

# NEWSLETTER

European Union Reference Laboratory for *Salmonella*

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**European Union Reference Laboratory for *Salmonella***

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

e-mail: [EURLSalmonella@rivm.nl](mailto:EURLSalmonella@rivm.nl)

website: [www.eurlsalmonella.eu](http://www.eurlsalmonella.eu)

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## Editorial Note

Bilthoven, 3 October 2022

Dear colleague,

Hopefully you have had a nice and relaxing summer? In the Netherlands it has been very warm and dry during the summer months and I heard that it was similar in the other parts of Europe. Currently autumn is coming round again with lower temperatures and heavy rainfall.

During the summer months we have performed a first analysis of the results of the **interlaboratory study (ILS) for determination of the performance characteristics** of draft ISO/DTS 6579-4 (Identification of monophasic *Salmonella* Typhimurium by PCR). We have asked the participants for additional technical data which may help us with further analysis of the results.

Currently the NRLs-*Salmonella* are performing the **combined PT on the detection of *Salmonella* in food and in samples from the primary production stage (PPS)**. The matrix under analysis are hygiene swabs. In this PT the NRLs-*Salmonella* for food as well as the NRLs-*Salmonella* for primary production participate. Resulting in a total of 70 participants! The deadline for reporting the results of this PT is 4 November 2022.

In November the **PT on typing of *Salmonella*** will be organised, containing an obligatory part on serotyping of *Salmonella*, and a voluntary part on cluster analysis. The timetable for this PT was included in the previous Newsletter, as well as in the current one.

Early September 2022, the following EURL-*Salmonella* report was published:

Pol-Hofstad, I.E. and Mooijman, K.A., 2022. EURL-*Salmonella* Proficiency Test Primary Production Stage 2021. Detection of *Salmonella* in chicken faeces adhering to boot socks. RIVM report 2021-0129. National Institute for Public Health and the Environment, Bilthoven, the Netherlands.  
<https://www.rivm.nl/bibliotheek/rapporten/2021-0129.pdf>

Best wishes,  
Kirsten Mooijman  
Coordinator EURL-*Salmonella*

## Contribution of the EURL-*Salmonella*

### Timetable EURL- *Salmonella* Proficiency Test Typing 2022 Serotyping and optional part Cluster Analysis

Week (2022)	Date	Subject
39	Week of 26 September	Emailing of the link to the registration form for the typing study. Please <b>register by 19 October 2022</b> at the latest.
43	Week of 24 October	Emailing of the protocol 2022.
45	Monday 7 November	Shipment of the parcels to the participants as Biological Substance Category B (UN 3373).
45	Week of 7 November	<i>Upon receipt:</i> Starting the identification of the strains, according to the usual practice of the laboratory. Sending the link for the result form on serotyping to the participants. Sending the link for the result form on cluster analysis to the participants in a separate email.
50	16 December 2022 at the latest	Deadline for completing the electronic submission of <b>serotyping</b> results: <b>16 December 2022.</b> After this deadline, the result form for serotyping will be closed.
	27 January 2023 at the latest	Deadline for completing the electronic submission of <b>cluster analysis</b> results: <b>27 January 2023.</b>
	February 2023	Serotyping: Evaluation of individual laboratory results and Interim summary report.
	April/May 2023	Cluster Analysis: Evaluation of individual laboratory results and Interim summary report.

If you have questions or remarks about this Proficiency Test, or in case of problems, please contact:

Wilma Jacobs

E-mail: [wilma.jacobs@rivm.nl](mailto:wilma.jacobs@rivm.nl)

Mobile number: +31 6 3114 2419

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

## From the Literature

### Salmonella-related Literature from Scopus: July – August 2022

**D'Angelo, L., Paradiso, R., Alfano, D., Riccardi, M.G., Borriello, G., Galiero, G.**  
*First Report on Abortion Caused by Salmonella enterica subsp. enterica Serovar Enteritidis in Water Buffalo (Bubalus bubalis)*

(2022) *Frontiers in Veterinary Science*, 9, art. no. 894154, .

**ABSTRACT:** *Salmonella enterica* subsp. *enterica* Serovar Enteritidis is one of the major pathogens associated with enteric diseases in animals and humans. Thus, due to the importance of *Salmonella* spp. infections for animal production and public health, the aim of the present study was to describe the first detection of *S. enteritidis* in an aborted water buffalo fetus in southern Italy by characterizing the phylogroup profile and the antimicrobial susceptibility of the isolated pathogenic strains. The different clinical manifestations of salmonellosis in animals include diarrhea, abortion, pneumonia, septic arthritis, meningitis, and others, depending on the virulence of the serovars, infectious dose, and host immunity. This study reports the first case of abortion caused by *Salmonella enterica* subsp. *enterica* serovar Enteritidis in water buffalo (*Bubalus bubalis*) in the Campania region, southern Italy. Complete necropsy was performed on the aborted water buffalo fetus under study, and samples and swabs from different organs were collected. Samples were processed by microbiological and molecular analyses to detect bacterial, viral, and protozoarian pathogens possibly responsible for abortion. Whole genome sequencing (WGS) was carried out to further characterize the isolated *S. Enteritidis* strain. Our findings highlight the crucial role of *S. Enteritidis* as a potential abortive agent in water buffalo and its presence should therefore be investigated in cases of bubaline abortion.

ISSN: 22971769

**Rocha, M.D., Chaves, R.D., Freire, L., Pia, A.K.R., Furtado, M.M., Alvarenga, V.O., Crucello, A., Lopes, L.S., Santos, A.F.M., Rodrigues, D.P., Sant'Ana, A.S.**

*Salmonella enterica* in soybean production chain: Occurrence, characterization, and survival during soybean storage

(2022) *International Journal of Food Microbiology*, 372, art. no. 109695, .

**ABSTRACT:** This study aimed to determine *Salmonella enterica* occurrence along the soybean meal production chain (raw material, in-processing samples, final products, and in the environment of five processing plants), characterize the isolates, and assess the survival of *Salmonella* Senftenberg 775W in soybeans stored under different temperature conditions. Among 713 samples analyzed, 12.9% (n = 92) were positive for *Salmonella enterica*. Dust collected inside and outside processing plants (n = 148) comprised the samples with the highest positivity for *Salmonella enterica*, 47.3%. The occurrence of *Salmonella enterica* varied among the different processing plants. Twenty-nine (n = 29) *Salmonella* serotypes were isolated, with *S. Mbandaka* as the most frequent serotype, whereas *S. Typhimurium* was mainly linked to final product samples (soybean meal). *S. Senftenberg* 775W did not survive for a long time in soybean stored at 20-37 °C, but at 20 °C, cells were viable for more than 60 days. This study suggests that soybean meal may harbor *Salmonella* serotypes related to foodborne disease outbreaks in humans and can be responsible for *Salmonella* introduction into livestock and, consequently, in foods of animal origin. This study provides crucial data on contamination pathways of *Salmonella* in the soybean production chain, contributing to the understanding of *Salmonella* epidemiology which is strategic for the development of preventive and control measures to reduce the burden of salmonellosis linked to products of animal origin. ISSN: 01681605

**Anis, N., Bonifait, L., Quesne, S., Baugé, L., Yassine, W., Guyard-Nicodème, M., Chemaly, M.**

*Survival of Campylobacter jejuni Co-Cultured with Salmonella spp. in Aerobic Conditions* (2022) *Pathogens*, 11 (7), art. no. 812, .

**ABSTRACT:** *Campylobacter* and *Salmonella* are responsible for the two major foodborne zoonotic diseases in Europe; poultry is the main infection source. *Campylobacter* cannot grow under aerobic conditions, but can show aerobic survival when co-cultured with other

microorganisms; however, its interaction with *Salmonella* has not been studied yet. In this study, these two bacteria were co-cultured under controlled aerobic conditions. Different concentrations and strains of *C. jejuni* were incubated with or without different *Salmonella* serotypes (10 CFU) at 37 °C for 16 h. *C. jejuni* did not grow after incubation with or without *Salmonella*. The survival of *C. jejuni* was observed only for the highest initial concentration of 6 log CFU/mL with or without *Salmonella*. However, its survival was significantly higher when co-cultured with *Salmonella*. No survival was observed at lower concentrations. *C. jejuni* survival was positively affected by the presence of *Salmonella* but depended on the *Salmonella* serotype, the *C. jejuni* strain and the initial concentration. On the other hand, the *Salmonella* enumerations were not affected by *C. jejuni*. Our results suggest potential interactions between *Salmonella* and *C. jejuni* that require further investigations for a clearer understanding of their behavior in natural habitats.  
ISSN: 20760817

**McMillan, E.A., Weinroth, M.D., Frye, J.G.**

*Increased Prevalence of Salmonella Infantis Isolated from Raw Chicken and Turkey Products in the United States Is Due to a Single Clonal Lineage Carrying the pESI Plasmid (2022) Microorganisms, 10 (7), art. no. 1478, .*

ABSTRACT: *Infantis* has recently become one of the most common serotypes of *Salmonella* isolated in the U.S. from raw meat samples collected in processing facilities and in retail stores. Investigations have determined that the majority of these isolates contain the pESI plasmid, but there has not been a large-scale investigation of the chromosome of these isolates. Here, we investigated 3276 whole-genome sequences of *Salmonella Infantis* with and without the pESI plasmid to understand chromosomal differences between plasmid carriage groups. *S. Infantis* genomes arranged into multiple clades with a single clade containing the isolates carrying the plasmid. Fifty-eight SNPs were identified in complete linkage disequilibrium between isolates that did and did not carry the plasmid. However, there were no unique genes present only in the genomes of isolates containing the plasmid. On average, isolates with the plasmid did contain more insertion sequences than those without ( $p < 0.05$ ). Given that *S. Infantis* isolates carrying pESI form a single clade, it can be inferred that the increase in carriage of this plasmid in the U.S. is due to rapid clonal expansion of a single strain rather than as a result of multiple transfer events. As this *S. Infantis* clone does not contain any unique chromosomal genes, its proliferation appears to be due to pESI plasmid-encoded genes that may be advantageous in the chickens and turkeys or in their environment. ISSN: 20762607

**Sithole, T.R., Ma, Y.-X., Qin, Z., Wang, X.-D., Liu, H.-M.**

*Peanut Butter Food Safety Concerns—Prevalence, Mitigation and Control of Salmonella spp., and Aflatoxins in Peanut Butter (2022) Foods, 11 (13), art. no. 1874, .*

ABSTRACT: Peanut butter has a very large and continuously increasing global market. The food safety risks associated with its consumption are also likely to have impacts on a correspondingly large global population. In terms of prevalence and potential magnitude of impact, contamination by *Salmonella* spp., and aflatoxins, are the major food safety risks associated with peanut butter consumption. The inherent nature of the *Salmonella* spp., coupled with the unique chemical composition and structure of peanut butter, present serious technical challenges when inactivating *Salmonella* spp. in contaminated peanut butter. Thermal treatment, microwave, radiofrequency, irradiation, and high-pressure processing all are of limited efficacy in inactivating *Salmonella* spp. in contaminated peanut butter. The removal of aflatoxins in contaminated peanut butter is equally problematic and for all practical purposes almost impossible at the moment. Adopting good manufacturing hygiene practices from farm to table and avoiding the processing of contaminated peanuts are probably some of the few practically viable strategies for minimising these peanut butter food safety risks. The purpose of this review is to highlight the nature of food safety risks associated with peanut butter and to discuss the effectiveness of the initiatives that are aimed at minimising these risks. ISSN: 23048158

**Ernholm, L., Sternberg-Lewerin, S., Ågren, E., Ståhl, K., Hultén, C.**

*First Detection of Salmonella enterica Serovar Choleraesuis in Free Ranging European Wild Boar in Sweden (2022) Pathogens, 11 (7), art. no. 723, .*

ABSTRACT: Following the first detection of *Salmonella enterica* subsp. *enterica*, serovar *Choleraesuis* (*S. Choleraesuis*) in a Swedish pig herd for more than 40 years and subsequent detection of the same serotype in an enclosure with kept wild boar, a national surveillance for *S. Choleraesuis* in free living wild boar was launched. A total of 633 wild boar sampled within the active and the enhanced passive surveillance were examined for

*Salmonella enterica* serovars by culture. Of these, 80 animals were culture positive for *S. Choleraesuis* var. Kunzendorf. All positive animals, including those in the original outbreaks, originated from counties located in the southern and eastern parts of Sweden. Fifty-eight isolates were selected for sequence typing, revealing a relatively homogenous population of *S. Choleraesuis* with two distinct genetic clusters containing isolates from the southern counties in one and the counties further northeast in the other. Sequenced isolates from domestic pig farms all clustered with wild boar in the same region. *S. Choleraesuis* appears highly contagious in dense wild boar populations, making it a relevant model for other infectious diseases that may be transmitted to pigs. The many potential routes of introduction and spread of *S. Choleraesuis* warrant further investigations in order to prepare for other disease threats. ISSN: 20760817

**Gosling, R., Oastler, C., Nichols, C., Jackson, G., Wales, A.D., Davies, R.H.**

*Investigations into Salmonella Contamination in Feed Mills Producing Rations for the Broiler Industry in Great Britain*

(2022) *Veterinary Sciences*, 9 (7), art. no. 307, .

ABSTRACT: Feed-associated *Salmonella* serovars continue to be reported in poultry flocks. A study was conducted to investigate *Salmonella* contamination in major commercial feed mills that produce rations for broiler chickens within Great Britain. Dust and large moist gauze swab samples (12,791) were collected from 22 feed mills on 31 visits. *Salmonella* was isolated from 20 mills, with 15 mills (75%) having fewer than 5% *Salmonella*-positive samples. Fifty-one *Salmonella* serovars were isolated, with a large proportion of isolates being *Salmonella* (*S.*) Kedougou (29.4%) or *S.* 13,23:i:- (21.4%). European Union-regulated *Salmonella* serovars (*Enteritidis*, *Infantis*, *Typhimurium* and its monophasic variants) were isolated from 12 mills, mostly from non-processing areas, accounting for 40 isolates (4.4% of all *Salmonella*-positive samples). Fifteen *Salmonella* serovars were only isolated once. In terms of individual sampling locations within the mill, the waste handling locations were significantly more likely to be *Salmonella*-positive than some other mill locations. When sampling locations were grouped, samples collected from finished product areas were significantly less likely to be *Salmonella*-positive for *Salmonella* than some other mill areas. In conclusion, this study found that most mills producing broiler rations showed low-level *Salmonella* contamination. ISSN: 23067381

**Samarasekera, U.**

*Salmonella Typhimurium* outbreak linked to chocolate

(2022) *The Lancet. Infectious diseases*, 22 (7), p. 947.

**Cabrera-Díaz, E., Castillo, A., Martínez-Chávez, L., Beltrán-Huerta, J., Gutiérrez-González, P., Orozco-García, A.G., García-Frutos, R., Martínez-González, N.E.**

*Attachment and Survival of Salmonella enterica and Listeria monocytogenes on Tomatoes (Solanum lycopersicum) as Affected by Relative Humidity, Temperature, and Storage Time* (2022) *Journal of food protection*, 85 (7), pp. 1044-1052.

ABSTRACT: ABSTRACT: Tomatoes (*Solanum lycopersicum*) are one of the most commonly consumed fruits worldwide. The fruit can become contaminated with *Salmonella* and *Listeria monocytogenes* at various stages of the production and supply chain, and these pathogens may survive under various storage conditions. The effects of relative humidity, temperature, and duration of storage on the attachment and survival of both pathogens on the surface of tomatoes were investigated. Fresh whole Roma tomatoes were inoculated with a cocktail of *Salmonella* or *L. monocytogenes* strains and stored at 5, 12, 25, 30, or 35°C for up to 10 days. Every day during storage, relative humidity and temperature were measured and tomatoes were removed to enumerate pathogen cells that were loosely attached (LA; cells were detached from the tomato surface by rinsing) and strongly attached (SA; sonication was required to detach cells from the tomato surface). The attachment strength (SR) was calculated to express the proportion of surviving SA cells on the tomato surface. The initial levels of *Salmonella* and *L. monocytogenes* on the tomato surface after inoculation were 6.6 and 6.5 log CFU per tomato for LA cells and 5.1 and 5.6 log CFU per tomato for SA cells, respectively. For both pathogens, the LA levels were higher ( $P < 0.05$ ) than the SA levels. The LA and SA levels differed significantly as a function of temperature, relative humidity, and duration of storage. The SR for *Salmonella* was affected by storage time but not temperature, whereas the SR for *L. monocytogenes* was affected by storage time and temperature and relative humidity ( $P < 0.05$ ). An understanding of the attachment and survival of *Salmonella* and *L. monocytogenes* on tomatoes stored under various temperature conditions may be useful for preventing or reducing the establishment of pathogens and for designing improved decontamination methods. ISSN: 19449097



**Rasamsetti, S., Berrang, M.E., Cox, N.A., Shariat, N.W.**

*Assessing Salmonella prevalence and complexity through processing using different culture methods*

(2022) *Poultry Science*, 101 (7), art. no. 101949, .

**ABSTRACT:** Conventional *Salmonella* surveillance requires a week for isolation, confirmation, and subsequent serotyping. We previously showed that this could be reduced by 24 h by combining the pre-enrichment and enrichment steps into a single selective pre-enrichment step and was tested on directly after picking. The goal of this study was 2-fold: 1) to evaluate the use of selective pre-enrichment through each step of processing, including postintervention when the *Salmonella* load is reduced, and 2) to assess any changes in serovar populations in *Salmonella* positive samples. Duplicate carcass drip samples, each representative of 500 broiler carcasses, were collected by catching processing water drip under moving carcass shackle lines in each of three commercial broiler slaughter plants. Samples were collected post-pick, post-inside-outside bird wash (IOBW), and post-chill; duplicate wing rinses were performed pre- and post-antimicrobial parts dip. Each processing plant was sampled 6 times for a total of 180 samples collected. The number of *Salmonella* positives identified with selective pre-enrichment conditions (48/180) was similar to traditional selective enrichment culture conditions (52/180), showed good concordance in recovery rate between the 2 culture methods (Fisher's exact test,  $P = 0.72$ ). We also found that the incidence of *Salmonella* reduced dramatically after antimicrobial intervention (post-pick 66.7% vs. post chill 8.3%). When serovar populations were evaluated in *Salmonella* positive samples using CRISPR-SeroSeq, we detected four different *Salmonella* serovars, Kentucky, Infantis, Schwarzengrund, and Typhimurium, and their incidence rose between post-pick and post-IOBW. The relative abundance of Infantis within individual samples increased between post-pick and post-IOBW while the relative abundance of the other 3 serovars decreased. These results suggest that a selective pre-enrichment step reduces the time required for *Salmonella* isolation without negatively affecting detection and serovar profiles in culture positive samples were not altered between culture conditions used. ISSN: 00325791

**Alves, Â., Santos-Ferreira, N., Magalhães, R., Ferreira, V., Teixeira, P.**

*From chicken to salad: Cooking salt as a potential vehicle of Salmonella spp. and Listeria monocytogenes cross-contamination*

(2022) *Food Control*, 137, art. no. 108959, .

**ABSTRACT:** Epidemiological studies show that improper food handling practices at home account for a significant portion of foodborne illness cases. Mishandling of raw meat during meal preparation is one of the most frequent hazardous behaviours reported in observational research studies that potentially contributes to illness occurrence, particularly through the transfer of microbial pathogens from the raw meat to ready-to-eat (RTE) foods. This study evaluated the transfer of two major foodborne pathogens, *Salmonella enterica* and *Listeria monocytogenes*, from artificially contaminated chicken meat to lettuce via cooking salt (used for seasoning) during simulated domestic handling practices. Pieces of chicken breast fillets were spiked with five different loads (from ca. 1 to 5 Log CFU/g) of a multi-strain cocktail of either *S. enterica* or *L. monocytogenes*. Hands of volunteers (gloved) contaminated by handling the chicken, stirred the cooking salt that was further used to season lettuce leaves. A total of 15 events of cross-contamination (three volunteers and five bacterial loads) were tested for each pathogen. Immediately after the events, *S. enterica* was isolated from all the cooking salt samples ( $n = 15$ ) and from 12 samples of seasoned lettuce; whereas *L. monocytogenes* was isolated from 13 salt samples and from all the seasoned lettuce samples ( $n = 15$ ). In addition, *S. enterica* and *L. monocytogenes* were able to survive in artificially contaminated salt (with a water activity of 0.49) for, at least, 146 days and 126 days, respectively. The ability of these foodborne pathogens to survive for a long time in cooking salt, make it a good vehicle for transmission and cross-contamination if consumers do not adopt good hygiene practices when preparing meals. ISSN: 09567135

**Mudadu, A.G., Spanu, C., Pantoja, J.C.F., Dos Santos, M.C., De Oliveira, C.D., Salza, S., Piras, G., Uda, M.T., Virgilio, S., Giagnoni, L., Pereira, J.G., Tedde, T.**

*Association between Escherichia coli and Salmonella spp. food safety criteria in live bivalve molluscs from wholesale and retail markets*

(2022) *Food Control*, 137, art. no. 108942, .

**ABSTRACT:** This study presents epidemiological data on the prevalence of *Salmonella* spp. and *E. coli* in bivalve molluscs marketed in Sardinia (Italy). *E. coli* enumeration and *Salmonella* spp. occurrence at batch level were used to verify the association between microbiological food safety criteria in place in the European Community. From 2017 to 2020 bivalve molluscs samples including 2115 mussels (*Mytilus galloprovincialis*), 150

oysters (*Crassostrea gigas*) and 65 clams (*Ruditapes decussatus*) were collected at wholesale market and retail stores from 8 provinces of Sardinia. All samples were collected during official control activities and analyzed for the enumeration of *E. coli* according to the Most Probable Number (MPN) method (ISO 16649-3) and for the detection of *Salmonella* spp. according to the reference method ISO 6579-1 (ISO, 2017). Evaluation of *E. coli* and *Salmonella* spp. contamination was conducted at sample unit level while satisfaction of microbiological criteria was evaluated at batch level (each batch was composed of 5 bivalve molluscs sample units). Logistic regression was used to estimate the chances of observing an *E. coli* unsatisfactory batch as a function of year of collection (2017, 2018, 2019 and 2020), molluscs species (mussels, oysters, and clams), season (winter, fall, spring, and summer), and type of market (wholesale or retail). The Chi-square test was used to investigate the association between the occurrence of *E. coli* and *Salmonella* spp. in the study sample. Overall, *E. coli* was <230 MPN/100 g in 97.6% of the samples while *Salmonella* spp. was detected in 0.6% of the samples. At batch level, unsatisfactory results for *E. coli* and *Salmonella* spp. were observed in 2.6% and 1.7% of the samples, respectively. Instances of values above the limit were all observed in mussel's samples. The chances of *E. coli* unsatisfactory results were not different among the years of collection, bivalve species, season, or market type. No significant association was observed between *E. coli* and *Salmonella* spp. at both sample unit and batch levels, revealing a poor association between the two criteria in the final product. Overall, the edible bivalve molluscs marketed in Sardinia demonstrated a high microbiological quality and compliance with European Union criteria. ISSN: 09567135

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

*Survivability of Salmonella and Enterococcus faecium in chili, cinnamon and black pepper powders during storage and isothermal treatments*  
(2022) *Food Control*, 137, art. no. 108935, .

ABSTRACT: Outbreaks and recalls associated with foods containing spices suggest a need for risk assessment of *Salmonella* in spices. In this study, the survivability of *Salmonella* Enteritidis PT 30, *Salmonella* cocktail (*S. Enteritidis* PT 30, *S. Tennessee* K4643 and *S. Agona* 447967), and *Enterococcus faecium* NRRL B-2354 in chili, cinnamon and black pepper at water activities (*aw*) 0.3 and 0.5 were evaluated during one-year storage at 21 °C. The thermal resistance of *Salmonella* cocktail in spices was also evaluated at 70 °C before and after storage. At *aw* 0.5, 4-month storage caused 5 log reduction of *Salmonella* cocktail in chili, while 8 months led to the same level of reduction in cinnamon. But only 3 log reduction were observed in black pepper over one year. Storage at *aw* 0.3 caused less reduction in *Salmonella* cocktail during the same storage periods. Less than 2 log reduction of *E. faecium* were observed over the one year storage at both *aw* levels, except for in chili stored at 0.5 *aw*. The D70°C-values for *Salmonella* cocktail in chili, cinnamon and black pepper of *aw* 0.3 before storage were 15.4, 20.8 and 36.6 min, respectively. 21–50% drops in the D70°C-value were obtained after two-month of storage, mostly in chili and least in black pepper. The high D70°C-value in black pepper persisted over one-year storage. Based on these results, chili powder showed the highest antimicrobial effect, followed by cinnamon and black pepper powders during storage and isothermal treatments. ISSN: 09567135

**Kosznik-Kwaśnicka, K., Podlacha, M., Grabowski, Ł., Stasiłojć, M., Nowak-Zaleska, A., Ciemińska, K., Cyske, Z., Dydecka, A., Gaffke, L., Mantej, J., Myślińska, D., Necel, A., Pierzynowska, K., Piotrowska, E., Radzanowska-Alenowicz, E., Rintz, E., Sitko, K., Topka-Bielecka, G., Węgrzyn, G., Węgrzyn, A.**

*Biological aspects of phage therapy versus antibiotics against Salmonella enterica serovar Typhimurium infection of chickens*  
(2022) *Frontiers in Cellular and Infection Microbiology*, 12, art. no. 941867, .

ABSTRACT: Phage therapy is a promising alternative treatment of bacterial infections in human and animals. Nevertheless, despite the appearance of many bacterial strains resistant to antibiotics, these drugs still remain important therapeutics used in human and veterinary medicine. Although experimental phage therapy of infections caused by *Salmonella enterica* was described previously by many groups, those studies focused solely on effects caused by bacteriophages. Here, we compared the use of phage therapy (employing a cocktail composed of two previously isolated and characterized bacteriophages, vB\_SenM-2 and vB\_Sen-TO17) and antibiotics (enrofloxacin and colistin) in chickens infected experimentally with *S. enterica* serovar Typhimurium. We found that the efficacies of both types of therapies (i.e. the use of antibiotics and phage cocktail) were high and very similar to one another when the treatment was applied shortly (one day) after the infection. Under these conditions, *S. Typhimurium* was quickly eliminated from the gastrointestinal tract (GIT), to the amount not detectable by the used methods.

However, later treatment (2 or 4 days after detection of *S. Typhimurium* in chicken feces) with the phage cocktail was significantly less effective. Bacteriophages remained in the GIT for up to 2-3 weeks, and then were absent in feces and cloaca swabs. Interestingly, both phages could be found in various organs of chickens though with a relatively low abundance. No development of resistance of *S. Typhimurium* to phages or antibiotics was detected during the experiment. Importantly, although antibiotics significantly changed the GIT microbiome of chickens in a long-term manner, analogous changes caused by phages were transient, and the microbiome normalized a few weeks after the treatment. In conclusion, phage therapy against *S. Typhimurium* infection in chickens appeared as effective as antibiotic therapy (with either enrofloxacin or colistin), and less invasive than the use of the antibiotics as fewer changes in the microbiome were observed. Copyright ISSN: 22352988

**Hollmann, I., Lingens, J.B., Wilke, V., Homann, C., Teich, K., Buch, J., Chuppava, B., Visscher, C.**

*Epidemiological Study on Salmonella Prevalence in Sow Herds Using Direct and Indirect Detection Methods*

(2022) *Microorganisms*, 10 (8), art. no. 1532, .

ABSTRACT: In piglet production, the beginning of pork production, *Salmonella* prevalence requires greater attention as having an impact on the subsequent production steps. The aim of this study was to investigate *Salmonella* prevalence in three sow herds with attached piglet rearing units. *Salmonella* prevalence was investigated either directly by boot swabs and feces or indirectly by serum samples taken during gilt integration, the periparturient period, and piglet rearing. Boot swabs and feces were analyzed by real-time PCR and subsequent microbiology. Results indicated that high biosecurity measures in sow husbandry do not necessarily result in a low *Salmonella* prevalence. Furthermore, the sow herds' *Salmonella* prevalence should not be used to infer the situation in the associated piglet rearing. The proportion of positive boot swabs was 10.5, 3.6, and 21.3% for sows (gilts and periparturient) with an inverse situation in piglet rearing with 50.0, 63.3, and 5.8% positive swabs for farms A, B, and C, respectively. Boot swabs are suitable as a direct sampling method to gain an overview of *Salmonella* prevalence in both sows and piglets. Indirect serum antibody testing can be useful, although it should be evaluated considering age-dependent levels of antibody titres. ISSN: 20762607

**Grisendi, A., Defilippo, F., Lucchetti, C., Listorti, V., Ottoboni, M., Dottori, M., Serraino, A., Pinotti, L., Bonilauri, P.**

*Fate of Salmonella enterica Typhimurium and Listeria monocytogenes in Black Soldier Fly (Hermetia illucens) Larvae Reared on Two Artificial Diets*

(2022) *Foods*, 11 (15), art. no. 2208, .

ABSTRACT: Ensuring food security is one of the main challenges facing the world over the next 30 years. There is, thus, an urgent need to significantly increase the supply of sustainable protein that can be transformed into animal feed. Proteins from insects offer a valuable alternative. This article presents the results of challenge tests conducted to investigate the dynamics of the microbial load of *Salmonella enterica Typhimurium* and *Listeria monocytogenes* in black soldier fly (*Hermetia illucens*) larvae grown on contaminated substrates. Four separate challenge tests were performed on two substrates: the Gainesville diet and a homemade diet. The challenge test procedure was carried out in accordance with ISO/DIS 20976-2 (under development). The results of this study show that, when grown on contaminated substrates, BSF larvae do not eliminate *Salmonella Typhimurium* or *L. monocytogenes*, but can reduce their microbial load. Sanitation processes downstream of the breeding of BSF larvae are, however, required to reduce the microbiological risks of this novel food. ISSN: 23048158

**Moulana, Z., Asgharpour, F.**

*Prevalence and Antimicrobial Resistance of Salmonella Enterica Serovar Infantis Isolates from Poultry: a review*

(2022) *Poultry Science Journal*, 10 (1), pp. 13-26.

ABSTRACT: *Salmonella Infantis* (*S. Infantis*) is one of the most important zoonotic bacteria, which has become one of the leading public health problems in the world, especially in developing countries. The prevalence of multi-drug resistant (MDR) *S. Infantis* strains has increased worldwide and can be prevented by controlling the use of antibiotics in poultry. The purpose of this review article is to discuss the status of *S. Infantis* antibiotic resistance, especially, its prevalence, detection methods and resistance mechanisms in isolates from poultry samples using search engines such as Web of Science, Scopus, and PubMed. Based on our review, *S. Infantis* was the most prevalent serovar in poultry accompanied by an enhancing number of resistance genes in these strains. The use of

different genotypic and genetic methods can rapidly detect the presence of *Salmonella* in suspicious specimens to prevent disease and epidemics. Genes such as *invA*, *hilA* and *fliC* were most commonly used genes in the detection of *Salmonella*, and other genes were *viaB*, *spv*, *fliJB*, *rflB* and 16Sr RNA. The results of studies emphasize that poultry could act as reservoirs of MDR with a high tendency for dissemination. Resistance to the beta-lactam family is an important issue, because antibiotics such as beta-lactams are the best candidates for the treatment of salmonellosis, and this has raised concerns in the treatment of invasive *Salmonella*. These findings highlight the need to find ways to manage and reduce the impact of antibiotic use in poultry and prevent the transmission of antibiotic-resistant *S. infantis* to the human food chain and to find potential alternatives to antibiotics. ISSN: 23456604

**Torrico, M., Casino, P., López, A., Peiró, S., Ríos, M., Ríos, S., Montes, M.J., Guillén, C., Nardi-Ricart, A., García-Montoya, E., Asensio, D., Marqués, A.M., Piqué, N.**

*Improvement of Mueller-Kauffman Tetrathionate-Novobiocin (MKTTn) enrichment medium for the detection of Salmonella enterica by the addition of ex situ-generated tetrathionate (2022) Journal of microbiological methods, 199, p. 106524.*

**ABSTRACT:** The detection of *Salmonella* in food is based on the use of a selective enrichment broth such as Muller-Kauffman Tetrathionate-Novobiocin (MKTTn), in which tetrathionate plays a key role by providing *Salmonella* with a growth advantage. As sodium tetrathionate is unstable, it is generated in situ by the addition of iodine (Lugol's solution) before seeding. This step is cumbersome as the solution is easily spilled, compromising the performance of the medium and hindering the work of technicians. The aim of this study was to optimize MKTTn broth by generating tetrathionate ex situ through an external reaction between iodine and thiosulphate followed by lyophilization. Quality control procedures were performed to compare the modified and original media, testing pure productivity (enrichment with 50-120 CFU of *Salmonella* *Thyphimurium* ATCC 14028 and *Salmonella* *Enteritidis* ATCC 13076 and plating on Xylose Lysine Deoxycholate agar, XLD), mixed productivity (50-120 CFU of *Salmonella* strains and *Pseudomonas aeruginosa* and *Escherichia coli* at  $\geq 10^4$  CFU and XLD plating) and selectivity ( $\geq 10^4$  CFU of *P. aeruginosa* and *Enterococcus faecalis* and plating on Tryptone Casein Soy agar, TSA). The modified MKTTn medium (S/L) performed comparably with the original medium in terms of growth of both *Salmonella* strains ( $> 300$  colonies in XLD), alone or with *P. aeruginosa* and *E. coli*. Quantitative assays showed no statistically significant differences in the number of colonies grown on XLD after 10-5 dilution ( $p = 0.7015$  with *S. Thyphimurium* ATCC 14028 and  $p = 0.2387$  with *S. Enteritidis* ATCC 13076; ANOVA test). MKTTn medium (S/L) was also selective against *E. coli* ( $\leq 100$  colonies) and *E. faecalis* ( $< 10$  colonies). These results suggest that adding tetrathionate as a lyophilisate (S/L) is a feasible alternative to the use of Lugol's solution for the preparation of MKTTn enrichment broth and does not affect the properties of the medium. ISSN: 18728359

**Shimajima, Y., Shimajima, H., Morita, Y.**

*Survival of Campylobacter jejuni, Salmonella, and Listeria monocytogenes and Temperature Change in Low-Temperature-Longtime-Cooked Chicken Meat (2022) Journal of food protection, 85 (8), pp. 1166-1171.*

**ABSTRACT:** ABSTRACT: Low-temperature and longtime (LT-LT) cooking, also known as sous vide cooking, is the process in which meat is sealed in a bag and cooked in hot water at a relatively low temperature of around 60°C. This cooking method has increased in popularity, and low-temperature cookers for home use are now commercially available. However, after LT-LT cooking, if any foodborne bacteria remain, they could cause infection and foodborne illnesses. Therefore, in the present study, the aim was to determine the appropriate LT-LT cooking methods for chicken by assessing temperature changes and studying the bacteria in LT-LT-cooked chicken meat. At set cooking temperatures of 60 and 65°C, the temperatures were measured at the surface and in the centers of single- and double-layer samples of 300 g of chicken breast meat. The times required to reach 50°C were 5 to 14 min at the surface, 25 min in the center of the single-layer sample, and 33 to 35 min in the center of the double-layer sample. The time taken to reach 50°C was fastest in the surface of single-layer chicken meat, followed by the center of single-layer and double-layer chicken meat ( $P < 0.05$ ). When the meat was LT-LT cooked at 60 and 65°C for 60 min, color changes in the meat and heating of the meat were observed all the way to the interior. *Campylobacter jejuni*, *Salmonella* O7, and *Listeria monocytogenes* were inoculated into chicken breasts, which were then cooked at set temperatures of 60 and 65°C for 15, 30, 60, 90, and 120 min. *C. jejuni* survived for up to 30 min of cooking, *Salmonella* O7 survived for up to 60 min of cooking at 60°C and 30 min at 65°C, and *L. monocytogenes* survived for up to 90 min of cooking at 60°C and 60 min at 65°C. Thus, to

prevent infection and illness caused by the three tested bacteria species, LT-LT cooking for 120 min at 60°C and 90 min at 65°C is recommended. ISSN: 19449097

**Marin, C., Cerdà-Cuéllar, M., González-Bodi, S., Lorenzo-Rebenaque, L., Vega, S.**  
*Research Note: Persistent Salmonella problems in slaughterhouses related to clones linked to poultry companies*

(2022) *Poultry Science*, 101 (8), art. no. 101968, .

ABSTRACT: Salmonellosis remains one of the main foodborne zoonoses in Europe, with poultry products as the main source of human infections. The slaughterhouse has been identified as a potential source for Salmonella contamination of poultry meat. Despite the mandatory programme of the EU, there are companies with persistent Salmonella that are unable to remove the bacteria from their processing environment, compromising the entire production line. In this context, an intensive sampling study was conducted to investigate a slaughterhouse with persistent Salmonella problems, establishing the genetic relationship among Salmonella strains isolated during the slaughter process. A total of 36 broiler flocks were sampled during processing at the slaughterhouse. Salmonella was identified based on ISO 6579-1:2017 (Annex D), serotyped by Kauffman-White-Le-Minor technique, and the genetic relationship was assessed with ERIC-PCR followed by PFGE. The outcomes showed that 69.4% of the batches sampled carried Salmonella upon arrival at the slaughterhouse and that 46.3% of the different samples from carcasses were contaminated with Salmonella. The two serovars isolated at the different steps in the slaughterhouse were Enteritidis (98.2%) and Kentucky (1.8%). Pulsed-field gel electrophoresis analysis revealed a low genetic diversity, with all *S. Enteritidis* isolates showing a nearly identical pulsotype (similarity >85%) and *S. Kentucky* strains showed the same XbaI PFGE profile (95.0% genetic similarity). The results of this study showed a high genetic relationship among isolates recovered from carcasses and environmental samples in the slaughterhouse from both Salmonella-positive and Salmonella-free flocks. Salmonella strains re-circulated across to poultry flocks and re-entered the slaughterhouse to survive on the processing line. Thus, it is necessary to implement molecular diagnosis methods in time at the field level to determine the Salmonella epidemiology of the flock, to make rapid decisions for the control of Salmonella and prevent entry into the slaughterhouse environment. ISSN: 00325791

**Georgalis, L., Psaroulaki, A., Aznar, A., Fernández, P.S., Garre, A.**

*Different model hypotheses are needed to account for qualitative variability in the response of two strains of Salmonella spp. under dynamic conditions*

(2022) *Food Research International*, 158, art. no. 111477, .

ABSTRACT: In this article, the thermal inactivation of two Salmonella strains (*Salmonella* Enteritidis CECT4300 and *Salmonella* Senftenberg CECT4565) was studied under both isothermal and dynamic conditions. We observed large differences between these two strains, with *S. Senftenberg* being much more resistant than *S. Enteritidis*. Under isothermal conditions, *S. Senftenberg* had non-linear survivor curves, whereas the response of *S. Enteritidis* was log-linear. Therefore, weibullian inactivation models were used to describe the response of *S. Senftenberg*, with the Mafart model being the more suitable one. For *S. Enteritidis*, the Bigelow (log-linear) inactivation model was successful at describing the isothermal response. Under dynamic conditions, a combination of the Peleg and Mafart models (secondary model of Mafart;  $t^*$  of Peleg) fitted to the isothermal data could predict the response of *S. Senftenberg* to the dynamic treatments tested (heating rates between 0.5 and 10 °C/min). This was not the case for *S. Enteritidis*, where the model predictions based on isothermal data underestimated the microbial concentrations. Therefore, a dynamic model that considers stress acclimation to one of the dynamic profiles was fitted, using the remaining profiles as validation. In light of this, besides its quantitative impact, variability between strains of bacterial species can also cause qualitative differences in microbial inactivation. This is demonstrated by *S. Enteritidis* being able to develop stress acclimation where *S. Senftenberg* could not. This has important implications for the development of microbial inactivation models to support process design, as every industrial treatment is dynamic. Consequently, it is crucial to consider different model hypotheses, and how they affect the model predictions both under isothermal and dynamic conditions. ISSN: 09639969

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

*Growth potential of Salmonella enterica in thirty-four different RTE vegetable salads during shelf-life*

(2022) *International Journal of Food Science and Technology*, 57 (8), pp. 5036-5047.

ABSTRACT: Thirty-four different ready-to eat (RTE) vegetable salads were inoculated with a cocktail of three *Salmonella enterica* strains, and stored under a modified atmosphere for

up to 168 h at 4, 7, 12 and 16°C. Eighteen (18) of the salad samples comprised of two or more vegetable ingredients (also referred to as MV RTE salads), and 16 were made up of single vegetable ingredients (SV RTE salads). Generally, the growth potential of inoculated *S. enterica* varied depending on temperature and type of RTE vegetable salad. The higher temperature was generally more favourable for the growth of *S. enterica*. Among all 34 salad samples, 5, 11, 18 and 24 salad samples supported the growth of *Salmonella* at 4, 7, 12 and 16°C, respectively. All salads consisting of multiple vegetable ingredients except two: one comprised of carrots, lettuce and beetroot and another comprised of white cabbage and purple cabbage, supported the growth of *Salmonella* at high temperatures (either 12 or 16 or both 12 and 16°C). Although the growth of *Salmonella* was variable in the different types of RTE salads, and growth was generally low at 4°C, *Salmonella* exhibited consistently minimal growth in some vegetable salads such as those comprised of carrots, lettuce and beetroot, carrots, beetroots, cabbage and cucumber, as well as one comprised of beetroot and corn at all temperature conditions tested. ISSN: 09505423

**Toro, M., Weller, D., Ramos, R., Diaz, L., Alvarez, F.P., Reyes-Jara, A., Moreno-Switt, A.I., Meng, J., Adell, A.D.**

*Environmental and anthropogenic factors associated with the likelihood of detecting Salmonella in agricultural watersheds*

(2022) *Environmental Pollution*, 306, art. no. 119298, .

ABSTRACT: Surface water is one of the primary sources of irrigation water for produce production; therefore, its contamination by foodborne pathogens, such as *Salmonella*, may substantially impact public health. In this study, we determined the presence of *Salmonella* in surface water and characterized the relationship between *Salmonella* detection and environmental and anthropogenic factors. From April 2019 to February 2020, 120 samples from 30 sites were collected monthly in four watersheds located in two different central Chile agricultural regions (N = 1080). Water samples from rivers, canals, streams, and ponds linked to each watershed were obtained. Surface water (10 L) was filtrated in situ, and samples were analyzed for the presence of *Salmonella*. *Salmonella* was detected every month in all watersheds, with a mean detection percentage of 28% (0%–90%) across sampling sites, regardless of the season. Overall, similar detection percentages were observed for both regions: 29.1% for Metropolitan and 27.0% for Maule. *Salmonella* was most often detected in summer (39.8% of all summer samples tested positive) and least often in winter (14.4% of winter samples). Random forest analysis showed that season, water source, and month, followed by latitude and river, were the most influential factors associated with *Salmonella* detection. The influences of water pH and temperature (categorized as environmental factors) and factors associated with human activity (categorized as anthropogenic factors) registered at the sampling site were weakly or not associated with *Salmonella* detection. In conclusion, *Salmonella* was detected in surface water potentially used for irrigation, and its presence was linked to season and water source factors. Interventions are necessary to prevent contamination of produce, such as water treatment before irrigation. ISSN: 02697491

**Hessel, C.T., de Freitas Costa, E., Boff, R.T., Pessoa, J.P., Tondo, E.C.**

*A systematic review and Bayesian meta-analysis about Salmonella spp. prevalence on raw chicken meat*

(2022) *Microbial Risk Analysis*, 21, art. no. 100205, .

ABSTRACT: Salmonellosis involving chicken meat is one of the most frequent foodborne diseases registered worldwide. Many studies report the prevalence of *Salmonella* spp. on chicken meat; however, data are limited or variable. To perform stochastic Quantitative Microbial Risk Analysis, it is essential to input reliable data to estimate the risks, and the Bayesian meta-analysis model allows incorporating the uncertainty of the data into parameters which increases the robustness of the model. In this manuscript, we conduct a systematic review and a logit-normal hierarchical Bayesian meta-analysis model to assess the posterior distribution of *Salmonella* spp. prevalence of raw chicken meat. The posterior distribution of *Salmonella* spp. was reported according to carcass processing (whole carcass or cuts); cold status (fresh meat or frozen); place of sampling (retail or slaughterhouse), and geographical region (Brazil, Latin America, North America, Africa, Asia, and Europe). To implement the posterior distribution as uncertainty in stochastic a model, parameters were obtained by linear combination of the posterior distributions of the model. The percentual of variation regarding the heterogeneity between studies is 33.93%. Carcass processing and cold status do not influence *Salmonella* spp. prevalence. Raw chicken meat collected at slaughterhouses had a 4% higher chance of being positive for *Salmonella* spp. than those taken at retail. However, this small difference seems to be of minor relevance given the large 95% credible interval around the parameter. The posterior distribution shows lower *Salmonella* spp. prevalence for Latin America, Brazil,

Africa, Europe when compared to North America and Asia. In the sensitivity analysis, the parameters  $\beta_{cold}$ ,  $\beta_{sample}$ , and  $\beta_{processing}$  were weakly influenced by the priors, however, the relevance of the priors was more evident for the geographic region related parameters. *Salmonella* Enteritidis was the most widespread serovar identified and only three studies verified the concentration of *Salmonella* spp. but we were not able to conduct a meta-analysis because the studies omitted the standard deviation. ISSN: 23523522

**Oastler, C.E., Nichols, C., Newton, K., Cawthraw, S., Gosling, R.J., Martelli, F., Wales, A.D., Davies, R.H.**

*Observations on the distribution and control of Salmonella in commercial broiler hatcheries in Great Britain*

(2022) *Zoonoses and Public Health*, 69 (5), pp. 487-498.

**ABSTRACT:** *Salmonella* can enter hatcheries via contaminated eggs and other breaches of biosecurity. The study examined the prevalence and distribution of *Salmonella* in commercial hatcheries and assessed the effects of providing advice on *Salmonella* control. Intensive swab sampling was performed throughout 23 broiler hatcheries in Great Britain (GB). Swabs were cultured using a modified ISO6579:2017 method. After each visit, tailored advice on biosecurity and cleaning and disinfection procedures was provided to the hatchery managers. Repeat sampling was carried out in 10 of the 23 hatcheries. *Salmonella* prevalence ranged between 0% and 33.5%, with the chick handling areas, hatcher areas, macerator area, tray wash/storage areas, external areas and other waste handling areas being more contaminated than the setter areas. *Salmonella* Senftenberg and *Salmonella* 13,23:i:- were the most commonly isolated serovars. There was a reduction in *Salmonella* prevalence at the second visit in eight out of 10 premises, but prevalence values had increased again in all of the improved hatcheries that were visited a third time. One hatchery harboured a difficult-to-control resident *Salmonella* 13,23:i:- strain and was visited six times; by the final visit, *Salmonella* prevalence was 2.3%, reduced from a high of 23.1%. In conclusion, the study found low-level *Salmonella* contamination in some GB broiler hatcheries, with certain hatcheries being more severely affected. Furthermore, it was shown that *Salmonella* typically is difficult to eradicate from contaminated hatcheries, but substantial reductions in prevalence are possible with improvements to biosecurity, cleaning and disinfection. ISSN: 18631959

**Pettengill, J.B., Rand, H., Wang, S.S., Kautter, D., Pightling, A., Wang, Y.**

*Transient and resident pathogens: Intra-facility genetic diversity of Listeria monocytogenes and Salmonella from food production environments*

(2022) *PLoS ONE*, 17 (9 September), art. no. e0268470, .

**ABSTRACT:** Food production facilities are often routinely tested over time for the presence of foodborne pathogens (e.g., *Listeria monocytogenes* or *Salmonella enterica* subsp. *enterica*). Strains detected in a single sampling event can be classified as transient; positive findings of the same strain across multiple sampling events can be classified as resident pathogens. We analyzed whole-genome sequence (WGS) data from 4,758 isolates (*L. monocytogenes* = 3,685; *Salmonella* = 1,073) from environmental samples taken by FDA from 536 U.S. facilities. Our primary objective was to determine the frequency of transient or resident pathogens within food production facilities. Strains were defined as isolates from the same facility that are less than 50 SNP (single-nucleotide polymorphisms) different from one another. Resident pathogens were defined as strains that had more than one isolate collected >59 days apart and from the same facility. We found 1,076 strains (median = 1 and maximum = 21 strains per facility); 180 were resident pathogens, 659 were transient, and 237 came from facilities that had only been sampled once. As a result, 21% of strains (180/839) from facilities with positive findings and that were sampled multiple times were found to be resident pathogens; nearly 1 in 4 (23%) of *L. monocytogenes* strains were found to be resident pathogens compared to 1 in 6 (16%) of *Salmonella* strains. Our results emphasize the critical importance of preventing the colonization of food production environments by foodborne pathogens, since when colonization does occur, there is an appreciable chance it will become a resident pathogen that presents an ongoing potential to contaminate product. ISSN: 19326203

**De Sousa Violante, M., Podeur, G., Michel, V., Guillier, L., Radomski, N., Lailier, R., Le Hello, S., Weill, F.-X., Mistou, M.-Y., Mallet, L.**

*A retrospective and regional approach assessing the genomic diversity of Salmonella Dublin*

(2022) *NAR Genomics and Bioinformatics*, 4 (3), art. no. lqac047, .

**ABSTRACT:** From a historically rare serotype, *Salmonella enterica* subsp. *enterica* Dublin slowly became one of the most prevalent *Salmonella* in cattle and raw milk cheese in some regions of France. We present a retrospective genomic analysis of 480 *S. Dublin* isolates to

address the context, evolutionary dynamics, local diversity and the genesis processes of regional *S. Dublin* outbreaks events between 2015 and 2017. Samples were clustered and assessed for correlation against metadata including isolation date, isolation matrices, geographical origin and epidemiological hypotheses. Significant findings can be drawn from this work. We found that the geographical distance was a major factor explaining genetic groups in the early stages of the cheese production processes (animals, farms) while down-the-line transformation steps were more likely to host genomic diversity. This supports the hypothesis of a generalised local persistence of strains from animal to finished products, with occasional migration. We also observed that the bacterial surveillance is representative of diversity, while targeted investigations without genomics evidence often included unrelated isolates. Combining both approaches in phylogeography methods allows a better representation of the dynamics, of outbreaks. ISSN: 26319268

**Cathcart, A., Smyth, B.M., Forbes, C., Lyons, G., Murray, S.T., Rooney, D., Johnston, C.R.**

*Effect of anaerobic digestate fuel pellet production on Enterobacteriaceae and Salmonella persistence*

(2022) *GCB Bioenergy*, 14 (9), pp. 1055-1064.

**ABSTRACT:** Production of digestate pellets for fuel has been identified as a promising circular economy approach to provide renewable energy and additional income to farms, while at the same time presenting the potential to divert raw digestate from nutrient-saturated land and reduce the risk to water quality. Although previous research has investigated the feasibility of pellet production, there has been little focus on the bio-safety aspects of the system. Little is currently known about the persistence of bacteria present in the digestate and the potential impacts on human health for those handling this product. The aim of the present research was to determine the effect that each step in the pellet production process has on bacteria numbers: anaerobic digestion, mechanical separation, solid drying, and pelletisation. Enterobacteriaceae enumeration by colony count method was used to quantify bacteria, and the presence of *Salmonella* at each stage was determined. The Enterobacteriaceae count reduced with each stage, and the final pelletisation step reduced bacteria numbers to below detectable levels (<10 colony forming units/g). *Salmonella* was only detected in the starting slurry and absent from digestate onwards. Storage of the pellets under winter and simulated summer conditions showed no reactivation of Enterobacteriaceae over time. The pelletisation process produces a digestate product with Enterobacteriaceae counts below the maximum threshold (PAS110 specification) for transport off the source farm, but care must still be taken when handling digestate pellets as complete sterilisation has not been confirmed. ISSN: 17571693

**Ayasi, H., Dastar, B., Ghoorchi, T., Hashemi, S.R., Tabaraei, A., Alemi, M.**

*Research Note: Effect of corn silage and alfalfa meal as alternative induced molt methods to improving Salmonella Enteritidis resistance in laying hens*

(2022) *Poultry Science*, 101 (9), art. no. 101984, .

**ABSTRACT:** This experiment was conducted to evaluate diets containing a high level of corn silage and alfalfa meal in inducing molt and reducing susceptibility to *Salmonella Enteritidis* (SE) colonization in laying hens. Thirty-two healthy hens were examined by cloacal swab samples to be free of *Salmonella*. Then they were weighed individually and distributed to 4 experimental groups containing 8 hens each, including Full-fed (control, FF); total feed withdrawal (positive control for molt induction, FW); 80% corn silage (CS) + 20% layer diet (CS80), and 80% alfalfa meal (AM) + 20% layer diet (AM80). The molting program was initiated at 71 wk of age. On d 4 of the experiment, all hens were inoculated with SE by oral gavage. All hens were first weighed at the ending molting period on d 10 and then euthanized by CO<sub>2</sub> gas. The internal organs including the ovary, oviduct, liver, and spleen, were excised aseptically and weighed. Cloacal swab and feed samples at the beginning and organ samples (liver, ovary, spleen, and cecum) were collected from each hen at the end of the experiment and examined for SE colonies. Molted birds lost roughly 14 to 27% of their body weight and had significantly lower organ weight and egg production compared to FF group ( $P < 0.05$ ). No significant difference was observed in the number of days to zero egg production between molted treatments. The SE positive organs did not significantly differ between CS80 and AM80 with FF treatment. Treatment CS80 had the lowest crop pH and differed substantially from treatment FW. In conclusion, results indicate that using corn silage and alfalfa meal, can improve resistance to *salmonella Enteritidis* during molt inducing compared to traditional feed withdrawal. ISSN: 00325791

**Weber, M., Zanolari, P., Ardüser, F., Stucki, D., Akarsu, H., Overesch, G.**

*Prevalence and antimicrobial resistance of Salmonella enterica subsp. diarizonae serovar 61:k:1,5,(7) in Swiss sheep flocks*



(2022) *Preventive Veterinary Medicine*, 206, art. no. 105697, .

**ABSTRACT:** *Salmonella* (*S.*) *enterica* subspecies *diarizonae* (IIIb) serovar 61:k:1,5,(7) (*S.* IIIb 61:k:1,5,(7)) is considered to be sheep-associated, as it can be found in the intestine, tonsils and nose of clinically healthy sheep, but it has also been described in separate clinical disorders in sheep. In particular, *S.* IIIb 61:k:1,5,(7) is described as the causative agent of chronic proliferative rhinitis (CPR) in sheep. In Switzerland, CPR in sheep due to *S.* IIIb 61:k:1,5,(7) was first described in 2017 in a flock of Texel sheep. Therefore, we assessed the prevalence of *S.* IIIb 61:k:1,5,(7) within the Swiss sheep population using a representative sampling strategy. From May 2017 to June 2018 a total of 681 nasal swabs from individual clinically healthy sheep of 141 different flocks throughout Switzerland were taken. Swabs were analysed by selective enrichment for the presence of *S.* IIIb 61:k:1,5,(7). Additionally, antimicrobial resistance of the isolates was determined by broth microdilution. A total of 146 out of 681 nasal swabs tested positive for *S.* IIIb 61:k:1,5,(7), which corresponds to a prevalence on animal level of 21% (95%CI 18%–25%). In 73 out of 141 flocks tested, at least one sheep tested positive for *S.* IIIb 61:k:1,5,(7), resulting in a minimal prevalence on flock level of 52% (95%CI 43%–60%). Positive flocks were found in all cantons except the canton of Jura. Adults were significantly more affected than sheep under one year/lambs and positive sheep were found in several breeds. No microbiologically resistant isolates were detected, except for one isolate showing resistance against ampicillin. Because of its widespread occurrence in the Swiss sheep population, further research should focus on the pathogenic impact of *S.* IIIb 61:k:1,5,(7) on the health status of sheep. ISSN: 01675877

**Um, M.M., Castonguay, M.-H., Arsenault, J., Bergeron, L., Côté, G., Fecteau, G., Francoz, D., Giguère, J., Amine, K.M., Morin, I., Dufour, S.**

*Estimation of the accuracy of an ELISA test applied to bulk tank milk for predicting herd-level status for Salmonella Dublin in dairy herds using Bayesian Latent Class Models* (2022) *Preventive Veterinary Medicine*, 206, art. no. 105699, .

**ABSTRACT:** Enzyme-Linked Immunosorbent Assay (ELISA) test is commonly used for detection of antibodies to *Salmonella* Dublin in individual bovine milk samples. However, little is known about its accuracy when used on bulk tank milk for determining herd-level *S.* Dublin status and when evaluated without assuming a perfect reference test. The objectives of this study were: i) to estimate the herd prevalence of *S.* Dublin among dairy cattle herds in Québec, Canada; ii) to estimate the herd sensitivity and specificity of a commercially available ELISA test when used on bulk milk; iii) to examine how the diagnostic test accuracy varies with different bulk milk ELISA cut-offs; and (iv) to assess the added value of combining ELISA screening of bulk milk and individual serum of 10 animals for determining *S.* Dublin herd status. A cohort of 302 dairy herds selected in three regions (population 1) and 58 herds that have already tested positive to *S.* Dublin (population 2) were recruited. A total of 715 bulk milk samples and 7150 individual blood samples from cattle over 3 months old (10 animals per herd) sampled on two occasions were collected. Testing was conducted using PrioCHECK™ *Salmonella* Ab bovine Dublin ELISA test for milk (Bmilk ELISA: test under investigation) and for serum of 10 individual animals (Serum10 ELISA: imperfect reference test) to determine the herd-level *S.* Dublin status. A latent class model for two populations, two tests, allowing for conditional dependence between tests was fit within a Bayesian framework. At cut-off PP %  $\geq 15$  for a Bmilk ELISA, which is used by provincial authorities, the herd prevalence of *S.* Dublin estimated using informative prior was 6.8 % (4.3–9.9) in population 1. The herd sensitivity and specificity estimates (95 % Bayesian Credibility Intervals) for Bmilk ELISA were 40.6 % (15.6–88.8) and 91.9 % (88.3–95.8), respectively. Positive and negative predictive values of Bmilk ELISA applied in population 1 were 26.4 % (8.5–60.2) and 95.8 % (92.1–99.2), respectively. Increasing Bmilk ELISA cut-offs had little influence on predictive values. The combination of both ELISA tests did not improve the diagnostic accuracy of *S.* Dublin. Our study shows that a test-positive herd based on a single bulk milk sample would require complementary tests for status confirmation. However, a test-negative herd could be classified as true negative with a high certainty. ISSN: 01675877

**Grivokostopoulos, N.C., Makariti, I.P., Tsadaris, S., Skandamis, P.N.**

*Impact of population density and stress adaptation on the internalization of Salmonella in leafy greens*

(2022) *Food Microbiology*, 106, art. no. 104053, .

**ABSTRACT:** *Salmonella enterica* is capable of entering the interior of leafy greens and establishing in the apoplasmic area, a phenomenon known as internalization. The ability of internalized bacteria to evade common disinfection practices poses a well-established risk. Our aim was to study the effect of: i) inoculum size and ii) prior adaptation of *Salmonella* to sublethal stresses, on the internalization of the pathogen in four leafy vegetables.

Spinach, lettuce, arugula and chicory were inoculated, by immersion for 2 min at room temperature with: i) *Salmonella* Enteritidis at 3.0, 4.0, 5.0, 6.0, 7.0 log CFU/mL and ii) non-adapted or adapted *S. Enteritidis* to acid (in TSB with 1% glucose, incubated for 24 h at 37 °C), cold (in TSB for 7 days at 4 °C), starvation (0.85% NaCl of pH 6.6, 48 h at 37 °C) or desiccation (1.5 h at 42 °C, 4 days at 21 °C) stress at appx 3.5 log CFU/mL). Inoculated leafy greens were subsequently stored at 5 °C and 20 °C for 2 h and 48 h (n = 2 × 2). Population of internalized *Salmonella*, after surface decontamination with 1% w/v AgNO<sub>3</sub>, was assessed on selective media. Even the lowest initial bacterial inoculum was adequate for internalization of *Salmonella* to occur in leafy vegetables. Non-adapted *Salmonella* inoculum of 7.0 (maximum) and 3.0 log CFU/mL (lowest inoculation level tested) after short storage (2 h) resulted in 3.7–4.3 and 1.3–1.5 log CFU/g internalized bacterial population, respectively. Colonization (including both attachment and internalization processes), as well as internalization process, were positively correlated to initial inoculum level. These processes reached a different plateau beyond which, no further increase in internalization was observed. Adaptation of the pathogen to mild stresses enhanced internalization (P < 0.05), with desiccation- and acid-adapted *Salmonella* demonstrating the highest internalization capacity, regardless of the vegetable and storage temperature. These findings could contribute to further elucidation of colonization capacity of *Salmonella* in leafy vegetables and assist in selecting the proper conditions that contribute to the prevention of fresh produce contamination with *Salmonella*. ISSN: 07400020