

NEWSLETTER

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Editorial Note

Bilthoven, 1 April 2025

Dear colleagues,

Recently we were informed that the **Grant Agreement for EURL-*Salmonella* 2025-2027** has been signed by both HaDEA and by the Director of our institute (RIVM). That is good news, meaning that we can continue with our activities for the coming 3 years.

Unfortunately, soon after starting the activities for the new period, we had to update the **planning of the EURL-*Salmonella* activities for 2025**. In February we informed you by e-mail about this updated planning and the same information is given below. We had to introduce these changes in the planning due to the fact that the move of our institute to the new building in Utrecht has been delayed for several months. This move was originally foreseen to start in April/May this year, but is now planned for autumn 2025. We foresee a very busy time for the relocation activities from September onwards, which influences the original planning of the EURL-*Salmonella* activities for the second half of this year. For that reason we had to reconsider the planning of the workshop and of two Proficiency Tests (PTs) for this year. I am sorry that this new planning will ask some flexibility from all of you. We also would have preferred to stick to the original planning, but unfortunately this is impossible for this year. An overview of the updated planning of the EURL-*Salmonella* activities for 2025 is given in the table published in this Newsletter on page 6 (with the new dates indicated in blue). Some details:

- **EURL-*Salmonella* workshop:** For this year it was already foreseen to organise the workshop as a fully online meeting, this remains unchanged. To suit the original planning of the moving in May, the workshop was postponed from May to autumn this year. However, now the relocation is postponed, we go back to the month May for the workshop. Therefore, we have now set the date for this years' workshop on Tuesday **20 May 2025**. Early March we have sent you a link for registration to the workshop, with a deadline by mid-April 2025. Soon we will prepare the draft programme of the workshop and if you still want to make suggestions for one or more presentations, please do so before mid-April.
- **EURL-*Salmonella* PT Primary Production Stage (PPS):** Instead of September/October, we will bring forward this PT and organise it in June this year. More details can be found in the time table enclosed in this Newsletter on page 7.
- **EURL-*Salmonella* PT Typing:** Instead of November/December, we will also bring forward this PT and organise it in the period August-October this year. More details can be found in the time table enclosed in this Newsletter on page 8. Additionally, to restrict the workload for us and for the NRLs, this years' PT will not contain an optional part on cluster analyses, but only the obligatory part on serotyping of *Salmonella*.

Also in view of the move of our institute, we organised this years' first PT a little bit earlier. The **combined Food-Feed PT 2025** was organised in February/March and the deadline for reporting the results of this PT was 25 March. Soon we will start the analyses of the results.

In November 2024 we organised the **2024 PT on typing of *Salmonella***, containing an obligatory part on serotyping of *Salmonella*, and a voluntary part on cluster analysis (NGS only). The evaluation of the serotyping results was performed in January/February and by the end of February 2025, the participants

received their own results as well as the interim summary report with the results of all participants. This interim summary report is also available at the EURL-*Salmonella* website: <https://www.eurilsalmonella.eu/en/documenten/interim-summary-report-eurl-salmonella-pt-serotyping-2024>. One participant did not meet the level of good performance and discussions are ongoing for organizing a dedicated training for this NRL. The results of the optional part on cluster analysis of this PT are under evaluation and will be reported separately in the coming months.

Not changed in the planning is the organization of the **joint EURLs training course on NGS**. This training is still foreseen in June 2025. The training is organised at the premises of EURL-Coagulase Positive Staphylococci and EURL-*Listeria monocytogenes*. The registration for this training was closed by mid-March 2025 and the selected candidates are informed early April 2025.

In February 2025, the **new EU Regulation related to the generation and sharing of WGS data during foodborne outbreaks** (EU) 2025/179 was published and the NRLs-*Salmonella* were informed accordingly. This new Regulation can be found via the following link: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ:L_202500179. By the end of March 2025, the EC and EFSA published Frequently Asked Questions (FAQs) on the use of Whole Genome Sequencing (WGS) within the framework of foodborne outbreak investigations, related to the recently published Regulation (EU) 2025/179. See: https://food.ec.europa.eu/document/download/bb6b197a-cce2-4ebf-aca2-ebb6696c803f_en

As you may know, on 23-25 June 2025, the 10th edition of the **International Symposium *Salmonella* and Salmonellosis (I3S)** will be organised in Saint-Malo, France. It is still possible to register to this symposium. For more details see: <https://i3scongress.innozh.fr/>

Concerning the ISO/CEN activities, I can inform you that the final version of **EN ISO 6579-4** 'Identification of monophasic *Salmonella* Typhimurium by PCR', was published in February 2025. Due to copyrights we are not allowed to distribute this ISO document, but you can order it at your National Standardisation Organisation.

At the same time of publication of EN ISO 6579-4, the EURL-*Salmonella* published the report on validation of ISO 6579-4:

Diddens, R.E., van Hoek, A.H.A.M., Malorny, B, Fischer, J. and Mooijman, K.A., 2025. Validation of ISO 6579-4 for identification of monophasic *Salmonella* Typhimurium (1,4,[5],12:i:-) by polymerase chain reaction (PCR). RIVM report 2023-0430. National Institute for Public Health and the Environment, Bilthoven, the Netherlands. <https://www.rivm.nl/bibliotheek/rapporten/2023-0430.pdf>

Also in February 2025, the following EURL-*Salmonella* report was published:

Jacobs-Reitsma, W.F., Verbruggen, A., Diddens, R.E., van Hoek, A.H.A.M., Mooijman, K.A., 2025. EURL-*Salmonella* Proficiency Test Typing 2023. National Institute for Public Health and the Environment, Bilthoven, the Netherlands. RIVM report no.: 2024-024. <https://www.rivm.nl/bibliotheek/rapporten/2024-0024.pdf>

Best wishes,
Kirsten Mooijman
Coordinator EURL-*Salmonella*

Contribution of the EURL-*Salmonella*

Updated EURL- *Salmonella* activities foreseen in 2025

New dates in blue

Date(s)	Activity
24 February – 25 March 2025	EURL- <i>Salmonella</i> combined Proficiency Test Food-Feed 2025; Detection of <i>Salmonella</i> in flaxseed
20 May 2025	Online workshop
2 June – 4 July 2025	EURL- <i>Salmonella</i> Proficiency Test Primary Production Stage (PPS)
17, 18 June 2025	Joint Training Course of the inter EURLs Working Group on NGS in France.
August – October 2025	EURL- <i>Salmonella</i> Proficiency Test Serotyping

Timetable EURL-*Salmonella* Proficiency Test Primary Production Stage (PPS) 2025; Detection of *Salmonella* in chicken faeces samples

Week	Date	Subject
10 - 15		E-mailing the link to the registration form for the Proficiency Test. Please register 11 April 2025 at the latest.
21	Week of 19 May 2025	E-mailing the protocol and the link for the result form, as well as instructions for completing the result form to the participants. Preparation of media by the participants.
23	Monday 2 June 2025	Shipment of the parcels to the participants as Biological Substance Category B (UN3373).
23 - 24	Immediately after receipt of the parcel and at the latest on 10 June 2025	Start performance of the PT PPS.
27	Friday 4 July 2025 at the latest	Deadline for completing the result form: 4 July 2025 (23:59h CET) After this deadline the result form will be closed.
	Fall 2025	Results tabulation and performance summary

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

Robin Diddens

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Timetable EURL-*Salmonella* Proficiency Test Serotyping 2025

Note that this PT Typing 2025 does not contain an optional part on cluster analyses, but only the obligatory part on serotyping of *Salmonella*.

Week	Date	Subject
25	Week of 16 June	Emailing of the link to the registration form for the PT Serotyping. Please register by 18 July 2025 at the latest.
31	Week of 28 July	Emailing of the protocol 2025.
33	Monday 11 August 2025	Shipment of the parcels to the participants as Biological Substance Category B (UN 3373).
33	Week of 11 August	<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.
40	Friday 3 October 2025 at the latest	Deadline for completing the electronic submission of serotyping results: 3 October 2025. After this deadline, the result form will be closed.
	November/December 2025	Evaluation of individual laboratory results and Interim summary report.

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

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From the Literature

Salmonella-related Literature selection from Scopus: January – March 2025

Ricchi M., Filippi A., Scaltriti E., Tambassi M., Pongolini S., Bolzoni L., Prosperi A., Torreggiani C., Cammi M., Chiatante A., Arrigoni N., Massella E., Luppi A., Garbarino C.

Outbreak of Salmonella enterica subsp. enterica Serovar Napoli on a Dairy Cow Farm (2025) Animals, 15 (1), art. no. 79

ABSTRACT: Salmonella is diffused worldwide, and Salmonella enterica subsp. enterica is spread worldwide with many serovars associated with the infection of domestic bovines. The most spread are S. Dublin, S. Typhimurium and S. Infantis. S. Napoli is, however very rarely reported in domestic ruminants. Here, we report an outbreak of S. Napoli on a dairy cow farm in Northern Italy (Piacenza). A total of 18 S. Napoli isolates were recovered from aborted fetuses, feces, tissues and environmental samples. Whole genome sequencing suggested that all isolates belonged to the same cluster. After the application of stringent biocontainment and biosecurity measures, no further cases were reported. However, four months after the first case, the serovar was still isolated in environmental samples, underlying the importance of adopting the correct biosecurity and biocontainment measures in order to prevent the circulation and transmission of Salmonella within the farm. ISSN: 20762615

Manzaneres-Pedrosa A., Ayats T., Antilles N., Sabaté S., Planell R., González R., Montalvo T., Cerdà-Cuéllar M.

Urban yellow-legged gull (Larus michahellis) and peri-urban Audouin's gull (Larus audouinii) as a source of Campylobacter and Salmonella of public health relevance (2025) Science of the Total Environment, 960, art. no. 178227

ABSTRACT: Campylobacter spp. and Salmonella spp. are the leading cause of human enteric infections in the European Union. Some gull species act as reservoirs and play an important role in the epidemiology of these zoonotic agents. To gain insight into Campylobacter and Salmonella epidemiology we studied colonies of Audouin's gull (*Larus audouinii*) and yellow-legged gull (*Larus michahellis*) in Barcelona metropolitan area, Catalonia (north-eastern Spain). We assessed the occurrence, genetic diversity, virulence potential, and antimicrobial susceptibility of Campylobacter and Salmonella isolates recovered from gull faeces in different time periods within 2009–2018. The occurrence of Campylobacter was higher compared to Salmonella in both gull species. Also, the occurrence of both pathogens was significantly higher in Audouin's gull (45 % for Campylobacter, 20 % for Salmonella), than in yellow-legged gull (13 % and 7 %, respectively). All but one individual carried *C. jejuni*; the remaining positive yellow-legged gull carried *C. lari*. Salmonella serovar Typhimurium (including its monophasic variant) was the most frequent in both hosts followed by ser. Bredeney. Other serovars frequently associated with human salmonellosis (*Infantis*, *London*, *Virchow*) were only isolated from yellow-legged gulls. Multilocus Sequence Typing analyses showed that yellow-legged gull and not Audouin's gull carried several Campylobacter genotypes associated with human enteritis. Campylobacter isolates from both gull species revealed a high virulence potential, as opposed to Salmonella isolates which showed a lower prevalence of virulence-associated genes, particularly in Audouin's gull. Overall, a moderate to high frequency of antimicrobial resistance (including multidrug resistance) was found in both pathogens from both gull species. Campylobacter and Salmonella from yellow-legged gull showed a higher frequency of isolates resistant to antimicrobials of relevance in human medicine. Overall, our results highlight the potential public health threat associated with these gull species, particularly yellow-legged gull, in densely human populated areas. ISSN: 00489697

Vice Z., Zhou Y., Chitlapilly Dass S., Wang R.

Microscopic Analysis of Temperature Effects on Surface Colonization and Biofilm Morphology of Salmonella enterica (2025) Foods, 14 (2), art. no. 268

ABSTRACT: Salmonella enterica represents a diverse group of pathogens commonly associated with food contamination including red meat. Even though pre- and post-harvest cleaning and sanitization procedures are widely implemented at meat processing plants to mitigate the hazard, *S. enterica* cells may escape the process by colonizing, on contact, surfaces in the form of a biofilm that functions as an aggregated microbial community to facilitate mutual protection, antimicrobial resistance, proliferation and dissemination.

Biofilm development is a complex process that can be affected by a variety of factors including environmental temperature. We developed methods using scanning electron microscopy and confocal microscopy with a novel image analysis software tool to investigate the temperature influence on *S. enterica* cell colonization and biofilm formation by directly visualizing and comparing the biofilm matrix's morphological differences under various temperatures. Cocktails of *S. enterica* strains belonging to serovars, commonly isolated from meat samples, were applied to develop biofilms on a stainless steel surface at 7, 15, or 37 °C. Results of the microscopy analysis showed that as temperature increased, better-defined biofilm structures with extracellular polymeric structures (EPS) could be identified. However, *S. enterica* colonization and aggregated bacterial biomass were clearly observed at the low temperature (7 °C) as well. These results demonstrate that the environmental temperature significantly contributes to *S. enterica* biofilm formation as the higher temperatures encourage bacterial active proliferation and biofilm maturation leading to the development of well-pronounced structures, while the lower temperature may promote cell attachment but, meanwhile, limit the EPS biosynthesis and biofilm maturation. Our study indicates that the mature *S. enterica* biofilms formed under favorable conditions may protect the pathogens with the well-developed 3-dimensional (3D) structure against routine treatment. Furthermore, the low temperatures commonly maintained at meat plants are not able to effectively prevent *S. enterica* colonization and biofilm formation since at such temperatures there could still be colonized biomass that can contaminate the products. Therefore, the temperature effect on pathogen colonization and biofilm development should be taken into consideration while evaluating hygiene standards and sanitization procedures at the processing facilities. ISSN: 23048158

Lin Z., He S., Liang Z., Li D.

Temperature cycling between 4 °C and 37 °C could reduce Salmonella viability in low-moisture foods

(2025) *International Journal of Food Microbiology*, 428, art. no. 110995

ABSTRACT: Low-moisture foods (LMFs) have been linked to Salmonella transmission due to the remarkable resilience of Salmonella against desiccation, allowing its survival for extended periods. Being metabolically inactive, Salmonella in LMFs exhibit extraordinary resistance to inactivation treatments. This study proposes a novel strategy for mitigating Salmonella in LMF products through a temperature cycling (TC) approach. Alternating the temperature between 4 °C and 37 °C on a daily basis reduced the viability of *S. Typhimurium* air-dried on surfaces by >4 log after 6 days. TC also diminished Salmonella resistance to acidity and reduced its virulence. The mechanism was elucidated through an integrated analysis of transcriptomics and proteomics data. Specifically, transcriptomic data revealed elevated levels of protein synthesis alongside active energy metabolism. Proteomic analysis demonstrated that these protein activities were associated primarily with the heat shock protein response. Taken together, the principal mechanism by which TC exerts its inhibitory effect appears to be the repeated induction of heat shock protein synthesis within Salmonella, ultimately leading to energy depletion. Finally, the efficacy of TC was validated on representative LMF samples, including flour, protein powder, and mixed spices. The most notable effect was observed in the mixed spices, with a reduction of 2.7 ± 0.2 log after 6 days ($P < 0.05$). In conclusion, the TC approach demonstrated in this study provides valuable insights into the management of foodborne pathogens in LMFs. ISSN: 01681605

Felix M.A., Han J., Khajanchi B.K., Sanad Y.M., Zhao S., Foley S.L.

Salmonella enterica serovar Schwarzengrund: Distribution, Virulence, and Antimicrobial Resistance

(2025) *Microorganisms*, 13 (1), art. no. 92

ABSTRACT: The global incidence of Salmonella enterica serovar Schwarzengrund has risen in recent years. This serotype has been isolated from poultry, retail meat, and other food products, leading to multiple outbreaks. Alongside the increase in infections, there are growing concerns about the increasing levels of antimicrobial resistance (AMR) among *S. Schwarzengrund* strains. This study aims to better understand the genetic factors possibly contributing to the rising prevalence of *S. Schwarzengrund* by analyzing the sequences of 2058 isolates from both human patients (N = 313) and food- and animal-associated sources, including chicken (N = 1145), turkey (N = 300), pork (N = 132), and other sources (N = 168). Data were obtained from GenBank and analyzed for AMR genes using AMRFinder. Additionally, putative virulence genes and plasmid transfer genes were assessed using the Virulence and AMR Plasmid Transfer Factor Database. AMR genes were found in 1269 (61.7%) of the isolates, with a total of 2478 AMR genes among the isolates, the most common being *aph(3'')-Ib* (N = 969, 47.1%), *tet(A)* (N = 190, 9.2%), and *sul2* (N = 150, 7.3%), which are responsible for resistance to aminoglycoside, tetracycline, and

sulfonamide, respectively. Additionally, 1060 (51.5%) isolates carried multiple plasmid transfer genes associated with IncFIB-FIC(FII) plasmids. Other plasmid types found in at least 1% of the strains included IncI1 (N = 101, 4.9%), IncHI2 (N = 62, 3.0%), or IncHI1 (N = 24, 1.2%). The virulence gene profiles of human isolates showed diversity but largely overlapped with those from different food sources. Notably, the aerobactin iron acquisition genes, associated with *Salmonella*'s virulence and colonization, were highly prevalent among chicken isolates (N = 1019, 89.0%) but less frequent in isolates from other sources (N = 65, 7.2%). IncFIB-FIC(FII) plasmids, commonly harboring the aerobactin operon, were highly prevalent among chicken-related isolates and present in about 10% of human isolates. The diverse plasmid, AMR, and virulence gene profiles in human-associated isolates suggest that multiple factors may contribute to the increased virulence in *S. Schwarzengrund*. ISSN: 20762607

Vinha M.B., Moro L.B., Lima I.D.M., Nascimento M.D.S.D., Pires G.P., de Oliveira J.P., Cassini S.T.A.

Salmonella in black pepper (Piper nigrum): From farm to processing
(2025) *International Journal of Food Microbiology*, 426, art. no. 110921

ABSTRACT: Contamination of black pepper (*Piper nigrum*) with *Salmonella* is a frequent problem in retail and imported shipments. However, there is scarce information about the prevalence of the pathogen in the initial stages of black pepper production chain. This study sought to bridge this gap in research by determining the prevalence, as well as quantifying, and identifying the main *Salmonella* serovars present during black pepper primary production and processing. Black pepper (233) and environmental (175) samples were collected from farms (354) and processing plants (54) in Espirito Santo, Brazil. The pathogen was detected in soil (16.7 %), drying waste (20.4 %), fallen berries (3.7 %), threshed berries (14.3 %), and dried peppercorns (22.2 %) collected from farms. *Salmonella* was also detected in samples of raw material (11.1 %), export products (16.7 %), and processing waste (16.7 %) collected from processing plants. A total of 12 serotypes were identified, and *Salmonella* Javiana showed the highest prevalence (38.8 %). According to the results, contamination occurring in the post-harvest phase is not eliminated or reduced during processing. Therefore, the adoption of good agricultural and manufacturing practices, supported by hazard analysis and critical control points (HACCP), is crucial to mitigate this kind of contamination. These practices should be combined with decontamination treatments to ensure the safety of the final product. ISSN: 01681605

Mohamed M.-Y.I., Khalifa H.O., Habib I.

Food Pathways of Salmonella and Its Ability to Cause Gastroenteritis in North Africa
(2025) *Foods*, 14 (2), art. no. 253

ABSTRACT: Infections caused by human pathogenic bacteria in food sources pose significant and widespread concerns, leading to substantial economic losses and adverse impacts on public health. This review seeks to shed light on the recent literature addressing the prevalence of *Salmonella* in the food supply chains of North African countries. Additionally, it aims to provide an overview of the available information regarding health-related concerns, such as virulence genes, and the presence of antibiotic resistance in *Salmonella*. This review highlights a gap in our comprehensive understanding of *Salmonella* prevalence in the food supply chains of North African nations, with limited molecular characterization efforts to identify its sources. Studies at the molecular level across the region have shown the diversity of *Salmonella* strains and their virulence profiles, thus, these results show the difficulty of controlling *Salmonella* infections in the region. In addition, the discussion of antibiotic resistance makes it clear that there is a need for the development of comprehensive strategies to fight the potential threat of antimicrobial resistance in *Salmonella* strains. Despite common reports on animal-derived foods in this region, this review underscores the persistent challenges that *Salmonella* may pose to food safety and public health in North African countries. ISSN: 23048158

Kiplagat E., Ramezani M., Malla S., Cisneros-Zevallos L., Joshi V., Castillo A.

Factors Affecting Growth and Survival of Salmonella in Onion Extracts and Onion Bulbs
(2025) *Foods*, 14 (1), art. no. 1

ABSTRACT: This study investigated the survival and growth of *Salmonella* in onion extracts and bulbs. The inhibition or retardation of *Salmonella* growth by extracts of red, white, and yellow onions was tested against the onion germplasm and exposure to different light spectra during curing. Separately, survival of *Salmonella* Newport was tested on red, white, and yellow onion bulbs on the external and internal onion layers with a syringe and needle. Onions exposed to blue, red, and white LED light during curing produced extracts with variable antimicrobial effects ($p < 0.05$), with those exposed to blue light showing the strongest inhibitory effect on red and white onions only. In survival studies, *Salmonella*

inoculated on the outer scale was reduced by 1.2, >2.7, and >2.4 logs on red, white, and yellow onions, respectively, within 3 days, whereas it grew by 2.4, 2.6, and 2.8 logs inside red, white, and yellow onion bulbs, respectively, over 18 days. In separate trials, the outer layer again did not support the survival of *Salmonella* Newport. The aw increased significantly from 0.51 to 0.58 in the outer scales and 0.96 to 0.98 for the fourth inner scales. Despite being rich in antimicrobial polyphenols, tissue integrity and water content may still promote *Salmonella* growth in onions. ISSN: 23048158

Alves J.M., Silva F.A., Silveira D.R., Massaut K.B., Fiorentini Â.M., Lopes G.V., Magnani M.

Understanding the potential of fresh produce as vehicles of Salmonella enterica
(2025) *Advances in Food and Nutrition Research*, 113, pp. 133 - 180

ABSTRACT: This chapter presents an overview of *Salmonella enterica* as a contaminant in fresh produce, exploring outbreaks and recalls linked to them. It also provides information on potential sources of *S. enterica* contamination throughout the entire production chain of these products and presents food safety tools and new approaches for controlling this pathogen. *S. enterica* is recognized worldwide as a pathogen responsible for foodborne outbreaks, and there has been an increase in reported cases of salmonellosis linked to fresh produce. These products are susceptible to contamination throughout various stages of the farm-to-fork process. The potential sources of contamination are present from pre-harvest and harvest stages (e.g., soil, blossoms, seeds, irrigation water and gray/blackwater, wild and domestic animals/organic fertilizers, and distinctive traits of the plant) to post-harvest stages (e.g., processing, packaging, storage/retail, and preparing for consumption). Thus, controlling *S. enterica* contamination is extremely important for ensuring the safe consumption of fresh produce. However, obtaining practical, efficient, low-cost, and sustainable solutions that ensure the products' sensorial, nutritional, and food quality is still a challenge. As an alternative to conventional methods, recent studies report the use of new technologies, such as neutral, acidic or low chlorine electrolyzed oxidizing water, ultraviolet light, ultrasound, microemulsion of essential oils, cold plasma, irradiation, bacteriophages, and other methods, which can be used alone or in combination with the conventional ones. Therefore, understanding the main sources of *S. enterica* contamination in fresh produce and the effective approach for controlling this pathogen is crucial to reducing future outbreaks. ISSN: 10434526, ISBN: 978-044315890-2

Panera-Martínez S., Rodríguez-Melcón C., Del Campo C., Alonso-Calleja C., Capita R.

Prevalence and levels of cells of Salmonella spp. and Listeria monocytogenes in various physiological states naturally present in chicken meat
(2025) *Food Control*, 167, art. no. 110770

ABSTRACT: A determination was made of the prevalence and concentrations of cells of *Salmonella* spp. and *Listeria monocytogenes* in varying physiological states, whether viable, viable and culturable (VC), viable but not culturable (VBNC), or inactivated, in 52 poultry carcasses obtained from two slaughterhouses in North-Western Spain. For this purpose, a combination of culture-dependent methods (ISO 6579-1:2017 and ISO 11290-1:2018 norms for *Salmonella* and *L. monocytogenes*, respectively), with a quantitative real-time polymerase chain reaction (q-PCR) technique, was used. In order to detect viable cells, a q-PCR with addition of the viability marker propidium monoazide (v-PCR) was performed. *Salmonella* cells were detected in 22 carcasses (42.3% of total), in all instances by q-PCR (in 5 carcasses *Salmonella* was also detected by v-PCR). The average levels, expressed as log₁₀ CFU/g, of total, VBNC and inactivated *Salmonella* cells were 4.11, 3.55, and 3.97, respectively. *L. monocytogenes* was found in 39 samples (75.0% of total), in 10 (19.2%) by plating methods, in 13 (25.0%) through q-PCR and in 16 (30.8%) by both techniques. In 16 carcasses (30.8%) *L. monocytogenes* was detected by v-PCR. Average levels (log₁₀ CFU/g) of *L. monocytogenes* were 4.01 for total cells, 3.21 for viable, 1.00 for VC, 3.20 for VBNC, and 3.93 for inactivated. The presence of cells in the VBNC state (27.70% of total cells and 99.72% of viable cells in the case of *Salmonella*, and 15.66% of total cells and 99.38% of viable cells in the case of *L. monocytogenes*), with the potential for causing human diseases, highlights the importance of combining culture-dependent and culture-independent methods in investigating *Salmonella* and *L. monocytogenes* in poultry meat. ISSN: 09567135

Tambassi M., Berni M., Bracchi C., Menozzi I., Dodi A., Mazzera L., Morganti M., Scaltriti E., Pongolini S.

Salmonella pathogenicity Island 1 undergoes decay in serovars adapted to swine and poultry
(2025) *Microbiology Spectrum*, 13 (1)

ABSTRACT: Human salmonellosis is a high-priority foodborne disease worldwide. The main reservoir of Salmonella is livestock, mainly swine and poultry that are infected both by generalist serovars and serovars adapted to them. The most widespread livestock-adapted serovars are attenuated in both their primary hosts and humans. We previously identified a lineage of the swine-adapted Salmonella Derby carrying mutations in Salmonella Pathogenicity Island-1 (SPI-1) giving attenuation. To evaluate if SPI-1 decay is a general feature of swine-adapted serovars, we analyzed the Enterobase global population of Salmonella serovars most frequently isolated from swine. We found that deleterious mutations in SPI-1 are accumulated more in swine-adapted (S. Derby and Salmonella Rissen) than in generalist (Salmonella Typhimurium, I 1,4,[5],12:i:-, and Salmonella Infantis) serovars. The genomes carrying such mutations are widespread in the population of S. Derby and S. Rissen, indicating that the occurrence and fixation of deleterious mutations in SPI-1 are frequent. Similarly, also poultry-adapted Salmonella Kentucky has accumulated deleterious mutations in SPI-1, leading to over 70% of mutated genomes of this serovar compared with negligible proportions of mutated genomes in the generalist serovars from poultry. Although in vitro attenuation of S. Derby carrying mutated SPI-1 had been previously demonstrated, we showed the same phenotype for mutated S. Rissen. Considering also the known attenuation of S. Kentucky, our population-scale findings provide evidence of progressive evolution toward the attenuation of serovars adapted to the main animal reservoirs of human salmonellosis through the food chain. Intensive farming with a high density of animals could be a possible driver of this evolution.

IMPORTANCE This study shows at the global population level of Salmonella that the main attenuated serovars adapted to food-producing animals are undergoing convergent evolution toward further attenuation through the decay of SPI-1, considered critically important for the intestinal phase of Salmonella infection. The drivers of this evolution are unknown, but they could be attributed, at least in part, to the intensive farming of livestock with its high densities. On one side, our results contribute to the knowledge of the interaction between livestock populations and their host-adapted serovars of Salmonella. On the other side, the study provides scientific background for reconsidering the microbiological criteria adopted by the food safety legislation of many countries that ask for the absence of Salmonella in foods, regardless of any virulence evaluation of the detected strain. In this respect, the study provides molecular bases to investigate the virulence of different lineages within these host-adapted serovars. ISSN: 21650497

Arrieta-Gisasola A., Martínez-Ballesteros I., Martínez-Malaxetxebarria I., Bikandi J., Laorden L.

Detection of mobile genetic elements conferring resistance to heavy metals in Salmonella 4,[5],12:i:- and Salmonella Typhimurium serovars and their association with antibiotic resistance

ABSTRACT: Salmonella enterica subsp. enterica serovar Typhimurium variant 4,[5],12:i:- (referred to as S. 4,[5],12:i:-) has emerged rapidly as the predominant Salmonella serovar in pigs, often associated with the acquisition of antibiotic resistance (ABR) and heavy metal resistance (HMR) genes. Our study analysed 78 strains of S. 4,[5],12:i:- (n = 57) and S. Typhimurium (n = 21), collected from 1999 to 2021, to investigate the evolution of mobile genetic elements (MGEs) containing HMR and ABR genes. Five MGEs harbouring HMR genes were identified: pUO-STVR2, pSTM45, pUO-STmRV1, SGI-4 and MREL. Among the strains, 91.23 % (52/57) of S. 4,[5],12:i:- carried at least one of these elements, compared to only 14.29 % (3/21) of S. Typhimurium. Since 2008, S. 4,[5],12:i:- have shifted from predominantly carrying pUO-STmRV1 to the emergence of SGI-4 and MREL, reducing ABR genes, reflecting the European Union ban on the use of antibiotics as feed additives. Increased resistance to copper and silver in S. 4,[5],12:i:-, conferred by SGI-4 and MREL, reflected that their acquisition was linked to the ongoing use of heavy metals in food-animal production. However, strains carrying SGI-4 and MREL still exhibit multidrug resistance, emphasising the need for targeted interventions to mitigate multidrug-resistant Salmonella spread in veterinary and public health settings. ISSN: 01681605

Nerney A., Reitz S., Kovacevic J., Waite-Cusic J.

Cross-contamination Risks in Dry Produce Packinghouses: Efficacy of Alcohol-based Sanitizers to Reduce Salmonella and Potential Surrogates on Relevant Surface Materials (2025) Journal of Food Protection, 88 (2), art. no. 100443

ABSTRACT: A 2020 Salmonella outbreak was epidemiologically linked to red onions; however, insufficient cleaning and sanitation in the packinghouse expanded the recall to include all onions handled by the packing house in the preceding 3 months. Our objective was to evaluate the efficacy of dry sanitizers to reduce cross-contamination risk on food contact surfaces (FCSs) found in postharvest packing areas. Transfer of Salmonella and potential surrogates (Escherichia coli, Enterococcus faecium) to and from onions to FCS

materials (high-density polypropylene, polyester-nylon conveyor belts, plywood) was quantified. Transfer rates from inoculated onions to FCS were highly variable, but did not differ by surface, averaging $-1.19 \log \%$. Transfer rates from contaminated FCS to uninoculated onions averaged $0.2 \log \%$, with 40% (31/81) of onions having no detectable transfer. Onion variety, surface type, and species did not influence the transfer rate ($p > 0.05$). Commercial sanitizers and alcohol solutions were tested for efficacy of reducing targeted bacteria on FCS. Reductions were quantified after 30 s and after drying (15 min). High alcohol sanitizers (~60%) achieved a 5-log reduction on HDPE after drying. Lower alcohol (<30%) products were ineffective (<2-log reduction). *E. coli* and *Salmonella* were comparable in sensitivity to sanitizers, but *E. faecium* was more sensitive ($p < 0.05$). Transfer and sanitizer evaluation was scaled to investigate cross-contamination in postharvest storage bins (plastic and wood). With no sanitation, inoculated plastic bin contact resulted in contamination of 49–71% of onions. Use of ~60% alcohol solutions reduced contamination to <4% of onions ($p < 0.05$). Low-alcohol product significantly increased cross-contamination to 86–100% of onions ($p < 0.05$). These findings highlight the importance of validating sanitizer efficacy to quantify microbial reduction and cross-contamination risk on produce contact surfaces. ISSN: 0362028X

Kenney S.M., M'ikanatha N.M., Ganda E.

Antimicrobial Resistance and Zoonotic Potential of Nontyphoidal Salmonella From Household Dogs

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ABSTRACT: Background: Companion animals, like household dogs, are an overlooked transmission point for zoonotic pathogens such as nontyphoidal *Salmonella* (NTS). Given the proximity of dogs to humans and the use of critically important antibiotics in companion animal medicine, household dogs represent a risk for the spread of antimicrobial-resistant (AMR) *Salmonella*. Methods and Results: To this end, we aimed to leverage existing biosurveillance infrastructure to investigate AMR and the zoonotic potential of NTS isolated from dogs and humans. We identified all NTS strains isolated from domestic dogs via the Veterinary Laboratory Investigation and Response Network between May 2017 and March 2023 ($N = 87$), and spatiotemporally matched strains isolated from humans in the NCBI Pathogen Isolate Browser ($N = 77$). These 164 strains, collected from 17 states in the United States, formed the basis of our analysis. Strains isolated from dogs comprised diverse serovars, with most being clinically relevant to human health. All strains possessed AMR determinants for drug classes deemed critically or highly important by the World Health Organization. We identified sixteen NTS isolates from humans closely related to ≥ 1 of six dog-associated strains. Conclusions: Collectively, our data emphasize the importance of antimicrobial stewardship and sustained biosurveillance beyond human- and agriculture-associated veterinary medicine, using a One-Health framework that accounts for all transmission points including companion animals. ISSN: 18631959

Draper A.D.K., Gerrell J., McKay S., Forrester J., Ordonez A., Meumann E., Baird R., Menouhos D., Basnet M., Creeper T., Cummins M., Krause V.

An outbreak of Salmonella Muenchen gastroenteritis after consuming wild hunted kangaroo, Northern Territory, Australia, 2024

(2025) *Communicable diseases intelligence* (2018), 49

ABSTRACT: Abstract: An outbreak of salmonellosis occurred in August 2024 after consuming wild hunted kangaroo in a remote area of the Northern Territory (NT), Australia. We conducted an outbreak investigation via telephone and face-to-face interviews, using a standardised questionnaire that recorded symptoms and exposures to foods and activities prior to onset of symptoms. A confirmed outbreak case was defined as anyone with laboratory confirmed *Salmonella Muenchen* infection who was part of a group of people who shared meals on 25-26 August 2024. A probable outbreak case was defined as anyone who was part of a group of people who shared meals on 25-26 August 2024 and subsequently experienced diarrhoea, in the absence of a laboratory test. Of the seven members of the group who shared meals, all became ill (attack rate 100%); three were confirmed cases and four were probable cases. The median age was 32 years (range 23-65 years); six (86%) were male. The median incubation period was 24 hours (range 6-30 hours). The most commonly reported symptoms were diarrhoea (100%, 7/7) and abdominal pain (86%, 6/7). Two cases were admitted to hospital, both for an overnight stay; all recovered. All seven cases consumed the same meal - a single, locally hunted and butchered kangaroo. Contamination likely occurred due to unsafe butchering, storage, transportation and insufficient cooking of the meat. This outbreak highlights the risks of contamination of game meat (in this case kangaroo) with *Salmonella*. Those preparing hunted meat should wash hands and knives regularly while butchering an animal to avoid

contamination; should store butchered meat below 5 °C to avoid bacterial growth and cook foods thoroughly to kill microbes. We estimate that the cost to society of this outbreak was 9,810 Australian dollars. ISSN: 22096051

Rosenbaum A.A., Murphy C.M., Wszelaki A.L., Hamilton A.M., Rideout S.L., Strawn L.K.

Survival of Salmonella on Biodegradable Mulch, Landscape Fabric, and Plastic Mulch
ABSTRACT: Ground covers are used in produce production to enhance plant growth and control diseases and pests. While various factors are considered when selecting commercial ground covers, food safety, particularly the survival of foodborne pathogens, is often overlooked. This study aimed to assess the survival of Salmonella on different ground covers, including biodegradable mulch, landscape fabric, and plastic mulch. New rolls of each ground cover were cut to fit a 100 × 15 mm petri dish and spot inoculated with a seven-strain Salmonella cocktail at approximately 6 log CFU/cm². The inoculated coupons were stored in a climate-controlled chamber (23°C, 55% relative humidity) and sampled at 0, 0.06 (1.5 h), 0.17 (4 h), 1, 2, 3, 5, 7, 30, 60, 90, and 140 days postinoculation (dpi). If counts dropped below the detection limit (<0.12 log CFU/cm²), enrichments were performed following the Food and Drug Administration's Bacteriological Analytical Manual Salmonella protocol. Salmonella survived 140 dpi on all tested ground covers, with reductions >5 log CFU/cm². Survival rates at 140 dpi were highest on landscape fabric (83%, 25/30) followed by plastic mulch (50%, 15/30) and biodegradable mulch (13%, 4/30) coupons (p < 0.05). During the first 30 dpi, biodegradable mulch exhibited the smallest reduction in Salmonella (2.47 ± 0.26 log CFU/cm²), compared to landscape fabric (3.07 ± 0.30 log CFU/cm²) and plastic mulch (3.86 ± 0.72 log CFU/cm²). After 60 dpi, Salmonella reductions stabilized across all materials (~4 log CFU/cm²) and by 90 dpi, no significant differences were observed between ground cover types (p > 0.05). Although Salmonella survival varied among ground covers in the short-term (0–30 dpi), a >5 log CFU/cm² reduction of Salmonella was observed among all materials by 140 dpi. Findings suggest that ground cover material influences Salmonella survival and should be factored into food safety risk management strategies, especially when ground covers are reused. ISSN: 0362028X

Zwally K.M., Holda E., Perez I., Kaufman P.E., Lyons B., Athrey G., Taylor T.M.

Detection and antimicrobial resistance profiles of Salmonella enterica recovered from house fly intestinal tracts and environments of selected broiler farms in Texas (2025) Letters in Applied Microbiology, 78 (2), art. no. ova007
ABSTRACT: The entry of drug-resistant Salmonella enterica into the food supply is a challenge to public health and food safety. One emerging concern is the role of synanthropic insects for moving microbial pathogens throughout poultry production systems, where insects commonly thrive. We investigated the presence and phenotypic antimicrobial susceptibility of S. enterica from insect and environmental samples from broiler farms. Insects were collected throughout the broiler house and adjacent compost barn. Environmental samples (poultry feed, drinking water, fresh litter, and feces) were collected simultaneously (n = 80). Insect gastrointestinal tracts were dissected and pooled (n = 57). Recovered Salmonella isolates were serotyped and subjected to antimicrobial susceptibility testing against 14 medically important antimicrobials. Overall, six isolates were recovered from 137 total samples (4.4%): 3.5% (2/57) from adult house flies (Musca domestica), 15% (3/20) from poultry feed, and 4.8% (1/21) from litter. Salmonella Montevideo (16.7%; 1/6), Typhimurium (33.3%; 2/6), and Kentucky (50%, 3/6) were identified. All but one Salmonella isolate (83.3%; 5/6) demonstrated resistance to at least one antimicrobial. Further research should investigate movement patterns between broiler operations and food processing facilities to establish efficient biosecurity measures to prevent any instances of foodborne pathogen transmission into human food systems. ISSN: 02668254

Di Taranto P., Petrucci F., Normanno G., Pendarra C., Occhiochiuso G., Faleo S., Didonna A., Galante D., Pace L., Rondinone V., Trisolini C., Del Sambro L., Beverelli M., Catanzariti R., Caruso M., Palazzo L., Di Castri A., Parisi A.

Prevalence and Antimicrobial Resistance of Salmonella Strains Isolated from Chicken Samples in Southern Italy (2025) Microorganisms, 13 (2), art. no. 270
ABSTRACT: Salmonellosis is one of the most frequent foodborne zoonoses, and the consumption of contaminated poultry meat is considered the main source of Salmonella infections in humans. From 2021 to 2023, 384 chicken samples were collected from retailers in Apulia and Basilicata regions and analysed at the Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata (IZSPB) laboratories. The Salmonella isolates

were investigated to evaluate their phenotypic characteristics of antimicrobial resistance. A total of 125 (32.55%) samples tested positive for Salmonella spp. Three samples were simultaneously infected with strains of two different serotypes. Strains were classified into nine serotypes. Out of 128 strains, 112 (87.5%) isolates showed multidrug-resistant (MDR) profiles. Moreover, 16 isolates (12.5%) were ESBL producers with MDR profiles. Our data confirm that chicken products are a common source of Salmonella and highlight how *S. Infantis* was the most prevalent serotype (85.93%). Furthermore, Salmonella isolates showed antimicrobial resistance, which is very worrisome for animals and consumers. The high rate of antibiotic resistance along with the appearance of numerous MDR isolates is considered an actual concern and requires a robust surveillance network in a One Health concept. ISSN: 20762607

Li L., Chousalkar K.K., Jenkins C., Jennison A., McWhorter A.R.

The culturability of acid-tolerant Salmonella in mayonnaise, a raw egg-based sauce (2025) International Journal of Food Microbiology, 429, art. no. 111000

ABSTRACT: Salmonella is one of the most common bacterial foodborne pathogens and is frequently found as a contaminant of raw egg-based foods. Food safety regulations recommend the use of food acids to mitigate the risk of Salmonella persistence in raw egg-based foods. Salmonella, however, can become tolerant to acidic environments and it is not known how this can affect bacterial persistence in food. This study investigated whether acid-tolerant strains of Salmonella persisted longer in mayonnaise compared with sensitive strains. Isolates of *S. typhimurium*, *S. infantis*, *S. enteritidis*, and *S. hessarek* were used in this project. Acid-tolerant Salmonella strains were generated using a three-day step-down method where pH was decreased every 24 h from pH 7 to pH 5. Growth curves were determined for both acid-sensitive and acid-tolerant strains. Time-kill experiments were conducted to compare the survivability of acid-sensitive and acid-tolerant Salmonella serotypes in mayonnaise stored at either 5 °C or 25 °C for 72 h. Salmonella exhibited an extended lag phase with increased acid concentration, and acid-tolerant strains recovered faster in media compared with acid-sensitive strains. Elevated biofilm formation was found in acid-habituated strains compared to sensitive strains, and this varied between serotypes. The culturability of Salmonella in mayonnaise stored at 5 °C declined slower than when stored at 25 °C. Acid-tolerant strains persisted longer in mayonnaise and there was a statistically significant difference in culturability ($P < 0.05$). In conclusion, the current safe food recommendations to control Salmonella in raw egg-based foods are not effective in eliminating it. ISSN: 01681605

Cawthraw S., Wales A., Guzinski J., Trew J., Ring I., Huby T., Hussaini A., Petrovska L., Martelli F.

Salmonella Infantis outbreak on six broiler units in Great Britain: investigation, epidemiology, and control (2025) Journal of Applied Microbiology, 136 (3), art. no. Ixaf040

ABSTRACT: Aims: To describe the analysis, epidemiology, and control of six contemporaneous and linked outbreaks of Salmonella enterica subsp. enterica serovar Infantis on British broiler farms. Salmonella Infantis is a potentially multidrug-resistant foodborne zoonosis and can persistently colonize poultry flocks and farms. Methods and results: Routine monitoring initially identified the organism, which was tracked to six farms associated with a single company. Extensive, repeat sampling identified widespread and, in some cases, persistent contamination. Salmonella Infantis was also isolated from three associated processing factories and catching crew equipment, but not from associated hatcheries and feed mills. Whole genome sequencing and resistance phenotyping revealed one strain was present in the processing plants and on five farms. However, on one of those farms, several highly genetically distinct strains were also detected, including one also found in one of the processing plants. The sixth farm had a strain that was genetically unrelated to strains collected from the other premises and which exhibited an extended spectrum beta-lactamase phenotype. Cleaning and disinfection were enhanced, and the organism was eventually cleared from all farms. Conclusions: There were multiple incursions of varied strains, with a possible link to processing factories. Elimination of *S. Infantis* from premises can be challenging but achievable. ISSN: 13645072

Sarjit A., Gatehouse J., Dykes G.A.

Transfer of Salmonella from Skin to Flesh and Peelers during Peeling of Baby Cucumbers (2025) Food Protection Trends, 45 (2), pp. 123 - 130

ABSTRACT: Salmonella is of concern in the fresh produce sector, and a recent outbreak of Salmonella infections was associated with baby cucumbers in Australia. Some consumers advocate peeling cucumbers to improve food safety. We investigated the transfer of Salmonella Typhimurium ($n = 2$) and Salmonella Newport ($n = 1$) from unrinsed and

rinsed cucumber skin to flesh and peelers (stainless steel or plastic) during peeling of baby cucumbers. Levels of *Salmonella* attached to cucumbers were ~ 7.64 to 7.87 log CFU/g (unrinsed) and ~ 6.75 to 7.40 log CFU/g (rinsed). Levels of *Salmonella* Typhimurium transferred onto the flesh were higher (~ 0.1 to 1.8 log %) than those of *Salmonella* Newport (~ -1.7 to 1.4 log %) irrespective of rinsing prior to peeling. Levels of *Salmonella* transferred to the stainless steel peeler were generally lower (~ -3.62 to 1.58 log %) than those transferred to the plastic peeler (~ -3.74 to -0.52 log %). Rinsing reduced attachment of *Salmonella* to unpeeled cucumber (~ -0.50 to 1.00 log CFU/g) and reduced transfer to the flesh (~ -1.71 to 1.11 log %) and peelers (~ -3.71 to -1.42 log %). *Salmonella* strains and peeler material may contribute to the degree of transfer. Stainless steel peelers may be a better option for consumers, but use of these peelers does not eliminate the risk of *Salmonella* transfer during peeling. ISSN: 15419576