

# NEWSLETTER

European Union Reference Laboratory for *Salmonella*

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## Editorial Note

Bilthoven, 5 January 2023

Dear colleagues,

First of all I would like to **wish you all the best for a very good and healthy 2024!** I hope you are all doing well and were able to have a good rest during the Christmas break. After a few weeks off-line, we start again with our activities with new energy. To help you with keeping an overview of the (main) EURL-*Salmonella* activities, we include in this Newsletter a table with the planning of our activities for 2024. Like in former years, we plan to organise 3 Proficiency Tests this year. Other main activities are the organisation of the workshop in May and a Joint Training Course of the inter EURLs Working Group on NGS in June. More details are given below.

At first I would like to look back to the Proficiency Tests (PTs) organised in the second half of 2023.

In September/October 2023 we organised the **PT on detection of *Salmonella* in samples from the primary production stage (PPS) 2023**. The laboratory own results, as well as the interim summary report of this PT was sent to the participants by early December 2023. In total 37 NRLs-*Salmonella* participated in this PT, of which 35 fulfilled the criteria of good performance. The two underscoring laboratories are contacted for additional explanation of their results and when needed a follow-up study will soon be organised.

In November the **2023 PT on typing of *Salmonella*** was organised. Like former years, this study contained an obligatory part on serotyping of *Salmonella*, and a voluntary part on cluster analysis (NGS only). The deadline for reporting the serotyping results was by mid-December 2023, and the deadline for the part on cluster analysis is 31 January 2024. Soon we will start the analysis of the results of the serotyping part, after which you will be informed about the results.

As indicated in the previous Newsletter, the first **PT to be organised in 2024** will focus on the **detection of *Salmonella* in live bivalve molluscs (LBM)**. By the end of November 2023, the relevant NRLs-*Salmonella* were informed about the planning of this PT, and the time table is also included in this Newsletter. The registration form is still open until 26 January 2024. So, if your laboratory is NRL-*Salmonella* for LBM, please make sure to complete the registration form before the deadline.

As the NRLs-*Salmonella* for live bivalve molluscs generally differ from the other NRLs for detection of *Salmonella* in food samples, we plan to organise in September/October 2024 **a combined PT for NRLs-*Salmonella* for Food and PPS**. For this PT we plan to introduce a few small changes. Firstly, we will no longer prescribe the use of control samples. As all NRLs have to be accredited, the use of control samples shall be part of their quality system. We may still ask you to report the details of your (own) control samples, but we no longer prescribe the use of specific control samples in the PT. Secondly, we will give the NRLs the opportunity to start the analysis of the PT samples immediately after receipt instead of prescribing a fixed date to start the analysis. Instead we will indicate what shall be the latest date to start the analysis (generally one week after sending the samples). This may provide a bit more flexibility with the performance of the PT.

By mid-December 2023 we have sent information to the NRLs-*Salmonella* about the organisation of the **EURL-*Salmonella* workshop in May 2024**. This workshop will be organised as a face-to-face meeting in Leiden, the Netherlands,

with limited facilities for online participation. The registration is still open until 26 January, so please make sure that you register on time. Additionally, we would very much welcome suggestions for interesting presentations.

Concerning the ISO/CEN activities, I can inform you that on 22 December 2023 the Draft International Standard **ISO/DIS 6579-4** ('Identification of monophasic *Salmonella* Typhimurium by PCR') was published for voting and comments until 15 March 2024. This extra voting round is needed, as it was decided by summer 2023 that the document will be published as a full ISO instead of a Technical Specification (TS). In ISO/DIS 6579-4 the comments given to draft ISO/DTS 6579-4 (of 12 May 2023) were incorporated. Several NRLs gave very fruitful comments to the draft ISO/DTS version, for which I want to thank them very much. The table with comments, including our observations will be shared with the NRLs. The official voting on ISO/DIS 6579-4 shall go through your national standardisation organisation.

Another activity in ISO related to *Salmonella* is **ISO/TC34/SC9-WG9** (ISO 6579-1 'Detection of *Salmonella*'). The main reason for re-activating this working group, is to complete the performance characteristics of ISO 6579-1 for the relevant product categories. In March 2023, WG9 held its first (virtual) meeting and by then it was agreed to at first look into the literature for possible validation data for missing product categories. In the past months we have gone through many validation studies and checked it for useful validation data for ISO 6579-1. Luckily, *Salmonella* is a popular micro-organism for development of alternative methods, resulting in many validation studies performed by validation organisations like Afnor and MicroVal. Although these studies concern the validation of an alternative method, the reference method to which the alternative method is compared to generally is ISO 6579-1. Therefore, the validation of an alternative method may give useful information on performance characteristics of ISO 6579-1 as well. The data collected will be discussed with the members of ISO-WG9 at the second meeting of this working group on 15 January 2024. At this meeting, also the next steps will be discussed.

By the end of 2023 we have also been busy with some improvements to our **website** <https://www.eurlsalmonella.eu/>. Before, the information on analytical methods was a bit hidden behind the button 'Publications'. Currently this has been changed by adding a new button 'Methods' to the ribbon at the top of the website. In this way, we hope that the information on methods will be easier to find. Please have a look at <https://www.eurlsalmonella.eu/methods> and let us know what you think of it. For example, the page 'NGS' (<https://www.eurlsalmonella.eu/methods/ngs>) now has a more clear subdivision into 'Guidance documents', 'Training courses' and 'Events'. On the page 'Guidance documents' you will find the links to the guidance documents on NGS, drafted by the inter EURL working group on NGS. On the page 'Training courses' you will find the presentations of the training courses on NGS given in 2022 and in 2023. Finally, on the page 'Events' you will find the links to the presentations of the 'Science meets Policy' conferences of 2020 and 2023, as well as the link to the presentations of the webinar on PTs on cluster analysis of 2023.

Speaking about NGS, I can inform you that the **inter EURLs working group on NGS** is planning to organise again a **joint EURLs (basic) training course on NGS** in June 2024. The set-up of the training will be similar to the previous two joint EURLs training courses and will be organised at the premises of the EURL-*Campylobacter* in Sweden. More details about this training will be shared with the NRL networks soon.

In fall 2023, the following EURL-*Salmonella* reports were published:  
Diddens, R.E. and Mooijman, K.A. EURL-*Salmonella* Proficiency Test Food-Feed 2023. Detection of *Salmonella* in flaxseed. National Institute for Public

Health and the Environment, Bilthoven, the Netherlands. RIVM Report no.: 2023-0340. <https://www.rivm.nl/bibliotheek/rapporten/2023-0340.pdf>

Jacobs-Reitsma, W.F., Verbruggen, A., Diddens, R.E., van Hoek, A.H.A.M., Mooijman, K.A., 2023. EURL-*Salmonella* Proficiency Test Typing 2022. National Institute for Public Health and the Environment, Bilthoven, the Netherlands. RIVM report no.: 2023-0339. <https://www.rivm.nl/bibliotheek/rapporten/2023-0339.pdf>

Mooijman, K.A. The 28<sup>th</sup> EURL-*Salmonella* workshop; 22 and 23 May 2023, Online. National Institute for Public Health and the Environment, Bilthoven, the Netherlands. RIVM Report no.: 2023-0341.

<https://www.rivm.nl/bibliotheek/rapporten/2023-0341.pdf>

Best wishes,  
Kirsten Mooijman  
Coordinator EURL-*Salmonella*

## Contribution of the EURL-*Salmonella*

### Planning of EURL- *Salmonella* activities in 2024

<b>Date(s)</b>	<b>Activity</b>
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* Proficiency Test Live Bivalve Molluscs 2024 - Detection of *Salmonella* in mussels**

<b>Week</b>	<b>Date</b>	<b>Subject</b>
4	Friday <b>26 January 2024</b> at the latest	Please register by <b>26 January 2024</b> at the latest.
6	Week of 5 February 2024	E-mailing the link for the result form to the participants. E-mailing the protocol and instructions for the result form to the NRLs.
7 – 8		Preparation of media by the NRLs.
9	Monday <b>26 February 2024</b>	Shipment of the parcel with mussels to the participants as Biological Substance Category B (UN3373). Shipment of the parcel with <i>Salmonella</i> reference materials to the participants as Biological Substance Category B (UN3373) and on dry-ice (UN1845).
9	<b>Immediately after receipt of the parcel and at the latest on 28 February 2024</b>	<b>Start performance of the Proficiency Test.</b>
12	Friday <b>22 March 2024</b> at the latest	Deadline for completing the result form: <b>22 March 2024 (23:59h CET)</b> After this deadline the result form will be closed.
	May 2024	Interim summary report

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

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## From the Literature

### Salmonella-related Literature selection from Scopus: October – December 2023

**Escobar C., Munoz L.R., Bailey M.A., Krehling J.T., Pacheco W.J., Hauck R., Buhr R.J., Macklin K.S.**

*Buffering Capacity Comparison of Tris Phosphate Carbonate and Buffered Peptone Water Salmonella Pre-Enrichments for Manufactured Feed and Feed Ingredients (2023) Animals, 13 (19), art. no. 3119*

ABSTRACT: Various culture-based methods to detect Salmonella in animal feed have been developed due to the impact of this bacterium on public and animal health. For this project, tris phosphate carbonate (TPC) and buffered peptone water (BPW) buffering capacities were compared as pre-enrichment mediums for the detection of Salmonella in feed ingredients. A total of 269 samples were collected from 6 feed mills and mixed with the pre-enrichments; pH was measured before and after a 24 h incubation. Differences were observed when comparing pH values by sample type; DDGS and poultry by-product meal presented lower initial pH values for TPC and BPW compared to the other samples. For both TPC and BPW, meat and bone meal presented higher final pH values, while soybean meal and peanut meal had lower final pH values. Furthermore, for BPW, post cooling, pellet loadout, and wheat middlings reported lower final pH values. Additionally, most feed ingredients presented significant differences in pH change after 24 h of incubation, except DDGS. From meat and bone meal samples, four Salmonella isolates were recovered and identified: three using BPW and one using TPC. TPC provided greater buffer capacity towards neutral pH compared to BPW, but BPW was more effective at recovering Salmonella. ISSN: 20762615

**Samper-Cativiela C., Prieto M.E., Collado S., De Frutos C., Branscum A.J., Saez J.L., Alvarez J.**

*Risk Factors for Salmonella Detection in Commercial Layer Flocks in Spain (2023) Animals, 13 (20), art. no. 3181*

ABSTRACT: Trends in Salmonella human infections are assumed to be related to the distribution of the pathogen in the animal reservoir/food products, and cases in humans are most often linked to poultry and poultry products (eggs, meat). Therefore, ongoing Salmonella national control programs (NCPs) in European Union Member States have the objective of monitoring and reducing its prevalence in commercial poultry flocks. Results from NCPs have shown certain factors (housing systems, season of sampling and if sampling is conducted by food business operators (FBOs) or competent authorities (CAs), among others) can influence detection rates, but associations are often not consistent. Here, we analyzed data from the Spanish NCP on 7216 laying hen flocks subjected to 36,193 sampling events over a six-year period to characterize its performance and identify variables influencing detection rates. Overall, 1205 sampling events were positive for Salmonella spp. (any serovar) and 132 for *S. Enteritidis*-*S. Typhimurium*/monophasic. Bayesian multivariable models adjusting for multiple covariates concluded that sampling events later in the year, in caged flocks with older animals and conducted by CAs had increased odds of positivity for Salmonella spp., revealing aspects linked with a differential estimation of Salmonella levels in laying hen flocks. ISSN: 20762615

**Torres-Boncompete J., Catalá-Gregori P., Garcia-Llorens J., Soriano J.M., Sevilla-Navarro S.**

*Simultaneous Salmonella and bacteriophage isolation on Modified Semisolid Rappaport Vassiliadis media (2023) Poultry Science, 102 (10), art. no. 102960*

ABSTRACT: Salmonella represents a food safety concern worldwide. Despite the application of National Control Programs (NCP) against Salmonella, regulated by the European Union, every year the European Food Safety Authority reports new cases. On the look for new alternatives to antibiotics, bacteriophages, or phages, rise as a promising alternative to treat multidrug resistance infections. Although they are known to be ubiquitous in the environment, their high specificity to host cells hinders their isolation and usage for phage therapy. The ISO 6579-1:2017 is performed as a reference method in the NCP and uses an unspecific media to enrich the sample the same way most phage isolation protocols do. Later, the protocol uses a more selective media to isolate the Salmonella, Modified Semisolid Rappaport Vassiliadis (MSRV). This paper aims to find out whether, due to the similarity between phage isolation protocols and the ISO 6579-1:2017, this last one

could be used as a protocol to also isolate phages against the same bacterium that is being simultaneously isolated. To do so, 2 experiments were performed to assess phage isolation from MSRV media in in-vivo conditions. The results from experiments 1 and 2 proved that the MSRV media was usable for simultaneous phage and pathogen isolation through a single procedure. Additionally, there is a correlation between the antigenic formulae from the bacteria and the phage's host range, seeming to be effective against bacteria with similar antigenic formulae. ISSN: 00325791

**Bettridge J.M., Snow L.C., Tang Y., Petrovska L., Lawes J., Smith R.P.**

*Using SNP addresses for Salmonella Typhimurium DT104 in routine veterinary outbreak detection*

(2023) *Epidemiology and Infection*, 151, art. no. e187

ABSTRACT: SNP addresses are a pathogen typing method based on whole-genome sequences (WGSs), assigning groups at seven different levels of genetic similarity. Public health surveillance uses it for several gastro-intestinal infections; this work trialed its use in veterinary surveillance for salmonella outbreak detection. Comparisons were made between temporal and spatio-temporal cluster detection models that either defined cases by their SNP address or by phage type, using historical data sets. Clusters of SNP incidents were effectively detected by both methods, but spatio-temporal models consistently detected these clusters earlier than the corresponding temporal models. Unlike phage type, SNP addresses appeared spatially and temporally limited, which facilitated the differentiation of novel, stable, or expanding clusters in spatio-temporal models. Furthermore, these models flagged spatio-temporal clusters containing only two to three cases at first detection, compared with a median of seven cases in phage-type models. The large number of SNP addresses will require automated methods to implement these detection models routinely. Further work is required to explore how temporal changes and different host species may impact the sensitivity and specificity of cluster detection. In conclusion, given validation with more sequencing data, SNP addresses are likely to be a valuable addition to early warning systems in veterinary surveillance. ISSN: 09502688

**Paradis A., Beaudet M.-F., Boisvert Moreau M., Huot C.**

*Investigation of a Salmonella Montevideo Outbreak Related to the Environmental Contamination of a Restaurant Kitchen Drainage System, Québec, Canada, 2020–2021*  
(2023) *Journal of Food Protection*, 86 (10), art. no. 100131

ABSTRACT: In May 2020, the Direction de santé publique du CIUSSS de la Capitale-Nationale (DSPu) received a report from the Laboratoire de santé publique du Québec of a cluster of three cases of *Salmonella enterica enterica*, serogroup C1, serotype Montevideo. The epidemiological investigation identified a total of 67 cases between January 1, 2020, and August 13, 2021, 66% of which were directly linked to a restaurant in the area. The *Salmonella* strains from most of these cases were found to be identical by whole-genome sequencing (cluster code 2005MontWGS-1QC). The initial inspection of the restaurant by the competent authorities (Ministère de l'agriculture, des pêcheries et de l'alimentation du Québec) – including the evaluation of hygiene and food safety, the search for cases of illness among workers and food sampling – was unable to establish the source of the outbreak. Environmental samples showed that the restaurant's kitchen drains were contaminated with the same strain of *Salmonella* Montevideo as the cases in the outbreak. Several cleaning and disinfection methods were used repeatedly. When environmental sampling at the restaurant sites was repeatedly and consecutively negative, cases in the community stopped. The prior occurrence of a fire in the kitchen may have played a role in the contamination of the restaurant drains. In conclusion, public health professionals should consider drainage systems (plumbing) and possible aerosolization of bacteria as a potential source of a restaurant-related salmonellosis outbreak. ISSN: 0362028X

**Soliani L., Rugna G., Prospero A., Chiapponi C., Luppi A.**

*Salmonella Infection in Pigs: Disease, Prevalence, and a Link between Swine and Human Health*

(2023) *Pathogens*, 12 (10), art. no. 1267

ABSTRACT: *Salmonella* is one of the most spread foodborne pathogens worldwide, and *Salmonella* infections in humans still represent a global health burden. The main source of *Salmonella* infections in humans is represented by contaminated animal-derived foodstuffs, with pork products being one of the most important players. *Salmonella* infection in swine is critical not only because it is one of the main causes of economic losses in the pork industry, but also because pigs can be infected by several *Salmonella* serovars, potentially contaminating the pig meat production chain and thus posing a significant threat to public health globally. As of now, in Europe and in the United States, swine-related *Salmonella* serovars, e.g., *Salmonella* Typhimurium and its monophasic variant *Salmonella* enterica

subsp. enterica 1,4,[5],12:i:-, are also frequently associated with human salmonellosis cases. Moreover, multiple outbreaks have been reported in the last few decades which were triggered by the consumption of *Salmonella*-contaminated pig meat. Throughout the years, changes and evolution across the pork industry may have acted as triggers for new issues and obstacles hindering *Salmonella* control along the food chain. Gathered evidence reinforces the importance of coordinating control measures and harmonizing monitoring programs for the efficient control of *Salmonella* in swine. This is necessary in order to manage outbreaks of clinical disease in pigs and also to protect pork consumers by controlling *Salmonella* subclinical carriage and shedding. This review provides an update on *Salmonella* infection in pigs, with insights on *Salmonella* ecology, focusing mainly on *Salmonella* Choleraesuis, *S. Typhimurium*, and *S. 1,4,[5],12:i:-*, and their correlation to human salmonellosis cases. An update on surveillance methods for epidemiological purposes of *Salmonella* infection in pigs and humans, in a "One Health" approach, will also be reported. ISSN: 20760817

**Turner M., Van Hulzen L., Pietri J.E.**

*The gut microbiota induces melanin deposits that act as substrates for fimA-mediated aggregation of Salmonella Typhimurium and enhance infection of the German cockroach vector*

(2023) *Microbiology Spectrum*, 11 (5)

ABSTRACT: When *Salmonella Typhimurium* is ingested by German cockroaches, the bacteria replicate in the gut and persist for at least 7 d, enabling transmission in the feces. However, the mechanisms that facilitate survival and persistence in the cockroach gut remain poorly detailed. We previously reported the formation of biofilm-like aggregate populations of *S. Typhimurium* in the gut of cockroaches upon ingestion. We also reported that deletion of the type-1 fimbrial subunit of *S. Typhimurium*, *fimA*, leads to a reduced bacterial load in the cockroach gut. Here, we link these observations and provide further insight into the mechanism and function of *S. Typhimurium* aggregation in the gut of the cockroach. We show that *S. Typhimurium* but not *Escherichia coli* forms aggregated populations in the cockroach gut, and that aggregate formation requires *fimA* but not the biofilm formation-related genes *csgA* and *csgD*. Furthermore, we show that *S. Typhimurium* aggregates are formed using small granular deposits present in the cockroach gut, which exhibit properties consistent with melanin, as substrates. These melanin deposits are prevalent in the guts of both immature and adult cockroaches from laboratory colonies and are correlated with increased gut bacterial density while being entirely absent in gnotobiotic cockroaches reared without exposure to environmental bacteria, indicating they are induced as a response to the gut microbiota. When cockroaches lacking melanin deposits in the gut are fed *S. Typhimurium*, they exhibit lower rates of infection than those harboring melanin deposits, demonstrating that microbiota-induced melanin deposits enhance infection of the gut of the vector. IMPORTANCE Cockroaches, including the German cockroach (*Blattella germanica*), can be both mechanical and biological vectors of pathogenic bacteria. Together, our data reveal a novel mechanism by which *S. Typhimurium* interacts with the cockroach gut and its microbiota that promotes infection of the vector. These findings exemplify the emerging but underappreciated complexity of the relationship between cockroaches and *S. Typhimurium*. ISSN: 21650497

**Mahmoud S., Aboul-Ella H., Marouf S., Armanious W.**

*Low Temperature-Survivability Behavior of Salmonella Enterica Subsp. Enterica Serovar Typhimurium and Salmonella Enterica Subsp. Enterica Serovar Enteritidis in a Minced Beef Meat Model as an Evaluation of the Cold Chain's Preserving-Effectiveness*  
(2023) *International Journal of Veterinary Science*, 12 (6), pp. 853 - 859

ABSTRACT: *Salmonella* spp. commonly existed in processed meat production and consumption environment. Hence, their transmission to meat products is of great concern. The industry has widely used cold chain low temperatures as a strategy to prevent the bacterial growth, and recently, refrigeration and freezing have been suggested as a preserving method to improve mechanical operations, quality, and safety of meat products. The purpose of this study was to evaluate the effect of the most used low temperatures in the cold chain, on the survival of *Salmonella enterica* subsp. enterica serovar *Typhimurium* (*Salmonella Typhimurium*) and *Salmonella enterica* subsp. enterica serovar *Enteritidis* (*Salmonella Enteritidis*) that were artificially inoculated on the raw minced meat products. Samples were refrigerated at (6°C) or completely frozen at (-20°C) for 9 weeks. The *Salmonella* spp. were recovered on appropriate selective and non-selective media. Log reductions and their reflection on the extent of bacterial cell injury were calculated and treatments were calculated and compared. No significant differences were observed in the bacterial count log reduction of refrigerated or frozen bacteria on

minced beef meat up to the most used minced meat low temperature keeping periods (14days). The average reduction for *Salmonella* Enteritidis was 0.5log CFU/mL, and for *Salmonella* Typhimurium 2logs CFU/mL; therefore, none of the final reductions were greater than (3logs). Bacterial cell injury was not significantly different among any of the treatments in the first 14days, but variant reductions have been recorded further. Data showed no practical significance for the initial bacterial count log reduction of these pathogens from refrigeration and freezing, thus, this technology should not be considered as a strategy for the reduction or elimination of *Salmonella* spp. ISSN: 23043075

**Alvarez D.M., Barrón-Montenegro R., Conejeros J., Rivera D., Undurraga E.A., Moreno-Switt A.I.**

*A review of the global emergence of multidrug-resistant Salmonella enterica subsp. enterica Serovar Infantis*

(2023) *International Journal of Food Microbiology*, 403, art. no. 110297

ABSTRACT: *Salmonella enterica* serovar *Infantis* is an emergent foodborne and zoonotic *Salmonella* serovar with critical implications for global health. In recent years, the prevalence of *S. Infantis* infections has increased in the United States, Europe, and Latin America, due to contaminated chicken and other foods. An essential trait of *S. Infantis* is its resistance to multiple antibiotics, including the critically important third-generation cephalosporins and quinolones, undermining effective medical treatment, particularly in low-resource settings. We describe the emergence of multidrug-resistant (MDR) *S. Infantis*, focusing on humans, animals, the environment, and food. We conducted a systematic review (1979-2021), selected 183 studies, and analyzed the origin, source, antimicrobial resistance, and presence of a conjugative plasmid of emerging *S. Infantis* (pESI) in reported isolates. *S. Infantis* has been detected worldwide, with a substantial increase since 2011. We found the highest number of isolations in the Americas (42.9 %), Europe (29.8 %), Western Pacific (17.2 %), Eastern Mediterranean (6.6 %), Africa (3.4 %), and South-East Asia (0.1 %). *S. Infantis* showed MDR patterns and numerous resistant genes in all sources. The primary source of MDR *S. Infantis* is broiler and their meat; however, this emerging pathogen is also present in other reservoirs such as food, wildlife, and the environment. Clinical cases of MDR *S. Infantis* have been reported in children and adults. The global emergence of *S. Infantis* is related to a plasmid (pESI) with antibiotic and arsenic- and mercury-resistance genes. Additionally, a new megaplasmid (pESI-like), carrying blaCTX-M-65 and antibiotic-resistant genes reported in an ancestral version, was detected in the broiler, human, and chicken meat isolates. Strains harboring pESI-like were primarily observed in the Americas and Europe. MDR *S. Infantis* has spread globally, potentially becoming a major public health threat, particularly in low- and middle-income countries. ISSN: 01681605

**Napoleoni M., Villa L., Barco L., Lucarelli C., Tiengo A., Baggio G., Dionisi A.M., Angellotti A., Ferretti E., Ruggeri S., Staffolani M., Rocchegiani E., Silenzi V., Morandi B., Blasi G.**

*Monophasic Variant of Salmonella Typhimurium 4,[5],12:i:- (ACSSuGmTmpSxt Type) Outbreak in Central Italy Linked to the Consumption of a Roasted Pork Product (Porchetta)*  
(2023) *Microorganisms*, 11 (10), art. no. 2567

ABSTRACT: The monophasic variant of *S. Typhimurium* 4,[5],12:i:- (MVST) is the third most commonly reported *Salmonella* serovar involved in human infections (8.8%) in the EU and ranks after *S. Enteritidis* (54.6%) and *S. Typhimurium* (11.4%). In Italy, in contrast, the MVST has achieved peculiar epidemiological and ecological success which has allowed it to be, since 2011, the serovar most frequently isolated from humans. In the summer of 2022, a foodborne outbreak of the MVST involving 63 people occurred in the Marche Region (Central Italy). A common food exposure source among some human cases was a roasted, ready-to-eat (RTE) pork product, porchetta, which is a typical product of Central Italy. This paper describes the results of investigations conducted to clarify this outbreak. The porchetta was produced by a local manufacturing plant and distributed to at least two local retail stores, one of which was the retail outlet for the manufacturing plant. The MVST was isolated from surface samples collected at the porchetta manufacturing plant and at both local retail stores via bacterial analysis, and the porchetta sampled at one store contained the MVST. These data confirm this type of RTE pork product can be a source of *Salmonella* infection in humans. ISSN: 20762607

**Jenkins E., Gardenhire I., Whitney B.M., Martin K.B., Schwensohn C., Gieraltowski L., Leeper M.M., McCurdy V., McClure M., Wellman A., Pightling A., Smith M., Swinford A., Hainstock L., Crosby A.J., Bazaco M.C., Viazis S.**

*An investigation of an outbreak of Salmonella Newport infections linked to melons – United States, 2020*

(2023) *Food Control*, 152, art. no. 109833

ABSTRACT: The United States are one of the world's leading consumers of melons. In 2020, the Food and Drug Administration (FDA), the Centers for Disease Control and Prevention (CDC), and state health and regulatory partners investigated an outbreak of *Salmonella* Newport infections linked to melons from southwest Indiana, resulting in 80 ill persons and 18 hospitalizations reported across 15 states. Epidemiologic and traceback data indicated melons as the vehicle for these infections, but the collinearity of melon varieties purchased and consumed together in combination with the traceback investigation that could not rule out either melon type, did not allow investigators to delineate whether the vehicle was cantaloupe alone or, both cantaloupe and watermelons. Analysis of traceback records for cantaloupe and/or watermelon exposures for 12 ill people indicated convergence on a grower in southwest Indiana which supplied cantaloupe to the nine of eleven points of service where ill people purchased cantaloupe; similar convergence was not observed for watermelon. While *Salmonella* isolates were recovered from environmental samples collected by FDA throughout the growing operation, they were not highly genetically related to the outbreak strain by whole genome sequencing analyses, i.e. greater than a 20 high quality single nucleotide polymorphisms difference. This outbreak illustrates the need for additional efforts to determine the source and extent of environmental contamination in the melon growing region of southwest Indiana and emphasizes the need for outreach and education efforts to help promote farm practices to reduce pathogen contamination of melons. ISSN: 09567135

**Marin C., Cerdà-Cuellar M., Rosario I., Lorenzo-Rebenaque L., Vega S., Manzanares A., Padilla D., Real F., Rodríguez-Ponce E., Acosta-Hernández B.**

*Impact of genetic diversity and antibiotic-resistance of Salmonella isolated from feral cats: One Health approach*

(2023) *Comparative Immunology, Microbiology and Infectious Diseases*, 101, art. no. 102043

ABSTRACT: Free-living cats usually live in colonies in urban areas, especially close to parks and neighbourhoods where people feed them without any sanitary control. This can pose a human, animal and environmental health concern due to the close contact between uncontrolled colonies, the population and other domestic and/or wild animals. Thus, this study aimed to assess the genetic diversity and antimicrobial resistance (AMR) among *Salmonella enterica* subsp. *enterica* strains isolated from feral cats in a previous epidemiological study in the Gran Canaria island (Spain). A total of nineteen *Salmonella* isolates were obtained from November 2018 to January 2019 in a *Salmonella* epidemiological study in feral cats. All isolates obtained were genotyped by pulsed-field gel electrophoresis (PFGE) and were tested for antimicrobial susceptibility, in accordance with Decision 2013/652/EU. PFGE analysis revealed isolates clustering by serovar, with identical clones for serovars Bredeney and Gran Canaria, while differing pulsotypes were observed for serovars Florida (88.89 % similarity) and Nima (83.23 % similarity). All but two isolates were resistant to at least one antimicrobial. The results obtained demonstrate that feral cats in the region investigated are a reservoir of *Salmonella* strains resistant to gentamicin (94.1 %) and of the critically important antimicrobial tigecycline (23.5 %). Hence, they could excrete AMR strains through their faeces and contaminate the environment, favoring the spread of such bacteria to cohabiting pets. Moreover, this widespread presence of AMR *Salmonella* clones across various serovars highlights the urgent need to implement efficient antimicrobial stewardship and control programs by the local governments due to the ongoing need to protect human and animal health under a One Health concept. ISSN: 01479571

**Sun S., Xie Y., Zhou X., Zhu M.-J., Sablani S., Tang J.**

*Survival and thermal resistance of Salmonella in chocolate products with different water activities*

(2023) *Food Research International*, 172, art. no. 113209

ABSTRACT: Contamination of *Salmonella* in chocolate products has caused worldwide outbreaks and recalls. There is a lack of information on the impact of water activity (*aw*) on the stability of *Salmonella* in chocolate products during storage and thermal treatments. In this research, the survival and thermal resistance of a *Salmonella* cocktail (*S. Enteritidis* PT30, *S. Tennessee* K4643, *S. Typhimurium* S544) was examined in different chocolate products (dark chocolate, white chocolate, milk chocolate) at two *aw* levels (0.25, 0.50) over 12 months at 22 °C. A reduction of 4.19 log<sub>10</sub> CFU/g of *Salmonella* was obtained in dark chocolate after 12 months (*aw* = 0.50, at 22 °C); less reductions were observed in white and milk chocolates. In all three products, more reductions were observed at *aw* = 0.50 than at *aw* = 0.25 over the 12-months storage. When treated at 80 °C, the D-values (time required to cause 1 log reduction) of the *Salmonella* cocktail in the chocolate

samples with initial aw of 0.25 were 35.7, 25.2 and 11.6 min in dark, white and milk chocolate, respectively, before the storage. The D80°C -values of Salmonella cocktail in the samples with initial aw of 0.50 were 6.45, 7.46, and 3.98 min in dark, white and milk chocolate, respectively. After 12 months of storage at 22 °C, the D80 °C-value of Salmonella cocktail decreased to 9.43 min ( $p < 0.05$ ) in milk chocolate but remained 22.7 min in white chocolate with an aw of 0.25 at 22 °C. The data suggests that Salmonella can survive in chocolate products for up to 12 months, and its thermal resistance remained relatively stable. Thus, Salmonella is resistant to desiccation in chocolates, particularly in milk and white chocolates, and its thermal resistance remains during one-year storage, which could pose a potential threat for future outbreaks. ISSN: 09639969

**Larivière-Gauthier G., Kerouanton A., Mompelat S., Bougeard S., Denis M., Fravallo P.**

*Monophasic Variant of Salmonella Typhimurium Infection Affects the Serum Metabolome in Swine*

(2023) *Microorganisms*, 11 (10), art. no. 2565

ABSTRACT: Salmonella is the most relevant foodborne zoonotic agent found in swine, and its presence in French herds is significant. Its carriage is asymptomatic, which makes it difficult to detect during rearing, thus increasing the risk of its presence on pork meat. Studies have shown that enteric infection in animals could be associated with changes in the serum metabolome composition, through the immune response or changes in the digestive microbiota composition. We hypothesized that these changes in the serum metabolome composition could be used as markers for the detection of asymptomatic animals infected by Salmonella. Using untargeted analysis by liquid chromatography coupled with mass spectrometry, we showed that significant differences in the composition of the serum metabolome could be detected between infected or noninfected animals both 1 and 21 days after experimental infection. This serum metabolome composition significantly changed during the 21 days postinfection in the infected animal groups, suggesting an evolution of the impact of infection with time. Despite this evolution, differences in the serum metabolome composition persisted between infected and noninfected animals 21 days after the initial infection. We also showed a possible difference between high-shedding and low-shedding animals 21 days postinfection. Finally, some of the variations in the metabolome were found to be significantly associated with variations of specific members of the fecal microbiota. Thus, excreting and asymptomatic animals, but also high-shedding animals, could be identified on the basis of their serum metabolome composition. ISSN: 20762607

**Vinueza-Burgos C., Medina-Santana J., Maldonado R., Vásquez Y., Lincango L., Villagomez E., Gómez C., Ron-Garrido L., Cevallos-Almeida M.B.**

*Evaluation of Virulence of Salmonella Infantis and Salmonella Enteritidis with In Vitro and In Vivo Models*

(2023) *Foodborne Pathogens and Disease*, 20 (11), pp. 484 - 491

ABSTRACT: Salmonella Infantis and Enteritidis serovars have been reported as important causes of salmonellosis in humans worldwide. However, the virulence of these two serovars has yet to be compared. To evaluate the virulence of Salmonella Infantis ( $n = 23$ ) and Salmonella Enteritidis ( $n = 7$ ), we used two models: the Caco2 cells model (in vitro) and the Galleria mellonella model (in vivo). Additionally, the virulence genes of all tested strains were contrasted with phenotypic outcomes. Results showed that adhesion means were 18.2% for Salmonella Enteritidis and 38.2% for Salmonella Infantis strains. Invasion means were 77.1% for Salmonella Enteritidis and 56.2% for Salmonella Infantis strains. Significant differences were found between serovars in adhesion and invasion assays. Mortality rates (58% for Salmonella Enteritidis and 62.6% for Salmonella Infantis) were not significantly different between serotypes. The distribution of virulence genes showed that genes fae (fimbrial adherence determinants) and shdA (nonfimbrial adherence determinants) were only found in Salmonella Infantis strains. On the other hand, the rck gene (invasion) and Plasmid-encoded fimbriae genes (pef A, B, C, D) were present in Salmonella Enteritidis exclusively. In conclusion, this study shows that Salmonella Enteritidis has a higher virulence potential under experimental conditions than Salmonella Infantis. However, more studies are needed to determine the risk that Salmonella Infantis could represent compared with Salmonella Enteritidis. Moreover, other in vivo models should be considered to assess the virulence of these serovars. ISSN: 15353141

**Fischer E.F., Müller R., Todte M., Taubert A., Hermosilla C.**

*Role of Free-Ranging Synanthropic Egyptian Geese (Alopochen aegyptiaca) as Natural Host Reservoirs for Salmonella spp. in Germany*

(2023) *Animals*, 13 (21), art. no. 3403

**ABSTRACT:** *Salmonella* is one of the most common and pathogenic bacteria worldwide, causing severe enteritis in humans and representing a relevant intestinal illness in One Health for young, old and immunosuppressed patients. Various *Salmonella* serovars have been described to be responsible for human salmonellosis. Birds represent natural carriers of different zoonotic-relevant *Salmonella* serovars and Anseriformes can not only transmit *Salmonella* spp. to humans but also manifest clinical salmonellosis. In this study, 138 scat samples (n = 138) of free-ranging Egyptian geese (EG; *Alopochen aegyptiaca*) were collected in Germany, including 83 scat samples from city parks, 30 samples from 14 public swimming pools and 25 fresh caecal samples of dead EG. Collected EG scat samples were examined for the presence of *Salmonella* spp. according either to the ISO 6579 (2017) norm or to a combination of bacterial pre-enrichment and specific PCR for detection of *Salmonella* DNA. All 138 analysed EG faecal samples resulted *Salmonella*-negative. Furthermore, the survival of *Salmonella enterica* subsp. *enterica* Serovar Anatum in spiked EG droppings was tested in four different concentrations of chlorinated pool water. In vitro testing demonstrated that *S. Anatum*-spiked EG droppings were still infectious for up to six hours in chlorinated pool water according to current German regulations for public swimming pools. This study is to be considered as a baseline investigation to clarify the role of synanthropic EG as natural carriers of zoonotic *Salmonella* in cities; nonetheless, large-scale epidemiological studies, including higher numbers of samples as well as more urban locations, are needed for final conclusions on the occurrence of this intestinal bacteria in neozootic EG. ISSN: 20762615

**Vinayaka A.C., Quyen T.L., Huynh V.N., Madsen M., Bang D.D., Wolff A.**

*Rapid detection of Salmonella enterica in primary production samples by eliminating DNA amplification inhibitors using an improved sample pre-treatment method (2023) Microbial Biotechnology, 16 (11), pp. 2105 - 2113*

**ABSTRACT:** Sensitive detection of pathogens in livestock farms is an integral part of the One Health Action Plan of the European Union (EU). Ensuring this requires on-site testing devices that are compatible with complex matrices such as primary production samples. Among all, faeces are considered the most challenging matrix type that makes it difficult to identify pathogens because of complexity in sample preparation for molecular testing. We have developed a loop-mediated isothermal amplification (LAMP) based veterinary point-of-care (POC) device (VETPOD) and adapted it to detect *Salmonella enterica* in primary production samples. Three different sampling methods (semi-wet chicken faeces, boot socks collection and dust samples from poultry shed) were iteratively tested to assess their nature of complexity and possibility for adapting them as suitable sampling methods for on-site testing. During the study, the sample preparation method that included a two-step centrifugation combined with washing of the enriched *Salmonella* cells was found crucial in eliminating amplification inhibitors originating from the faecal matrices. A total of 90 samples were tested that included 60 samples for sensitivity study and 30 samples for relative level of detection (RLOD, a level of detection in comparison to ISO 6579:1 reference method). Overall, the VETPOD had a sensitivity of 90%, 84.62% and 81.82% for boot sock, faecal and dust samples, respectively. The RLOD was 2.23 CFU/25 g which was found to be 1.33 times higher than the ISO 6579:1. Performing with an excellent agreement with ISO 6579:1, the VETPOD proved as a promising alternative to detect *Salmonella* spp. in primary production and animal husbandry samples. ISSN: 17517915

**Barua S., Bailey M., Zhong K., Iduu N., Dormitorio T., Macklin K., Bourassa D., Price S., Hauck R., Krehling J., Kitchens S., Kyriakis C., Buhr R.J., Wang C.**

*Research Note: Role of darkling beetles (Alphitobius diaperinus) and litter in spreading and maintaining Salmonella Enteritidis and Campylobacter jejuni in chicken flocks (2023) Poultry Science, 102 (11), art. no. 103061*

**ABSTRACT:** *Salmonella* and *Campylobacter* are common foodborne pathogens in chickens, but their persistence mechanisms within flocks are not fully understood. In this study, 4 groups of SPF Leghorn chickens (n = 50) were orally inoculated with 108 *Salmonella* Enteritidis and 108 *Campylobacter jejuni*, housed in BSL-2 rooms inside containers with autoclaved bedding and beetles (n = 200). Phase I (wk 1-3): the infected chickens remained in the containers and were then euthanized while beetles and litter remained in the container (group A), beetles were removed and litter remained in the container (group B), beetles remained and litter was removed (group C), and beetles and litter were removed (group D). Phase II (wk 5-7): autoclaved bedding was added to containers in groups C and D, and new SPF chickens (n = 50) were introduced and kept. Phase III (wk 8-20): all chickens were euthanized, and the litter and/or beetles remained in the containers for 17 wk. The prevalence of *Salmonella* Enteritidis and *Campylobacter* was significantly higher when detected by PCR compared to culture. In phase II, when infected chickens were removed and new chickens were introduced, 1 fecal sample in group B and

3 litter samples in groups B and C were found positive for *Salmonella* Enteritidis, and *Campylobacter* was still detected in groups A, B, and C litter samples, but not in beetles. In phase III, when all chickens were removed, *Salmonella* Enteritidis was identified in beetle samples from group A and the litter samples of all tested groups A, B, and C, and *C. jejuni* was positive in litter samples from groups A and B but not in the beetle. Sixty-nine days after removing infected chickens, culturable *Salmonella* was still found in beetles. *Salmonella* and *Campylobacter* were detectable in litter up to 127 d after removing infected chickens. This study highlights the transmission of *Salmonella* and *Campylobacter* via beetles and litter to new flocks in successive rearing cycles. Intensive control programs should target insect exclusion and implement strict poultry litter management or litter changes between flocks. ISSN: 00325791

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

*Fate and biofilm formation of Salmonella enterica subsp. enterica serovar Thompson on fresh strawberries stored under refrigeration and room temperatures (2023) Food Control, 153, art. no. 109906*

ABSTRACT: Fresh strawberry is a popular, frequently fruit consumed, which is largely appreciated for its organoleptic characteristics and health benefits. However, different outbreaks caused by food-borne pathogens have been attributed to strawberry consumption. The persistence of bacteria on fruits like strawberries could be related to their ability to adhere and form biofilms on the surface. The present study aimed to evaluate the fate and biofilm formation capacity of *Salmonella enterica* subsp. *enterica* serovar Thompson in post-harvest strawberries stored at 4 and 7 °C for 192 h and 20 °C for 72 h. Fresh strawberries were spot inoculated to achieve an initial concentration of around 5 log CFU/g of *S. Thompson*. During storage at 20 °C, a 2-log reduction was observed. At 7 and 4 °C, the *S. Thompson* population decreased 1.7 and 2.0 log, respectively, after 192 h of storage. A more marked decrease of the pathogen was observed at 20 °C, in comparison with the other temperatures tested. On the other hand, the observations with scanning electron microscopy and confocal laser scanning microscopy demonstrated the formation of biofilm on the epidermis of the strawberry stored at all temperatures. This could be considered a mechanism adopted by the bacteria against stress during storage. Prevention of biofilm formation and disruption of biofilm in strawberries become essential for food safety. ISSN: 09567135

**Miranda C., Batista S., Mateus T.L., Vieira-Pinto M., Ribeiro V., Dantas R., Brito N.V.**

*A Preliminary Investigation of Salmonella Populations in Indigenous Portuguese Layer Hen Breeds (2023) Animals, 13 (21), art. no. 3389*

ABSTRACT: The sustainability of agroecological systems, biodiversity protection, animal welfare, and consumer demand for higher quality products from alternative and extensive farming methods have reinforced interest in local breeds that are well adapted to low-input environments. However, food safety needs to be safeguarded to reinforce consumer confidence. The aim of this study was to conduct a preliminary investigation on the occurrence of *Salmonella* spp. in eggshells, hen's cloaca, and litter materials from autochthonous Portuguese laying hens raised in a semi-extensive system for small-scale production. A total of 279 samples from 31 flocks belonging to 12 farms were obtained, with 63 samples from the "Preta Lusitânica" breed, and 72 samples each from the remaining autochthonous breeds, namely, "Branca", "Amarela", and "Pedrês Portuguesa". None (0%) of the samples analyzed were positive for *Salmonella* spp. To the best of our knowledge, these are the first results of *Salmonella* evaluation from hen's cloaca, eggshells, and litter materials in autochthonous Portuguese chickens, suggesting that a semi-extensive production system can contribute to better food security and a lower risk to public health and the environment. ISSN: 20762615

**Mao Y., Zeineldin M., Usmani M., Jutla A., Shisler J.L., Whitaker R.J., Nguyen T.H.**

*Local and Environmental Reservoirs of Salmonella enterica After Hurricane Florence Flooding (2023) GeoHealth, 7 (11), art. no. e2023GH000877*

ABSTRACT: In many regions of the world, including the United States, human and animal fecal genetic markers have been found in flood waters. In this study, we use high-resolution whole genomic sequencing to examine the origin and distribution of *Salmonella enterica* after the 2018 Hurricane Florence flooding. We specifically asked whether *S. enterica* isolated from water samples collected near swine farms in North Carolina shortly after Hurricane Florence had evidence of swine origin. To investigate this, we isolated and fully sequenced 18 independent *S. enterica* strains from 10 locations (five flooded and five



unflooded). We found that all strains have extremely similar chromosomes with only five single nucleotide polymorphisms (SNPs) and possessed two plasmids assigned bioinformatically to the incompatibility groups IncFIB and IncFII. The chromosomal core genome and the IncFIB plasmid are most closely related to environmental *Salmonella* strains isolated previously from the southeastern US. In contrast, the IncFII plasmid was found in environmental *S. enterica* strains whose genomes were more divergent, suggesting the IncFII plasmid is more promiscuous than the IncFIB type. We identified 65 antibiotic resistance genes (ARGs) in each of our 18 *S. enterica* isolates. All ARGs were located on the *Salmonella* chromosome, similar to other previously characterized environmental isolates. All isolates with different SNPs were resistant to a panel of commonly used antibiotics. These results highlight the importance of environmental sources of antibiotic-resistant *S. enterica* after extreme flood events. ISSN: 24711403

**Shaji S., Selvaraj R.K., Shanmugasundaram R.**

*Salmonella Infection in Poultry: A Review on the Pathogen and Control Strategies (2023) Microorganisms, 11 (11), art. no. 2814*

ABSTRACT: *Salmonella* is the leading cause of food-borne zoonotic disease worldwide. Non-typhoidal *Salmonella* serotypes are the primary etiological agents associated with salmonellosis in poultry. Contaminated poultry eggs and meat products are the major sources of human *Salmonella* infection. Horizontal and vertical transmission are the primary routes of infection in chickens. The principal virulence genes linked to *Salmonella* pathogenesis in poultry are located in *Salmonella* pathogenicity islands 1 and 2 (SPI-1 and SPI-2). Cell-mediated and humoral immune responses are involved in the defense against *Salmonella* invasion in poultry. Vaccination of chickens and supplementation of feed additives like prebiotics, probiotics, postbiotics, synbiotics, and bacteriophages are currently being used to mitigate the *Salmonella* load in poultry. Despite the existence of various control measures, there is still a need for a broad, safe, and well-defined strategy that can confer long-term protection from *Salmonella* in poultry flocks. This review examines the current knowledge on the etiology, transmission, cell wall structure, nomenclature, pathogenesis, immune response, and efficacy of preventative approaches to *Salmonella*. ISSN: 20762607

**Babayi Khomaraki M., Ownagh A., Enferadi A.**

*Molecular Identification of Salmonella spp. in Ticks Isolated from Domestic Animals in Zanjan Province*

*(2023) Journal of Zoonotic Diseases, 7 (4), pp. 385 - 392*

ABSTRACT: *Salmonella* is a Gram-negative Bacteria that is commonly found in most environments and organisms and is a causative agent of disease. *Salmonella* spp. is one of the most common foodborne illnesses. Salmonellosis is a common infectious disease in humans and animals that manifests with gastrointestinal or hepatic symptoms and can lead to various clinical symptoms such as diarrhea in infants, fetal abortion, orchitis, pneumonia, and septicemia. In the current study, 412 hard ticks were classified and identified to investigate *Salmonella* spp. based on diagnostic keys. In total, 412 hard ticks, including 208 *Hyalomma* species and 204 *Rhipicephalus* species, were identified. The samples were divided into 82 pools according to the tick genus, and DNA was extracted from the ticks. Pathogens transmitted by ticks were diagnosed using PCR, and samples were examined for the presence of *Salmonella* spp. bacteria. In the study, a total of 208 *Hyalomma* tick samples and 204 *Rhipicephalus* tick samples were collected and were separated by gender in pools of five. Out of these, 51 male pools and 30 female pools were identified. *Rhipicephalus* ticks had 27 male and 14 female pools, while *Hyalomma* ticks had 24 males and 16 female pools. The study found that 8 out of 40 (20%; 95% CI: 10.5%-34.76%) *Hyalomma* ticks, and 12 out of 41 (29.27%; 95% CI: 17.61%-44.48%) *Rhipicephalus* ticks were carriers of the pathogens, indicating that these pathogens can be transmitted by different species of hard ticks. Ticks and tick-borne diseases are a significant public health concern worldwide. ISSN: 2476535X

**Petrović J., Mirčeta J., Prodanov-Radulović J.**

*Prevalence and main factors for Salmonella spreading in wild boars - a risk for food safety (2023) Meat Technology, 64 (2), pp. 339 - 343*

ABSTRACT: *Salmonella* is not a priority pathogen for wild boar health. However, it poses a hazard for meat safety. This paper presents the results of our multi-year research on the prevalence and epidemiology of *Salmonella* in hunting grounds in Vojvodina, Serbia. In total, 425 wild boars (25.3% of the total population) were studied. The overall *Salmonella* prevalence in Vojvodina boars was not high (3.1%) and was quite similar to findings from Spain, Germany and Japan. However, the prevalence in some hunting grounds was very high (13.3-33.3%). The anthropogenic impact is significant, as the prevalence is

statistically significantly higher in open hunting grounds where animals have contact with domestic animals and access to animal waste. The pulsotype (PFGE) profiles confirmed a link between isolates from wild pigs and domestic animals. The category of wild boars in which *Salmonella* was most commonly found was sows older than 36 months and weighing more than 75 kg, which is a direct consequence of their increased need for protein during the lactation period when they exhibit scavenging and cannibalistic behaviour. The measures taken against *Salmonella* in hunting grounds need to incorporate biosecurity measures that prevent anthropogenic influence. The hygienic and sanitary measures for the control of caught animals should also include enhanced measures when processing risk categories. ISSN: 24664812

**Patel K., Stapleton G.S., Trevejo R.T., Tellier W.T., Higa J., Adams J.K., Hernandez S.M., Sanchez S., Nemeth N.M., Debess E.E., Rogers K.H., Mete A., Watson K.D., Foss L., Low M.S.F., Gollarza L., Nichols M.**

*Human Salmonellosis Outbreak Linked to Salmonella Typhimurium Epidemic in Wild Songbirds, United States, 2020–2021*

(2023) *Emerging Infectious Diseases*, 29 (11), pp. 2298 - 2306

ABSTRACT: *Salmonella* infection causes epidemic death in wild songbirds, with potential to spread to humans. In February 2021, public health officials in Oregon and Washington, USA, isolated a strain of *Salmonella enterica* serovar Typhimurium from humans and a wild songbird. Investigation by public health partners ultimately identified 30 illnesses in 12 states linked to an epidemic of *Salmonella* Typhimurium in songbirds. We report a multistate outbreak of human salmonellosis associated with songbirds, resulting from direct handling of sick and dead birds or indirect contact with contaminated birdfeeders. Companion animals might have contributed to the spread of *Salmonella* between songbirds and patients; the outbreak strain was detected in 1 ill dog, and a cat became ill after contact with a wild bird. This outbreak highlights a One Health issue where actions like regular cleaning of birdfeeders might reduce the health risk to wildlife, companion animals, and humans. ISSN: 10806040

**Perry K.V., Kelton D.F., Dufour S., Miltenburg C., Umana Sedo S.G., Renaud D.L.**

*Risk factors for Salmonella Dublin on dairy farms in Ontario, Canada*

(2023) *Journal of Dairy Science*, 106 (12), pp. 9426 - 9439

ABSTRACT: *Salmonella* Dublin is an emerging pathogen on dairy farms in Canada. In Ontario, *Salmonella* Dublin has been increasingly isolated from diagnostic laboratory samples. The objective of this observational cross-sectional study was to identify management practices associated with herd positivity for *Salmonella* Dublin. A convenience sample of 100 dairy farms was visited in Ontario, Canada, from April to August 2022. Farms were visited once to collect blood samples from 20 heifers between 4 and 24 mo old, sample bulk tank milk, and administer an in-person questionnaire on management practices. An additional bulk tank milk sample was collected before the visit by milk transporters. All bulk tank and serum samples underwent ELISA testing to determine *Salmonella* Dublin positivity ( $\geq 35\%$  positivity on ELISA). Of the 1,990 heifers sampled, 44 (2.2%) animals were seropositive for *Salmonella* Dublin. At least one seropositive heifer was identified on 24% of participating farms. Based on the bulk tank milk samples collected during both sampling periods, 4% of farms were positive for *Salmonella* Dublin. Overall, of the 100 farms visited, 25% were classified as *Salmonella* Dublin positive, meaning at least one serum or bulk tank sample was interpreted as positive. A multivariable logistic regression model identified 5 factors associated with herd-level positivity for *Salmonella* Dublin. Specifically, introducing purchased animals within the last 2 years increased the likelihood that farms were positive for *Salmonella* Dublin (odds ratio [OR] = 4.6). Farms that had at least one animal leave the premises for a cattle show, embryo collection center, or loan to another farm and return within the last 2 years were also at a higher risk for *Salmonella* Dublin (OR = 4.9). Farms that removed manure from the surface of bedding in calving pens twice per month or after every calving were at greater risk for *Salmonella* Dublin than farms that removed manure less frequently (OR = 8.5). Farms that added bedding material to calving areas once or twice weekly were at lower risk for *Salmonella* Dublin compared with farms that added bedding less than once weekly (OR = 0.1). In addition, farms that kept 3 cows or less per pen in the calving area were at lower risk for *Salmonella* Dublin. Test positivity for *Salmonella* Dublin among Ontario dairy farms sampled is high, and dairy producers should consider avoiding management practices that are associated with an increased risk of *Salmonella* Dublin infection. ISSN: 00220302

**Pradhan J., Pradhan D., Sahu J.K., Mishra S., Mallick S., Das S., Negi V.D.**

*A novel rspA gene regulates biofilm formation and virulence of Salmonella Typhimurium (2023) Microbial Pathogenesis, 185, art. no. 106432*

ABSTRACT: *Salmonella* spp. are facultative anaerobic, Gram-negative, rod-shaped bacteria and belongs to the Enterobacteriaceae family. Although much has been known about *Salmonella* pathogenesis, the functional characterizations of certain genes are yet to be explored. The *rspA* (STM14\_1818) is one such gene with putative dehydratase function, and its role in pathogenesis is unknown. The background information showed that *rspA* gene is upregulated in *Salmonella* when it resides inside macrophages, which led us to investigate its role in *Salmonella* pathogenesis. We generated the *rspA* knockout strain and complement strain in *S. Typhimurium* 14028. Ex-vivo and in-vivo infectivity was looked at macrophage and epithelial cell lines and *Caenorhabditis elegans* (*C. elegans*). The mutant strain differentially formed the biofilm at different temperatures by altering the expression of genes involved in the synthesis of cellulose and curli. Besides, the mutant strain is hyperproliferative intracellularly and showed increased bacterial burden in *C. elegans*. The mutant strain became more infectious and lethal, causing faster death of the worms than the wild type, and also modulates the worm's innate immunity. Thus, we found that the *rspA* deletion mutant was more pathogenic. In this study, we concluded that the *rspA* gene differentially regulates the biofilm formation in a temperature dependent manner by modulating the genes involved in the synthesis of cellulose and curli and negatively regulates the *Salmonella* virulence for longer persistence inside the host. ISSN: 08824010

**Tomás Fornés D., De Benito A., Ruiz B., Klijn A.**

*Use of Neutralizers to Improve Salmonella spp. Detection in Inhibitory Food Matrices (2023) Food Analytical Methods, 16 (11-12), pp. 1680 - 1689*

ABSTRACT: The use of right sample preparation protocols is critical when analyzing challenging matrices containing inhibitory compounds inhibiting *Salmonella* growth during pre-enrichment step. The use of right sample preparation protocols will not only avoid a false negative result, but it can also have a huge positive impact on laboratory operations, as it is used for every type of method (cultural, ELISA, or PCR), every day in every laboratory. Sample preparation instructions to overcome the inhibitory effects are limited and for most cases an increase in the dilution factor (i.e., an increase of the volume of pre-enrichment broth). In this study, two neutralizers were identified as suitable alternatives for *Salmonella* detection in spices, natural flavors, and other food items (coffee, tea). Pre-enrichment with buffered peptone water and activated charcoal is considered the preferred solution, since it is cheaper to use and applicable to a broad range of food items, including coffee, vanilla, allspice, onion, tea, and peppermint with limit of detection between 0.63 and 2.45 cfu/25g whereas other food items like cinnamon and mustard were inactivated by buffered peptone water with cocktail of neutralizers (limit of detection between 0.35 and 1.05 cfu/25g). The use of neutralizers can reduce the analytical cost for *Salmonella* analysis as well as reducing the amount of reagents used (ranging from 1 L up to 4.5 L per 25-g sample) which will have a positive impact on laboratory operation workload as well as ergonomics and the environment. ISSN: 19369751

**Galán-Relaño &Á., Valero Díaz A., Huerta Lorenzo B., Gómez-Gascón L., Mena Rodríguez M.&Á., Carrasco Jiménez E., Pérez Rodríguez F., Astorga Márquez R.J.**

*Salmonella and Salmonellosis: An Update on Public Health Implications and Control Strategies (2023) Animals, 13 (23), art. no. 3666*

ABSTRACT: Salmonellosis is globally recognized as one of the leading causes of acute human bacterial gastroenteritis resulting from the consumption of animal-derived products, particularly those derived from the poultry and pig industry. *Salmonella* spp. is generally associated with self-limiting gastrointestinal symptoms, lasting between 2 and 7 days, which can vary from mild to severe. The bacteria can also spread in the bloodstream, causing sepsis and requiring effective antimicrobial therapy; however, sepsis rarely occurs. Salmonellosis control strategies are based on two fundamental aspects: (a) the reduction of prevalence levels in animals by means of health, biosecurity, or food strategies and (b) protection against infection in humans. At the food chain level, the prevention of salmonellosis requires a comprehensive approach at farm, manufacturing, distribution, and consumer levels. Proper handling of food, avoiding cross-contamination, and thorough cooking can reduce the risk and ensure the safety of food. Efforts to reduce transmission of *Salmonella* by food and other routes must be implemented using a One Health approach. Therefore, in this review we provide an update on *Salmonella*, one of the main zoonotic pathogens, emphasizing its relationship with animal and public health. We carry out a review on different topics about *Salmonella* and salmonellosis, with a special emphasis on

epidemiology and public health, microbial behavior along the food chain, predictive microbiology principles, antimicrobial resistance, and control strategies. ISSN: 20762615

**Rodríguez A., Sacristán C., Iglesias I., de la Torre A.**

*Salmonella* assessment along the Spanish food chain: Likelihood of *Salmonella* occurrence in poultry and pig products is maintained across the food chain stages (2023) *Zoonoses and Public Health*, 70 (8), pp. 665 - 673

ABSTRACT: Salmonellosis is one of the most important foodborne diseases worldwide, including the European Union. Despite the One Health approach measures for risk assessment and risk management implemented by the European Union, the occurrence of disease and disease outbreaks remains high (e.g. 694 outbreaks were reported in 2020), highlighting the need of new assessment methods. Herein we applied machine learning using the random forests method to evaluate and identify key points regarding the occurrence of *Salmonella* sp. along the Spanish food chain during 2015–2020, using data provided by the Spanish Agency for Food Safety and Nutrition. We compared the role of the three categorical variables [product (20 categories), region (18 categories) and stage (11 categories)]. *Salmonella* presence was influenced by the three explanatory variables considered: first by product, followed by region and stage. The most determinant product for *Salmonella* probability was 'meat', while the most important stage was 'slaughterhouse'. Specifically, the highest values were found in pig and poultry meats. In these products, the *Salmonella* probability was high at the early and final stages of the food chain, although not at intermediate stages. The presence of *Salmonella* in the final stages (retail) of the food chain is of concern, as it can cause human cases of salmonellosis, including outbreaks. This study demonstrates the utility of the random forest method to identify key points and evaluate the control efforts. We recommend improving the surveillance and control measures, especially in the product and stages pointed out by our analysis, and enhancing the data collection harmonization among the different autonomous communities. ISSN: 18631959

**Fjelkner J., Hultén C., Jacobson M., Nörregård E., Young B.**

*Salmonella enterica* subspecies *enterica* serovar *Choleraesuis* in a Swedish gilt-producing herd, a case report (2023) *Porcine Health Management*, 9 (1), art. no. 35

ABSTRACT: Background: When *Salmonella enterica* subspecies *enterica* serovar *Choleraesuis* (*S. Choleraesuis*) was detected in faecal samples collected within the Swedish *Salmonella* surveillance program from a gilt multiplying herd in September 2020, *S. Choleraesuis* had not been detected in domestic pigs or wild boar in Sweden for over 40 years. This report describes the subsequent investigation, identification of possible entry routes and measures undertaken to eliminate the pathogen from the herd. Case presentation: In accordance with Swedish regulations, pig movements to and from the farm were restricted, internal biosecurity measures were enhanced, and a test-and-remove strategy was implemented. Testing included repeated faecal sampling, tissue samplings from all dead or euthanized pigs, and serological sampling of replacement gilts. Epidemiological investigations included scrutinising of production records, employee interviews, analysing feed and environmental samples, faecal samples from the herd's purebred gilt supplier, and tissue and faecal samples from wild boars in the adjacent area. Testing of in-contact herds receiving gilts (n = 15) or 30-kg pigs (n = 7) from the multiplier included whole-herd faecal sampling and tissue cultures from pigs that died with signs of septicaemia. In total, *S. Choleraesuis* was detected in 12/4200 faecal and 5/1350 tissue samples from the herd, and the corresponding groups of pigs were euthanized. All feed and environmental samples as well as samples from the gilt supplier were negative. Testing of contact herds resulted in the identification and culling of one group of *S. Choleraesuis*-positive gilts. Replacement gilts introduced to the herd from January until May 2021 remained serologically negative during a surveillance-period of five months. Conclusion: Although speculative, the epidemiological investigation identified indirect transmission from wild boar as possible source of introduction to the herd. Whole-genome sequencing of *S. Choleraesuis* isolates from wild boar in the area showed that they clustered with isolates from the herd. Repeated testing of the herd indicated that the test-and-remove strategy was successful. In August 2021, all restrictions were removed, and the herd was re-instated as a gilt producing herd. Compensation from the Swedish state to the farmer for production losses, culled animals and extra costs associated with the elimination cost totalled SEK 7 992 234. ISSN: 20555660

**Reta G.G., Lopes S.M., Martins de Aquino N.S., Tondo E.C.**

*Quantification of Salmonella transfer in cross-contamination scenarios found in chicken slaughterhouses*

(2023) *Food Microbiology*, 116, art. no. 104347

ABSTRACT: Chickens are among the main reservoirs of Salmonella, and slaughterhouses have been identified as key sites for cross-contamination of this pathogen. This study aimed to quantify the transfer rate of Salmonella in different cross-contamination scenarios found in chicken slaughterhouses. To this end, a pool of Salmonella spp. was inoculated onto chicken carcasses and thighs, reaching out concentrations of 2–5 log<sub>10</sub> CFU/g. After inoculation, carcasses and thighs were used to reproduce four cross-contamination scenarios based on industrial reality as follows: 1. Transfer of Salmonella from chicken carcasses to stainless steel and polyethylene surfaces; 2. Transfer of Salmonella between hanging chicken carcasses; 3. Transfer of Salmonella from stainless steel surfaces to chicken carcasses, and 4. Transfer of Salmonella from thighs to stainless steel and polyethylene surfaces. The results showed that the transfer rates (TR) of Salmonella on the chicken carcass to stainless steel and polyethylene were 25.77 ± 22.63% and 24.71 ± 13.93%, respectively, while the TR between hanging chicken carcasses was 5.11 ± 1.71%. When sliding carcasses through a stainless steel ramp, 41.47 ± 1.32% of the Salmonella present on the ramp adhered to the chicken carcasses, and the greater transfer seems to be linked to the wet surfaces. The transfer rates from the thighs to the stainless steel and polyethylene were 1.81 ± 0.66% and 9.0 ± 1.34%, respectively. Cross-contamination occurred regardless of the sample weight, time of contact, and amount of inoculum. ISSN: 07400020

**Solís D., Cordero N., Quezada-Reyes M., Escobar-Astete C., Toro M., Navarrete P., Reyes-Jara A.**

*Prevalence of Salmonella in Eggs from Conventional and Cage-Free Egg Production Systems and the Role of Consumers in Reducing Household Contamination*

(2023) *Foods*, 12 (23), art. no. 4300

ABSTRACT: Salmonella is one of the leading causes of foodborne disease worldwide, usually related to contaminated poultry or poultry products, such as eggs. Since egg contamination with Salmonella depends on multiple factors that make it challenging to control, consumers' knowledge about food safety and the proper handling of eggs is crucial. The aims of the study were (1) to determine the prevalence of Salmonella in eggs from conventional and alternative production systems, (2) to characterize the Salmonella isolates according to phenotypic-genotypic and antimicrobial-resistant traits, and (3) to understand how consumers manage the hazards related to egg contamination in the household. A total of 426 egg samples were analyzed (conventional systems = 240; alternative systems = 186). Culture-based and molecular microbiological methods were used to identify Salmonella and bioinformatics analysis of whole genome sequences was used to determine the serotype and antimicrobial-resistant genes. Salmonella enterica serotype Enteritidis was detected only in eggs from alternative systems (1.1%, 2/186). Isolates showed resistance to nalidixic acid (100%, 2/2), and the aac(6')-Iaa gene and a mutation in the gyrA gene were identified in both isolates. Overall, consumers demonstrated knowledge regarding food safety; however, many still engage in practices that pose a risk of acquiring foodborne illnesses. ISSN: 23048158

**Wierup M.**

*The Importance of Hazard Analysis by Critical Control Point for Effective Pathogen Control in Animal Feed: Assessment of Salmonella Control in Feed Production in Sweden, 1982–2005*

(2023) *Foodborne Pathogens and Disease*, 20 (12), pp. 545 - 552

ABSTRACT: This study is the first to show that Hazard Analysis by Critical Control Point (HACCP)-based monitoring can be an effective tool for ensuring Salmonella-safe feed, by virtually eliminating feedborne Salmonella infection even in broiler production. Data from the control of Salmonella in feed and food animal production during 1982–2005, showed that conventional endpoint testing in feed mills did not ensure a Salmonella-safe feed, and in one feed mill failed to detect Salmonella contamination, resulting in the feed infecting 80 out of 197 (40.6%) recipient broiler flocks. Following implementation in 1991 of a HACCP-based control in feed mills, the annual number of samples tested at specified critical control points during a 15-year period increased from ~4400 to 10,000, while the proportion of Salmonella-contaminated samples decreased from 2.0% to 0.3%. Thus, introduction of HACCP was followed by a dramatic decrease, from 40 to <5, in the annual number of Salmonella-infected broiler flocks identified by preslaughter monitoring. Incidence has generally remained at that low level, despite production since 1980 increasing from 39 to 112 million chickens per year. Feed mills start using soy meal with an

unsafe Salmonella status and possibly with a suboptimal HACCP control, increased their level of Salmonella-contaminated HACCP samples, and their feed subsequently infected 78 swine-producing herds. The results also show that the HACCP concept can be an effective tool to supply feed mills with Salmonella-safe feed ingredients as demonstrated for a soybean crushing plant, which produced Salmonella-safe soymeal over a 19-year period despite frequent (34%) and highly varied (92 different serovars) Salmonella contamination in samples from incoming soybean. Similar results are reported for a plant producing rapeseed meal. It is emphasized that the achievements described through use of the HACCP required interventions of relevant preventive biosecurity measures and corrective actions when the HACCP-based monitoring identified Salmonella contamination. ISSN: 15353141

**Harris C.E., Josselson L.N.B., Buhr R.J.**

*Recovery of Salmonella Enteritidis and Campylobacter coli injected into hatching eggs that are cold-stored and incubated through day 18 of incubation*

*(2023) Journal of Applied Poultry Research, 32 (4), art. no. 100372*

ABSTRACT: Salmonella and Campylobacter spp. are important foodborne pathogens of concern. Both horizontal and vertical transmission of Salmonella into poultry flocks is well documented to occur, whereas only horizontal transmission of Campylobacter has been confirmed. The objective of this study was to evaluate the ability of Salmonella and Campylobacter to translocate from egg contents into the embryo during incubation. Three experiments were performed comparing Salmonella recovery from eggs that were cold-stored (48 h) and incubated up to D15 or D18 of incubation. Four experiments were performed comparing Campylobacter recovery from eggs that were cold-stored and incubated up to D15 or D18 of incubation. Eggs were injected with 10<sup>2</sup>-3 CFU Salmonella Enteritidis or 10<sup>3</sup> CFU Campylobacter coli into the albumen or yolk, and sampling of the egg contents and viable embryos was performed periodically during cold-storage and incubation. In the Salmonella experiments, egg contents from yolk injected eggs sampled during incubation were 100% positive, and no viable embryos were present. In contrast, Salmonella albumen injected eggs sampled on D18 had 51% positive egg contents and 37% positive embryos. In the Campylobacter experiments, 15% of embryos of yolk injected eggs were positive at D15, but no egg contents or embryos were positive at D18 of incubation. Results indicate that the albumen is likely to be the site for contamination than the yolk for forming eggs resulting in Salmonella positive embryos. Additionally, Campylobacter vertical transmission appears to be unlikely, but if it does occur, the location of the contamination is more likely the yolk contents. ISSN: 10566171

**Pinarelli Fazio J., Marzoli F., Pezzuto A., Bertola M., Antonelli P., Dolzan B., Barco L., Belluco S.**

*A systematic review of experimental studies on Salmonella persistence in insects*

*(2023) npj Science of Food, 7 (1), art. no. 44*

ABSTRACT: The consumption of insects as food and feed has been recently suggested as a possible alternative to the rising global food need, thus it is crucial to monitor any potential food safety hazards in the insect supply chain. The aims of this systematic review were to collect, select, and evaluate studies investigating the persistence of Salmonella in insects. We searched PUBMED, EMBASE, WEB of Science Core Collection, and Food Science and Technology Abstracts. In total, 36 papers investigating the persistence of Salmonella in insects (both holometabolous and heterometabolous) were included after screening. Regarding complete metamorphosis insects, the longest Salmonella persistence was reported in *Phormia regina*, in which the pathogen persisted for 29 days at 5 °C. Similarly, Salmonella persisted in the feces of *Alphitobius diaperinus* for 28 days. The incomplete metamorphosis insect showing the longest Salmonella persistence (>10 months) was *Blattella germanica*. *Periplaneta americana* excreted Salmonella via feces for 44 days until all the insects were dead. The retrieved data on the persistence of Salmonella can be useful for further analysis by risk assessors and decision-makers involved in the safety of insect-based food, contributing to defining the sanitary requirements and risk mitigation measures along the supply chain. The review protocol is registered in PROSPERO database (CRD42022329213). ISSN: 23968370