

# NEWSLETTER

European Union Reference Laboratory for *Salmonella*

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## Editorial Note

Bilthoven, 5 July 2022

Dear colleague,

By mid-June 2022 the International Symposium *Salmonella* and Salmonellosis (**IS**) was organised in Saint-Malo, France and for me it was the first time in more than 2 years that I travelled abroad and met foreign colleagues in person again. It was really nice that this was possible now and I enjoyed it a lot! It was a well organised and interesting symposium and I was happy to be able to join it.

We had hoped that we could organise this years' **workshop** also as an in person meeting again. Unfortunately that was not feasible due to the fact that many participants found it still too risky to travel abroad in May, which we fully understood. Therefore we organised the EURL-*Salmonella* workshop, for the third time, online on 23 and 24 May 2022. It seems that we get used to these online meetings. Still I hope we can meet again in person next year.

The results of the cluster analysis, using MLVA and/or WGS data, of the **PT on typing of *Salmonella* 2021** were evaluated and reported before the end of May 2022. The summary report can be found at the EURL-*Salmonella* website: <https://www.eurlsalmonella.eu/documenten/interim-summary-report-eurl-salmonella-pt-cluster-analysis-2021>

In May-July 2022 the **interlaboratory study (ILS) for determination of the performance characteristics** of draft ISO/DTS 6579-4 (identification of monophasic *Salmonella* Typhimurium by PCR) was organised. The deadline for reporting the results was 1 July 2022 and in the coming months (after the summer holidays) we will perform the analysis of the results.

In September 2022 we will organise a **combined PT on the detection of *Salmonella* in food and in samples from the primary production stage (PPS)**. The matrix under analysis will be hygiene swabs. As no PT for food was organised this spring (due to the organisation of the ILS) this combined PT will be obligatory for NRLs-*Salmonella* analysing PPS samples as well as for the NRLs-*Salmonella* analysing food samples. The timetable of this combined PT was published in the previous Newsletter and is included in this Newsletter as well.

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

I would like to wish you a nice, sunny, relaxing and Covid-free summer!

Best wishes,  
Kirsten Mooijman  
Coordinator EURL-*Salmonella*

## Contribution of the EURL-*Salmonella*

### Timetable EURL-*Salmonella* combined Proficiency Test Primary Production Stage-Food 2022 Detection of *Salmonella* in hygiene sponges

Week (2022)	Date	Subject
27-35		E-mailing the link to the registration form for the detection study. Please <b>register by 31 August 2022</b> at the latest.
39		E-mailing the link for the result form to the participants. E-mailing the protocol and instructions for the result form to the NRLs. Preparation of media by the NRLs.
39	Monday 26 September 2022	Shipment of the parcels to the participants as Biological Substance Category B (UN 3373).
40	Monday 3 October 2022	Performance of the Proficiency Test.
44	4 November 2022 at the latest	Deadline for completing the result form: <b>4 November 2022</b> (23:59h CET) After this deadline the result form will be closed.
	December 2022	Interim summary report.

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

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## 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 14 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:

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## From the Literature

### Salmonella-related Literature from Scopus: April – June 2022

#### **Grzymajlo, K.**

*The Game for Three: Salmonella–Host–Microbiota Interaction Models (2022) Frontiers in Microbiology, 13, art. no. 854112, .*

ABSTRACT: Colonization of the gastrointestinal (GI) tract by enteric pathogens occurs in a context strongly determined by host-specific gut microbiota, which can significantly affect the outcome of infection. The complex gameplay between the trillions of microbes that inhabit the GI tract, the host, and the infecting pathogen defines a specific triangle of interaction; therefore, a complete model of infection should consider all of these elements. Many different infection models have been developed to explain the complexity of these interactions. This review sheds light on current knowledge, along with the strengths and limitations of in vitro and in vivo models utilized in the study of Salmonella–host–microbiome interactions. These models range from the simplest experiment simulating environmental conditions using dedicated growth media through in vitro interaction with cell lines and 3-D organoid structure, and sophisticated “gut on a chip” systems, ending in various animal models. Finally, the challenges facing this field of research and the important future directions are outlined. ISSN: 1664302X

#### **Carraturo, F., Panico, A., Giordano, A., Libralato, G., Aliberti, F., Galdiero, E., Guida, M.**

*Hygienic assessment of digestate from a high solids anaerobic co-digestion of sewage sludge with biowaste by testing Salmonella Typhimurium, Escherichia coli and SARS-CoV-2 (2022) Environmental Research, 206, art. no. 112585, .*

ABSTRACT: Anaerobic digestion is a consolidated technology to convert sewage sludge and other organic wastes into biogas and a nutrient-rich fertilizer (i.e. digestate). The origin of sewage sludge does not exclude the potential presence of pathogens (e.g. Salmonella spp. and SARS-CoV-2) in mature digestate that hence could represent a source of sanitary concerns when it is spread on soil for agriculture purpose. Therefore, an experimental study aimed at proving the sanitizing effect of a full scale thermophilic high solids anaerobic digestion process was conducted by monitoring the hygienic characteristics of mature digestate. Although Salmonella spp. was detected in the sewage sludge fed to the full scale plant, the anaerobic digestion treatment demonstrated sanitization capacity since the monitored pathogens were never found in the mature digestate over the entire duration of the monitoring survey. Furthermore, tests on the regrowth of Salmonella Typhimurium and Escherichia coli, artificially inoculated on mature digestate, were also conducted under both anaerobic and aerobic conditions with the aim to assess the effectiveness of mature digestate as microbial growth medium. Concentrations of Salmonella Typhimurium and Escherichia coli were drastically reduced after a short time of incubation under anaerobic process and the two microorganisms already resulted undetectable after 24–48 h, whereas, under aerobic conditions, two microorganisms' concentrations were stably high for longer than 10 days. The combination of no free oxygen, high temperature, anaerobic metabolites (e.g. total ammonium nitrogen, and volatile fatty acids) production, bacteria competition and lack of nutritional elements in mature digestate considerably reduced in 24–48 h the sanitary risks associated to accidentally contaminated digestate. Furthermore, a SARS-CoV-2 monitoring survey on mature digestate during 13 months, resulted in the absence of the virus RNA in the analyzed digestate. ISSN: 00139351

**Larkin, L., de la Gandara, M.P., Hoban, A., Pulford, C., Silva, N.J.-D., de Valk, H., Browning, L., Falkenhorst, G., Simon, S., Lachmann, R., Dryselius, R., Karamehmedovic, N., Börjesson, S., van Cauteren, D., Laisnez, V., Mattheus, W., Pijnacker, R., van den Beld, M., Mossong, J., Ragimbeau, C., Vergison, A., Brandal, L.T., Lange, H., Garvey, P., Nielsen, C.S., León, S.H., Varela, C., Chattaway, M., Weill, F.-X., Brown, D., McKeown, P.**

*Investigation of an international outbreak of multidrug-resistant monophasic Salmonella Typhimurium associated with chocolate products, EU/EEA and United Kingdom, February to April 2022*

(2022) *Eurosurveillance*, 27 (15), .

ABSTRACT: An extensive multi-country outbreak of multidrug-resistant monophasic Salmonella Typhimurium infection in 10 countries with 150 reported cases, predominantly affecting young children, has been linked to chocolate products produced by a large multinational company. Extensive withdrawals and recalls of multiple product lines have been undertaken. With Easter approaching, widespread product distribution and the vulnerability of the affected population, early and effective real-time sharing of microbiological and epidemiological information has been of critical importance in effectively managing this serious food-borne incident. ISSN: 1025496X

**Petrin, S., Mancin, M., Losasso, C., Deotto, S., Olsen, J.E., Barco, L.**

*Effect of pH and Salinity on the Ability of Salmonella Serotypes to Form Biofilm*

(2022) *Frontiers in Microbiology*, 13, art. no. 821679, .

ABSTRACT: Salmonella is a major cause of food-borne infections in Europe, and the majority of human infections are caused by only a few serotypes, among them are Salmonella enterica subsp. enterica serotype Enteritidis (hereafter Salmonella Enteritidis), Salmonella Typhimurium, and the monophasic variant of S. Typhimurium. The reason for this is not fully understood, but could include virulence factors as well as increased ability to transfer via the external environment. Formation of biofilm is considered an adaptation strategy used by bacteria to overcome environmental stresses. In order to assess the capability of different Salmonella serotypes to produce biofilm and establish whether this is affected by pH and salinity, 88 Salmonella isolates collected from animal, food, and human sources and belonging to 15 serotypes, including those most frequently responsible for human infections, were tested. Strains were grown in tryptic soy broth (TSB), TSB with 4% NaCl pH 4.5, TSB with 10% NaCl pH 4.5, TSB with 4% NaCl pH 7, or TSB with 10% NaCl pH 7, and biofilm production was assessed after 24 h at 37°C using crystal violet staining. A linear mixed effect model was applied to compare results from the different experimental conditions. Among the tested serotypes, S. Dublin showed the greatest ability to form biofilm even at pH 4.5, which inhibited biofilm production in the other tested serotypes. Salmonella Senftenberg and the monophasic variant of S. Typhimurium showed the highest biofilm production in TSB with 10% NaCl pH 7. In general, pH had a high influence on the ability to form biofilm, and most of the tested strains were not able to produce biofilm at pH 4.5. In contrast, salinity only had a limited influence on biofilm production. In general, serotypes causing the highest number of human infections showed a limited ability to produce biofilm in the tested conditions, indicating that biofilm formation is not a crucial factor in the success of these clones. Copyright ISSN: 1664302X

**Lin, Y., Yang, L., Qiu, S., Yang, C., Wang, K., Li, J., Jia, L., Li, P., Song, H.**

*Rapid Identification and Source Tracing of a Salmonella Typhimurium Outbreak in China by Metagenomic and Whole-Genome Sequencing*

(2022) *Foodborne Pathogens and Disease*, 19 (4), pp. 259-265.

ABSTRACT: Salmonella spp. are among the most prevalent foodborne pathogens. Rapid identification of etiologic agents during foodborne outbreaks is of great importance. In this study, we report a traceback investigation of a Salmonella outbreak in China. Metagenomic sequencing of suspected food samples was performed on MinION and MiSeq platforms. Real-time nanopore sequencing analysis identified reads belonging to the Enterobacteriaceae family. MiSeq sequencing identified 63 reads specifically mapped to Salmonella. Conventional methods including quantitative-PCR and culture-based isolation confirmed as Salmonella enterica serovar Typhimurium. The foodborne outbreak of Salmonella Typhimurium was further recognized by whole-genome sequencing and pulsed-field gel electrophoresis analysis. Our study demonstrates the ability of metagenomic sequencing to rapidly identify enteric pathogens directly from food samples. These results highlight the capacity of metagenomic sequencing to deliver actionable information rapidly and to expedite the tracing and identification of etiologic agents during foodborne outbreaks. ISSN: 15353141

**Harrison, O.L., Gebhardt, J.T., Paulk, C.B., Plattner, B.L., Woodworth, J.C., Rensing, S., Jones, C.K., Trinetta, V.**

*Inoculation of Weaned Pigs by Feed, Water, and Airborne Transmission of Salmonella enterica Serotype 4,[5],12:i:*

(2022) *Journal of food protection*, 85 (4), pp. 693-700.

ABSTRACT: ABSTRACT: Salmonella enterica serotype 4,[5],12:i:- (STM) has become an increasing problem for food safety and has been often detected in swine products.

Weanling pigs were exposed to STM-contaminated feed, water, or air to determine possible STM transmission routes. A control group of pigs was included. STM was monitored daily in feces and rectal and nasal swabs. STM colonization was most prevalent in tissues from tonsil, lower intestine, and mesenteric lymph nodes. No differences in lesion severity were observed between inoculated and control pigs. Contaminated feed, water, and aerosolized particles caused infection in weaned pigs; however, no STM colonization was observed in skeletal muscle destined for human consumption. Based on the results from this study, STM contamination in pork products most likely results from cross-contamination of meat by digesta or lymph node tissue during processing. ISSN: 19449097

**Alessiani, A., Goffredo, E., Mancini, M., Occhiochiuso, G., Faleo, S., Didonna, A., Fischetto, R., Suglia, F., De Vito, D., Stallone, A., D'attoli, L., Donatiello, A.**

*Evaluation of Antimicrobial Resistance in Salmonella Strains Isolated from Food, Animal and Human Samples between 2017 and 2021 in Southern Italy*  
(2022) *Microorganisms*, 10 (4), art. no. 812, .

ABSTRACT: *Salmonella enterica* is one of the most common causes of foodborne infection in the world, and the most common one in Italy. Italy collaborates with the other EU member states to survey the antimicrobial resistance of *Salmonella* on a large scale. This study on the situation in Apulia and Basilicata provides a more focused point of view on the territory, and anticipates the data reported in future Italian reports. Antimicrobial resistance was detected using the MIC detection method, with EUVSEC® plates, on the strains collected between 2017 and 2021. The results of serotyping showed that *Salmonella* Infantis is the serovar that has increased the most over time in veterinary samples, while *Salmonella* Typhimurium and its monophasic variant are the most isolated in human samples. The results of the antimicrobial resistance study comply with European data, showing high resistance to quinolones, tetracyclines, ampicillin and trimethoprim, and low resistance to colistin and cepheims. The significant exception was that all strains were resistant to sulphametoxazole. The presence of MDRs, which was 85% in veterinary and 77.4% in human strains, often included critically important antibiotics, which is a sign that more study and action is needed to manage the use of antibiotics. ISSN: 20762607

**Wang, R., King, D.A., Kalchayanand, N.**

*Evaluation of Salmonella Biofilm Cell Transfer from Common Food Contact Surfaces to Beef Products*

(2022) *Journal of food protection*, 85 (4), pp. 632-638.

ABSTRACT: Meat contamination by *Salmonella enterica* is a serious public health concern. Available data have suggested that biofilm formation at processing plants and contaminated contact surfaces might contribute to meat contamination. Because transfer from contact surfaces to food products via direct contact has been deemed the most common bacteria transmission route that can lead to contamination, we evaluated the effect of *Salmonella* biofilm forming ability, contact surface material, and beef surface tissue type on *Salmonella* biofilm transfer from hard surfaces to beef products. *Salmonella* biofilms developed on the common contact surfaces stainless steel (SS) and polyvinylchloride (PVC) were transferred consecutively via 30 s of direct contact to either lean muscle or adipose tissue surfaces of 15 pieces of beef trim. The *Salmonella* biofilm cells could be effectively transferred multiple times from the contact surfaces to the beef trim as indicated by quantifiable *Salmonella* cells on most meat samples. Biofilm forming ability had the most significant impact ( $P < 0.05$ ) on transfer efficiency. More cells of *Salmonella* strains that formed strong biofilms were transferred after each contact and contaminated more meat samples with quantifiable cells compared with strains that formed weak biofilms. Contact surface materials also affected transferability. *Salmonella* biofilms on SS transferred more efficiently than did those on PVC. In contrast, the two types of meat surface tissues were not significantly different ( $P > 0.05$ ) in biofilm transfer efficiency. Beef trim samples that were in contact with biofilms but did not have quantifiable *Salmonella* cells were positive for *Salmonella* after enrichment culture. Our results indicate the high potential of *Salmonella* biofilms on common contact surfaces in meat processing plants to cause product cross-contamination. Published 2022 by the International Association for Food Protection. ISSN: 19449097

**Flock, G., Richardson, M., Pacitto-Reilly, D., Anderson, N., Chen, F., Ahnrud, G., Mendoza, A., Senecal, A.**

*Survival of Salmonella enterica in Military Low-Moisture Food Products during Long-Term Storage at 4, 25, and 40°C*

(2022) *Journal of food protection*, 85 (4), pp. 544-552.

ABSTRACT: *Salmonella enterica* has been increasingly implicated in foodborne outbreaks involving low-moisture foods (LMF) during the recent decade. This study aimed

to investigate the potential for persistence of *S. enterica* in a range of LMF during storage at three temperatures. LMF products, boil-in-bag eggs (freeze-dried product), chocolate protein drink, cran-raspberry First Strike bars, mocha dessert bar, and peanut butter, were inoculated with a five-strain cocktail of *S. enterica* and stored at 4, 25, or 40°C for 36 months. *Salmonella* populations remained above 7 log CFU/g in all products stored at 4°C and above 6 log CFU/g in products stored at 25°C, excluding the cran-raspberry First Strike bars. Storage at 40°C resulted in *Salmonella* populations above 5.5 log CFU/g in boil-in-bag eggs after 36 months and demonstrated survivability for 12 months or less in the other five products. Additionally, a mocha bar production temperature profile study identified rapid cooling of bars in which the temperatures reached would have no measurable impact on *Salmonella* populations. The results indicate the ability of *Salmonella* to survive in a variety of LMF category foods, even under adverse storage conditions and identifies how the food matrix may affect *Salmonella* survivability. The data indicate the importance of establishing food processing procedures that adequately mitigate the presence of *Salmonella* throughout food processing systems, while also increasing comprehensive understanding of *Salmonella* survivability mechanisms. Published 2022 by the International Association for Food Protection. ISSN: 19449097

**Gambino, D., Gargano, V., Butera, G., Sciortino, S., Pizzo, M., Oliveri, G., Cardamone, C., Piraino, C., Cassata, G., Vicari, D., Costa, A.**

*Food Is Reservoir of MDR Salmonella: Prevalence of ESBLs Profiles and Resistance Genes in Strains Isolated from Food*

(2022) *Microorganisms*, 10 (4), art. no. 780, .

ABSTRACT: *Salmonella* spp. are among the most frequent causes of foodborne diseases, and the increasing occurrence of MDR strains is an additional cause for concern. In the three-year period 2019–2021, we collected *Salmonella* spp. strains isolated from different food categories analysed in the context of Regulation (EC) No 2073/2005 in order to assess their antibiotic susceptibility profiles and ESBL production. To determine the susceptibility profiles and identify MDR strains, we used the Kirby–Bauer method to test 17 antibiotics. Double-disc and PCR testing then allowed us to assess the production of ESBLs and the presence of beta-lactamase resistance genes. Phenotypic tests showed that 36 out of 67 strains were MDR and 52.7% of these were ESBL producers. Finally, molecular investigations conducted on ESBL-producing strains revealed the presence of blaSHV, blaCTX-M and blaTEM genes. Our results confirmed the prevalence of *S. Infantis*, an MDR strain and ESBL producer, in chicken meat. This suggests that further research on the prevalence of antibiotic resistance genes (ARGs) in foodborne strains is needed, especially from a One Health perspective. ISSN: 20762607

**Persad, A.K., Fahmy, H.A., Anderson, N., Cui, J., Topalcengiz, Z., Jearnsripong, S., Spanniger, P.M., Buchanan, R.L., Kniel, K.E., Jay-Russell, M.T., Danyluk, M.D., Rajashekara, G., Lejeune, J.T.**

*Identification and Subtyping of Salmonella Isolates Using Matrix-Assisted Laser Desorption-Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF)*

(2022) *Microorganisms*, 10 (4), art. no. 688, .

ABSTRACT: Subtyping of bacterial isolates of the same genus and species is an important tool in epidemiological investigations. A number of phenotypic and genotypic subtyping methods are available; however, most of these methods are labor-intensive and time-consuming and require considerable operator skill and a wealth of reagents. Matrix-Assisted Laser Desorption–Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF), an alternative to conventional subtyping methods, offers a rapid, reproducible method for bacterial identification with a high sensitivity and specificity and at minimal cost. The purpose of this study was to determine the feasibility of using MALDI-TOF to differentiate between six *Salmonella* serovars recovered from experimental microcosms inoculated with known strains of *Salmonella*. Following the establishment of a MALDI-TOF reference library for this project, the identity of 843 *Salmonella* isolates recovered from these microcosms was assessed using both MALDI-TOF and conventional methods (serotyping/PCR). All 843 isolates were identified as being *Salmonella* species. Overall, 803/843 (95%) of these isolates were identified similarly using the two different methods. Positive percent agreement at the serovar level ranged from 79 to 100%, and negative percent agreement for all serovars was greater than 98%. Cohen’s kappa ranged from 0.85 to 0.98 for the different serovars. This study demonstrates that MALDI-TOF is a viable alternative for the rapid identification and differentiation of *Salmonella* serovars. ISSN: 20762607

**Wang, Z., Zhang, Y., Xu, H., Chu, C., Wang, J., Jiao, X., Li, Q.**

*Whole-genome sequencing analysis reveals pig as the main reservoir for persistent evolution of Salmonella enterica serovar Rissen causing human salmonellosis*

(2022) *Food Research International*, 154, art. no. 111007, .

ABSTRACT: *Salmonella enterica* serovar Rissen is recognized as one of the most common serotypes in pigs and pig products in many countries and can be transmitted to human through the consumption of contaminated food. To reveal the genetic characteristics of *S. Rissen*, 39 isolates from human and animal food were subjected to whole-genome sequencing analysis combined with 337 genome sequences downloaded from the NCBI Assembly database. Core genome single nucleotide polymorphism (cgSNP) divided these *S. Rissen* isolates into two clusters, of which cluster A included 78.3% of American isolates, while cluster B was mainly composed of isolates from China, Asia, and the UK. The 39 *S. Rissen* isolates located in cluster B were further divided into two subclusters with cluster B-1 of 26 isolates from both humans and food, while cluster B-2 consisted of 13 isolates from animal food, mainly pork. CRISPR typing and cgMLST of the 39 isolates showed perfect correspondence to cgSNP results of their phylogenetic relationship. Virulence factors analysis revealed that ABZJ\_0085 and ABZJ\_0086 genes presenting in cluster B-2 were lost in cluster B-1. Additionally, antimicrobial resistance gene profiles showed *qnrS1*, *cmlA1*, and *tet(M)* could be detected in cluster B-2 other than cluster B-1. The findings of the divergent difference between cluster B-1 and cluster B-2 demonstrated that *S. Rissen* was continuously evolved through the pork production chain. In conclusion, pigs are the main reservoir for *S. Rissen* capable of causing human nontyphoid salmonellosis. ISSN: 09639969

**Harrison, O.L., Rensing, S., Jones, C.K., Trinetta, V.**

*Salmonella enterica* 4,[5],12:i:-, an Emerging Threat for the Swine Feed and Pork Production Industry

(2022) *Journal of food protection*, 85 (4), pp. 660-663.

ABSTRACT: *Salmonella* continues to be a significant cause of foodborne illnesses in human medicine. The Centers for Disease Control and Prevention reported *Salmonella* as the second leading cause of foodborne illness in the United States and the leading cause of both hospitalizations and deaths. *Salmonella enterica* 4,[5],12:i:- (STM) is a monophasic variant of *Salmonella Typhimurium*, and it is an emerging threat to both human and animal health. STM was first identified in the 1980s from poultry products and has become increasingly prevalent in meat products including pork. STM has also been identified in swine farms as well as in feed manufacturing environments and feed itself. Similar pulse-field gel electrophoresis profiles have been observed between human clinical cases and the STM samples originating from swine feed. These related profiles suggest a link between ingestion of contaminated feed by swine and the source of foodborne illness in human. The objective of this article was to better understand the history of STM and the possible pathway from swine feed to table. Continued research is necessary to better understand how STM can enter both the feed supply chain and the pork production chain to avoid contamination of pork products destined for human consumption. ISSN: 19449097

**Sacchini, L., Ricci, L., Zilli, K., Romantini, R., Di Donato, G., Neri, D., Persiani, T., Di Giannatale, E.**

*Characterization of Salmonella Typhimurium and monophasic Salmonella Typhimurium isolated in Abruzzo and Molise regions, Italy, from 2012 to 2017*

(2022) *Veterinaria Italiana*, 57 (4), pp. 297-304.

ABSTRACT: Summary Salmonellosis is currently the second most common zoonosis in European Union but in the 6-years periods, between 2012 and 2017, there has been a significant decrease trend in the yearly number of infections caused by *Salmonella*. In Italy, *S. Typhimurium* and monophasic *S. Typhimurium* represent the most prevalent serotypes. In this paper, we investigated these two serovars isolated from 2012 to 2017 in Abruzzo and Molise regions. A set of 345 strains isolated from human sporadic cases, surface water, food and animals were collected and analyzed. Monophasic *S. Typhimurium* strains were found to be resistant to streptomycin, sulfisoxazole, ampicillin, tetracycline and nalidixic acid, while *S. Typhimurium* isolates showed high levels of resistance to tetracycline, sulfisoxazole and ampicillin. The 5-loci Multilocus Variable-Number Tandem Repeat Analysis (MLVA) identified 88 genotypes (GT), six of which were common for the two serovars. Several MLVA profiles were shared by human and non-human isolates. MLVA had sufficient typing resolution for epidemiological studies of *S. Typhimurium* but demonstrated poor discriminatory in trace-back study of monophasic *S. Typhimurium*. ISSN: 0505401X

**Castillo-Contreras, R., Marín, M., López-Olvera, J.R., Ayats, T., Fernandez Aguilar, X., Lavín, S., Mentaberre, G., Cerdà-Cuéllar, M.**

*Zoonotic Campylobacter spp. and Salmonella spp. carried by wild boars in a metropolitan area: occurrence, antimicrobial susceptibility and public health relevance* (2022) *Science of the Total Environment*, 822, art. no. 153444, .

**ABSTRACT:** *Campylobacter* spp. and *Salmonella* spp. are the most reported zoonotic agents in Europe. They can be transmitted from wildlife to humans, and wild boars (*Sus scrofa*) can harbour them. In the Metropolitan Area of Barcelona (MAB, NE Spain) wild boars are found in urbanized areas. To assess the potential public health risk of this increasing wild boar population, we collected stool samples from 130 wild boars from the MAB (June 2015 – February 2016), to determine the *Campylobacter* and *Salmonella* occurrence and the antimicrobial susceptibility of the isolates. We also investigated the genetic diversity and virulence potential of *Campylobacter*. *Campylobacter* prevalence in wild boars was 61%. Forty six percent of wild boars carried *Campylobacter lanienae*, 16% carried *Campylobacter coli*, and 1% carried *Campylobacter hyointestinalis*; 4% carried both *C. lanienae* and *C. coli*, and 1% carried both *C. lanienae* and *C. hyointestinalis*. This is the first report of *C. hyointestinalis* in wildlife in Spain. Using pulse-field gel electrophoresis and multilocus sequence typing, we observed a high genetic diversity of *Campylobacter* and identified new sequence types. Thirty-three percent of *C. coli* and 14% of *C. lanienae* isolates showed a high virulence potential. All of the *Campylobacter* isolates analysed were resistant to at least one antimicrobial agent. Multidrug resistance was only detected in *C. coli* (67%). *Salmonella enterica* subsp. *enterica* was detected in four wild boars (3%) and included a *S. Enteritidis* serovar (1/4 wild boars) and a multidrug-resistant (ASSuT) monophasic *S. Typhimurium* serovar (1/4 wild boars) which is associated with human infections and pig meat in Europe. The characteristics of some of the *Campylobacter* and *Salmonella* isolates recovered suggest an anthropogenic origin. Wild boars are a reservoir of *Campylobacter* and have the potential to spread antimicrobial resistant *Campylobacter* and *Salmonella* in urbanized areas in the MAB. ISSN: 00489697

**Cabrera-Díaz, E., Martínez-Chávez, L., Gutiérrez-González, P., Pérez-Montaño, J.A., Rodríguez-García, M.O., Martínez-González, N.E.**

*Effect of storage temperature and time on the behavior of Salmonella, Listeria monocytogenes, and background microbiota on whole fresh avocados (Persea americana var Hass)*

(2022) *International Journal of Food Microbiology*, 369, art. no. 109614, .

**ABSTRACT:** Avocados are popular fruits; however, contamination of whole fresh avocados and avocado products with foodborne pathogens has raised concern about their safety. Recalls and import alerts of avocado products due to contamination with *Listeria monocytogenes* cause important economic losses. The behavior of *Salmonella*, *L. monocytogenes*, and background microbiota on whole fresh avocados at 5 and 25 °C as affected by temperature and time of storage was investigated. Whole fresh avocados were inoculated by immersion in suspensions containing six rifampicin-resistant strains of *Salmonella* or *L. monocytogenes*, and stored at 5 °C for 48 d, or at 25 °C for 11 d. At selected sampling times, avocados were removed from storage and pathogens enumerated. The log counts of both pathogens at each temperature were fitted to the Weibull distribution nonlinear model to estimate kinetic parameters including the time for the first 1-log reduction ( $\delta$ ), the shape of the curve ( $\rho$ ), and the time for two (2-D) and three (3-D) log reductions. *Salmonella* and *L. monocytogenes* initial populations (approx. 7 log CFU/avocado) decreased during storage at 5 and 25 °C; *L. monocytogenes* mean counts were higher than those observed for *Salmonella* ( $P < 0.05$ ). *L. monocytogenes* showed a lower rate of decline at 5 °C when compared to *Salmonella*. In general, the ability of both pathogens to survive on the surface of avocados stored at room temperature was similar. *Salmonella* and *L. monocytogenes* counts decline over time on the epicarp of whole avocados; however, if the initial number of cells is large enough, the pathogens could be present for large periods of time. Simultaneously, psychrotrophic microorganisms (PM), aerobic plate count (APC), coliforms (C) and yeasts/molds (Y/M) were enumerated from non-inoculated avocados stored at 5 and 25 °C. Initial mean counts for PM, APC, C and Y/M ranged from 6.1 to 6.6 log CFU/avocado and showed no change ( $P > 0.05$ ) during storage at both temperatures. Good agricultural and handling practices from farm to fork are crucial to prevent or minimize contamination of whole avocados; otherwise, if large numbers of pathogens contaminate the fruit, they could survive and be transferred to the pulp, or to other ready to eat foods, representing a risk for consumers. ISSN: 01681605

**Jørgensen, F., McLauchlin, J., Verlander, N.Q., Aird, H., Balasegaram, S., Chattaway, M.A., Dallman, T., Herdman, M.T., Hoban, A., Lai, S., Larkin, L., McCormick, J., Reeves, L.S., Willis, C.**

*Levels and genotypes of Salmonella and levels of Escherichia coli in frozen ready-to-cook chicken and turkey products in England tested in 2020 in relation to an outbreak of S. Enteritidis*

(2022) *International Journal of Food Microbiology*, 369, art. no. 109609, .

ABSTRACT: Frozen reformulated (FR) breaded chicken products have previously been implicated in causing human salmonellosis. A multi-country *Salmonella enterica* serovar Enteritidis outbreak involving several strains with >400 reported human cases in the UK occurred in 2020. Initially *S. Infantis* was detected in one sample from a case home but *S. Enteritidis* was then also isolated using a *S. Enteritidis* specific PCR in combination with isolation via a Craigie-tube. This prompted a survey to examine the presence and levels of *Salmonella* and *E. coli* in ready-to-cook FR poultry products in England in 2020. From a total of 483 samples, including two from cases' homes, *Salmonella* was detected in 42 chicken samples, these originated from six out of 53 production plants recorded. *Salmonella* detection was associated with elevated levels of generic *E. coli* (OR = 6.63). *S. Enteritidis* was detected in 17 samples, *S. Infantis* in 25, *S. Newport* in four and *S. Java*, *S. Livingstone* and *S. Senftenberg* in one each. The highest levels of *Salmonella* were 54 MPN/g for *S. Infantis* and 28 MPN/g for *S. Enteritidis*; 60% of the *Salmonella*-positive samples had <1.0 MPN/g. *S. Enteritidis* was detected together with *S. Infantis* in five samples and with *S. Livingstone* in one. Where *S. Enteritidis* was detected with other *Salmonella*, the former was present at between 2 and 100-fold lower concentrations. The *Salmonella* contamination was homogeneously distributed amongst chicken pieces from a single pack and present in both the outer coating and inner content. The *S. Enteritidis* were all outbreak strains and detected in six products that were linked to four production plants which implicated a Polish origin of contamination. Despite *S. Infantis* being most prevalent in these products, *S. Infantis* from only two contemporaneous human cases in the UK fell into the same cluster as isolates detected in one product. Except for one human case falling into the same cluster as one of the *S. Newport* strains from the chicken, no further isolates from human cases fell into clusters with any of the other serovars detected in the chicken samples. This study found that higher *E. coli* levels indicated a higher probability of *Salmonella* contamination in FR chicken products. The results also highlight the importance of recognising co-contamination of foods with multiple *Salmonella* types and has provided essential information for detecting and understanding outbreaks where multiple strains are involved. ISSN: 01681605

**Zacher, B., Czogiel, I.**

*Supervised learning using routine surveillance data improves outbreak detection of Salmonella and Campylobacter infections in Germany*

(2022) *PLoS ONE*, 17 (5 May), art. no. e0267510, .

ABSTRACT: The early detection of infectious disease outbreaks is a crucial task to protect population health. To this end, public health surveillance systems have been established to systematically collect and analyse infectious disease data. A variety of statistical tools are available, which detect potential outbreaks as aberrations from an expected endemic level using these data. Here, we present supervised hidden Markov models for disease outbreak detection, which use reported outbreaks that are routinely collected in the German infectious disease surveillance system and have not been leveraged so far. This allows to directly integrate labeled outbreak data in a statistical time series model for outbreak detection. We evaluate our model using real *Salmonella* and *Campylobacter* data, as well as simulations. The proposed supervised learning approach performs substantially better than unsupervised learning and on par with or better than a state-of-the-art approach, which is applied in multiple European countries including Germany. ISSN: 19326203

**Klose, C., Scuda, N., Ziegler, T., Eisenberger, D., Hanczaruk, M., Riehm, J.M.**

*Whole-Genome Investigation of Salmonella Dublin Considering Mountain Pastures as Reservoirs in Southern Bavaria, Germany*

(2022) *Microorganisms*, 10 (5), art. no. 885, .

ABSTRACT: Worldwide, *Salmonella* Dublin (*S. Dublin*) is responsible for clinical disease in cattle and also in humans. In Southern Bavaria, Germany, the serovar was identified as a causative agent for 54 animal disease outbreaks in herds between 2017 and 2021. Most of these emerged from cattle herds (n = 50). Two occurred in pig farms and two in bovine herds other than cattle. Genomic analysis of 88 *S. Dublin* strains isolated during these animal disease outbreaks revealed 7 clusters with 3 different MLST-based sequence types and 16 subordinate cgMLST-based complex types. Antimicrobial susceptibility investigation revealed one resistant and three intermediate strains. Furthermore, only a few genes coding for bacterial virulence were found among the isolates. Genome analysis enables pathogen identification and antimicrobial susceptibility, serotyping, phylogeny, and follow-up traceback analysis. Mountain pastures turned out to be the most likely locations for

transmission between cattle of different herd origins, as indicated by epidemiological data and genomic traceback analyses. In this context, *S. Dublin* shedding was also detected in asymptomatic herding dogs. Due to the high prevalence of *S. Dublin* in Upper Bavaria over the years, we suggest referring to this administrative region as "endemic". Consequently, cattle should be screened for salmonellosis before and after mountain pasturing.  
ISSN: 20762607

**Farahani, R.K., Meskini, M., Langeroudi, A.G., Gharibzadeh, S., Ghosh, S., Farahani, A.H.K.**

*Evaluation of the different methods to detect Salmonella in poultry feces samples (2022) Archives of Microbiology, 204 (5), art. no. 269, .*

ABSTRACT: *Salmonella* is one of the most common causes of foodborne outbreaks and infection worldwide. The gold-standard detection method of *Salmonella* is cultivation. There is a need to investigate rapid and accurate processes with time-consuming cultivation. The study evaluated different approaches to detect *Salmonella* in poultry feces samples. Poultry farm feces samples from 21 cities in Iran were collected from January 2016 to December 2019. Microbiological cultures, serological assays, and multiplex PCR (m-PCR) were used to detect and characterize *Salmonella* spp. isolates. Serological assays and m-PCR were used to determine the serogroups A, B, C1, C2, D1, E, H, and FliC. The m-PCR was used to detect seven *Salmonella* serovars, and a Chi-square test was performed to compare the discriminatory power of the methods. Of 2300 poultry feces samples, 173 (7.5%) and 166 (7.2%) samples were detected as *Salmonella* spp. by cultivation and m-PCR, respectively. The sensitivity of the molecular method was equal to cultivation at 0.96 (CI = 95%). Assessment of H antigenic subgroups showed the same for both m-PCR and serological tests. Therefore, the matching rate of the two methods for detecting all H antigenic subgroups was 100%. Thus, the relationship between the results obtained from both methods was significant in the contingency table test ( $P < 0.01$ ). The PCR-based approach confirmed the detection of *Salmonella* in a shorter period (24–36 h) compared to the conventional microbiological approach (3–8 days). ISSN: 03028933

**Zhang, Y., Pérez-Reyes, M.E., Qin, W., Hu, B., Wu, Q., Liu, S.**

*Modeling the effect of protein and fat on the thermal resistance of Salmonella enterica Enteritidis PT 30 in egg powders*

*(2022) Food Research International, 155, art. no. 111098, .*

ABSTRACT: Microorganisms in low-moisture foods (LMFs) exhibit prolonged survivability and high heat resistance. Various external factors (water, food texture, nutritional compounds, etc.) influence the microbial heat resistance in LMFs; yet, the influential degree of each factor is not fully understood. In this study, the thermal resistance parameters (D and z values) of *Salmonella enterica* Enteritidis PT 30 (*S. Enteritidis*) at 80, 85, and 90 °C at the room-temperature water activity ( $a_w$ , 25°C) of  $0.32 \pm 0.02$  were measured. A series of egg powders with different fat and protein ratios (obtained by mixing egg white and yolk powders) were chosen as the model foods. Primary and secondary models were built from the isothermal inactivation kinetics of *S. Enteritidis* in the tested samples. The importance of fat and protein was then confirmed by controlling the water activity at the treatment temperature ( $a_w$ , treatment temperature) via thermal water activity cells. The survivor curves of *S. Enteritidis* fitted well with the Weibull-type and log-linear models. The D values of *S. Enteritidis* increased with increasing fat (0–56.7%, w.b.) and decreasing protein contents (83.59–31.81%, w.b.). Incorporating the modified Bigelow model into the log-linear model yielded the  $z_{fat}$  and  $z_{protein}$  of 58.96 and 57.14, respectively. At the controlled  $a_w$ , 90°C of  $0.32 \pm 0.02$ , the D<sub>90°C</sub> values of *S. Enteritidis* increased remarkably ( $P < 0.05$ ), but the values in egg white, whole egg, and egg yolk powders ( $11.73 \pm 1.24$ ,  $23.82 \pm 2.0$ , and  $60.0 \pm 2.4$  min) were remarkably different. Our study identified that the influential degrees of fat, protein ( $z_{fat}$  and  $z_{protein}$  values), and  $a_w$  on the thermal resistance of *S. Enteritidis* in egg powders is in the order:  $a_w$ , treatment temperature > fat > protein. Fat considerably increased the thermal resistance of *S. Enteritidis* even at the same  $a_w$ , treatment temperature. This study quantified the effect of fat and protein on the thermal resistance of *S. Enteritidis* and emphasized the non-negligible effects of food components in LMFs' microbial safety.  
ISSN: 09639969

**Rosario, I., Calcines, M.I., Rodríguez-Ponce, E., Déniz, S., Real, F., Vega, S., Marin, C., Padilla, D., Martín, J.L., Acosta-Hernández, B.**

*Salmonella enterica subsp. enterica serotypes isolated for the first time in feral cats: The impact on public health*

*(2022) Comparative Immunology, Microbiology and Infectious Diseases, 84, art. no. 101792, .*

**ABSTRACT:** Stray cat populations can represent a significant threat of the transmission of zoonotic diseases such as salmonellosis. The objective of this study was to assess *Salmonella* carriage by free-living cats in Gran Canaria island and the *Salmonella* serovars involved, in order to inform to those responsible for the colonies about the possible risk factors. One hundred rectal swabs of feral cats were taken. *Salmonella* strains were serotyped in accordance with Kauffman-White-Le-Minor technique. Of a total of 100 animals under study, 19% were found to be positive to *Salmonella* spp. This is the first report that described the zoonotic serovars S. Nima, S. Bredeney, S. Grancanaria and S. Kottbus in cats. The present study demonstrates that feral cats may represent a source of risk for the spread of different *Salmonella* zoonotic serovars. It has been reported that there is a certain correlation between *Salmonella* isolates from pets and wild animals. Further studies are needed from other animal species and environmental sources to make this correlation. ISSN: 01479571

**Szpinak, V., Ganz, M., Yaron, S.**

*Factors affecting the thermal resistance of Salmonella Typhimurium in tahini*  
(2022) *Food Research International*, 155, art. no. 111088, .

**ABSTRACT:** *Salmonella enterica* is a leading human pathogen responsible for foodborne outbreaks worldwide. In the last decade, foods with low water activity (aw) and high-fat content have been involved in an increased occurrence of foodborne outbreaks. This research focuses on the foodstuff tahini, which is often linked to *Salmonella* infection outbreaks and recalls. Thermal treatments are suggested to reduce microbial populations in tahini, but little is known about its effectiveness against *Salmonella*. Our major objectives were to study the survival of *Salmonella Typhimurium* in tahini treated at temperatures  $\geq 70$  °C, and to identify food related factors that could influence its survival. Based on our experimental results the thermal treatments at 70 °C, 80 °C and 90 °C are suitable to inactivate only a partial population of *Salmonella*. The death of *Salmonella* in tahini matches a biphasic logarithmic inactivation model, with a maximal 3-log reduction after 1 h at 90 °C. Moreover, we observed that a second thermal treatment the day after the first treatment, is significantly less effective compared with the first thermal treatment. The inactivation rates of *Salmonella* in 100% tahini are almost 4-log lower than in water/tahini emulsions at 70 °C, with negative linear correlation between D-value and aw, and the *Salmonella* susceptibility to heat in sesame oil/tahini emulsions is affected by the matrix of pre-acclimation. Bacteria that had been acclimated in tahini kept their heat resistance, while acclimation in sesame oil before mixing in the preheated oil/tahini emulsions resulted in a sharp decline within 2 min at 70 °C. According to these findings, tahini producers' current pasteurization processes are not sufficient to achieve the required 5-log reduction. Furthermore, we suggest that due to the tahini heterogeneity, the aw in the micro-environment of each bacterium, which is shaped by the tahini substances, plays an essential role in *Salmonella*'s survival in tahini at temperatures  $\geq 70$  °C. ISSN: 09639969

**Mengarda Buosi, D.T., de Moraes, J.O., Cheng, Y., Cheng, R.A., Moraru, C.I., Carciofi, B.A.M.**

*Effective pulsed light treatments for inactivating Salmonella enterica serotypes*  
(2022) *Food Control*, 135, art. no. 108776, .

**ABSTRACT:** Pulsed light (PL) is a nonthermal treatment that kills microorganisms by exposing them to short-duration pulses of high-intensity broad-spectrum light. This study evaluated the PL inactivation of different serotypes of *Salmonella enterica* subsp. *enterica* (Typhimurium, Enteritidis, Heidelberg, and Minnesota) to specifically assess how pulse width and voltage can affect treatment effectiveness. Bacterial suspensions were spread onto solid agar plates, used as a model food surface, and then treated with PL at different treatment parameters (voltage, pulse width, and fluence). Surviving cells were recovered, enumerated, and the proportion of surviving cells were determined. The log-linear model was used to characterize inactivation kinetics. Three independent replicates were performed for all treatments, and data were analyzed statistically. The results obtained with *S. Typhimurium* highlighted that the inactivation effectiveness of PL treatment could be enhanced by selecting the right combination of parameters within the practical limitations of the equipment. Higher voltages delivered more energy with pulse width, (i.e., a higher irradiance) resulting in a more lethal process. PL was very effective for the inactivation of *Salmonella*, and a 6 to 7 log reduction was achieved for all four serotypes and their cocktail, after a single light pulse with a fluence of 338 mJ/cm<sup>2</sup> and 280 mJ/cm<sup>2</sup>, at 2500 V (200  $\mu$ s) and 3000 V (100  $\mu$ s), respectively. The results demonstrated similar log reductions for individual serotypes and the cocktail. ISSN: 09567135

**Whitehill, F.M., Stapleton, G.S., Koski, L., Sievert, D., Nichols, M.**

*Risk factors for hospitalization among adults aged ≥65 years with non-typhoidal Salmonella infection linked to backyard poultry contact (2022) Zoonoses and Public Health, 69 (3), pp. 215-223.*

ABSTRACT: Objective: We describe the epidemiology of live poultry-associated salmonellosis (LPAS) and investigate potential risk factors associated with hospitalization among adults aged ≥65 years in the United States during 2008–2017. LPAS is a public health concern in the United States, especially among people with increased risk for hospitalization, such as older adults. Sample: We analysed data from people aged ≥65 years with non-typhoidal salmonellosis who reported live poultry contact within seven days prior to illness onset. Procedure: We used logistic regression to estimate the odds of hospitalization associated with several risk factors including types of live poultry contact exposures. Results: LPAS among older adults in this analysis resulted in high hospitalization rates. Salmonella Hadar infection was associated with increased hospitalization. Among older adults with LPAS, 109 individuals of 127 (86%) reported contact with live poultry at their or someone else's residence, and 85 of 105 with available information (81%) reported owning poultry. Conclusions and Clinical Relevance: Additional infection prevention information and education targeted at poultry-owning older adults are needed to prevent illness and hospitalization. ISSN: 18631959

**Hoff, C., Nichols, M., Gollarza, L., Scheffel, J., Adams, J., Tagg, K.A., Francois Watkins, L., Poissant, T., Stapleton, G.S., Morningstar-Shaw, B., Signs, K., Bidol, S., Donovan, D., Basler, C.**

*Multistate outbreak of Salmonella Typhimurium linked to pet hedgehogs, United States, 2018–2019 (2022) Zoonoses and Public Health, 69 (3), pp. 167-174.*

ABSTRACT: In December 2018, PulseNet, the national laboratory network for enteric disease surveillance, identified an increase in Salmonella Typhimurium isolates with an uncommon pulsed-field gel electrophoresis pattern which was previously isolated from hedgehogs. CDC, state, and local health partners interviewed patients with a questionnaire that focused on hedgehog exposures, conducted traceback of patients' hedgehog purchases, and collected hedgehog faecal pellets and environmental samples. Isolates in this outbreak were analysed using core-genome multi-locus sequence typing (cgMLST) and compared to sequence data from historic clinical isolates from a 2011–2013 outbreak of Salmonella Typhimurium illnesses linked to pet hedgehogs. Fifty-four illnesses in 23 states were identified between October 2018 and September 2019. Patients ranged from <1 to 95 years, and 65% were female. Eight patients were hospitalized. Eighty-one per cent (29/36) of patients interviewed reported contact with a hedgehog before becoming ill; of these, 21 (72%) reported owning a hedgehog. Analysis of 53 clinical, 11 hedgehog, and two hedgehog bedding isolates from this outbreak, seven hedgehog isolates obtained prior to this outbreak, and two clinical isolates from the 2011–2013 outbreak fell into three distinct groupings (37 isolates in Clade 1 [0–10 alleles], 28 isolates in Clade 2 [0–7 alleles], and eight isolates in Clade 3 [0–12 alleles]) and were collectively related within 0–31 alleles by cgMLST. Purchase information available from 20 patients showed hedgehogs were purchased from multiple breeders across nine states, a pet store, and through an online social media website; a single source of hedgehogs was not identified. This outbreak highlights the ability of genetic sequencing analysis to link historic and ongoing Salmonella illness outbreaks and demonstrates the strain of Salmonella linked to hedgehogs might continue to be a health risk to hedgehog owners unless measures are taken to prevent transmission. ISSN: 18631959

**da Silva, R.T., Schaffner, D.W., de Souza Pedrosa, G.T., de Albuquerque, T.M.R., Maciel, J.F., de Souza, E.L., Alvarenga, V.O., Magnani, M.**

*Survival kinetics, membrane integrity and metabolic activity of Salmonella enterica in conventionally and osmotically dehydrated coconut flakes (2022) International Journal of Food Microbiology, 370, art. no. 109669, .*

ABSTRACT: Many outbreaks involving Salmonella enterica in dehydrated coconut have been reported. Little is known about the survival of S. enterica in dehydrated coconut flakes at common retail or domestic storage conditions. This study evaluated the behavior of a S. enterica cocktail (S. Enteritidis PT4, S. Typhimurium PT4, S. Bredeney, S. Muenster and S. Agona) in conventionally and osmotically dehydrated coconut flakes under four storage regimes: 25 °C for 120 days, 25 °C for 30 days then 7 °C for 90 days, 7 °C for 30 days then 25 °C for 90 days, and 7 °C for 120 days. S. enterica membrane integrity (using with propidium iodide and bis-1,3-dibutylbarbutyric acid) and metabolic activity (using 5-cyano-2,3-ditolyl tetrazolium chloride) were assessed by flow cytometry analysis after dehydration and storage at 7 °C or 25 °C for 120 days. Lower S. enterica inactivation rates

( $k_{max}$  0.02 to 0.04 1/days) were observed in conventionally dehydrated coconut flakes compared to osmotically dehydrated coconut flakes ( $k_{max}$  0.16 to 0.20 1/days). Changes in storage temperature did not affect the behavior of *S. enterica* in conventionally or osmotically dehydrated coconut flakes. Results show that *S. enterica* inactivation in conventionally dehydrated coconut flakes could be described by log-linear with tail models. *S. enterica* inactivation in osmotically dehydrated coconut flakes could be described by log-linear with shoulder and tail models. Subpopulations of *S. enterica* cells with damaged membranes and without metabolic activity were larger in conventionally (32.1% and 90.9%, respectively) than osmotically dehydrated coconut (18.5% and 82.2%, respectively) flakes at the beginning of the storage. Subpopulations of *S. enterica* cells with damaged membrane decreased by 9.4–14.4%, while cells with membrane potential and intact membrane increased by 23.7 and 24.2% in conventionally dehydrated coconut flakes after 120 days of storage at 7 °C or 25 °C, respectively. Subpopulations of *S. enterica* with damaged membranes did not change significantly in osmotically dehydrated coconut flakes. Our findings suggest that *S. enterica* populations decline during storage occurs due in part to membrane integrity losses. These data can contribute to the development of risk management strategies for *S. enterica* in dehydrated coconut flakes. ISSN: 01681605

**Vittal, R., Mohanraj, J.R., Chakraborty, G.**

*Rapid Detection of Salmonella spp from Meat: Loop Mediated Isothermal Amplification (LAMP)*

(2022) *Journal of Pure and Applied Microbiology*, 16 (2), pp. 929-936.

ABSTRACT: Loop-mediated isothermal amplification (LAMP) is a novel, high specific and sensitive method which amplifies nucleic acid under isothermal conditions. *Salmonella* is considered one of the threatening pathogens in food industries and these species are associated with distinct food poisoning called salmonellosis. Four primers (two outer and two inner primers) were designed to target six distinct regions on the target gene *invA* which is conserved in *Salmonella* species. The reaction was optimised for 60 mins at 65 °C. The sensitivity of the LAMP and PCR assay for *Salmonella* was 10 CFU/ml and 100 CFU/ml respectively. Artificial spiking of chicken meat shows detection of *Salmonella* even at dilution to extinction (<1 CFU/ml) immediately after spiking as well after 48hr enrichment. All the LAMP experiments were compared to PCR method. This study reports the development of a highly sensitive, specific and a rapid diagnostic assay for the detection of *Salmonella* from food. The developed method could be very useful for routine pathogens point of care (POC) diagnostics. ISSN: 09737510

**Mihalache, O.A., Monteiro, M.J., Dumitrascu, L., Neagu, C., Ferreira, V., Guimarães, M., Borda, D., Teixeira, P., Nicolau, A.I.**

*Pasteurised eggs - A food safety solution against Salmonella backed by sensorial analysis of dishes traditionally containing raw or undercooked eggs*

(2022) *International Journal of Gastronomy and Food Science*, 28, art. no. 100547, .

ABSTRACT: This study evaluates the potential of pasteurised eggs to be used as a replacement for raw eggs in recipes where eggs remain uncooked or undercooked. Sensory tests were performed by 178 untrained panellists (143 from Portugal and 35 from Romania) using a 9-point hedonic scale. The tested recipes were tiramisu, chocolate mousse, eggnog, hollandaise sauce, and condensed milk mousse (Baba de camelo). While in Portugal the colour and texture of the chocolate mousse prepared with pasteurised and unpasteurised eggs differed significantly, no significant differences in regards to the organoleptic attributes were recorded in Romania. Nonetheless, the results revealed comparable distribution scores regarding the overall acceptability for all the recipes indicating an agreement among panellists for both raw and pasteurised eggs dishes in both countries. The regression analysis showed that the main drivers of consumers' acceptance of dishes made with pasteurised eggs are flavour and texture. This research demonstrates that five dishes prepared with liquid pasteurised eggs are accepted by consumers as they cannot clearly be distinguished from their counterparts prepared with raw eggs. As *Salmonella*-contaminated eggs are the most frequent cause of salmonellosis, pasteurised eggs can be used as a safer ingredient to substitute shell eggs in raw egg-based dishes without significant reduction of the sensory quality of the dish. Since liquid pasteurised eggs are already widely used, especially by industry and restaurants, we provide reasons supporting pasteurisation of eggs in shell as a more convenient solution for consumers. Pasteurised eggs, either liquid or in shell, constitute a way to reduce the burden of foodborne diseases by diminishing the number of cases of salmonellosis associated with cooking at home. Researchers and food safety authorities can use our results as a starting point for future studies or intervention strategies. ISSN: 1878450X

**Morasi, R.M., Rall, V.L.M., Dantas, S.T.A., Alonso, V.P.P., Silva, N.C.C.**

*Salmonella* spp. in low water activity food: Occurrence, survival mechanisms, and thermoresistance

(2022) *Journal of Food Science*, 87 (6), pp. 2310-2323.

ABSTRACT: The occurrence of disease outbreaks involving low-water-activity (aw) foods has gained increased prominence due in part to the fact that reducing free water in these foods is normally a measure that controls the growth and multiplication of pathogenic microorganisms. *Salmonella*, one of the main bacteria involved in these outbreaks, represents a major public health problem worldwide and in Brazil, which highlights the importance of good manufacturing and handling practices for food quality. The virulence of this pathogen, associated with its high ability to persist in the environment, makes *Salmonella* one of the main challenges for the food industry. The objectives of this article are to present the general characteristics, virulence, thermoresistance, control, and relevance of *Salmonella* in foodborne diseases, and describe the so-called low-water-activity foods and the salmonellosis outbreaks involving them. ISSN: 00221147

**Cortés, V., Sevilla-Navarro, S., García, C., Marín, C., Catalá-Gregori, P.**

*Monitoring antimicrobial resistance trends in Salmonella spp. from poultry in Eastern Spain* (2022) *Poultry Science*, 101 (6), art. no. 101832, .

ABSTRACT: *Salmonella* spp. is one of the most important zoonotic pathogens with economic impact in public health worldwide. The relevance of *Salmonella* increases with the appearance of resistant strains. The aim of this study was to determine the level of antimicrobial resistance in 332 *Salmonella* isolates selected from 3 different poultry productive orientations in Eastern Spain during 3 yr (2015–2017). Antimicrobial susceptibility was evaluated by broth microdilution method using 14 antibiotics. Epidemiological cut-off values (ECOFF) were used to evaluate the microbiological resistance to antibiotics. The rates of *Salmonella* resistance at least to one antibiotic were 96, 98, and 56% in broilers, turkeys, and layers, respectively. Regarding multidrug resistance, all productive orientations seem to present a decreasing trend along the study, being the mean rates 80% in turkeys followed by broilers (40%) and layers (6%). Throughout the study, the highest percentage of resistance was found to sulfamethoxazole in all productive orientations. Strains from broilers showed the highest resistance rates to sulfamethoxazole (73%), gentamicin (57%), ciprofloxacin (50%), nalidixic acid (29%), and tetracycline (24%). Relative to turkeys the highest resistance rates were to sulfamethoxazole (76%), ciprofloxacin (69%), tetracycline (75%), nalidixic acid (63%), and ampicillin (63%). Layers presented the most elevated resistance rates to sulfamethoxazole (39%) and tetracycline (13%). Regarding serovars the most MDR common serovars to the 3 productive orientations were *S. Kentucky* and *S. Hadar*. In the other hand, high MDR rates were found in other serovars like *S. Infantis* and *S. Typhimurium* in broilers and turkeys. Results shown in the present study suggest that the reduction in the use of antibiotics begins to be reflected in the reduction of the number of MDRs, especially in layers, with no MDR *Salmonella* strains in the last period. However, the level of resistances found in this study suggests the necessity of continuing working on the limitation of the use of antimicrobials in poultry to achieve (as in layers) the control of MDRs. ISSN: 00325791

**Shariat, N.W., Larsen, B.R., Schaeffer, C., Richardson, K.E.**

*Animal feed contains diverse populations of Salmonella*

(2022) *Journal of Applied Microbiology*, 132 (6), pp. 4476-4485.

ABSTRACT: Aims: In food animals, *Salmonella* can exist as multiseroovar populations, and the goal of this study was to determine whether *Salmonella*-positive animal feed samples also consist of multiseroovar populations. Methods and Results: In all, 50 *Salmonella*-positive samples, collected from 10 countries, were cultured using three different media for *Salmonella* isolation: universal pre-enrichment broth, Rappaport-Vassiliadis (RV) broth and tetrathionate (TT) broth. The samples included 25 samples from feed ingredients, 13 from complete feed and 12 feed mill dust samples. Samples from pelleted overnight cultures were analysed by CRISPR-SeroSeq to examine serovar populations in individual samples. Serovars Anatum and Mbandaka were the most commonly identified and were found in feed, feed ingredients and feed environments. Serovars commonly associated with human illness were also identified, and included serovars Enteritidis, Typhimurium and Infantis. Overall, we detected 12 different serogroups (37 different serovars), with eight serovars belonging to the O:7 serogroup (C1). Over half (56%) of the samples contained two or more serovars, with 11 serovars found in one sample. Feed ingredients exhibited higher serovar diversity, with an average of three serovars. Across paired samples of pre-enriched and enriched populations, the Bray-Curtis dissimilarity metric showed that 83% of serovar populations were a strong match. Conclusions: The data presented show that serovars

belonging to the O:7 serogroup are commonly found in feed, and that feed can contain multiple serovars. The serovar populations across different *Salmonella* media were largely concordant. Significance and Impact of Study: The presence of *Salmonella* in animal feed is considered a transmission route into meat and poultry products and this study demonstrates that animal feed can contain multiple *Salmonella* serovars.  
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**Groat, E.F., Williams, N.J., Pinchbeck, G., Warner, B., Simpson, A., Schmidt, V.M.**  
*UK dogs eating raw meat diets have higher risk of Salmonella and antimicrobial-resistant Escherichia coli faecal carriage*  
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**ABSTRACT:** Objectives: To compare detection of *Salmonella* species and antimicrobial-resistant *Escherichia coli* in the faeces of dogs eating raw meat or non-raw diets and examine risk factors for their carriage. Materials and Methods: Canine faecal samples (raw fed n=114; non-raw fed n=76) were collected from May to July 2015 from across the UK. Enrichment and selective culture and biochemical and PCR assays were used to identify isolates. *Escherichia coli* underwent susceptibility testing to a range of antimicrobials, including third-generation cephalosporins; PCR assays were used to detect antimicrobial-resistant genes. Questionnaires were used to collect data on independent variables as risks for antimicrobial-resistant (resistant to  $\geq 1$  tested antimicrobial), multi-drug-resistant (resistant to  $\geq 3$  antimicrobial classes) and third-generation cephalosporin resistant *Escherichia coli*. Results: Antimicrobial-resistant, multi-drug-resistant and third-generation cephalosporin resistant *Escherichia coli* were significantly more likely to be detected in raw fed (54, 25 and 31%, respectively) compared to non-raw fed (17, 4 and 4%, respectively) dogs; *Salmonella* species were detected in eight (4%) raw fed dogs only. Clinical Significance: Raw fed dogs may be a source of *Salmonella* species and *Escherichia coli*, resistant to highest priority critically important antimicrobials, representing a potential animal welfare and public health issue. Owners should be aware of the risks, especially households with members, both human and canine, who are very young, elderly or immunocompromised. ISSN: 00224510