevalence and antimicrobial resistance of Salmonella in pet dogs and cats in Turkey

(2024) *Veterinary Medicine and Science*, 10 (4), art. no. e1513

ABSTRACT: Background: Although salmonellosis is considered to be a foodborne zoonotic disease, pets can play a significant role in the dissemination of antimicrobial-resistant Salmonella organisms to humans because of close contact with their owners. Objectives: To determine the prevalence, risk factors, virulence factors, serotypes, and antimicrobial resistance profile of Salmonella in pet dogs and cats in Turkey and to assess the public health risk. Furthermore, to perform macroscopic comparison of lactic acid bacteria (LAB) in Salmonella-positive and Salmonella-negative animals. Methods: International Standards Organization (ISO) 6579-1:2017 and Food and Drug Administration (FDA) methods were used to compare the effectiveness of culture methods in the identification of Salmonella in 348 rectal swabs. Positive isolates were serotyped using the slide agglutination method according to the White-Kauffmann-Le Minor scheme and the presence of virulence genes (*invA* and *stn*) were evaluated by polymerase chain reaction (PCR). Antimicrobial activity was tested by Kirby–Bauer disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Results: Salmonella prevalence was 5.73% (9/157) in dogs and 0.0% (0/191) in cats. Eight (8/9) isolates were cultured with the ISO method and 5 (5/9) isolates were cultured with the FDA method. Macroscopic results revealed that Salmonella agents had no effect on LAB. Three different serotypes were detected and all isolates were positive for virulence genes. Antibiotic resistance profiling indicated that 11.1% of the isolates were MDR and the highest resistance was found for ciprofloxacin. MDR-resistant *S. Virchow* and carbapenem-resistant *S. Enteritidis* were detected from dog isolates. There was a significant difference between raw meat consumption and Salmonella carriage ($p < 0.01$). Conclusions: Dogs could be potential carriers of Salmonella infection. The isolation of Salmonella in healthy dogs instead of dogs suffering from diarrhoea indicates that attention should be paid to asymptomatic carriage. The emergence of resistance among zoonotic Salmonella isolates poses a significant threat to public health. ISSN: 20531095

Akgun Z., Coskun A.G., Cetin E., Temelli S., Eyigor A.

Salmonella carriage and change in serovar distribution in broiler giblets at slaughterhouse level in Turkiye: first report using ISO 6579-1:2017 and ISO 6579-3:2014

(2024) *Poultry Science*, 103 (7), art. no. 103805

ABSTRACT: This study aimed to determine the prevalence and serovar distribution of salmonellae in liver, heart, and spleen (LHS) and gizzard (G) of slaughtered broilers. For this, a total of 60 sample units, comprised of 30 LHS and 30 G collected from 3 slaughterhouses, were analysed by reference methods for detection and serotyping as revised ISO 6579-1:2017 and ISO 6579-3:2014, respectively. Also, Salmonella-specific real-time PCR (Salm-PCR) was used for species confirmation, while Salmonella Enteritidis (*S. Enteritidis*) and Salmonella Typhimurium (*S. Typhimurium*) specific real-time PCR (SE/ST-PCR) was evaluated to determine its efficiency for rapid detection of the serovars mandated in current legal regulations compared to standard serotyping. All LHS (100%–30/30) and 90% (27/30) of G samples harbored Salmonella with an overall prevalence of 95% (57/60) in samples examined, where all isolates were confirmed as Salmonella by Salm-PCR. The most prevalent serovar in broiler giblets was *S. Virchow* (80.70%–46/57) followed by *S. Enteritidis* (19.30%–11/57). SE/ST-PCR (%17.54–10/57) could not detect one G isolate, which was serotyped as *S. Enteritidis* by standard serotyping. High relative accuracy (98.25%), sensitivity (100%) and specificity (100%), and agreement between methods (κ : 0.94) verified SE/ST-PCR's potential to be used as an alternative in rapid detection of *S. Enteritidis* and *S. Typhimurium*. Data on high Salmonella prevalence in broiler giblets of slaughterhouse origin, and detection of the pathogen by the implementation of all requirements indicated in the revised ISO 6579-1:2017 standard method, enabling the determination of actual prevalence in the samples with high sensitivity and specificity is of significance for public health. Additionally, identification of *S. Virchow* as the dominant serovar followed by *S. Enteritidis* with a relatively lower prevalence, and absence of *S. Typhimurium* in broiler giblets are important findings for Turkiye. This up to date data, obtained by strict application of ISO 6579-3:2014 procedures, indicated a shift in circulating serovars in the broiler industry. The objective findings in this study would bring awareness to national/international literature, and may be of use in future improvements in legal regulations. ISSN: 00325791

**Silva B.S., Amorim-Neto D.P., Pia A.K.R., Campagnollo F.B., Furtado M.M.,
Carvalho A.C.B.R., Oteiza J.M., Sant'Ana A.S.**

*The fate of Salmonella enterica and Listeria monocytogenes in the pulp of eight native
Brazilian and exotic fruits*

(2024) *International Journal of Food Microbiology*, 420, art. no. 110783

ABSTRACT: Despite the wide variety of native and exotic fruits in Brazil, there is limited understanding of their ability to support pathogens during storage. This study aimed to evaluate the behavior of *Salmonella enterica* and *Listeria monocytogenes* inoculated into the pulp of eight fruits native and exotic to Brazil: Jenipapo (*Genipa americana* L.), Umbu (*Spondias tuberosa* Arruda), Maná (*Solanum sessiliflorum*), Cajá-manga (*Spondias dulcis*), Physalis (*Physalis angulata* L.), Feijoa (*Acca sellowiana*), Cupuaçu (*Theobroma grandiflorum*) (average pH < 3.3) and in a low acidic fruit: Abiu (*Pouteria caimito*) (pH 6.11). The pathogens were inoculated into the different fruits and stored at 10, 20, 30 and 37 °C for up to 12 h and 6 days, respectively. Among the fruits evaluated, Abiu was the only one that allowed *Salmonella* growth, showing higher δ -values at 20 and 30 °C (5.6 log CFU/g for both temperatures). For Physalis and Feijoa, there was a small reduction in the pathogen concentration (<1 log-cycle), mainly at 10 and 20 °C, indicating its ability to remain in the matrices. For the other fruits, notable negative δ -values were obtained, indicating a tendency towards microbial inactivation. The survival potential was significantly affected by temperature in Abiu, Maná, Cupuaçu, and Cajá-manga ($p < 0.05$). The same phenomena regarding δ -value were observed for *L. monocytogenes* population, with the greatest survival potential observed at 20 °C in Abiu (3.3 log CFU/g). Regarding the exponential growth rates in Abiu, the highest values were observed at 30 and 37 °C, both for *Salmonella* (4.6 and 4.9 log (CFU/g)/day, respectively) and for *L. monocytogenes* (2.8 and 2.7 log (CFU/g)/day, respectively), with no significant difference between both temperatures. Regarding microbial inactivation, *L. monocytogenes* showed greater resistance than *Salmonella* in practically all matrices. Jenipapo and Umbu were the pulps that, in general, had the greatest effect on reducing the population of pathogens. Furthermore, the increase in storage temperature seems to favor the increase on inactivation rates. In conclusion, *Salmonella* and *L. monocytogenes* can grow only in Abiu pulp, although they can survive in some acidic tropical fruits kept at refrigeration and abusive temperatures. ISSN: 01681605

**Lehti S.-M., Andersen O., Leppäaho-Lakka J., Suominen E., Vainio A., Matsinen M.,
Kuronen H., Rimhanen-Finne R.**

*Salmonella Typhimurium caused an unprecedentedly large foodborne outbreak in Finland
in 2021*

(2024) *Zoonoses and Public Health*, 71 (5), pp. 560 - 567

ABSTRACT: Aims: *Salmonella* infections are significant causes of foodborne outbreaks in the European Union. This study investigates a sudden increase in gastroenteritis patients in the hospital district of Central Finland in June 2021. The primary aim was to study the outbreak's magnitude and source of the outbreak. Methods and Results: Epidemiological, microbiological, environmental and traceback investigations were conducted. Over 700 persons fell ill during the outbreak caused by *Salmonella* Typhimurium associated with a daycare lunch. Similar *S. Typhimurium* was found in the patients and a vegetable mix containing iceberg lettuce, cucumber and peas served during lunch. The traceback investigation revealed that the batch information of vegetables from the wholesaler was not complete. The wholesaler had received quality complaints about the iceberg lettuce from the central kitchen. The manufacturer did not test the suspected batch for *Salmonella* since the production plant had given a certificate declaring it *Salmonella* negative. Conclusions: The most suspect ingredient was one batch of iceberg lettuce due to quality complaints. The lettuce had not been served in two daycare centres without cases. We recommend that in order to enable thorough microbiological investigation, institutional kitchens store the food samples separately as part of the internal quality control and that food items should always be tested when *Salmonella* contamination in an outbreak is suspected. ISSN: 18631959

Singh S., Koo O.K.

*A Comprehensive Review Exploring the Protective Role of Specific Commensal Gut Bacteria
against Salmonella*

(2024) *Pathogens*, 13 (8), art. no. 642

ABSTRACT: Gut microbiota is a diverse community of microorganisms that constantly work to protect the gut against pathogens. *Salmonella* stands out as a notorious foodborne pathogen that interacts with gut microbes, causing an imbalance in the overall composition of microbiota and leading to dysbiosis. This review focuses on the interactions between *Salmonella* and the key commensal bacteria such as *E. coli*, *Lactobacillus*, *Clostridium*,

Akkermansia, and Bacteroides. The review highlights the role of these gut bacteria and their synergy in combating *Salmonella* through several mechanistic interactions. These include the production of siderophores, which compete with *Salmonella* for essential iron; the synthesis of short-chain fatty acids (SCFAs), which exert antimicrobial effects and modulate the gut environment; the secretion of bacteriocins, which directly inhibit *Salmonella* growth; and the modulation of cytokine responses, which influences the host's immune reaction to infection. While much research has explored *Salmonella*, this review aims to better understand how specific gut bacteria engage with the pathogen, revealing distinct defense mechanisms tailored to each species and how their synergy may lead to enhanced protection against *Salmonella*. Furthermore, the combination of these commensal bacteria could offer promising avenues for bacteria-mediated therapy during *Salmonella*-induced gut infections in the future. ISSN: 20760817

Woodford L., Fellows R., White H.L., Ormsby M.J., Pow C.J., Quilliam R.S.

Survival and transfer potential of Salmonella enterica serovar Typhimurium colonising polyethylene microplastics in contaminated agricultural soils
(2024) *Environmental Science and Pollution Research*, 31 (39), pp. 51353 - 51363

ABSTRACT: Agricultural environments are becoming increasingly contaminated with plastic pollution. Plastics in the environment can also provide a unique habitat for microbial biofilm, termed the 'plastisphere', which can also support the persistence of human pathogens such as *Salmonella*. Human enteric *Salmonella enterica* serovar Typhimurium can enter agricultural environments via flooding or from irrigation with contaminated water. Using soil mesocosms we quantified the ability of *S. Typhimurium* to persist on microplastic beads in two agriculturally relevant soils, under ambient and repeat flood scenarios. *S. Typhimurium* persisted in the plastisphere for 35 days in both podzol and loamy soils; while during multiple flood events was able to survive in the plastisphere for up to 21 days. *S. Typhimurium* could dissociate from the plastisphere during flooding events and migrate through soil in leachate, and importantly could colonise new plastic particles in the soil, suggesting that plastic pollution in agricultural soils can aid *S. Typhimurium* persistence and facilitate further dissemination within the environment. The potential for increased survival of enteric human pathogens in agricultural and food production environments due to plastic contamination poses a significant public health risk, particularly in potato or root vegetable systems where there is the potential for direct contact with crops. ISSN: 09441344

Sun S., Yang R., Xie Y., Zhu M.-J., Sablani S., Tang J.

The effect of water activity on thermal resistance of Salmonella in chocolate products with different fat contents
(2024) *Food Control*, 162, art. no. 110443

ABSTRACT: Several instances of *Salmonella* outbreaks have been documented in association with chocolates. Conching, an important chocolate manufacturing step, may be designed for control of *Salmonella* if proper product temperature is maintained for sufficient time. A better understanding of thermal inactivation kinetics of *Salmonella* in different chocolate products is, however, highly desirable in evaluating the effectiveness of an existing conching process for *Salmonella* reduction or assessing the need for modification of manufacturing operations. Our previous study on milk chocolate (41.8% fat content) noted that *aw* and temperature are two key factors influencing *Salmonella* inactivation in a thermal process. In this research we studied the effect of different fat levels on *Salmonella* inactivation in chocolates at 80 °C. White and dark chocolates (fat content: 53.0% and 74.3%) were used in this study. The samples were conditioned to three water activity (*aw*) levels, 0.23, 0.33, and 0.43, at room temperature. The changes of *aw* in these samples were quantified when heated to 70 °C and 80 °C to reflect the real relative humidity conditions during the process. The data indicated an increase in the *aw* in the chocolate products, correlating with elevated temperatures. The thermal inactivation tests showed that D-values (time to achieve one log reduction at a fixed temperature) of *Salmonella* were between 33.9 and 46.5 min when treated at 80 °C, the corresponding *aw*, 80 °C were 0.29 and 0.28 in white and dark chocolate samples, respectively. Our data suggests that the higher fat content contributed to a less increase in *aw*, leading to a higher heat tolerance of bacteria in the chocolates. The research results may offer useful insights for the chocolate manufactures to utilize conching process for the pathogen control of chocolate products made with different recipes. ISSN: 09567135

Sheng L., Wang H., Harris L.J., Wang L.

Survival of Listeria monocytogenes and Salmonella in finishing waxes used for fresh citrus fruits
(2024) *Food Control*, 162, art. no. 110394

ABSTRACT: Citrus fruits are typically waxed before packaging to prevent water loss and extend shelf-life. The survival of *Listeria monocytogenes* and *Salmonella* was evaluated in 15 different commercial finishing waxes used for fresh citrus in California. Survival of *L. monocytogenes* and *Salmonella* in citrus finishing waxes depended on wax type. Over 24 h at 4 and 22 °C, populations of *L. monocytogenes* declined more slowly than or equal to *Salmonella* in all finishing waxes except wax F8 (organic, beeswax based, non-morpholine). Over 24 h of storage at 4 °C, *L. monocytogenes* populations declined by >4 log (below the detection limit by enrichment, 0.3 log CFU/mL) in waxes F4, F7, F8, and F9. Population declines of 1.2–2.8 log were observed in waxes F5, F10, F11, F13, and F14 and of <1.0 log in waxes F1, F2, F3, F6, F12, and F15. With the exception of waxes F2 (polyethylene based, non-morpholine) and F6 (shellac based, non-morpholine), greater declines of *L. monocytogenes* were observed over 24 h in most waxes when the storage temperature was increased to 22 °C. During long-term storage at 4 °C, *L. monocytogenes* declined by >5 log after 5, 10, 20, 30, and 135 days in waxes F3, F1, F15, F12, and F6, respectively. *L. monocytogenes* populations in all waxes stored at 22 °C fell below the detection limit by enrichment within 20 days except for wax F2. In two separate lots of wax F2, *L. monocytogenes* populations inoculated at ~4 or ~6 log CFU/mL declined by 1.6–2.6 log by day 10 and then increased to 5.4 to 5.7 log CFU/mL on day 20. Citrus packinghouse hazard assessments and food safety plans should consider the survival of foodborne pathogens in commercial citrus finishing waxes. ISSN: 09567135

Szmolka A., Lancz Z.S., Rapcsák F., Egyed L.

Emergence and Comparative Genome Analysis of Salmonella Ohio Strains from Brown Rats, Poultry, and Swine in Hungary

ABSTRACT: Rats are particularly important from an epidemiological point of view, because they are regarded as reservoirs for diverse zoonotic pathogens including enteric bacteria. This study is the first to report the emergence of *Salmonella* serovar Ohio in brown rats (*Rattus norvegicus*) and food-producing animals in Hungary. We first reveal the genomic diversity of the strains and their phylogenomic relationships in the context of the international collection of *S. Ohio* genomes. This pathogen was detected in 4.3% (4/92) of rats, captured from multiple sites in Hungary. A whole-genome-based genotype comparison of *S. Ohio*, *Infantis*, *Enteritidis*, and *Typhimurium* strains showed that 76.4% (117/153) of the virulence and antimicrobial resistance genes were conserved among these serovars, and none of the genes were specific to *S. Ohio*. All *S. Ohio* strains lacked virulence and resistance plasmids. The cgMLST phylogenomic comparison highlighted a close genetic relationship between rat and poultry strains of *S. Ohio* from Hungary. These strains clustered together with the international *S. Ohio* genomes from aquatic environments. Overall, this study contributes to our understanding of the epidemiology of *Salmonella* spp. in brown rats and highlights the importance of monitoring to minimize the public health risk of rodent populations. However, further research is needed to understand the route of infection and evolution of this serovar. ISSN: 16616596

Trees E., Carleton H.A., Folster J.P., Gieraltowski L., Hise K., Leeper M., Nguyen T.-A., Poates A., Sabol A., Tagg K.A., Tolar B., Vasser M., Webb H.E., Wise M., Lindsey R.L.

Genetic Diversity in Salmonella enterica in Outbreaks of Foodborne and Zoonotic Origin in the USA in 2006–2017

(2024) *Microorganisms*, 12 (8), art. no. 1563

ABSTRACT: Whole genome sequencing is replacing traditional laboratory surveillance methods as the primary tool to track and characterize clusters and outbreaks of the foodborne and zoonotic pathogen *Salmonella enterica* (*S. enterica*). In this study, 438 *S. enterica* isolates representing 35 serovars and 13 broad vehicle categories from one hundred epidemiologically confirmed outbreaks were evaluated for genetic variation to develop epidemiologically relevant interpretation guidelines for *Salmonella* disease cluster detection. The Illumina sequences were analyzed by core genome multi-locus sequence typing (cgMLST) and screened for antimicrobial resistance (AR) determinants and plasmids. Ninety-three of the one hundred outbreaks exhibited a close allele range (less than 10 allele differences with a subset closer than 5). The remaining seven outbreaks showed increased variation, of which three were considered polyclonal. A total of 16 and 28 outbreaks, respectively, showed variations in the AR and plasmid profiles. The serovars Newport and I 4,[5],12:i:-, as well as the zoonotic and poultry product vehicles, were overrepresented among the outbreaks, showing increased variation. A close allele range in cgMLST profiles can be considered a reliable proxy for epidemiological relatedness for the vast majority of *S. enterica* outbreak investigations. Variations associated with mobile elements happen relatively frequently during outbreaks and could be reflective of changing selective pressures. ISSN: 20762607

Kaboudari A., Aliakbarlu J., Mehdizadeh T.

Interactive effects of osmotic, acid, heat, cold, and freezing stresses on the biofilm formation ability of Salmonella serotypes

(2024) *Journal of Food Safety*, 44 (4), art. no. e13156

ABSTRACT: Food-related stresses such as heating and freezing may influence the biofilm formation ability of bacteria. This study aimed to investigate the main and interactive effects of food-related stresses on the biofilm formation potential of *Salmonella* strains isolated from meat. *Salmonella enteritidis*, *Salmonella typhi*, and *Salmonella typhimurium* were subjected to osmotic, acid, heat, cold, and freezing stresses. The colorimetric microtiter plate method was used to measure the biofilm formation ability as a response to the stresses. Among the main effects, freezing time had the most significant effect on the biofilm formation responses of three *Salmonella* serovars. Freezing reduced the biofilm formation ability of *Salmonella typhimurium* and *Salmonella enteritidis* but increased that of *Salmonella typhi* ($p < 0.05$). Among the interaction effects, the most significant effect on the biofilm formation response of *Salmonella typhi* was the interaction between pH and heat, which had a negative effect on the biofilm formation response. In contrast, the interaction between osmotic pressure and cold stresses was the most significant interactive effect on the biofilm formation responses of *Salmonella enteritidis* and *Salmonella typhimurium*, which had an increasing effect. This study concluded that the food-related stresses could change the biofilm formation capacity of *Salmonella* serotypes, and each serotype might show different biofilm formation abilities in response to different stresses. ISSN: 01496085

Omac B.

Modeling the growth behavior of Salmonella spp. in grated carrots inoculated with different inoculum levels stored at various temperatures

(2024) *Journal of Food Safety*, 44 (4), art. no. e13150

ABSTRACT: The consumption of fresh and fresh-cut fruits and vegetables, such as carrots, has increased for the last decades for a healthy life and an adequate diet, but concerns regarding the microbial safety of them have been raised. The present study was conducted to develop predictive models for *Salmonella* spp. in grated carrots. The results showed that *Salmonella* spp. did not display growth at 5°C, but it grew in grated carrots at other temperatures (10, 15, 20, 25, and 37°C) for both inoculum levels. Also, the inoculum levels affected the growth of this pathogen in grated carrots when the storage temperatures ranged from 15 to 25°C. The theoretical minimum temperatures calculated using the Huang model were 3.48 and 5.79°C for inoculum levels of 10¹ and 10² CFU/g, respectively. The primary and secondary models performed well in terms of agreement between experimental and estimated values. Furthermore, compared to the Ratkowsky model, the theoretical minimum temperature was given a more reasonable value using the Huang model. The models developed in the present study will be a useful input for future quantitative microbial risk assessment to appraise the proliferation of *Salmonella* spp. in grated carrots throughout the production process, storage, and distribution. ISSN: 01496085

Conrady B., Dervic E.H., Klimek P., Pedersen L., Reimert M.M., Rasmussen P., Apenteng O.O., Nielsen L.R.

Social network analysis reveals the failure of between-farm movement restrictions to reduce Salmonella transmission

(2024) *Journal of Dairy Science*, 107 (9), pp. 6930 - 6944

ABSTRACT: An increasing number of countries are investigating options to stop the spread of the emerging zoonotic infection *Salmonella* Dublin (S. Dublin), which mainly spreads among bovines and with cattle manure. Detailed surveillance and cattle movement data from an 11-yr period in Denmark provided an opportunity to gain new knowledge for mitigation options through a combined social network and simulation modeling approach. The analysis revealed similar network trends for noninfected and infected cattle farms despite stringent cattle movement restrictions imposed on infected farms in the national control program. The strongest predictive factor for farms becoming infected was their cattle movement activities in the previous month, with twice the effect of local transmission. The simulation model indicated an endemic S. Dublin occurrence, with peaks in outbreak probabilities and sizes around observed cattle movement activities. Therefore, pre- and postmovement measures within a 1-mo time window may help reduce S. Dublin spread. ISSN: 00220302

Hofer K., Trockenbacher B., Sodoma E., Khol J.L., Dünser M., Wittek T.

Establishing a surveillance programme for Salmonella Dublin in Austrian dairy herds by comparing herd-level vs. individual animal detection methods (2024) Preventive Veterinary Medicine, 230, art. no. 106277

ABSTRACT: Due to its increasing occurrence in cattle farms in various countries, leading to significant economic losses in affected livestock, *Salmonella enterica* subspecies *enterica* serovar Dublin (*S. Dublin*) has become a highly investigated pathogen in cattle production. In Austria, there have been occasional human cases of *S. Dublin* as well as an increase in laboratory-confirmed cases in cattle, indicating the need for a screening programme to determine the current status in Austria. The aims of this study were, firstly, to determine the seroprevalence of *S. Dublin* in dairy herds through bulk milk screenings in two federal states (Salzburg, Tyrol) of Austria. Secondly, the study aimed to identify the infection status of the herds through individual animal and herd level detection, comparing microbiological, molecular and serological detection methods. The results of the study will allow the development of a sampling strategy for a surveillance programme in Austria. A total of 6973 dairy farms were tested through serological bulk milk screening. The seroprevalence for the federal state of Tyrol was 14.8 % and for Salzburg it was 18.2 %, resulting in an average seroprevalence of 16.5 %. At an individual animal level, 205 (11.3 %) animals tested positive for shedding of *S. Dublin* in the faeces through microbiological detection, and 268 (17.0 %) animals had positive values (ct value ≤ 38) by qPCR. The association between microbiological and molecular detection was statistically significant ($p < 0.001$), with a calculated kappa value of 0.65 ± 0.27 ($p \leq 0.001$), assuming a substantial level of agreement. In 17 herds, where an individual animal tested positive for shedding of *S. Dublin*, environmental sampling and testing were carried out. At a herd level 16 (94.1 %) out of the 17 participating herds, tested positive for *S. Dublin* either microbiologically or by molecular assay in boot swab samples. Bulk milk samples from 14 out of the 17 participating herds were analysed for antibodies to *S. Dublin* and 12 samples (85.7 %) were positive. In total 111 (18.9 %) out of 587 blood samples tested positive for *S. Dublin* antibodies, demonstrating a statistically significant correlation ($p < 0.001$) both with microbiological ($\kappa = 0.32 \pm 0.49$; $p \leq 0.001$) and molecular ($\kappa = 0.23 \pm 0.06$; $p \leq 0.001$) findings. It was possible to identify *S. Dublin* by culture from boot swabs in 14 (82.4 %) out of 17 herds and by molecular assay using qPCR in 15 (88.2 %) out of 17 herds, indicating a suitable sample type for screening on a herd level-basis for acute infections, but not for identifying chronic infections or asymptomatic carriers. Other environmental samples, such as sponge-sticks, are only suitable to a limited extent for the detection of *S. Dublin*. The results of this study demonstrate a moderate *S. Dublin* prevalence in dairy herds in the selected Austrian regions, signalling further screening and management programmes for the future. ISSN: 01675877

Zhao X., Jacxsens L., Tzeneva V., Kokken M., Winkler A., Vadier C., de Toledo N., Seliworstow T., Uyttendaele M.

Salmonella Prevalence in Raw Cocoa Beans and a Microbiological Risk Assessment to Evaluate the Impact of Cocoa Liquor Processing on the Reduction of Salmonella (2024) Journal of Food Protection, 87 (9), art. no. 100327

ABSTRACT: *Salmonella* in raw cocoa beans ($n = 870$) from main sourcing areas over nine months was analyzed. It was detected in 71 (ca. 8.2%) samples, with a contamination level of 0.3–46 MPN/g except for one sample (4.1×10^4 CFU/g). Using prevalence and concentration data as input, the impact of thermal treatment in cocoa processing on the risk estimate of acquiring salmonellosis by a random Belgian chocolate consumer was calculated by a quantitative microbiological risk assessment (QMRA) approach. A modular process risk model from raw cocoa beans to cocoa liquor up to a hypothetical final product (70–90% dark chocolate tablet) was set up to understand changes in *Salmonella* concentrations following the production process. Different thermal treatments during bean or nib steam, nib roasting, or liquor sterilization (achieving a 0–6 log reduction of *Salmonella*) were simulated. Based on the generic FAO/WHO *Salmonella* dose–response model and the chocolate consumption data in Belgium, salmonellosis risk per serving and cases per year at population level were estimated. When a 5 log reduction of *Salmonella* was achieved, the estimated mean risk per serving was 3.35×10^{-8} (95% CI: 3.27×10^{-10} – 1.59×10^{-7}), and estimated salmonellosis cases per year (11.7 million population) was 88 (95% CI: <1–418). The estimated mean risk per serving was 3.35×10^{-9} (95% CI: 3.27×10^{-11} – 1.59×10^{-8}), and the estimated salmonellosis cases per year was 9 (95% CI: <1–42), for a 6 log reduction. The current QMRA model solely considered *Salmonella* reduction in a single-step thermal treatment in the cocoa process. Inactivation obtained during other process steps (e.g. grinding) might occur but was not considered. As the purpose was to use QMRA as a tool to evaluate the log reduction in the cocoa processing, no postcontamination from the processing environment and ingredients

was included. A minimum of 5 log reduction of *Salmonella* in the single-step thermal treatment of cocoa process was considered to be adequate. ISSN: 0362028X

Ismael B., Wilson M., Miller D., Pietri J.E.

Differences in Salmonella Typhimurium infection and excretion among laboratory and field strains of the German cockroach suggest a genomic basis for vector competence (2024) Infection, Genetics and Evolution, 123, art. no. 105624

ABSTRACT: The German cockroach, *Blattella germanica*, can be a vector of human enteric bacterial pathogens, including *Salmonella enterica* serovar Typhimurium (*S. Typhimurium*). Transmission of such pathogens by cockroaches has largely been considered a passive mechanical process, but recent studies have argued against this dogma by demonstrating bacterial proliferation within the cockroach gut and the necessity of specific bacterial genes for successful transmission in the feces, revealing unappreciated biological complexity in the vector-pathogen relationship between cockroaches and *S. Typhimurium*. However, the influence of naturally occurring variation among cockroach populations on pathogen infection and dissemination has not been investigated. Thus, this study aimed to examine whether distinct strains of *B. germanica* exhibit differences in their ability to become infected by and disseminate *S. Typhimurium*. We performed controlled infections of one long-term laboratory strain and three recently field-collected strains reared under identical conditions, then compared bacterial loads in the body and excreta of individual insects. Separately, we also compared rates of necrophagy, a behavior known to contribute to the horizontal spread of *S. Typhimurium* among cockroaches. Our data show significant differences in infection susceptibility, pathogen shedding in the excreta, and necrophagy between laboratory and field strains as well as between some field strains. These observations represent the first evidence that genomic variation among cockroach populations may influence their ability to become infected by and disseminate pathogens, providing further support for the hypothesis that German cockroaches are active biological vectors rather than passive mechanical vectors of *S. Typhimurium*. Additional studies are needed to identify the genomic drivers of vector competence for *S. Typhimurium* in *B. germanica*. ISSN: 15671348