

European Union Reference Laboratory for Salmonella

Vol. 30 No. 1 March 2024

ISSN 2211-6877



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

Produced by

European Union Reference Laboratory for Salmonella

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

e-mail: <u>EURLSalmonella@rivm.nl</u>

website: www.eurlsalmonella.eu

This activity is co-funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the granting authority European Health and Digital Executive Agency (HaDEA). Neither the European Union nor the granting authority can be held responsible for them.





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Editorial Note

Bilthoven, 4 April 2024

Dear colleagues,

When starting to draft this editorial note, I realised that the current Newsletter is already the thirtieth volume! It is hard to believe that we are already publishing Newsletters for 30 years! The **first Newsletter** must have been published in 1995, but by that time it was published only on paper and unfortunately these very first editions are lost. Probably only a few copies were printed and we have not found a copy of this first edition in our archives. If your organisation happens to be part of the EURL/NRL-*Salmonella* (or by that time CRL/NRL) network since 1995 and you are the lucky owner of the first volume of the Newsletter, we would be very happy to receive a copy or a scan of it. All other volumes can be found in the archive of our website. If you are interested in some history of the EURL-*Salmonella*, please have a look at https://rivm.sitearchief.nl/?subsite=eurl-salmonella#archive.

Back to our current activities, I can inform you that (finally) the **Grant Agreement** for the EURL-*Salmonella* activities 2023-2024 has been signed by the EC and by our organisation mid-March 2024. It is no typo that this agreement is retrospective for 2023 as well. This is due to the fact that the SMP (Single Market Programme Regulation) Work Programme for 2023-2024 was adopted by the European Commission only by February 2023. Hence, the formal call to all (approx. 50) EURLs to submit their proposals for work programmes 2023-2024 was only made by early May 2023. Next it took until early this year before all work programmes and budget proposals were evaluated and updated and all relevant information was properly entered into the e-portal of HaDEA. Luckily, the work programme for the activities of our EURL-*Salmonella* could be continued as proposed. A table, summarising the main activities planned for 2024 was published in the previous Newsletter and included again in this Newsletter.

As usual, an important activity is the organisation of the EURL-*Salmonella* Proficiency Tests (PTs):

In November 2023, the **2023 PT on typing of** *Salmonella* was organised, containing an obligatory part on serotyping of *Salmonella*, and a voluntary part on cluster analysis (NGS only). The evaluation of the serotyping results was performed in the past months and by the end of March 2024, the participants received their own results as well as the interim summary report with the results of all participants. This interim summary report is also available at the EURL-*Salmonella* website:

<u>https://www.eurlsalmonella.eu/publications/proficiency-test-reports</u> - Reports PTs Typing of *Salmonella*. One participant did not meet the level of good performance and a follow-up study will soon be organised. The results on the optional part on cluster analysis of this PT are under evaluation and will be reported separately in the coming months.

In February-March 2024, the **2024 PT** on the **detection of** *Salmonella* **in live bivalve molluscs (LBM)** was organised. The deadline for completing the result form was 22 March 2024 and we are currently analysing the results.

In the previous Newsletter we already informed you that we plan to organise in September/October 2024 **a combined PT for NRLs-Salmonella for Food and for PPS**. This PT will focus on the detection of *Salmonella* in environmental samples from the food and animal production. In addition, we have discussed with the ISO subgroup on statistics and validation if it would be possible to also use the results of this PT for the generation of (missing) performance characteristics for ISO 6579-1. However, the aim and therefore the set-up of a study to test the performance of a method (interlaboratory study – ILS) differs from a study to test the performance of laboratories (PT). As a result, we had to find a compromise to use one study for both options. By now, I can inform you that we have found an acceptable compromise. To keep the study 'as blind as possible', I can only inform you that the number of samples is comparable to former PTs, but that somewhat more variation in contamination levels will be introduced. The registration for this combined PT will probably follow in June.

More about the **ISO/CEN activities**:

In January 2024, an online meeting was organised for ISO/TC34/SC9-WG9 ('Detection of Salmonella'). At this meeting, the missing performance characteristics of ISO 6579-1 were discussed. Currently, ISO 6579-1 contains performance characteristics for 3 food categories and for samples from the primary production stage. It is requested by SC9 to complete the performance characteristics of ISO 6579-1 for all relevant product categories, being a broad range of food (at least 5 food categories), pet food and animal feed, environmental samples and samples from the primary production stage. For generating missing performance characteristics in ISO 6579-1, WG 9 made a literature review of mainly alternative method validation reports of AFNOR, MicroVal and Nordval. In total, 10 studies showed data of possible use for generation of missing performance characteristics of ISO 6579-1. These 10 studies comprise performance characteristics of 6 different categories, being 5 food categories and the (one) category pet food and animal feed. For generation of performance characteristics for the one missing category 'environmental samples from the food or feed production', EURL-Salmonella made a proposal for a PT-ILS combination (see information above). After this, only performance characteristics for larger test portion sizes (>25 g) are still lacking. A call was made to find an organiser for an ILS with these larger test portion sizes.

The other ISO working group on *Salmonella*, **ISO/TC34/SC9-WG10** ('Typing of *Salmonella*'), is in charge of the development of ISO 6579-4 ('Identification of monophasic *Salmonella* Typhimurium by PCR'). the Draft International Standard, **ISO/DIS 6579-4**, was published in December 2023, for voting and comments until 15 March 2024. The document was also sent to the NRLs-*Salmonella* and several useful comments were received. Currently, we are elaborating all comments and updating draft ISO 6579-4 accordingly. The comments and updated ISO document will be discussed with WG10 in an online meeting on 23 April 2024.

Another important activity is the organisation of the **EURL-Salmonella workshop in May 2024**. After organising only online workshops for 4 years in a row, we are very glad that we finally are able to organise a physical workshop again. It seems that most NRLs are also happy to meet each other again, as despite the fact that this workshop is organised as an hybrid meeting, the majority of the 74 participants (43) will participate physically. We are currently preparing the draft programme of the workshop and we hope to send you the final version soon. In the meantime, our secretariat has been very busy with booking the flights for the ones participating physically for our budget. Almost all flights are now booked and information was sent to the participants. In case of questions/problems, do not hesitate to contact us.

In the previous Newsletter and through e-mail communications you were informed about the fact that the **inter EURLs working group on NGS** is organising again a **joint EURLs (basic) training course on NGS** in June 2024. The training is organised at the premises of the EURL-*Campylobacter* and of the EURL-foodborne viruses in Sweden. The registration for this training was closed by mid-March 2024 and the selected candidates were informed by the end of March. We were glad to notice that there is still a large interest in this training course from all (8) different EURL/NRL networks.

Best wishes, Kirsten Mooijman Coordinator EURL-Salmonella

Contribution of the EURL-Salmonella

Date(s)	Activity
26 February – 22 March 2024	EURL-Salmonella Proficiency Test Live Bivalve Molluscs; Detection of Salmonella in mussels.
28 – 29 May 2024	EURL-Salmonella workshop in Leiden, the Netherlands.
June 2024	Joint Training Course of the inter EURLs Working Group on NGS in Sweden.
September – October 2024	Combined EURL-Salmonella Proficiency Test PPS-Food.
November 2024 – January 2025	EURL-Salmonella Proficiency Test Typing; Serotyping and (optional) NGS Cluster analysis.

Planning of EURL- Salmonella activities in 2024

From the Literature

Salmonella-related Literature selection from Scopus: January - March 2024

Moore H.L., Aabye M., Hoban A., Rosner B., Lefevre S.K., Litrup E., Müller L.,

Ethelberg S., Simon S., Balasegaram S., Larkin L., Jernberg C., Takkinen J. *Multinational collaboration in solving a European Salmonella Braenderup outbreak linked to imported melons, 2021*

(2024) Eurosurveillance, 29 (1)

ABSTRACT: A genomic cluster of Salmonella Braenderup ST22, a serovar of Salmonella enterica subsp. enterica which causes symptoms of gastrointestinal illness, was notified by Danish authorities to the European Centre for Disease Prevention and Control (ECDC) on 3 May 2021. By 6 July 2021, S. Braenderup outbreak cases (n = 348) had been reported from 12 countries in the European Union/European Economic Area (EU/EEA) and the United Kingdom (UK), including 68 hospitalised cases. With support from affected EU/EEA countries, and in partnership with the European Food Safety Authority (EFSA), ECDC established an international outbreak investigation team to rapidly identify the source and prevent outbreak spread. Consumption information was shared with affected countries through a standard line list, revealing that 124 of 197 cases (63%) reported having eaten (any) melons within 7 days prior to disease onset. The speed and completeness of the investigation, which identified the outbreak vehicle as galia melons imported from Honduras in June 2021, was a direct result of extensive collaboration and information sharing between countries national food safety and public health authorities. This article describes the outbreak and the benefits, successes, and challenges of multi-country collaboration for consideration in future large foodborne outbreaks across Europe. ISSN: 1025496X

Benefo E.O., Karanth S., Pradhan A.K.

A machine learning approach to identifying Salmonella stress response genes in isolates from poultry processing

(2024) Food Research International, 175, art. no. 113635

ABSTRACT: We explored the potential of machine learning to identify significant genes associated with Salmonella stress response during poultry processing using whole genome sequencing (WGS) data. The Salmonella isolates (n = 177) used in this study were obtained from various chicken sources (skin before chiller, chicken carcass before chiller, frozen chicken, and post-chill chicken carcass). Six machine learning algorithms (random forest, neural network, cost-sensitive learning, logit boost, and support vector machine linear and radial kernels) were trained on Salmonella WGS data, and model fit was assessed using standard evaluation metrics such as the area under the receiver operating characteristic (AUROC) curve and confusion matrix statistics. All models achieved high performances based on the AUROC metric, with logit boost showing the best performance with an AUROC score of 0.904, sensitivity of 0.889, and specificity of 0.920. The significant genes identified included ybtX, which encodes a Yersiniabactin-associated zinc transporter, and the transferase-encoding genes yccK and thiS. Additionally, genes coding for cold (cspA, cspD, and cspE) and heat shock (rpoH and rpoE) responses were identified. Other significant genes included those involved in lipopolysaccharide biosynthesis (irp1, waaD, rfc, and rfbX), DNA repair and replication (traI), biofilm formation (ccdA and fyuA), and cellular metabolism (irtA). ISSN: 09639969

Ollinger M., Lim K.H., Knott T.

Incentives for Salmonella control in chicken broilers: Why the sampling protocol matters (2024) *Food Control, 155, art. no. 110083*

ABSTRACT: Better control of Salmonella in chicken has been a public health priority at the Food Safety Inspection Service (FSIS) in the U.S. and public health agencies worldwide. Testing for Salmonella in chicken carcasses and enforcement of a standard has been a central feature of the FSIS Salmonella-control program. In recent years, FSIS began disclosing the identities of the performance of establishments on Salmonella tests and reduced its tolerance for Salmonella by half. Both led to reductions in Salmonella, but some establishments still had consistently poor performance on Salmonella tests relative to others, leading FSIS to change the sampling protocol for Salmonella from a set-based to

a moving average approach in 2015. This paper examines the impact of this change in sampling protocol on establishment-level positive tests for Salmonella per year as a share of samples tested in chicken. Results indicate that establishments that had historically weak performance on tests for Salmonella before the change in sampling protocol converged in performance on Salmonella tests towards the Salmonella share of the better-performing establishments over 2012–2017. The establishments with the worst historical performance on tests for Salmonella had the greatest improvement. These results are consistent across three measures of performance on Salmonella tests. ISSN: 09567135

Gupta P.C., Divya K.H., De S., Johari S., Jayapalan G., Gupta M.K., Gupta A., Reddy J.S.

Microbiology proficiency testing in fish and fishery products: detection of Listeria monocytogenes and Salmonella spp.

(2024) Journal of Food Science and Technology, 61 (1), pp. 62 - 68 ABSTRACT: This paper presents the results of two proficiency testing (PT) rounds conducted by the Export Inspection Agency (EIA) Chennai laboratory in 2021 for food testing laboratories in India. The PT program was designed in accordance with ISO/TS 22117, a standard for proficiency testing in food microbiology, and targeted Listeria monocytogenes and Salmonella spp as the organisms of focus. The samples were found to be stable and recoverable during the analysis, and all PT sample packages were delivered to participant laboratories in good condition. The participant laboratories reported high sensitivity rates of 100% for PT round 061021 M and 96.49% for PT round 050721 M. The accuracy rate in PT round 061021 M was 91.89% and 92.10% in case of PT round 050721 M. However, there were some false positive and false negative results reported by some participant laboratories in both PT rounds, which may have been caused by operational errors or inconsistencies in analysis. During the PT round 061021 M, out of a total of 38 participant laboratories, five laboratories reported false positive results and one laboratory reported a false negative result. Similarly, during the PT round 050721 M, six laboratories reported false positive results which resulted in their results being deemed unsatisfactory. ISSN: 00221155

Logue C.M., De Cesare A., Tast-Lahti E., Chemaly M., Payen C., LeJeune J., Zhou K. Salmonella spp. in poultry production—A review of the role of interventions along the production continuum

(2024) Advances in Food and Nutrition Research, 108, pp. 289 - 341

ABSTRACT: Salmonella is a significant pathogen of human and animal health and poultry are one of the most common sources linked with foodborne illness worldwide. Global production of poultry meat and products has increased significantly over the last decade or more as a result of consumer demand and the changing demographics of the world's population, where poultry meat forms a greater part of the diet. In addition, the relatively fast growth rate of birds which is significantly higher than other meat species also plays a role in how poultry production has intensified. In an effort to meet the greater demand for poultry meat and products, modern poultry production and processing practices have changed and practices to target control and reduction of foodborne pathogens such as Salmonella have been implemented. These strategies are implemented along the continuum from parent and grandparent flocks to breeders, the farm and finished broilers to transport and processing and finally from retail to the consumer. This review focuses on common practices, interventions and strategies that have potential impact for the control of Salmonella along the poultry production continuum from farm to plate. ISSN: 10434526

Wilson R., Barrs V.R.

Salmonella enterica subspecies enterica serotype Typhimurium induced pyelonephritis and suspected multifocal myositis in a cat

(2024) Journal of Feline Medicine and Surgery Open Reports, 10 (1)

ABSTRACT: Case summary: A 2-year-old male neutered domestic shorthair cat presented with an acute onset of muscular pain, ataxia and fever. Serological tests for Toxoplasma gondii IgM and IgG, cryptococcal antigen, feline immune deficiency virus antibody and feline leukaemia virus antigen were all negative. Brain and spinal MRI showed evidence of myositis and bilateral renal parenchymal abnormalities and pyelectasis. Salmonella enterica subspecies enterica serotype Typhimurium 1,4, [5],12:i:1,2 was isolated from urine and was susceptible to amoxycillin, amoxycillin–clavulanic acid, enrofloxacin and trimethoprim–sulfonamide. All clinical signs resolved after a 2-week treatment course with oral amoxycillin–clavulanate. A repeat urine culture 7 days after completing the antimicrobial course was negative. Relevance and novel information: Infection with Salmonella species is uncommon in cats and has not previously been reported in association with pyelonephritis or generalised myositis. The importance of performing urine

culture in the initial diagnostic investigation of cats with pyrexia is highlighted in this case report. ISSN: 20551169

Ahmad A.R., Ridgeway S., Shibl A.A., Idaghdour Y., Jha A.R.

Falcon gut microbiota is shaped by diet and enriched in Salmonella (2024) PLoS ONE, 19 (1 January), art. no. e0293895

ABSTRACT: The gut microbiome is increasingly being appreciated as a master regulator of animal health. However, avian gut microbiome studies commonly focus on birds of economic importance and the gut microbiomes of raptors remain underexplored. Here we examine the gut microbiota of 29 captive falcons-raptors of historic importance-in the context of avian evolution by sequencing the V4 region of the 16S rRNA gene. Our results reveal that evolutionary histories and diet are significantly associated with avian gut microbiota in general, whereas diet plays a major role in shaping the falcon gut microbiota. Multiple analyses revealed that gut microbial diversity, composition, and relative abundance of key diet-discriminating bacterial genera in the falcon gut closely resemble those of carnivorous raptors rather than those of their closest phylogenetic relatives. Furthermore, the falcon microbiota is dominated by Firmicutes and contains Salmonella at appreciable levels. Salmonella presence was associated with altered functional capacity of the falcon gut microbiota as its abundance is associated with depletion of multiple predicted metabolic pathways involved in protein mass buildup, muscle maintenance, and enrichment of antimicrobial compound degradation, thus increasing the pathogenic potential of the falcon gut. Our results point to the necessity of screening for Salmonella and other human pathogens in captive birds to safeguard both the health of falcons and individuals who come in contact with these birds. ISSN: 19326203

Rodrigues I.C., Cristal A.P., Ribeiro-Almeida M., Silveira L., Prata J.C., Simões R., Vaz-Pires P., Pista Â., Martins da Costa P.

Gulls in Porto Coastline as Reservoirs for Salmonella spp.: Findings from 2008 and 2023 (2024) Microorganisms, 12 (1), art. no. 59

ABSTRACT: Gulls act as intermediaries in the exchange of microorganisms between the environment and human settlements, including Salmonella spp. This study assessed the antimicrobial resistance and molecular profiles of Salmonella spp. isolates obtained from fecal samples of gulls in the city of Porto, Portugal, in 2008 and 2023 and from water samples in 2023. Antimicrobial susceptibility profiling revealed an improvement in the prevalence (71% to 17%) and antimicrobial resistance between the two collection dates. Two isolate collections from both 2008 and 2023 underwent serotyping and whole-genome sequencing, revealing genotypic changes, including an increased frequency in the monophasic variant of S. Typhimurium. qacE was identified in 2008 and 2023 in both water and fecal samples, with most isolates exhibiting an MDR profile. The most frequently observed plasmid types were IncF in 2008 (23%), while IncQ1 predominated in 2023 (43%). Findings suggest that Salmonella spp. circulate between humans, animals, and the environment. However, the genetic heterogeneity among the isolates from the gulls' feces and the surface water may indicate a complex ecological and evolutionary dynamic shaped by changing conditions. The observed improvements are likely due to measures to reduce biological contamination and antimicrobial resistance. Nevertheless, additional strategies must be implemented to reduce the public health risk modeled by the dissemination of pathogens by gulls. ISSN: 20762607

Russo I., Fischer J., Uelze L., Napoleoni M., Schiavano G.F., Andreoni F., Brandi G., Amagliani G.

From farm to fork: Spread of a multidrug resistant Salmonella Infantis clone encoding blaCTX-M-1 on pESI-like plasmids in Central Italy

(2024) International Journal of Food Microbiology, 410, art. no. 110490 ABSTRACT: Salmonella enterica subsp. enterica serovar Infantis (S. Infantis) is one of the "top five Salmonella serovars" of clinical significance in the European Union (EU). Antimicrobial resistant and extended spectrum β -lactamase (ESBL) AmpC-producing S. Infantis have been described in food production systems and human clinical samples in Italy. Recently, an increase of MDR S. Infantis carrying blaCTX-M genes involved in 3rd generation cephalosporin resistance was noticed in the EU, including Italy, mainly due to the spread of S. Infantis harboring a pESI-like plasmid. The aim of this study was to investigate the occurrence of the S. Infantis pESI-like plasmid among antibiotic resistant S. Infantis strains isolated at different points of the food chain, and to provide a phylogenetic analysis to gain further insight on their transmission pathways from 'farm to fork'. MDR S. Infantis strains (n. 35) isolated from 2016 to 2021 at different stages of the food chain (animals, food, food-related environments, and humans) were investigated with in depth molecular characterization using real-time PCR, S1 nuclease pulsed-field gel electrophoresis (S1-PFGE) and whole genome sequencing (WGS). Our study reported the occurrence of S. Infantis strains harboring pESI-like plasmids, carrying blaCTX-M-1 genes, in Central Italy, at different sampling points along the food chain. Results confirmed the presence of a plasmid with a molecular size around 224–310 kb, thus consistent with the pESI-like, in 97 % of the 35 samples investigated. Two variants of S. Infantis pESI-like IncFIB(K)_1_Kpn3 were detected, one associated with the European clone carrying blaCTX-M-1 (21 isolates) and the other associated with U.S. isolates carrying blaCTX-M-65 (2 isolates, pESI-like U.S. variant). The majority was resistant to 3rd generation cephalosporins but none of the strains tested positive for the carbapenemase encoding genes. A total of 118 virulence genes were identified in isolates harboring the pESI-like plasmid. cgMLST and SNP-based analysis revealed the presence of one main cluster, composed by strains isolated from the environment, animals, food and humans. The results of this investigation underline the importance of phylogenetic studies to monitor and understand pathogen and AMR spread in a One Health approach. ISSN: 01681605

Ormsby M.J., White H.L., Metcalf R., Oliver D.M., Feasey N.A., Quilliam R.S.

Enduring pathogenicity of African strains of Salmonella on plastics and glass in simulated peri-urban environmental waste piles

(2024) Journal of Hazardous Materials, 461, art. no. 132439

ABSTRACT: In low- and middle-income countries, plastic has become a major constituent of landfills and urban dump sites. Environmental plastic pollution can also provide a novel surface for the formation of microbial biofilm, which often includes pathogenic bacteria and viruses. Here, under conditions simulating a peri-urban waste pile typical of an African informal settlement, we aimed to determine if pathogenic Salmonella spp. can retain their virulence following a prolonged period of desiccation on the surfaces of environmental plastic and glass. We show that clinically (and environmentally) relevant strains of Salmonella including S. Enteritidis, S. Typhimurium and S. Typhi can persist on plastic and glass for at least 28-days and that temperature (which increases with the depth of an urban waste pile) is a key determinant of this survival. All three strains of Salmonella retained their pathogenicity (determined by using a Galleria mellonella model of infection) following their recovery from the plastisphere indicating that plastics in the environment can act as reservoirs for human pathogens and could facilitate their persistence for extended periods of time. Pathogens colonising environmental plastic waste therefore pose a heightened public health risk, particularly in areas where people are frequently exposed to plastic pollution. ISSN: 03043894

Lin Z., Jiang S., Zwe Y.H., Zhang K., Li D.

Glycogen plays a key role in survival of Salmonella Typhimurium on dry surfaces and in low-moisture foods

(2024) Food Research International, 175, art. no. 113714

ABSTRACT: Salmonella enterica is known to survive in desiccate environments and is often associated with low-moisture foods (LMFs). In this work, S. Typhimurium ATCC 14028 was found to survive better by achieving the least reductions $(3.17 \pm 0.20 \text{ Log CFU reduction})$ compared to S. Tennessee ATCC 10722 (3.82 ± 0.13 Log CFU reduction) and S. Newport ATCC 6962 (6.03 \pm 0.36 Log CFU reduction) after 30 days on surfaces with a relative humidity of 49% at ambient temperature. A metabolomic analysis revealed that S. Typhimurium was still active in energy metabolism after 24 h in the desiccate environment and glycogen, an energy reserve, was drastically reduced. We followed up on the glycogen levels over 30 days and found indeed a sharp decline on the first day. However, the glycogens detected on day 7 were significantly higher (P < 0.05) and thereafter remained stable above the original levels until day 30. The expression levels of both glycogen anabolism- and catabolism-related genes (csrA, glgA, glgC, glgX) were significantly upregulated at all tested points (P < 0.05). The glgA and glgC insertion mutants displayed weaker survivability on both dry surfaces and in representative LMFs (flour and milk powder) compared to the wild-type strain. This work highlights the role of glycogen during different periods of desiccation, which may bring novel insight into mitigating Salmonella by disrupting glycogen metabolism. ISSN: 09639969

Marzoli F., Bertola M., Pinarelli Fazion J., Cento G., Antonelli P., Dolzan B., Barco L., Belluco S.

A systematic review on the occurrence of Salmonella in farmed Tenebrio molitor and Acheta domesticus or their derived products

(2024) International Journal of Food Microbiology, 410, art. no. 110464

ABSTRACT: Insects represent a sustainable and protein-rich food source. This new supply chain requires the study and monitoring of pathogens' presence and impact, as for other farmed animals. Among pathogens, Salmonella is of interest due to the well-established possibility for insects to harbor it. Since Acheta domesticus (cricket) and Tenebrio molitor (mealworm) are the most sold and farmed insect species, the present systematic review aimed to collect, select, and evaluate, in the available scientific literature, studies investigating the occurrence of Salmonella in these species sampled. All available studies published in peer-reviewed journals in English, French, Italian, Portuguese, German, and Spanish were considered. No time limits were imposed. We searched PUBMED, EMBASE, WEB of Science Core Collection, and Food Science and Technology Abstracts. The first date searched was May 10th, 2022; an update of the search was conducted on May 5th, 2023. The data synthesis was presented in tables reporting the number of positives on the number of total analyzed samples with other relevant characteristics of the study. The quality assessment was carried out considering relevant aspects for sampling and the method of analysis for Salmonella detection. At the end of the screening process, 10 and nine studies conducted on crickets and mealworms, respectively, were included for data extraction. The S. serovar Wandsworth and S. serovar Stanley were isolated only in one sample of ready-to-eat crickets. A second study detected OTUs related to S. enterica in cricket and mealworm powders. No studies detected Salmonella in mealworms according to cultural methods. The limitations of the present review are that few studies were retrieved and that included studies had important limitations in terms of study design as sampling was mostly based on convenience and not on a sound statistical basis. The present systematic review underlines the need to obtain reliable data about Salmonella presence in insects considering the growing market and the scaling up of existing farms. This research was funded by the Italian Ministry of Health – Ricerca Corrente IZSVe 03/21. The review protocol was published on the Systematic Reviews for Animals and Food (SYREAF) Web site (https://syreaf.org/protocols/). ISSN: 01681605

Bolzoni L., Conter M., Lamperti L., Scaltriti E., Morganti M., Poeta A., Vecchi M., Paglioli S., Rampini A., Ramoni P., De Vita D., Bacci C., Rega M., Andriani L., Pongolini S., Bonardi S.

Salmonella in horses at slaughter and public health effects in Italy (2024) International Journal of Food Microbiology, 408, art. no. 110429 ABSTRACT: The study assessed the role of equids at slaughter as faecal carriers of Salmonella enterica and the occurrence of contaminated equid carcasses during the slaughter process in Northern Italy (Emilia-Romagna Region). From June to November 2021, 152 equids (146 horses, 5 donkeys and 1 mule) were tested for Salmonella both in caecal contents and through carcass swabs. Antimicrobial resistance (AMR) of recovered strains was tested against 15 antimicrobials. Salmonella was detected in 3/152 of the caecal contents (2.0 %), while all carcass samples were negative. S. enterica serovars Enteriditis, Typhimurium and Stanleyville were identified. The only AMR isolate was S. Typhimurium with AMR profile AmCStxT. Considering the consumption of raw horse meat (i.e., minced raw meat named "pesto di cavallo" and dried and smoked strips named "sfilacci di cavallo") in different areas of Northern Italy, we also investigated the possible link between horse meat eating and salmonellosis cases in the human population in the same area. Specifically, we compared the Salmonella strains collected during the study with those routinely processed in the laboratory surveillance system for human salmonellosis in Emilia-Romagna (a region with about 4.5 million inhabitants). The comparison was based on whole genome sequencing data through core genome multilocus sequence typing (cgMLST) used in routine surveillance. A genomic match in cgMLST was found between the strain of S. enterica serovar Enteritidis isolated from a horse caecal content and an enduring outbreak of 17 human cases in Emilia-Romagna during the study period. The consequent epidemiological investigation highlighted that a number of cases with known food history reported the consumption of horse meat and traced different batches of the consumed meat, released weeks apart from each other, to the slaughter investigated in the study. The results of the epidemiological investigation suggested the role of horses in the S. enterica serovar Enteritidis outbreak affecting raw horse meat consumers. This study shows that, despite the low prevalence on equid carcasses, S. enterica in horse meat can represent a risk to consumers. From the perspective of the slaughter activities, this highlights the need to maintain a high level of hygiene during the entire process, starting from the hygiene at lairage up to the slaughtering phase and dressing of carcasses. ISSN: 01681605

De Bene A.F., Russini V., Corradini C., Vita S., Pecchi S., De Marchis M.L., Terracciano G., Focardi C., Montemaggiori A., Zuffi M.A.L., Weill F.-X., Bossù T. *An extremely rare serovar of Salmonella enterica (Yopougon) discovered in a Western Whip Snake (Hierophis viridiflavus) from Montecristo Island, Italy: case report and review* (2024) Archives of Microbiology, 206 (1), art. no. 49 ABSTRACT: Reptiles, including snakes, can be asymptomatically infected with multiple pathogen microorganisms, including Salmonella spp., which is considered an important concern for public and animal health. Small and uninhabited isles are quite ecologically different from mainland and represent interesting fields of study, to discover unexpected biological and microbiological aspects of their wild inhabitants. This work reports the presence of the very rare Salmonella enterica serovar Yopougon, isolated in a carcass of a native wild snake (Hierophis viridiflavus) from an Italian uninhabited island of Mediterranean Sea, Montecristo. To our knowledge, S. enterica serovar Yopougon was previously isolated only once 34 years earlier in Ivory Coast, from a human fecal sample. In the present study, we present the genomic characterization of the new isolate, the phylogenetic comparison with the previously isolated S. enterica serovar Yopougon strain of human origin and with other sequences available in public databases. In addition, an extensive review of available data in the literature and from our case history is provided. Our finding represents an example of the ability of some pathogens to travel for very long distances within their hosts and then to infect others, even from different taxa. ISSN: 03028933

Zheng J., Reed E., Maounounen-Laasri A., Deng X., Wang S.S., Ramachandran P., Ferreira C., Bell R., Brown E.W., Hammack T.S., Wang H.

Evaluation of universal preenrichment broth and comparison of rapid molecular methods for the detection of Salmonella from spent sprout irrigation water (SSIW) (2024) International Journal of Food Microbiology, 411, art. no. 110527 ABSTRACT: Sprouts and spent sprout irrigation water (SSIW) present unique challenges for the development of a Salmonella detection method in food matrices. This study aimed to compare universal preenrichment broth (UPB) and lactose broth (LB) as preenrichment media for cultural and rapid screening methods and to compare their abilities to recover Salmonella in SSIW samples from different sprout varieties (i.e., alfalfa, broccoli, and mung bean sprouts). The associated co-enriched microbiota with different sprout varieties using different preenrichment media were also examined using a quasi-metagenomic approach. The performance of media and detection methods was compared using the relative level of detection (RLOD) value, as recommended by ISO 16140-2:2016. The level of detection (LOD) for Salmonella culture method with UPB was similar to that with LB in low aerobic plate count (APC) background samples (the relative LOD, i.e., RLOD, was nearly 1 after adjusting for the effects of SSIW variety and serovar), but significantly lower than that with LB in high APC background samples (RLOD = 0.32). The LOD for Salmonella with selected rapid methods was comparable to each other (RLOD from 0.97 to 1.50) and to the culture method (RLOD from 0.69 to 1.03), and no significant difference was detected between preenrichment broths in low APC background samples with RLOD values between 0.76 and 1.04. In samples with a high APC background, however, a drastic difference in LOD was observed between methods and between preenrichment broths for each method. The RLOD ranged from 0.03 to 0.32 when UPB was compared to LB as preenrichment broth. The composition and relative abundance (RA) of co-enriched microbiota was affected by multiple factors including food matrices, preenrichment media and Salmonella contamination. Altogether, this study validated UPB as a better preenrichment broth than LB for the detection of Salmonella enterica from SSIW. This study also suggested UPB may also be an optimal preenrichment medium for rapid screening methods when APC level is high. The observation of potential exclusion of Salmonella in preenrichment through the overgrowth of competitive microflora from the quasi-metagenomic study provided novel information that may be used to further optimize preenrichment formulations. ISSN: 01681605

Politi L., Mellou K., Chrysostomou A., Mandilara G., Spiliopoulou I., Theofilou A., Polemis M., Tryfinopoulou K., Sideroglou T.

A Community Waterborne Salmonella Bovismorbificans Outbreak in Greece (2024) International Journal of Environmental Research and Public Health, 21 (2), art. no. 167

ABSTRACT: Background: In August 2022, the Hellenic National Public Health Organisation was notified about a gastroenteritis outbreak in town A in Southern Greece. Investigations aimed to identify the source and implement control measures. Methods: Case definition categories were used in a 1:3 case-control study. Cases and controls were interviewed about various exposures. Cases' stool samples were cultured on agar plates and characterised by serotyping, antimicrobial susceptibility testing and Pulse Field Gel Electrophoresis (PFGE). Environmental investigations included tap water sampling for microbiological and chemical analysis in town A and inspection of the water supply system. Results: We identified 33 cases (median age: 17 years). Tap water consumption was the only significant risk factor for gastroenteritis (OR = 5.46, 95% CI = 1.02–53.95).

Salmonella (S.) Bovismorbificans isolated from eight stool and one tap water samples had identical PFGE profiles. No resistant isolates were identified. Residual chlorine levels were lower than the acceptable limits before and during the outbreak. We advised consumption of bottled water and adherence to strict hand hygiene rules until tap water was declared suitable for drinking. Conclusions: Epidemiological and molecular data revealed a waterborne S. Bovismorbificans outbreak in town A. We recommend local water safety authorities to ensure that residual chlorine levels comply with the legislation towards water safety planning, to mitigate risks. ISSN: 16617827

Reina M., McConnell A.D., Figueroa J.C., Riggs M.R., Buhr R.J., Price S.B., Macklin K.S., Bourassa D.V.

Quantification of Salmonella Infantis transfer from transport drawer flooring to broiler chickens during holding

(2024) Poultry Science, 103 (2), art. no. 103277

ABSTRACT: Transportation is a potential point of cross-contamination before broiler chickens arrive at the processing plant for slaughter. Previous studies have associated the use of uncleaned transport containers with the introduction of pathogenic bacteria onto uncontaminated broilers. The objective of this study was to quantify the transfer of Salmonella from transport drawer perforated flooring to broiler chickens during different holding times. For traceability, the flooring of each drawer was inoculated with fecal content slurry containing a marker strain of Salmonella Infantis. Three drawers per treatment were used, and each drawer was subjected to one of the following treatments: pressure wash, disinfectant, and pressure wash (A), pressurized steam followed by forced hot air (B), or no cleaning (C). Drawers were classified as top, middle, or bottom based on their relative position with each other. After treatment, broilers were introduced to each drawer and held for 2, 4, or 6 h. At each timepoint, broilers were removed from drawers, euthanized, and carcasses rinsed to obtain Salmonella counts. Samples under the limit of direct plating detection were enriched, plated, and later confirmed positive or negative. Differences were observed per treatment, holding time, and drawer relative position (P <0.0001). Broilers placed in transport containers that underwent a cleaning procedure (A or B) had lower levels of Salmonella when compared to broilers placed in noncleaned containers (C). However, most of the samples below the limit of detection were positive after enrichment, indicating that both procedures evaluated need improvement for efficient pathogen inactivation. A decrease in Salmonella transfer was observed after 6 h in rinsates obtained from broilers placed in noncleaned containers (C). Rinsates obtained from top drawers had less Salmonella than the middle or bottom drawers when broilers were placed in transport containers that underwent a cleaning procedure (A and B). The application of pressurized steam and forced hot air was comparable to the use of water washes and disinfectant indicating a potential role in cleaning poultry transport containers. ISSN: 00325791

Stathas L., Aspridou Z., Koutsoumanis K.

Quantitative microbial risk assessment of Salmonella in fresh chicken patties (2024) *Food Research International, 178, art. no. 113960*

ABSTRACT: Quantitative microbial risk assessment (QMRA) has witnessed rapid development within the context of food safety in recent years. As a means of contributing to these advancements, a QMRA for Salmonella spp. in fresh chicken patties for the general European Union (EU) population was developed. A two-dimensional (Second Order) Monte-Carlo simulation method was used for separating variability and uncertainty of model's parameters. The stages of industrial processing, retail storage, domestic storage, and cooking in the domestic environment were considered in the exposure assessment. For hazard characterization, a dose-response model was developed by combining 8 published dose-response models using a Pert distribution for describing uncertainty. The QMRA model predicted a mean probability of illness of 1.19*10-4 (5.28*10-5 - 3.57*10-4 95 % C.I.), and a mean annual number of illnesses per 100,000 people of 2.13 (0.96 - 6.59 95 % C.I.). Moreover, sensitivity analysis was performed, and variability in cooking preferences was found to be the most influential model parameter (r = -0.39), followed by dose-response related variability (r = 0.22), and variability in the concentration of Salmonella spp. at the time of introduction at the processing facility (r =0.11). Various mitigation strategy scenarios were tested, from which, "increasing the internal temperature of cooking" and "decreasing shelf life" were estimated to be the most effective in reducing the predicted risk of illness. Salmonella-related illnesses exhibit particularly high severity, making them some of the most prominent zoonotic diseases in the EU. Regular monitoring of this hazard in order to further highlight its related parameters and causes is a necessary procedure. This study not only provides an updated assessment of Salmonella spp. risk associated with chicken patties, but also facilitates the

identification of crucial targets for scientific investigation and implementation of real-world intervention strategies. ISSN: 09639969

Obe T., Kiess A.S., Nannapaneni R.

Antimicrobial Tolerance in Salmonella: Contributions to Survival and Persistence in Processing Environments

(2024) Animals, 14 (4), art. no. 578

ABSTRACT: Salmonella remains a top bacterial pathogen implicated in several food-borne outbreaks, despite the use of antimicrobials and sanitizers during production and processing. While these chemicals have been effective, Salmonella has shown the ability to survive and persist in poultry processing environments. This can be credited to its microbial ability to adapt and develop/acquire tolerance and/or resistance to different antimicrobial agents including oxidizers, acids (organic and inorganic), phenols, and surfactants. Moreover, there are several factors in processing environments that can limit the efficacy of these antimicrobial activity of common disinfectants/sanitizers used in poultry processing environments and the ability of Salmonella to respond with innate or acquired tolerance and survive exposure to persists in such environments. Instead of relying on a single antimicrobial agent, the right combination of different disinfectants needs to be developed to target multiple pathways within Salmonella. ISSN: 20762615

Leão C., Silveira L., Usié A., Gião J., Clemente L., Themudo P., Amaro A., Pista A.

Genetic Diversity of Salmonella enterica subsp. enterica Serovar Enteritidis from Human and Non-Human Sources in Portugal

(2024) Pathogens, 13 (2), art. no. 112

ABSTRACT: Salmonella enterica subsp. enterica serovar Enteritidis (S. Enteritidis) is one of the leading causes of foodborne infections associated with broilers and laying hens. Portugal has had the lowest notification rates of salmonellosis in recent years, due to the vaccinations of layer and breeder flocks and strict compliance with biosecurity measures. However, data about the genetic diversity of S. Enteritidis in Portugal are scarce. In this study, 102 S. Enteritidis isolates selected from human (n = 63) and non-human sources (n = 39) were characterized by serotyping, antimicrobial susceptibility, and whole genome sequencing. The S. Enteritidis population was mainly resistant to fluoroquinolones, and a sole isolate showed resistance to extended-spectrum cephalosporins. ST11 was the most frequent sequence type, and three novel STs from human isolates (ST9236, ST4457, and ST9995) were assigned. Several Salmonella pathogenic islands (SPI) and Putative SPI were present in the genomes, namely SPI-1, 2, 3, 4, 5, 9, 10, 12, 13, and 14, C63PI, CS54_island, and 170 virulence genes were identified. The phylogenetic analysis revealed that strains from Portugal are genetically heterogeneous regarding sample type, collection date, and genetic content. This study increases the available data, essential to a better characterization of strains in a global context. ISSN: 20760817

Schwensohn C., Schneider B., Jenkins E., Wellman A., Federman S.S., Oni O., Stone N., Adams J., Gieraltowski L.

Rapidly Linking an Outbreak of Salmonella Typhimurium Infections to Domestically Grown Cantaloupes Through Early Collaboration - United States, 2022 (2024) Morbidity and Mortality Weekly Report, 73 (5), pp. 114 - 115 ISSN: 01492195

Olsen J.E., Frees D., Kyvsgaard N.C., Barco L.

Lack of correlation between growth, stress, and virulence phenotypes in strains of Salmonella enterica serovar Enteritidis, S. Typhimurium DT104, S. 4,12, b:- and S. Liverpool

(2024) Letters in Applied Microbiology, 77 (2), art. no. ovae015

ABSTRACT: Strains of Salmonella Enteritidis (SEnt, n = 10) and S. Typhimurium (STm, n = 11), representing clones with high impact on human health, and strains of S. 4,12: b:-(S412B n = 11) and S. Liverpool (SLiv, n = 4), representing clones with minor impact on human health were characterized for 16 growth, stress, and virulence phenotypes to investigate whether systematic differences exist in their performance in these phenotypes and whether there was correlation between performance in different phenotypes. The term serotype was not found to be predictive of a certain type of performance in any phenotype, and surprisingly, on average, strains of SEnt and STm were not significantly better in adhering to and invading cultured intestinal cells than the less pathogenic types. Forest analysis identified desiccation tolerance and the ability to grow at 42°C with high salt as the characters that separated serovars with low human health impact (S412B/SLiv) from serovars with high human health impact (SEnt/STm). The study showed that variation in

phenotypes was high even within serovars and correlation between phenotypes was low, i.e. the way that a strain performed phenotypically in one of the tested conditions had a low predictive value for the performance of the strain in other conditions. ISSN: 02668254

Han M., Zarkani A.A., Duan Y., Grimm M., Trotereau J., Virlogeux-Payant I., Schikora A.

Bidirectional Comparisons Revealed Functional Patterns in Interaction between Salmonella enterica and Plants

(2024) Plants, 13 (3), art. no. 414

ABSTRACT: Plants may harbor the human pathogen Salmonella enterica. Interactions between S. enterica and different plant species have been studied in individual reports. However, disparities arising from the distinct experimental conditions may render a meaningful comparison very difficult. This study explored interaction patterns between different S. enterica strains including serovars Typhimurium 14028s and LT2 and serovar Senftenberg, and different plants (Arabidopsis, lettuce, and tomato) in one approach. Better persistence of S. enterica serovar Typhimurium strains was observed in all tested plants, whereas the resulting symptoms varied depending on plant species. Genes encoding pathogenesis-related proteins were upregulated in plants inoculated with Salmonella. Furthermore, transcriptome of tomato indicated dynamic responses to Salmonella, with strong and specific responses already 24 h after inoculation. By comparing with publicly accessible Arabidopsis and lettuce transcriptome results generated in a similar manner, constants and variables were displayed. Plants responded to Salmonella with metabolic and physiological adjustments, albeit with variability in reprogrammed orthologues. At the same time, Salmonella adapted to plant leaf-mimicking media with changes in biosynthesis of cellular components and adjusted metabolism. This study provides insights into the Salmonella-plant interaction, allowing for a direct comparison of responses and adaptations in both organisms. ISSN: 22237747

Bainotti M.B., Colás-Medà P., Viñas I., Garza S., Alegre I.

The Survival of Salmonella enterica Strains in Ready-to-Eat Fruit Purees under Different Storage Temperatures

(2024) Beverages, 10 (1), art. no. 17

ABSTRACT: Salmonella enterica, known for its resilience to acidic environments, has been linked to foodborne outbreaks of illness from fruit derivatives. This study aimed to assess the survival of five serovars of Salmonella enterica subsp. enterica in various fruit purees subjected to different storage temperatures. Among the studied serovars, S. enteritidis exhibited the most significant population decrease in all fruit purees. In contrast, S. Agona, S. Gaminara, S. Michigan, and S. Montevideo survived in peach puree at 4 °C for at least 3 days, and S. Agona, S. Gaminara, and S. Montevideo maintained their initial levels in pear puree under the same time/temperature conditions. However, none of the strains were detectable in plum and black currant purees after 2 days at 4, 15, or 25 °C. These findings highlight variations in the behaviour of S. enterica serovars within different fruit purees. Likewise, low-temperature conditions prolonged the survival of the tested strains in all fruit purees analysed. ISSN: 23065710

Cohen E., Azriel S., Auster O., Gal A., Mikhlin S., Crauwels S., Rahav G., Gal-Mor O.

A new Salmonella enterica serovar that was isolated from a wild sparrow presents a distinct genetic, metabolic and virulence profile

(2024) Microbes and Infection, 26 (3), art. no. 105249

ABSTRACT: Salmonella enterica is a ubiquitous and clinically-important bacterial pathogen, able to infect and cause different diseases in a wide range of hosts. Here, we report the isolation and characterization of a new S. enterica serovar (13,23:i:-; S. Tirat-Zvi), belonging to the Havana supper-lineage that was isolated from a wild house sparrow (Passer domesticus) in Israel. Whole genome sequencing and complete assembly of its genome indicated a plasmid-free, 4.7 Mb genome that carries the Salmonella pathogenicity islands 1–6, 9, 19 and an integrative and conjugative element (ICE), encoding arsenic resistance genes. Phenotypically, S. Tirat-Zvi isolate TZ282 was motile, readily formed biofilm, more versatile in carbon source utilization than S. Typhimurium and highly tolerant to arsenic, but impaired in host cell invasion. In-vivo infection studies indicated that while S. Tirat-Zvi was able to infect and cause an acute inflammatory enterocolitis in young chicks, it was compromised in mice colonization and did not cause an inflammatory colitis in mice compared to S. Typhimurium. We suggest that these phenotypes reflect the distinctive ecological niche of this new serovar and its evolutionary adaptation to passerine birds, as a permissive host. Moreover, these results further illuminate the genetic, phenotypic and ecological diversity of S. enterica pathovars. ISSN: 12864579

Fischerstrom K., Dryselius R., Lindblad M., Kuhlmann-Berenzon S., Karamehmedovic N., Borjesson S., Hashemi N., Gunn I., Gustavsson A.-M., Lindroos N., Nederby-Ohd J., Widerstrom M., Rimhanen-Finne R., Vainio A., Rehn M.

Outbreak of Salmonella Typhimurium linked to Swedish pre-washed rocket salad, Sweden, September to November 2022

(2024) Eurosurveillance, 29 (10), art. no. 2300299

ABSTRACT: In September 2022, the Public Health Agency of Sweden observed an increase in domestic Salmonella Typhimurium cases through the Swedish electronic notification system, and an outbreak strain was identified with whole genome sequencing. Overall, 109 cases with symptom onset between 17 September and 24 November 2022 were reported from 20 of 21 Swedish regions. The median age of cases was 52 years (range 4-87 years) and 62% were female. A case-control study found cases to be associated with consumption of rocket salad (adjusted odds ratio (aOR) = 4.9; 95% confidence interval (CI): 2.4-10, p value < 0.001) and bagged mixed salad (aOR = 4.0; 95% CI: 1.9-8.1, p value < 0.001). Traceback, supported by Finnish authorities who identified the Swedish outbreak strain in a Finnish cluster during the same time period, identified rocket salad, cultivated, pre-washed and pre-packed in Sweden as the likely source of the outbreak. No microbiological analyses of rocket salad were performed. Our investigation indicates that bagged leafy greens such as rocket salad, regardless of pre-washing procedures in the production chain, may contain Salmonella and cause outbreaks, posing a health risk to consumers. We emphasise the need for primary producers of leafy greens to identify possible contamination points to prevent outbreaks. ISSN: 1025496X

Woyda R., Oladeinde A., Endale D., Strickland T., Plumblee Lawrence J., Abdo Z.

Genetic Characteristics of Salmonella Isolates Recovered From Reused Broiler Litter Over Three Successive Flocks

(2024) Journal of Food Protection, 87 (3), art. no. 100236

ABSTRACT: Salmonella infections are a leading cause of bacterial food-borne illness worldwide. Infections are highly associated with the consumption of contaminated food, and in particular, chicken meat. The severity of Salmonella infections depends on the presence of antimicrobial resistance genes and virulence factors. While there are many studies which have investigated Salmonella strains isolated from postharvest chicken samples, there is a gap in our understanding of the genetic properties that influence the persistence of Salmonella in preharvest and in particular their makeup of antimicrobial resistance genes and virulence factors. We used whole genome sequencing and hierarchical clustering to characterize and classify the genetic diversity of Salmonella enterica isolates (n = 55) recovered from the litter of commercial broiler chicken raised in four colocated broiler houses of one integrated farm over three consecutive flocks. The chicken were raised under a newly adopted "No Antibiotics Ever" program, and copper sulfate was administered via drinking water. In-silico serovar prediction identified three S. enterica serovars: Enteritidis (n = 12), Kentucky (n = 40), and Senftenberg (n = 3). Antimicrobial susceptibility testing revealed that only one S. Kentucky isolate was resistant to streptomycin, while the remaining isolates were susceptible to all antibiotics tested. Metal resistance operons, including copper and silver, were identified chromosomally and on plasmids in serovar Senftenberg and Kentucky isolates, respectively, while serovar Enteritidis carried several virulence factors on plasmids. Serovar Kentucky isolates harboring metal resistance operons were the only Salmonella isolates recovered from the litter of third flock cohort. These results suggest that there might be environmental selection for Salmonella strains carrying plasmid-associated metal resistance and virulence genes, which could play a role in their persistence in litter. ISSN: 0362028X

Powell M.R.

Trends in reported illness due to poultry- and nonpoultry associated Salmonella serotypes; United States 1996–2019

(2024) Risk Analysis, 44 (3), pp. 641 - 649

ABSTRACT: Retrospective review is a key to designing effective food safety measures. Despite the reported reduction of Salmonella prevalence in poultry products, there has not been a concomitant reduction of the overall incidence of Salmonella illnesses reported to the US Foodborne Diseases Active Surveillance Network (FoodNet) since 1996. However, there have been significant annual trends among Salmonella serotypes. This analysis examines trends in the reported incidence of illness due to poultry- and nonpoultry associated Salmonella serotypes. Overall, the findings indicate declining trends in illness due to the poultry-associated serotypes and increasing trends in illness due to Salmonella serotypes not associated with poultry. Published 2023. This article is a U.S. Government work and is in the public domain in the USA. ISSN: 02724332