

NEWSLETTER

European Union Reference Laboratory for *Salmonella*

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Editorial Note

Bilthoven, 5 January 2023

Dear colleague,

I hope you have had a nice and relaxing Christmas break and a good start of the new year. I would like to **wish you all a very good and healthy 2023!** Last year January we were still in the middle of the SARS-CoV-2 pandemic and many countries were in lockdown. Currently we are luckily more or less back to normal. However, what is 'normal'? This pandemic also has learned us that it is possible to work from home for many of us and that online meetings are well feasible, so that traveling is not always necessary. Still, physical meetings are important to retain good contact with colleagues, also something we learned from this pandemic.

The end of the year is also a moment to look back and to realise that time is slipping through your fingers. Especially when dear colleagues, with whom you have been working for many years are retiring, or much worse, pass away. For this I want to have a moment of reflection to remember some dear colleagues of NRLs-*Salmonella* who passed away last year: Maria Emmanuel from Cyprus and Jim McLaughlin from United Kingdom. Very sad losses for family, friends and colleagues.

Still we have to go on, as *Salmonella* is never far away. At first looking back to the Proficiency Tests and interlaboratory study organised in 2022.

In May-June 2022 we organised the **interlaboratory study (ILS) for determination of the performance characteristics** of draft ISO/DTS 6579-4 (Identification of monophasic *Salmonella* Typhimurium by PCR). The analysis of the results was more complicated than expected, but shortly before the Christmas break we could inform the participants about the (draft) results. The draft performance characteristics will be discussed with the members of ISO/TC34/SC9-WG10 in February 2023 and will be introduced in the next draft of ISO/TS 6579-4. The results of the methods evaluation study as well as of the ILS will also be summarised in more detail in a report which we are currently drafting. As soon as the next draft version of ISO/TS 6579-4 will become available and when the report about the validation studies of ISO/TS 6579-4 is published, all NRLs-*Salmonella* will be informed.

In October 2022, the **combined PT on the detection of *Salmonella* in food and in samples from the primary production stage (PPS)** was organised. Also the results of this study were a bit more complicated than 'usual'. Unexpectedly, *Salmonella* was detected in approx. 10% of the total number of negative samples by several laboratories. Investigations to the source of contamination is still ongoing and for that reason, the EURL-*Salmonella* has decided not to evaluate the results of the negative samples of this study. The results per laboratory, as well as the interim summary report were sent to the participants shortly before the Christmas break.

In November the **PT on typing of *Salmonella*** was organised, containing an obligatory part on serotyping of *Salmonella*, and a voluntary part on cluster analysis. The deadline for reporting the serotyping results was by mid-December 2022, and the deadline for the part on cluster analysis is 27 January 2023. Soon we will start the analysis of the results of the serotyping part, after which you will be informed about the results.

The first Proficiency Test of 2023 will be a combined PT for Food-Food on detection of *Salmonella* in seeds. The time table of this PT was sent

to the NRLs-*Salmonella* in December 2022 and is included in this Newsletter as well.

In November 2022 we informed you about the fact that at the annual meeting of ISO/TC34/SC9 (June 2022), it was decided to 'reactivate' the **ISO working group (WG9) on detection of *Salmonella* (ISO 6579-1)**. The aims for reactivating ISO/TC34/SC9-WG9 are:

- To complete the performance characteristics of ISO 6579-1 ('Detection of *Salmonella*) for all relevant product categories (broad range of food, animal feed, environmental samples and samples from the primary production stage). Although ISO 6579-1 does contain performance characteristics, these are not complete for all categories. To have ISO 6579-1 validated for a broad range of food, at least 2 additional food categories need to be tested. Additionally, performance characteristics for animal feed and environmental samples are lacking. To determine the performance characteristics for these (additional) categories it may be necessary to organise one or more interlaboratory studies.
- To consider the comments to ISO 6579-1:2017 from the systematic review of 2022.

If you want to become a member of ISO/TC34/SC9-WG9, you are advised to contact your National standards body. Most likely, the standards body follows the work of ISO/TC 34/SC 9 'Microbiology' and/or CEN/TC 463 'Microbiology of the food chain' and has a national standards committee on microbiology of the food chain.

In December 2022 we were informed that the SMP (Single Market Programme Regulation) Work Programme for 2023-2024 was not yet adopted by the European Commission, resulting in the fact that the EURLs were not yet invited to submit their **work programmes for 2023-2024**. The call for submitting the work programmes is expected in the first quarter of 2023 and the grant agreements for 2023-2024, for all EURLs, is not expected to take place before the second semester 2023. Currently the EURLs are invited to prepare their individual (draft) work programmes for 2023-2024 and discuss this with the desk officers at DG SANTE before the end of January 2023.

Due to the late formal decision about the EURL-*Salmonella* work programme and budget for this year and next year, we discussed with the desk officer of DG SANTE if we could take the 'risk' to organise a physical workshop in May 2023. We were advised to either organise an online workshop in May or move the organisation of a physical workshop to the second half of 2023. However, organisation of the workshop in e.g. October would still mean that booking of flight tickets and agreements with a meeting location will be done before the grant decision is taken and thus still 'risky'. For that reason we decided that this years' workshop will again be an **online workshop in May**, but for 2024 we will do our utmost to organise a physical workshop. I am very sorry that for the fourth time we have to organise a remote workshop, this time for financial reasons and (probably) not for pandemic reasons. More details about the date(s) of the workshop will follow as soon as possible.

In October 2022, the following EURL-*Salmonella* report was published: Mooijman, K.A. The 27th EURL-*Salmonella* workshop; 23 and 24 May 2022, Online. National Institute for Public Health and the Environment, Bilthoven, the Netherlands. RIVM Report no.: 2022-0107.

<https://www.rivm.nl/bibliotheek/rapporten/2022-0107.pdf>

Best wishes,
Kirsten Mooijman
Coordinator EURL-*Salmonella*

Contribution of the EURL-*Salmonella*

Timetable EURL- *Salmonella* Proficiency Food-Feed 2023 Detection of *Salmonella* in seeds

Week	Date	Subject
8	24 February 2023	E-mailing the link to the registration form for the detecting study. Please register by 24 February 2023 at the latest.
10 - 11	06 March 2023 – 17 March 2023	E-mailing the link for the result form to the participants. E-mailing the protocol and instructions for the result form to the NRLs.
12	Monday 20 March 2023	Shipment of the parcels to the participants as Biological Substance Category B (UN3373). Preparation of media by the NRLs.
13	Monday 27 March 2023	Performance of the Proficiency Test.
16	19 April 2023 at the latest	Deadline for completing the result form: 19 April 2023 (23:59h CET) After this deadline the result form will be closed.
	May 2023	Interim summary report

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

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RIVM / Z&O (internal Pb 63) EURL- *Salmonella*

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From the Literature

Salmonella-related Literature from Scopus: October – December 2022

Rosauer, M.L., Lopez-Velasco, G., Silbernagel, K.M., Horine, L., Tudor, A.

Validation of the 3MTM Molecular Detection Assay 2-Salmonella for detection of Salmonella in Dried Cannabis Flower and Dried Hemp Flower: AOAC Performance Tested MethodsSM 091501

(2022) *Journal of AOAC International*, 105 (6), pp. 1684-1690.

ABSTRACT: BACKGROUND: The 3M™ Molecular Detection Assay 2-Salmonella method is based on real-time loop-mediated isothermal amplification when used with the 3M Molecular Detection System for the rapid and specific detection of Salmonella in enriched products. The 3M Molecular Detection Assay 2-Salmonella was approved as AOAC INTERNATIONAL (AOAC) Performance Tested MethodSM (PTM) Certificate No. 091501 and as AOAC Official Method of AnalysisSM2016.01. OBJECTIVE: This matrix extension study evaluated the 3M Molecular Detection Assay 2-Salmonella for detection of Salmonella in dried cannabis flower [$>0.3\%$ delta 9-tetrahydrocannabinol (THC)] and dried hemp flower ($\leq 0.3\%$ THC) at a 10 g test portion size. METHOD: Matrix studies in dried cannabis and hemp flowers followed procedures outlined in 3M Molecular Detection Assay 2-Salmonella product instructions and Standard Method Performance Requirement (SMPR®) for Detection of Salmonella species in Cannabis and Cannabis Products (AOAC SMPR 2020.002). The method was evaluated at low, high, and non-contaminated levels. RESULTS: Results showed no statistically significant difference between the presumptive positive 3M Molecular Detection Assay 2-Salmonella results and the SMPR 2020.002 recommended cultural confirmations. CONCLUSIONS: This study demonstrates that the 3M Molecular Detection Assay 2-Salmonella is a reliable method for the rapid and specific detection of Salmonella in dried cannabis flower and dried hemp flower. HIGHLIGHTS: The 3M Molecular Detection Assay 2-Salmonella method is suitable for the rapid and specific detection of Salmonella in dried cannabis flower and dried hemp flower. ISSN: 19447922

Kääriäinen, S., Obach, D., Paspaliari, D.K., Tofferi, M., Nieminen, A., Pihlajasaari, A., Kuronen, H., Vainio, A., Rimhanen-Finne, R.

Salmonella Typhimurium outbreak associated with frozen tomato cubes at a restaurant in western Finland, January to February 2021

(2022) *Eurosurveillance*, 27 (41), .

ABSTRACT: Several individuals reported gastrointestinal symptoms following meals consumed in late January 2021 at a restaurant in western Finland. We conducted a retrospective cohort study and defined a case as a person who ate at the lunch restaurant between 27 and 29 January 2021 and had stomach pain, vomiting or diarrhoea and/or a laboratory-confirmed Salmonella Typhimurium infection within 2 weeks after the exposure. We collected faecal and food samples for microbiological analysis. Salmonella isolates were characterised in detail using whole genome sequencing (WGS) and cluster analysis by core genome multilocus sequence typing (cgMLST). Altogether, 393 meals were sold and 101 people (who ate 142 meals) participated in the cohort study. There were 49 cases; 23 were laboratory-confirmed infections with a multidrug-resistant S. Typhimurium. The S. Typhimurium isolates from cases and frozen tomato cubes used uncooked in salads were closely related and clustered together in cgMLST comparison. These salads were consumed by 76% of the cases. Based on the cgMLST clustering, they were the suggested source of the outbreak. Statistical association was not significant between eating the salads and being a case. Following the outbreak investigation, the producer decided to recommend cooking of their frozen tomato products before consumption. ISSN: 1025496X

Peruzy, M.F., Proroga, Y.T.R., Capuano, F., Mancusi, A., Montone, A.M.I., Cristiano, D., Balestrieri, A., Murru, N.

Occurrence and distribution of Salmonella serovars in carcasses and foods in southern Italy: Eleven-year monitoring (2011–2021)

(2022) *Frontiers in Microbiology*, 13, art. no. 1005035, .

ABSTRACT: Salmonella is one of the most common agents of foodborne illness. The genus Salmonella includes two species (Salmonella bongori and S. enterica) and six subspecies (enterica I, salamae II, arizonae IIIa, diarizonae IIIb, houtenae IV, and indica VI), each of

which contains multiple serotypes associated with animal and human infections. The aim of the study was to evaluate the presence of *Salmonella* spp. in carcasses of food-producing animals and foods in southern Italy and the serovar distribution among different sources. From 2011 to 2021, a total of 12,246 foods and 982 samples from animal carcasses were collected and analyzed. The overall percentage of positive samples was 5.84% (N = 773) and a significant increase in prevalence was observed by comparing the years 2011–2015 (257, 3.27%) and 2016–2021 (516, 9.61%; $p < 0.05$). The highest percentage of positive food samples was observed in “Meat and Meat Products” (N = 327/2,438, 13.41%) followed by “Fish and fishery products” (N = 115/1,915, 6.01%). In carcasses, the highest percentage of positive samples was reported from broilers (N = 42/81, 51.85%) followed by buffalo (N = 50/101, 49.50%) and pork (N = 140/380, 36.84%). After typing, the isolates were assigned to the species *S. enterica* and to the subspecies: *enterica* (N = 760, 98.32%), *diarizonae* (N = 8, 1.03%), *salamae* (N = 3, 0.39%) and *houtenae* (N = 2, 0.26%). *S. infantis* was the most frequently detected (N = 177, 24.76%), followed by *S. Derby* (N = 77, 10.77%), monophasic *S. Typhimurium* (N = 63, 8.81%), *S. Typhimurium* (N = 54, 7.55%), and *S. Rissen* (N = 47, 6.57%). By comparing the sampling period 2011–2015 with that of 2016–2021, an increase in the prevalence of *S. infantis* and monophasic *S. Typhimurium* and a decrease of *S. Typhimurium* were recorded ($p < 0.05$). Thus, present data suggest that, despite the implementation of national and European control strategies to protect against *Salmonella*, the prevalence of this pathogen in southern Italy is still increasing and a change of national control programs to protect against *Salmonella* are necessary. ISSN: 1664302X

Sheets, T.R., Wickware, C.L., Snyder, A.M., Weimer, S.L., Johnson, T.A.

Ileal and cecal microbiota response to Salmonella Typhimurium challenge in conventional and slow-growing broilers

(2022) *Frontiers in Physiology*, 13, art. no. 971255, .

ABSTRACT: Despite the negative impacts of *Salmonella* intestinal colonization on human health, *Salmonella* is a natural colonizer of the gastrointestinal tract and is not overtly pathogenic to the avian host. It is of interest to understand the impacts and colonization rates of *Salmonella* across selected genetic lines such as slow-growing (SG) and conventional (CONV) broilers. The objective of this study was to characterize the relationship between *Salmonella enterica* serovar *Typhimurium* challenge and selected broiler genetic lines on the ileal and cecal microbiome. Male chicks of two broiler breeds (n = 156/breed) were cohoused in an open floor pen until day 7. On day 13, the chicks were then separated into 12 isolators per breed (4 rooms, 6 isolators/room, 11 chicks/isolator). On day 14, chicks in the 12 treatment isolators (6 isolators/breed, 108 total) were challenged with *Salmonella Typhimurium* (ST) ($1 \times$ (Formula presented.) CFU/ml) via oral gavage while the remaining chicks (n = 108) were given an oral gavage of sterile tryptic soy broth control (C). Ileal and cecal contents were collected on day 7 from 24 chicks of each breed, and on days 13, 17, 21, and 24 from two chicks per isolator. Samples underwent DNA extraction and PCR amplification to obtain 16S rRNA amplicons that were sequenced with Illumina MiSeq. *Salmonella Typhimurium* colonization in the cecum was not different in the two broiler breeds. The main effect of breed had the greatest impact on the ileum microbiota of broilers 7 days of age where SG broilers had significantly lower diversity and richness compared to CONV broilers ($p < 0.05$). *Salmonella Typhimurium* challenge consistently caused a change in beta diversity. Regardless of day or intestinal location, challenged broilers had many amplicon sequence variants (ASVs) with decreased abundance of likely beneficial bacteria such as *Mollicutes RF39*, *Shuttleworthia*, *Flavonifractor*, and *Oscillibacter* compared to broilers that were unchallenged with *Salmonella Typhimurium* ($p < 0.05$). Additionally, there was a difference in the timing of when the microbiota alpha and beta diversity of each breed responded to *Salmonella Typhimurium* challenge. Thus, both broiler breed and *Salmonella Typhimurium* can impact the intestinal microbiota. ISSN: 1664042X

Williams, M.S., Ebel, E.D., Golden, N.J., Saini, G., Nyirabahizi, E., Clinch, N.

Assessing the effectiveness of performance standards for Salmonella contamination of chicken parts

(2022) *International Journal of Food Microbiology*, 378, art. no. 109801, .

ABSTRACT: The United States Department of Agriculture's Food Safety and Inspection Service implemented *Salmonella* performance standards for establishments producing chicken parts in 2016. The standards were chosen based on the assumption that a 30 % reduction in the occurrence of *Salmonella*-contaminated chicken parts samples (i.e., legs, breasts or wings) would result following implementation of the performance standard program. The derivation of the performance standards was based on data collected prior to the implementation of the standards and in the intervening years, so overall changes in

the *Salmonella* contamination of this product can be assessed. This study presents a historical review of changes in *Salmonella* contamination on chicken parts as these changes relate to the performance standard. The analysis demonstrates that the reduction in *Salmonella* contaminated chicken parts samples was more than 75 %, so the FSIS risk assessment significantly underestimated the actual reduction in *Salmonella* contamination. An analysis of chicken parts samples collected at retail demonstrates reductions of a similar magnitude. Changes in the characteristics of *Salmonella* contamination that are potentially relevant to the occurrence or severity of human illness, such as seasonal changes in contamination, the composition of serotypes and changes in antimicrobial resistance, are also assessed. Small but significant seasonal increases in contamination were observed, with the peaks occurring in late winter rather than the more traditional late summer peak. Rapid changes in both the five most common serotypes and antimicrobial resistance patterns were also observed. ISSN: 01681605

Kim, G.R., Kim, S.H., Kim, E.-Y., Park, E.H., Hwang, I.Y., Jeong, S.H., Kim, H.S., Kim, Y.A., Uh, Y., Shin, K.S., Kim, Y.R., Ryoo, N., Shin, J.H., Shin, J.H.

Performance of MALDI-TOF Mass Spectrometry (VITEK MS) in the Identification of Salmonella Species

(2022) *Microorganisms*, 10 (10), art. no. 1974, .

ABSTRACT: *Salmonella* is a major pathogen causing foodborne infections in humans. *Salmonella* isolates are identified using biochemical and serological tests, including automated systems such as the VITEK2 system. However, there are few reports on *Salmonella* identification using VITEK MS. Therefore, we aimed to evaluate the usefulness of MALDI-TOF VITEK MS for *Salmonella* identification. A total of 1389 *Salmonella* isolates were identified using VITEK MS ver3.0 or ver3.2. All *Salmonella* isolates were confirmed by serotyping using the Kauffmann-White scheme, and the results were compared with the VITEK MS results. A total of 1389 *Salmonella* isolates, including 66 serotypes, were correctly identified at the genus level by VITEK MS. However, these systems failed to correctly identify typhoidal *Salmonella*. Among the five *Salmonella enterica* ssp. *diarizonae* isolates, only one was correctly identified, whereas one and three isolates were partially identified and misidentified, respectively. On the other hand, the VITEK2 system successfully identified all typhoidal *Salmonella* (Typhi and Paratyphi A) and *Salmonella enterica* ssp. *diarizonae* isolates. VITEK MS was useful for identifying *Salmonella* species isolated from clinical specimens; however, additional biochemical tests, such as the VITEK2 System, should be considered to accurately identify *Salmonella* ser. Typhi, and *Salmonella* ser. Paratyphi A. ISSN: 20762607

Dec, M., Zając, M., Puchalski, A., Szczepaniak, K., Urban-Chmiel, R.

Pet Reptiles in Poland as a Potential Source of Transmission of Salmonella

(2022) *Pathogens*, 11 (10), art. no. 1125, .

ABSTRACT: Reptiles are considered a potential source of *Salmonella* transmission to humans. The aim of this research was to determine the incidence of *Salmonella* in pet reptiles in Poland and to examine *Salmonella* isolates with regard to their biochemical characteristics, serotype, antimicrobial susceptibility, and pathogenic and zoonotic potential. The research material consisted of 67 reptile faeces samples. The taxonomic affiliation of the *Salmonella* isolates was determined by MALDI-TOF mass spectrometry, biochemical analyses, and serotyping; whole genome sequencing (WGS) analysis was performed on three isolates whose serotype could not be determined by agglutination. The antimicrobial susceptibility of the *Salmonella* isolates was determined by the broth dilution method, and in the case of some antimicrobials by the disk diffusion method. The pathogenic and zoonotic potential of the identified serotypes was estimated based on available reports and case studies. The presence of *Salmonella* was confirmed in 71.6% of faecal samples, with the highest incidence (87.1%) recorded for snakes, followed by lizards (77.8%) and turtles (38.9%). All isolates (n = 51) belonged to the species *S. enterica*, predominantly to subspecies I (66.7%) and IIIb (25.5%). Among these, 25 serotypes were identified, including 10 that had previously been confirmed to cause reptile-associated salmonellosis (RAS). *Salmonella* isolates were susceptible to all antimicrobial substances used except streptomycin, to which 9.8% of the strains showed resistance. None of the strains contained corresponding resistance genes. The study demonstrates that pet reptiles kept in Poland are a significant reservoir of *Salmonella* and contribute to knowledge of the characteristics of reptilian *Salmonella* strains. Due to the risk of salmonellosis, contact with these animals requires special hygiene rules. ISSN: 20760817

Fagbamila, I.O., Hernandez-Segura, A., van den Beld, M., Mooijman, K., Orsini, M., Ajayi, O.T., Ngulukun, S., Jambalang, A.R., Sati, N., Emennaa, P., Ankeli, P.I., Muhammad, M., Barco, L.

Salmonella enterica Newserovar Abeokuta Genome Sequence, Strain OG19FER4 Isolated from Poultry Feed in Nigeria

(2022) *Microbiology Resource Announcements*, 11 (10), .

ABSTRACT: This report announces the genome of a newly confirmed *Salmonella* serovar (*Salmonella enterica* serovar Abeokuta) that was isolated from a poultry feed sample collected on a farm in Abeokuta, capital of Ogun State in Nigeria. *Salmonella* Abeokuta has not been identified outside Nigeria, nor does it appear to be a cause for concern for animal and human health. ISSN: 2576098X

Berghaus, R.D., Baxter, V.A., Jones, M.K., Hofacre, C.L.

Intra-cluster correlations for ceca Salmonella prevalence and enumeration from 40 experimental floor pen trials in broiler chickens using a seeder bird challenge model

(2022) *Poultry Science*, 101 (10), art. no. 102102, .

ABSTRACT: Floor pen trials are an efficient way to evaluate the effectiveness of potential *Salmonella* control interventions in broiler chickens. When treatments are allocated at the pen level, and outcomes are measured at the individual bird level, floor pen studies are considered to be cluster randomized trials. Estimating the sample size required to achieve a desired level of statistical power for a cluster randomized trial requires an estimate of the intra-cluster correlation (ICC) as an input. In this study, ICCs were estimated for the untreated challenged control group from 40 broiler chicken *Salmonella* pen trials performed using a seeder bird challenge model. The ICCs for ceca *Salmonella* prevalences ranged from 0.00 to 0.64, with a median of 0.17. The ICCs for ceca *Salmonella* log₁₀(MPN/g + 1) ranged from 0.00 to 0.52, with a median of 0.14. These findings indicate that the effect of pen-level clustering is substantial in *Salmonella* floor pen trials, and it must be considered during both the study design and analysis. In a multivariable regression analysis, ICCs for ceca *Salmonella* prevalences were associated with the challenge status of sampled birds, age of birds at the time of challenge, and *Salmonella* serovar. ICCs were lower for studies in which a combination of direct (seeder) and indirect (horizontal) challenged birds were sampled, and for studies in which birds were challenged on the day of hatch or at one day of age. ICCs were higher for studies in which *Salmonella* Heidelberg was used as the challenge strain. These findings may be useful for investigators that are planning pen trials to evaluate *Salmonella* control interventions in broiler chickens. Choosing study design elements associated with a lower ICC may improve efficiency by leading to a larger effective sample size for the same number of experimental units. ISSN: 00325791

Lund, S., Tahir, M., Vohra, L.I., Hamdana, A.H., Ahmad, S.

Outbreak of monophasic Salmonella Typhimurium Sequence Type 34 linked to chocolate products

(2022) *Annals of Medicine and Surgery*, 82, art. no. 104597, .

ABSTRACT: As of 3rd June 2022, 445 cases of monophasic *Salmonella* Typhimurium sequence type 34 infection had been reported globally. The outbreak was caused by two novel strains of monophasic *S. Typhimurium* with unusual multi-drug resistance. The majority of these cases involved children aged 10 or younger, and they had a hospitalization rate higher than most previous outbreaks of monophasic *S. Typhimurium*, but no fatalities were recorded. The infection was traced to certain Belgian chocolate products after extensive microbiological and epidemiological research. Public health officials took immediate action to recall all the contaminated products, and the risk of exposure was reduced. The common symptoms are bloody diarrhea, acute onset of fever, abdominal pain, and vomiting. This article aims to thoroughly review the recent outbreak of monophasic *Salmonella* Typhimurium ST-34, including its epidemiology and comparison with ongoing outbreaks. We also highlighted past chocolate-related salmonella outbreaks and current control and prevention guidelines and recommendations. ISSN: 20490801

Davies, N., Jørgensen, F., Willis, C., McLauchlin, J., Chattaway, M.A.

Whole genome sequencing reveals antimicrobial resistance determinants (AMR genes) of Salmonella enterica recovered from raw chicken and ready-to-eat leaves imported into England between 2014 and 2019

(2022) *Journal of Applied Microbiology*, 133 (4), pp. 2569-2582.

ABSTRACT: Aims: To compare the antimicrobial resistance (AMR) genes in a genetically diverse group of *Salmonella enterica* recovered from foods imported into England between 2014 and 2018. Methods and Results: Whole genome sequence was used to detect AMR genes or chromosomal mutations associated with AMR in *Salmonella* recovered from edible

leaves imported from Asia (n = 115) as compared to *Salmonella* (n = 231) isolated from raw chicken, 74% originated from South America. Among isolates from edible leaves, three (3%) showed resistance to at least one antimicrobial agent, two (2%) of which were multidrug resistant (MDR, resistance to three or more antimicrobial classes). Resistance to at least one antimicrobial agent was detected in 214 (93%) in the chicken isolates, with 164 (71%) showing MDR. Genetic diversity and AMR profiles were highly heterogeneous across the different serovars. Conclusions: Resistance was rare among the *Salmonella* isolates from edible leaves but common (including MDR) among those from raw chicken. Significance and Impact of the Study: Surveillance of AMR in imported foods is essential for monitoring the risk of transmission of resistance from the food chain to humans and provides added public health value to pre-existing controls of the food chain. ISSN: 13645072

Cohn, A.R., Orsi, R.H., Carroll, L.M., Liao, J., Wiedmann, M., Cheng, R.A.

Salmonella enterica serovar Cerro displays a phylogenetic structure and genomic features consistent with virulence attenuation and adaptation to cattle (2022) *Frontiers in Microbiology*, 13, art. no. 1005215, .

ABSTRACT: *Salmonella enterica* subsp. *enterica* (S.) serovar Cerro is rarely isolated from human clinical cases of salmonellosis but represents the most common serovar isolated from cattle without clinical signs of illness in the United States. In this study, using a large, diverse set of 316 isolates, we utilized genomic methods to further elucidate the evolutionary history of S. Cerro and to identify genomic features associated with its apparent virulence attenuation in humans. Phylogenetic analyses showed that within this polyphyletic serovar, 98.4% of isolates (311/316) represent a monophyletic clade within section Typhi and the remaining 1.6% of isolates (5/316) form a monophyletic clade within subspecies *enterica* Clade A1. Of the section Typhi S. Cerro isolates, 93.2% of isolates (290/311) clustered into a large clonal clade comprised of predominantly sequence type (ST) 367 cattle and environmental isolates, while the remaining 6.8% of isolates (21/311), primarily from human clinical sources, clustered outside of this clonal clade. A tip-dated phylogeny of S. Cerro ST367 identified two major clades (I and II), one of which overwhelmingly consisted of cattle isolates that share a most recent common ancestor that existed circa 1975. Gene presence/absence and rarefaction curve analyses suggested that the pangenome of section Typhi S. Cerro is open, potentially reflecting the gain/loss of prophage; human isolates contained the most open pangenome, while cattle isolates had the least open pangenome. Hypothetically disrupted coding sequences (HDCs) displayed clade-specific losses of intact *speC* and *sopA* virulence genes within the large clonal S. Cerro clade, while loss of intact *vgrG*, *araH*, and *vapC* occurred in all section Typhi S. Cerro isolates. Further phenotypic analysis suggested that the presence of a premature stop codon in *speC* does not abolish ornithine decarboxylase activity in S. Cerro, likely due to the activity of the second ornithine decarboxylase encoded by *speF*, which remained intact in all isolates. Overall, our study identifies specific genomic features associated with S. Cerro's infrequent isolation from humans and its apparent adaptation to cattle, which has broader implications for informing our understanding of the evolutionary events facilitating host adaptation in *Salmonella*. ISSN: 1664302X

Papić, B., Kušar, D., Mićunović, J., Pirš, M., Ocepek, M., Avberšek, J.

Clonal Spread of pESI-Positive Multidrug-Resistant ST32 Salmonella enterica Serovar Infantis Isolates among Broilers and Humans in Slovenia (2022) *Microbiology Spectrum*, 10 (6), .

ABSTRACT: *Salmonella enterica* subsp. *enterica* serovar *Infantis* is the most prevalent serovar found in broilers and broiler meat and is among the top five serovars responsible for human infections in Europe. In 2008, a multidrug-resistant S. *Infantis* isolate emerged in Israel with a mosaic megaplasmid named pESI, associated with increased virulence, biofilm formation, and multidrug resistance. Since then, S. *Infantis* clones with pESI-like plasmids have been reported worldwide, replacing pESI-free clones. Here, we typed 161 S. *Infantis* isolates of poultry (n = 133) and human clinical (n = 28) origin using whole-genome sequencing. The isolates were collected between 2007 and 2021. In addition, we performed PacBio/Illumina sequencing for two representative pESI-like plasmids and compared them with publicly available sequences. All isolates belonged to sequence type 32 (ST32), except for one isolate that represented a novel single-locus variant of ST32. Core genome MLST (cgMLST) analysis revealed 14 clusters of genetically closely related isolates, of which four suggested broiler-to-human transmission of S. *Infantis*. pESI-like plasmids were present in 148/161 (91.9%) isolates; all were highly similar to the publicly available pESI-like sequences but lacked extended-spectrum beta-lactamase (ESBL) genes. PacBio/Illumina hybrid assembly allowed the reconstruction of two novel complete pESI variants. The present study revealed that the multidrug-resistant, pESI-positive S.

Infantis clone became the predominant *S. Infantis* clone in Slovenian broilers and humans during the last decade. Continued surveillance of resistant *S. Infantis* clones along the food chain is needed to guide public health efforts. ISSN: 21650497

Pista, A., Silveira, L., Ribeiro, S., Fontes, M., Castro, R., Coelho, A., Furtado, R., Lopes, T., Maia, C., Mixão, V., Borges, V., Sá, A., Soeiro, V., Correia, C.B., Gomes, J.P., Saraiva, M., Oleastro, M., Batista, R.

Pathogenic Escherichia coli, Salmonella spp. and Campylobacter spp. in Two Natural Conservation Centers of Wildlife in Portugal: Genotypic and Phenotypic Characterization (2022) Microorganisms, 10 (11), art. no. 2132, .

ABSTRACT: Human-wildlife coexistence may increase the potential risk of direct transmission of emergent or re-emergent zoonotic pathogens to humans. Intending to assess the occurrence of three important foodborne pathogens in wild animals of two wildlife conservation centers in Portugal, we investigated 132 fecal samples for the presence of *Escherichia coli* (Shiga toxin-producing *E. coli* (STEC) and non-STEC), *Salmonella* spp. and *Campylobacter* spp. A genotypic search for genes having virulence and antimicrobial resistance (AMR) was performed by means of PCR and Whole-Genome Sequencing (WGS) and phenotypic (serotyping and AMR profiles) characterization. Overall, 62 samples tested positive for at least one of these species: 27.3% for STEC, 11.4% for non-STEC, 3.0% for *Salmonella* spp. and 6.8% for *Campylobacter* spp. AMR was detected in four *E. coli* isolates and the only *Campylobacter coli* isolated in this study. WGS analysis revealed that 57.7% (30/52) of pathogenic *E. coli* integrated genetic clusters of highly closely related isolates (often involving different animal species), supporting the circulation and transmission of different pathogenic *E. coli* strains in the studied areas. These results support the idea that the health of humans, animals and ecosystems are interconnected, reinforcing the importance of a One Health approach to better monitor and control public health threats. ISSN: 20762607

Silva, J.L.D., Vieira, B.S., Carvalho, F.T., Carvalho, R.C.T., Figueiredo, E.E.D.S.

Salmonella Behavior in Meat during Cool Storage: A Systematic Review and Meta-Analysis (2022) Animals, 12 (21), art. no. 2902, .
9267d8e42db64

ABSTRACT: The aim of the present study was to investigate *Salmonella* behavior in meat stored in cool conditions (between 0 °C and 7.5 °C), by employing a systematic review and meta-analysis. The data were obtained from research articles published in SciELO, PubMed, the Web of Science, and Scopus databases. The results of the retrieved studies were obtained from meat (beef, chicken, pork, poultry, and turkey), fish, shellfish, and broth media samples. The data were extracted as sample size (n), initial concentration (Xi), final concentration (Xf), standard deviation (SD), standard error (SE), and microbial behavior effects (reduction or growth). A meta-analysis was carried out using the metaphor package from R software. A total of 654 articles were initially retrieved. After applying the exclusion criteria, 83 articles were selected for the systematic review, and 61 of these were used for the meta-analysis. Most studies were conducted at 0 °C to 4.4 °C storage temperatures under normal atmosphere package conditions. *Salmonella* Typhimurium, *S. Enteritidis*, and a cocktail (strain mixture) were inoculated at 5.0 and 6.0 log CFU mL⁻¹. Articles both with and without the addition of antimicrobial compounds were found. *Salmonella* concentration decreases were observed in most studies, estimated for all study combinations as -0.8429 ± 0.0931 log CFU g⁻¹ (95% CI; $-1.0254, -0.6604$) ($p < 0.001$), varying for each subgroup analysis. According to this survey, *Salmonella* concentration decreases are frequent during cool storage, although concentration increases and no bacterial inactivation were observed in some studies. ISSN: 20762615

Forgaciu, A., Tabaran, A., Colobatiu, L., Mihaiu, R., Dan, S.D., Mihaiu, M.

Concerning Increase in Antimicrobial Resistance Patterns of Pathogenic Strains of Salmonella Isolated in Poultry Meat Products (2022) Antibiotics, 11 (11), art. no. 1469, .

ABSTRACT: *Salmonella* is considered to be one of the major foodborne pathogens associated with the consumption of contaminated poultry meat products. To the best of our knowledge this is the first extended research performed on a number of *Salmonella* strains isolated during 2011–2021 from poultry meat products in Romania. The aim of this study was to characterize the prevalence of pathogenic *Salmonella* serovars, antimicrobial susceptibility, and antimicrobial resistance genes in 112 *Salmonella* isolates recovered from raw poultry meat products. The results showed that *Salmonella enterica* serovars Enteritidis and Typhimurium were the common serotypes (56%; 25%). Overall, the majority of the isolates were resistant to at least three tested antimicrobials. High

resistance was observed for tetracycline (84%), nalidixic acid (78%), and ampicillin (78%) in pathogenic *Salmonella* isolated during the period 2016–2021. All the pathogenic *Salmonella* isolated during 2016–2021 tested positive to at least one resistance gene encoding for tetracycline resistance, with the *tetA* gene being the most prevalent (62%). In addition, 64% (24/37) of the *Salmonella* isolates carried at least one of the genes (*bla*CMY-2, *bla*SHV1, *bla*TEM1) that code for β -Lactams resistance. The findings in this study showed a high prevalence of multi-drug resistant (MDR) *Salmonella* serovars in poultry meat products and a concerning increase of resistance patterns. The continuous occurrence of more resistant strains implies that effective measures should be strictly applied in this particular food chain in order to prevent their spread and guarantee microbial safety. ISSN: 20796382

Micallef, S.A., Han, S., Martinez, L.

Tomato Cultivar Nyagous Fruit Surface Metabolite Changes during Ripening Affect Salmonella Newport

(2022) *Journal of food protection*, 85 (11), pp. 1604-1613.

ABSTRACT: ABSTRACT: Tomatoes are a valuable crop consumed year-round. Ripe fruit is picked for local sale, whereas tomatoes intended for transit may be harvested at late mature green or breaker stages when fruit firmness preserves quality. In this study, we evaluated *Solanum lycopersicum* cv. BHN602 association with three *Salmonella* serotypes and *S. lycopersicum* cv. Nyagous with *Salmonella* Newport using fruit at two ripeness stages. Counts of *Salmonella* Javiana and Typhimurium were higher from red ripe fruit surfaces of BHN602, and counts of *Salmonella* Newport were higher from ripe Nyagous fruit than from mature green fruit ($P < 0.05$). Aqueous fruit washes containing fruit surface compounds collected from ripe Nyagous fruit supported more *Salmonella* Newport growth than green fruit washes ($P < 0.05$). Growth curve analysis showed that between 2 and 6 h, *Salmonella* Newport grew at a rate of 0.25 log CFU/h in red fruit wash compared with 0.17 log CFU/h in green fruit wash ($P < 0.05$). The parallel trend in *Salmonella* interaction between fruit and wash suggested that surface metabolite differences between unripe and ripe fruit affect *Salmonella* dynamics. Untargeted phytochemical profiling of tomato fruit surface washes with gas chromatography time-of-flight mass spectrometry showed that ripe fruit had threefold-lower amino acid and fourfold-higher sugar (fructose, glucose, and xylose) levels than green fruit. Green fruit had higher levels of lauric, palmitic, margaric, and arachidic acids, whereas red fruit had more capric acid. The phenolics ferulic, chlorogenic, and vanillic acid, as well as tyrosol, also decreased with ripening. Although limitations of this study preclude conclusions on how specific compounds affect *Salmonella*, our study highlights the complexity of the plant niche for foodborne pathogens and the importance of understanding the metabolite landscape *Salmonella* encounters on fresh produce. Fruit surface phytochemical profiling generated testable hypotheses for future studies exploring the differential *Salmonella* interactions with tomato varieties and fruit at various ripeness stages. ISSN: 19449097

Oguadinma, I.C., Mishra, A., Kumar, G.D.

Sunlight Parameters Influence the Survival and Decline of Salmonella and Escherichia coli in Water

(2022) *Journal of food protection*, 85 (11), pp. 1614-1624.

ABSTRACT: ABSTRACT: The effect of variations in temperature, UV radiation, and sunlight intensity on *Escherichia coli*, *E. coli* O157:H7, *Salmonella* Newport, and antibiotic resistant (ABR) variants of *E. coli* O157:H7 and *Salmonella* Newport exposed to sunlight was evaluated. Bacterial strains suspended in sterile deionized water at a concentration of 8 log CFU/mL were exposed to sunlight on three different days for 180 min; control treatments were stored in the dark. The mean temperature of 30.08 and 26.57°C on day 1 and day 3, respectively, was significantly different ($P < 0.05$). The UV intensity was significantly different on all 3 days, and sunlight intensity significantly differed on day 3 ($P < 0.05$). Bacterial population decline positively correlated with temperature, sunlight, and UV intensity. Differences in bacterial population declines differed among species, ABR profile, and day of exposure ($P < 0.05$). On day 1 and day 2, the populations of *E. coli* dropped below the limit of detection (1 log CFU/mL), whereas the percentage of live cells was 67 and 6.6%, respectively. The artificial neural network model developed to predict bacterial survival under different environmental conditions suggested that *Salmonella* cells were more resistant than *E. coli* cells. The ABR strains had significantly higher numbers of viable cells after sunlight exposure ($P < 0.05$). Sunlight-exposed cells resuscitated in tryptic soy broth varied in maximum population density and maximum specific growth rate based on bacterial species and presence of ABR. Morphological changes such as viable but nonculturable state transition and filament formation were detected in subpopulations of

sunlight-exposed bacteria. Daily fluctuations in UV and sunlight intensity can result in significant variations in bacterial decline and recovery. ISSN: 19449097

Henderson, K., Mason, C., Brülisauer, F., Williams, P.

Determining the prevalence of antibodies to Salmonella Dublin in dairy herds in Great Britain by quarterly bulk tank testing

(2022) *Preventive Veterinary Medicine*, 208, art. no. 105776, .

ABSTRACT: *Salmonella enterica* subspecies *enterica* serovar Dublin has been the most common *Salmonella* serovar isolated from cattle in Great Britain for the previous 22 years. It can cause a wide variety of clinical presentations and result in significant welfare and productivity concerns in infected herds. Bulk tank antibody testing undertaken every three or four months forms the basis of eradication and monitoring programmes in Denmark and the Netherlands and has been shown to be a sensitive, specific and cost-effective way of establishing seroprevalence and monitoring infection at a herd level. A prevalence estimate based on quarterly bulk tank testing has not been previously carried out in Great Britain. This study recruited 410 herds across Great Britain, who submitted milk samples on a quarterly basis for screening by an ELISA for *Salmonella* Dublin antibody. Classifying herds according to the Danish eradication scheme classification gave an apparent prevalence of 38% (95% confidence intervals 34–43%) and an estimated true prevalence of 40% (95% confidence intervals 35–45%), taking into account the test sensitivity and specificity. Of the 401 herds which completed the quarterly bulk tank testing, 45% had one or more positive bulk tank results. ISSN: 01675877

Lorenzo-Rebenaque, L., Malik, D.J., Catalá-Gregori, P., Torres-Boncompte, J., Marin, C., Sevilla-Navarro, S.

Microencapsulated bacteriophages incorporated in feed for Salmonella control in broilers

(2022) *Veterinary Microbiology*, 274, art. no. 109579, .

ABSTRACT: Bacteriophage inclusion as a feed additive could offer the prospect of its en masse application and reduce the intestinal carriage of *Salmonella* by broiler chickens. The objective of this study was to evaluate the application of microencapsulated phages as a strategy to control *Salmonella* and assess the impact of their use during the broiler rearing period. One-hundred one-day-old chicks were randomly divided in two identical poultry houses according to the two experimental groups (control vs Φ -treated group) simulating field production conditions. Half of the animals in each experimental group, were challenged with 10⁵ CFU/bird of *Salmonella* Enteritidis per os. In the Φ -treated group, microencapsulated *Salmonella*-phage encapsulated in Eudragit®L100 (a pH-responsive formulation) were incorporated in the starter diet feed. To assess *Salmonella* colonization, excretion and diffusion, cecum samples, cloacal swabs and boot swabs were taken weekly. *Salmonella* detection was based on ISO 6579-1:2017 (Annex D). *Salmonella* colonization was significantly reduced in most of the rearing period, meanwhile the excretion was significantly reduced on the 2nd, 4th and 5th week of rearing. Moreover, *Salmonella* contamination of the farm environment was eliminated at the end of the cycle. This study provides important insights into the potential use of phages as a preventative and biocontrol strategy against *Salmonella* infection from farm-to-table. ISSN: 03781135

Kotian, A., Aditya, V., Sheikh, J., Saikrishnan, S., Rai, P., Chakraborty, A., Karunasagar, I., Deekshit, V.K.

Effect of NaCl, high iron, iron chelator and antibiotics on growth, virulence gene expression and drug susceptibility in non-typhoidal Salmonella: an in vitro fitness study

(2022) *Archives of Microbiology*, 204 (11), art. no. 667, .

ABSTRACT: *Salmonella* is one among the most versatile and resilient enteric pathogens that is known to have developed various survival strategies within the host system. The ability of the bacteria to circumvent the physiological parameters as well as dodge the antimicrobial stress environment within the host is one of the most crucial steps in establishing an infection. With an alarming rise in multi-drug resistant serovars of non-typhoidal *Salmonella* and lack of vaccine for combatting the infections, behaviour of the bacteria in the presence of host physiological conditions (NaCl, high and low iron) and antibiotics will help in understanding the survival strategies as well as mechanisms of resistance. Two multi-drug resistant and two sensitive serovars of *Salmonella* Weltevreden and *Salmonella* Newport isolated from poultry and seafood were used for growth kinetics and virulence gene expression study. The results obtained revealed that despite similar resistance pattern, effect of individual class of antibiotics on the growth of serovars varied. On the contrary, no significant difference was observed in growth pattern on exposure to these in vitro experimental conditions. Nevertheless, coupling these conditions with antibiotics drastically reduced the minimum inhibitory concentration (MIC) of antibiotics in resistant strains. A first of its kind study that draws attention on the significant effect of

antibiotics and physiological conditions on MIC between resistant and sensitive nontyphoidal Salmonella serovars and expression of virulence genes from Salmonella pathogenicity island (SPI) 1 and 2 (invA, hilC, fliC2, sseA and ssrB). ISSN: 03028933

Gast, R.K., Jones, D.R., Guraya, R., Garcia, J.S., Karcher, D.M.

Research Note: Internal organ colonization by Salmonella Enteritidis in experimentally infected layer pullets reared at different stocking densities in indoor cage-free housing (2022) Poultry Science, 101 (11), art. no. 102104, .

ABSTRACT: Contamination of eggs by Salmonella has often been identified as a source of food-borne human illness. S. Enteritidis is deposited inside developing eggs when invasive infections of laying hens reach the reproductive organs. The susceptibility of hens in cage-based housing systems to S. Enteritidis has been associated with their stocking density, but the applicability of this information to extensive (cage-free) systems is uncertain. The present study assessed internal organ colonization by S. Enteritidis in egg-type pullets reared at 2 different stocking densities in cage-free housing. Pullets were reared at either 374 cm² or 929 cm² of floor space per bird. At 16 wk of age, 4 groups of 72 pullets were moved into isolation rooms simulating commercial cage-free barns; 1/3 of the pullets in 2 rooms were orally inoculated with S. Enteritidis immediately after transfer and pullets in 2 rooms were similarly infected at 19 wk. At 6 and 12 d postinoculation, the pullets were euthanized and samples of liver, spleen, and intestinal tract were removed for bacteriologic culturing. No significant differences ($P > 0.05$) in S. Enteritidis isolation frequencies from any tissue were observed between high and low density rearing groups following infection at either age. However, S. Enteritidis was found significantly ($P < 0.05$) more frequently among pullets infected orally at 19 wk than at 16 wk in spleens and intestines. Likewise, the frequency of S. Enteritidis isolation from all birds (inoculated plus contact-exposed) at 19 wk was significantly higher than at 16 wk in livers and spleens. This increased susceptibility to invasive S. Enteritidis infection at reproductive maturity emphasizes the importance of risk reduction at a critical stage in the egg production cycle. ISSN: 00325791

Morita, T., Ohyagi, N., Matsuura, J., Kawaguchi, T., Ishizaki, N.

Salmonella contamination and hazard analysis in a storage facility for feed materials in Japan

(2022) Journal of Applied Microbiology, 133 (5), pp. 2966-2978.

ABSTRACT: Aims: To reduce Salmonella contamination of animal feeds, Salmonella contamination in a feed materials storage facility was investigated, and hazard analysis was performed to acquire the basic information necessary to establish a control method for Salmonella. The result is that managers of feed materials storage facilities will be able to introduce the Hazard Analysis and Critical Control Point (HACCP) systems to quickly and efficiently. Methods and Results: A total of 472 samples, including deposits, adhesions, environment samples and materials, were investigated. The detection rate in the storage facility was 5.1% (24/472) over the three-year investigation period. The serovars of detected Salmonella were Agona, Anatum, Bron (or Agbeni), Chester, Infantis, Mbandaka, Minnesota, Muenster, Sinstorf, Typhimurium, O18:- and O1,3,19:-. As a result of hazard analysis after confirming the multiplication factors and detection rate, important hazards were judged to be the outdoor equipment and dust collector. Conclusions: The risk of Salmonella contamination in storage facilities was clarified. Important Salmonella hazards in storage facilities are Salmonella-contaminated feed materials, multiplication factors such as rainwater and dew condensation, and Salmonella-accumulated dust in dust collectors for fine-powder recovery. These results suggest that proper control of these hazards could reduce the risk of Salmonella in storage facilities. Significance and Impact of the Study: This is the first report describing actual Salmonella contamination conditions and risk assessment using the hazard analysis at a feed materials storage facility in Japan. This study will consider implications for the feed industry because the hazard analysis clarified the sites of Salmonella contamination, the tendency of contamination and the points for implementing control measures in the feed materials storage facility. ISSN: 13645072

Jung, J., Schaffner, D.W.

Role of Salmonella Newport cell surface structures on bacterial attachment and transfer during cucumber peeling

(2022) Letters in Applied Microbiology, 75 (5), pp. 1246-1253.

ABSTRACT: Fresh cucumbers have been recognized as a vehicle in foodborne disease outbreaks since several multistate outbreaks of salmonellosis linked to fresh cucumbers occurred in the United States. Little is known about how microbial cell surface characteristics that are known to affect adhesion can influence bacterial cross-contamination and transfer. This study investigated the role of S. Newport cell surface

components on bacterial attachment and transfer in cucumbers. Wild type *Salmonella* Newport and its transposon mutants were used to inoculate cucumbers. Attachment strength of *S. Newport* wild type to cucumber was not significantly different than that of mutants. Log₁₀ percent transfer of mutant strains to edible flesh was not different from the wild type. Significantly less wild type *Salmonella* remained on the peel and transferred to the peeler than one mutant did, but not the other. Our results suggest that while curli and cellulose enhance *Salmonella* attachment to surface of cucumbers, there appear to be other mechanisms and factors that govern *Salmonella* transfer in cucumbers.

ISSN: 02668254

Namli, S., Samut, H., Soyer, Y.

Microbial growth and attachment of Salmonella and enterohemorrhagic and enteroaggregative Escherichia coli strains on cress microgreens grown in peat soil system (2022) British Food Journal, 124 (11), pp. 3765-3782.

ABSTRACT: Purpose: This study aimed to investigate how enteric pathogens and their biofilm populations on fresh produce survive according to time that contamination has occurred on leaves and contamination route: seed irrigation water.

Design/methodology/approach: Cress was contaminated in two different ways: contamination of seeds and irrigation water with 8-log MPN/mL bacterial load, *Salmonella* Newport, *Escherichia coli* O157:H7, O104:H4 or O78:H2. While contaminated seeds were cultivated for seed contamination, contaminated irrigation was applied at the end of each week to separate groups of samples obtained from cultivated surface-sterile seeds to understand how long these pathogens could survive until harvest. **Findings:** The results indicated these pathogens survived until harvest, and formed biofilms on cress leaves grown using both contaminated seeds and irrigation water. No significant difference was observed among populations of *Salmonella* and *E. coli* groups in terms of survival (~4.5–6.0 log MPN/g) and biofilm formation (~4.4–5.7 log MPN/g) for contamination by seed. Also, SEM images revealed biofilm-like structures, the proofs of the attachment of these pathogens on leaf surfaces. **Originality/value:** From our knowledge this is the first study focusing on the survival and biofilm formation of one *Salmonella* serotype (Newport) and three *E. coli* serotypes (O157:H7, O104:H4, and O78:H2), representing enterohemorrhagic and enteroaggregative *E. coli* pathogenic subgroups, under the same irrigation and growth schemes. Furthermore, this study mimics the contamination of seeds and irrigation water with sewage or wastewater and may shed light on contamination of fresh produce grown using poor wastewater treatment. ISSN: 0007070X

van Elsland, D.M., Duijster, J.W., Zhang, J., Stévenin, V., Zhang, Y., Zha, L., Xia, Y., Franz, E., Sun, J., Mughini-Gras, L., Neefjes, J.

Repetitive non-typhoidal Salmonella exposure is an environmental risk factor for colon cancer and tumor growth (2022) Cell Reports Medicine, 3 (12), art. no. 100852, .

ABSTRACT: During infection, *Salmonella* hijacks essential host signaling pathways. These molecular manipulations disrupt cellular integrity and may induce oncogenic transformation. Systemic *S. Typhi* infections are linked to gallbladder cancer, whereas severe non-typhoidal *Salmonella* (NTS) infections are associated with colon cancer (CC). These diagnosed infections, however, represent only a small fraction of all NTS infections as many infections are mild and go unnoticed. To assess the overall impact of NTS infections, we performed a retrospective serological study on NTS exposure in patients with CC. The magnitude of exposure to NTS, as measured by serum antibody titer, is significantly positively associated with CC. Repetitively infecting mice with low NTS exposure showed similar accelerated tumor growth to that observed after high NTS exposure. At the cellular level, NTS preferably infects (pre-)transformed cells, and each infection round exponentially increases the rate of transformed cells. Thus, repetitive exposure to NTS associates with CC risk and accelerates tumor growth. ISSN: 26663791

Galán-Relaño, Á., Sánchez-Carvajal, J.M., Gómez-Gascón, L., Vera, E., Huerta, B., Cardoso-Toset, F., Gómez-Laguna, J., Astorga, R.J.

Phenotypic and genotypic antibiotic resistance patterns in Salmonella Typhimurium and its monophasic variant from pigs in southern Spain (2022) Research in Veterinary Science, 152, pp. 596-603.

ABSTRACT: The high incidence of human salmonellosis and multi-drug resistant (MDR) strains of *Salmonella* Typhimurium (ST) is of concern to global public and animal health. Our research, by means of the broth microdilution method, evaluated the Minimum Inhibitory Concentration (MIC) distribution of 12 antimicrobials against a collection of 73 ST and mST and *S. typhimurium* monophasic variant 4,[5],12:i:- (mST) isolates from slaughtered pigs reared in extensive systems in southern Spain, and also 12 resistance-

associated genes or antimicrobial resistance (AMR) determinants using qPCR. Our data revealed that 98.6% of strains were MDR, with resistance to cephalothin/tetracycline/sulfamethoxazole-trimethoprim/ampicillin/chloramphenicol being the most common pattern (55.6%). Regarding AMR determinants, the most significantly ($p < 0.05$) genes detected by qPCR were *sul1* and *aadA2* (89% of strains positive), *aadA1* and *dfrA12* (87.7%), and *blaTEM* and *tet(B)* (86.3% and 84.9%, respectively). Up to date information on ST antimicrobial resistance patterns is essential for epidemiological surveillance programs to support animal and public health. The high number of MDR isolates and variability regarding resistance determinants revealed in this study highlights the role of animals reared in extensive systems as a source of resistant *Salmonella* strains. ISSN: 00345288

Russini, V., Corradini, C., Rasile, E., Terracciano, G., Senese, M., Bellagamba, F., Amoroso, R., Bottoni, F., De Santis, P., Bilei, S., De Marchis, M.L., Bossù, T.

A Familiar Outbreak of Monophasic Salmonella serovar Typhimurium (ST34) Involving Three Dogs and Their Owner's Children (2022) Pathogens, 11 (12), art. no. 1500, .

ABSTRACT: *Salmonella* is a Gram-negative enteric bacterium responsible for the foodborne and waterborne disease salmonellosis, which is the second most reported bacterial zoonosis in humans. Many animals are potential sources of salmonellosis, including dogs, cats, and other pets. We report the case of an outbreak of salmonellosis in a family in central Italy, affecting two children and involving their three dogs as carriers. One of the children needed medical care and hospitalisation. Isolation and analysis of stool samples from the sibling and the animals present in the house were carried out. Serotyping allowed the identification of *S. enterica* subsp. *enterica* serovar Typhimurium in its monophasic variant for all the isolates. The results of whole-genome sequencing confirmed that the strains were tightly related. The minimum inhibitory concentration (MIC) test documented the resistance to ampicillin, sulfamethoxazole, and tetracycline. The origin of the zoonotic outbreak could not be assessed; however, the case study showed a clear passage of the pathogen between the human and non-human members of the family. The possibility of a transmission from a dog to a human suggests the need for further studies on the potential ways of transmission of salmonellosis through standard and alternative feed. ISSN: 20760817

Listorti, V., Garcia-Vozmediano, A., Pitti, M., Maurella, C., Adriano, D., Ercolini, C., Dellepiane, M., Guardone, L., Razzuoli, E.

Antimicrobial Resistance of Salmonella Strains Isolated from Human, Wild Boar, and Environmental Samples in 2018–2020 in the Northwest of Italy (2022) Pathogens, 11 (12), art. no. 1446, .

ABSTRACT: Antimicrobial resistance is one of the most challenging public health problems worldwide, and integrated surveillance is a key aspect in a One Health control strategy. Additionally, *Salmonella* is the second most common zoonosis in Europe. We aimed to investigate the circulation of *Salmonella* strains and their related antimicrobial resistance in human, environmental, and wild boar samples from the northwest of Italy, from 2018 to 2020, to obtain a more comprehensive epidemiological picture. *Salmonella* Typhimurium 1,4,[5],12:i:-, *S. Venezia* and *S. Newport* were the most common serotypes occurring in humans, the environment, and wild boar, respectively. Antimicrobial resistance was rather common in *Salmonella* isolates, with those from human displaying the highest degree of resistance against sulfadiazine–sulfamerazine–sulfamethazine (>90% of resistance). Moreover, resistance against azithromycin were exclusively observed in environmental samples, while only 7.7% (95% CI = 1.6–20.8) of wild boar isolates experienced resistance against trimethoprim–sulfamethoxazole. Multidrug resistance concurrently involved up to seven antimicrobial classes in human isolates, including third-generation cephalosporins and fluoroquinolones. *Salmonella* Typhimurium in humans and serotypes Goldcoast and Rissen from environmental sources showed the highest levels of resistance. This study shows diverse antimicrobial resistance patterns in *Salmonella* strains isolated from different sources and gives a broad picture of antimicrobial resistance spread in wild animals, humans, and the environment. ISSN: 20760817

Gambi, L., Ravaioli, V., Rossini, R., Tranquillo, V., Boscarino, A., Mattei, S., D'Incau, M., Tosi, G., Fiorentini, L., Donato, A.D.

Prevalence of Different Salmonella enterica Subspecies and Serotypes in Wild Carnivores in Emilia-Romagna Region, Italy (2022) Animals, 12 (23), art. no. 3368, .

ABSTRACT: *Salmonella* is a pathogen of considerable health concern, given its zoonotic potential, and, in Italy, is the most frequently reported causative agent for foodborne

outbreaks. Wild animals and in particular wild carnivores may be carriers of different *Salmonella enterica* subspecies and serotypes. Given their potential role as reservoirs, surveillance activities are necessary. This study aims to investigate the presence of different *Salmonella* subspecies and serotypes in wild carnivores in the Emilia-Romagna Region. A total of 718 fox (*Vulpes vulpes*), 182 badger (*Meles meles*) and 27 wolf (*Canis lupus*) carcasses, submitted between 2016–2022, were included for the present work. Gender and age data were collected along with geographical coordinates of carcass' discovery site. Contents of the large intestine were sampled and cultured according to ISO 6579-1 and both serogroup and serotype identification were performed according to ISO/TR 6579-3:2014. *Salmonella* was retrieved from 42 foxes (6%), 21 badgers (12%) and 3 wolves (12%), respectively. Isolated *Salmonella enterica* strains belonged to 4 different subspecies and 25 different serotypes. *S. veneziana* and *S. typhimurium* were the most frequent serotypes found (11/67 and 10/67, respectively). In conclusion, zoonotic serotypes were found in all these species of wildlife, thus confirming their potential role in the ecology of *Salmonella* spp. ISSN: 20762615

Surya, T., Jeyasekaran, G., Shakila, R.J., Sivaraman, B., Shalini, R., Sundhar, S., Arisekar, U.

Prevalence of biofilm forming Salmonella in different seafood contact surfaces of fishing boats, fish landing centres, fish markets and seafood processing plants (2022) Marine Pollution Bulletin, 185, art. no. 114285, .

ABSTRACT: The prevalence of biofilm forming *Salmonella* on different seafood contact surfaces was investigated. Out of 384 swab samples, 16.14 % and 1 % were confirmed biochemically and molecularly as *Salmonella* respectively. One out of four isolates was from the boat deck, and three were from the seafood processing plant. *Salmonella* was more prevalent in January, June, and September months. Different assays investigated the biofilm forming ability of isolates. Two out of four isolates have shown strong biofilms, and the others were moderate biofilm formers by microtitre plate assay. In the CRA assay, three isolates showed 'rdar' morphotype, and one showed 'bdar' morphotype. All isolates were positive for *gcpA* gene (~1700 bp), a critical gene found in *Salmonella* biofilms. The microbial load of *Salmonella* biofilms on different contact surfaces were determined, stainless steel and HDPE were found prone to biofilms. With this, a suitable mechanism shall be formulated to control the biofilms of *Salmonella*. ISSN: 0025326X

Wang, Y., Yang, H.

Metabolomics elucidating the effect of water activity on the thermal resistance of Salmonella in wheat flour (2022) Food Research International, 162, art. no. 112203, .

ABSTRACT: With mounting evidence indicating an enhanced thermal resistance of *Salmonella* at lower *aw*, the effectiveness of thermal treatment in wheat flour decontamination is challenged. Therefore, this study was carried out to evaluate the thermal resistance of three *Salmonella* strains, including Enteritidis (ATCC 13076), Typhimurium (ATCC 14028) and Newport (ATCC 6962), at 65 °C in wheat flour at three *aw* levels (0.33, 0.53 and 0.69), and to explore the mechanisms of the difference in thermal resistance via nuclear magnetic resonance (NMR)-based metabolomics. The results showed that except for the insignificant difference between the reductions of *S. Newport* at 0.53 and 0.69 flour *aw* ($P > 0.05$), a remarkable decreasing trend in *Salmonella* cell reduction with decreasing flour *aw* was observed after the 20-min thermal treatment. By comparing the metabolic profiles of each strain recovered from the lower-*aw* (0.33 or 0.53) flour with that from the *aw*-0.69 flour, the metabolic differences implying more efficient misfolded protein degradation, higher availability of amino acids as osmoprotectants, larger throughput of energy production by ATP synthase as well as wiser glucose allocation in the metabolic network were suspected to contribute to the strains' enhanced thermal resistance. Overall, the study adds to the evidence for the effect of lower *aw* in increasing the thermal resistance of *Salmonella* in wheat flour. Meanwhile, the identified discriminative metabolic pathways may be artificially modified in the future to help ease *Salmonella* inhibition during cooking or any types of thermal treatments. ISSN: 09639969

Teunis, P.F.M.

Dose response for Salmonella Typhimurium and Enteritidis and other nontyphoid enteric salmonellae (2022) Epidemics, 41, art. no. 100653, .

ABSTRACT: This dose response assessment combines data from 6 human challenge studies and 44 outbreaks to determine infectivity and pathogenicity of several serotypes of nontyphoid *Salmonella*. Outcomes focus on the major serotypes *Salmonella* Enteritidis and

Typhimurium, showing that Typhimurium is less infectious and has a lower probability of causing acute illness in infected subjects. The dose response relation of *Salmonella* Enteritidis is less steep than that of Typhimurium, indicating greater heterogeneity in infectivity and pathogenicity. This study revisits an older study with less flexible methods that could not combine the widely different outcomes of challenge studies and outbreaks, and had limited capability for dealing with missing information. Reported outcomes are in a format that allows use in calculations of uncertainty for quantitative risk assessment.
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Harris, C.E., Josselson, L.N.B., Bourassa, D.V., Buhr, R.J.

Examination of the impact of eggshell cuticle and membranes on Salmonella Enteritidis or Typhimurium recovery from inoculated and stored eggs

(2022) *Journal of Applied Poultry Research*, 31 (4), art. no. 100297, .

ABSTRACT: The effect of altering eggshell cuticle, as well as the impact of eggshell membranes and egg contents, on inoculated *Salmonella* recovery was examined. Four experiments were performed with Experiments 1 and 2 utilizing *Salmonella* Enteritidis and Experiments 3 and 4 utilizing both *Salmonella* Enteritidis and Typhimurium. Eggs were inoculated with 10⁵ to 10⁶ CFU of *Salmonella* after the following treatments were applied: mechanically stripped cuticle, chemically stripped cuticle, eggshells empty of egg contents, inoculation into the air cell, and unaltered eggshell positive control. Post-inoculation eggs were stored at room temperature (22–25°C) and eggshells were sampled at 1, 6, and 24 h for recovery of *Salmonella*. Mechanically and chemically stripping eggshell cuticle from this study did not impact *Salmonella* recovery compared to unaltered positive control eggs. Eggshells with contents removed had significantly higher recovery of *Salmonella* over 24 h storage, as well as eggs that were inoculated in the air cell ($P \leq 0.05$). For all experiments, *Salmonella* recovery decreased as holding times increased (1–24 h), which is consistent with literature where inoculated eggs were stored at room temperature. Results from Experiment 3 indicated that *S. Typhimurium* had higher recovery for all treatments and holding times compared to *S. Enteritidis*, except for eggshells without contents with 100% recovery at 1 h for both serotypes. Recovery of both serotypes was similar in Experiment 4. Overall, this study highlights the importance of eggshell cuticle, membranes, and albumen on the integrity of egg contents and the recovery of inoculated *Salmonella* from eggshells. ISSN: 10566171

Teng, K.T.-Y., Aerts, M., Jaspers, S., Ugarte-Ruiz, M., Moreno, M.A., Saez, J.L., Collado, S., de Frutos, C., Dominguez, L., Alvarez, J.

Patterns of antimicrobial resistance in Salmonella isolates from fattening pigs in Spain

(2022) *BMC Veterinary Research*, 18 (1), art. no. 333, .

ABSTRACT: Background: Swine are considered a major source of foodborne salmonellosis, a public health issue further complicated by the circulation of multidrug-resistant *Salmonella* strains that threaten the safety of the food chain. The current study aimed to identify patterns that can help to understand the epidemiology of antimicrobial resistance (AMR) in *Salmonella* in pigs in Spain through the application of several multivariate statistical methods to data from the AMR national surveillance programs from 2001 to 2017. Results: A total of 1,318 pig *Salmonella* isolates belonging to 63 different serotypes were isolated and their AMR profiles were determined. Tetracycline resistance across provinces in Spain was the highest among all antimicrobials and ranged from 66.7% to 95.8%, followed by sulfamethoxazole resistance (range: 42.5% – 77.8%), streptomycin resistance (range: 45.7% – 76.7%), ampicillin resistance (range: 24.3% – 66.7%, with a lower percentage of resistance in the South-East of Spain), and chloramphenicol resistance (range: 8.5% – 41.1%). A significant increase in the percentage of resistant isolates to chloramphenicol, sulfamethoxazole, ampicillin and trimethoprim from 2013 to 2017 was observed. Bayesian network analysis showed the existence of dependencies between resistance to antimicrobials of the same but also different families, with chloramphenicol and sulfamethoxazole in the centre of the networks. In the networks, the conditional probability for an isolate susceptible to ciprofloxacin that was also susceptible to nalidixic acid was 0.999 but for an isolate resistant to ciprofloxacin that was also resistant to nalidixic acid was only 0.779. An isolate susceptible to florfenicol would be expected to be susceptible to chloramphenicol, whereas an isolate resistant to chloramphenicol had a conditional probability of being resistant to florfenicol at only 0.221. Hierarchical clustering further demonstrated the linkage between certain resistances (and serotypes). For example, a higher likelihood of multidrug-resistance in isolates belonging to 1,4,[5],12:i:- serotype was found, and in the cluster where all isolates were resistant to tetracycline, chloramphenicol and florfenicol, 86.9% (n = 53) of the isolates were Typhimurium. Conclusion: Our study demonstrated the power of multivariate statistical methods in

discovering trends and patterns of AMR and found the existence of serotype-specific AMR patterns for serotypes of public health concern in *Salmonella* isolates in pigs in Spain.
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da Silva, M.R., Alves de Almeida, F., Coelho, A.Í.M., da Silva, F.L., Vanetti, M.C.D.
*Enhancing cell resistance for production of mixed microbiological reference materials with *Salmonella* and coliforms by freeze-drying*
(2022) *Brazilian Journal of Microbiology*, 53 (4), pp. 2107-2119.

ABSTRACT: The reference material (RM) is a technical requirement for the quality assurance of analytical results and proficiency tests or interlaboratory comparisons. Microbiological RMs are most available in the dehydrated form, mainly by freeze-drying, and maintaining bacterial survival after preparation is a challenge. Thus, obtaining the most resistant cells is essential. Considering that bacteria present cross-response to dehydration after being submitted to an array of stress conditions, this study aimed to evaluate the influence of growth conditions on enterobacteria for the production of mixed microbiological RMs by freeze-drying in skim milk powder. *Salmonella enterica* serovar Enteritidis, *Cronobacter sakazakii*, *Escherichia coli*, and *Citrobacter freundii* were grown in a minimal medium with 0.5 M NaCl and 0 to 5.0 mM of manganese sulfate (MnSO₄) until stationary phase. *Salmonella* Enteritidis presented an increased resistance to dehydration in the presence of Mn, while *C. sakazakii* was the most resistant to freeze-drying and further storage for 90 days. Mixed microbiological RMs were produced by freeze-drying and containing *Salmonella* Enteritidis and coliforms in skim milk powder with 100 mM of trehalose and the *Salmonella* survival rate was 91.2 to 93.6%. The mixed RM was stable after 30 days at -20 °C, and *Salmonella* and coliforms were detected by different methods being, the Rambach Agar the best for the bacterial differentiation. The results showed that the culture conditions applied in this study resulted in bacterial cells being more resistant to dehydration, freeze-drying, and stabilization for the production of mixed microbiological RMs more stable and homogeneous. ISSN: 15178382

Deane, A., Murphy, D., Leonard, F.C., Byrne, W., Clegg, T., Madigan, G., Griffin, M., Egan, J., Prendergast, D.M.
*Prevalence of *Salmonella* spp. in slaughter pigs and carcasses in Irish abattoirs and their antimicrobial resistance*

(2022) *Irish Veterinary Journal*, 75 (1), art. no. 4, .

ABSTRACT: Background: *Salmonella* is an important zoonotic pathogen and is one of the main causes of foodborne outbreaks and infections in the European Union. Pigs are a significant reservoir and are frequently subclinical carriers of this organism. *Salmonella* can be shed in the faeces allowing infection to spread to other pigs, the environment, transport vehicles, lairages and other areas. Inadvertent spillage of gut contents during the slaughter process also leads to contamination. A pig *Salmonella* control programme has operated in Ireland since 2002 but many local surveys and an EUMS baseline survey in 2008 continued to indicate high levels of the organism in the pig sector. The objectives of this study were to generate updated information on the prevalence of *Salmonella* spp. in slaughter pigs and carcasses in Irish abattoirs. Five pigs from each of 164 herds were randomly sampled over a 14-week period during 2016. One sample from each of the five pigs of; caecal content, ileo-caecal lymph nodes and carcass swabs (pre-chill) were collected. The five caeca and lymph node samples from each herd were processed as one pool of caecal samples and one pool of lymph node samples, respectively, while the five carcass swabs were tested as individual samples. All isolates were characterised by serotyping and antimicrobial susceptibility. Results: In total, 235 *Salmonella* spp. were isolated from 820 individual carcass swabs, 164 pooled lymph nodes and 164 caecal contents. *Salmonella* spp. were isolated from 54.3% of the caecal contents and from 31.7% of the ileo-caecal lymph node sample pools. A total of 11.5% of carcass-swab samples yielded *Salmonella* spp. *S. Typhimurium* 4,[5],12:i:1,2 or its monophasic variant 4,[5],12:i:-: predominated among isolates from all positive samples; accounting for 73% of lymph nodes, 68% of caecal contents and 56% of carcass swab isolates. *S. London* and *S. Derby* were the next most common isolated serotypes. Conclusions: These results confirm continuing high levels of *Salmonella* in fattening pigs in Ireland although reductions in carcass contamination compared to previous surveys were noted. A high prevalence of *Salmonella* in lymph nodes suggests that it remains a significant problem pre slaughter and a challenge to abattoirs in adhering to process hygiene requirements. The high prevalence of monophasic *S. Typhimurium* 4,[5],12:i:-: is of serious concern. Therefore, it is important to identify contributing factors in the dissemination of this pathogen in the pork industry in order to minimise the risk of human salmonellosis cases.
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Usmael, B., Abraha, B., Alemu, S., Mammed, B., Hiko, A., Abdurehman, A.

Isolation, antimicrobial susceptibility patterns, and risk factors assessment of non-typhoidal Salmonella from apparently healthy and diarrheic dogs (2022) BMC Veterinary Research, 18 (1), art. no. 37, .

ABSTRACT: Background: Dogs are one of the important asymptomatic carriers of antimicrobial resistant and potentially pathogenic strains of *Salmonella*. They can harbor large bacterial load in the intestines and mesenteric lymph nodes which can be shed in their feces with the possibility of transmission to humans. Therefore, a cross-sectional study was conducted with the objectives of estimating the prevalence of non-typhoidal *Salmonella*, assessing the risk factors for dog's *Salmonella* carriage, and profiling the antimicrobial resistance pattern of *Salmonella* isolates among housed dogs in Harar town, Eastern Ethiopia. A total of 415 rectal swab samples were collected from randomly selected dogs. Samples were examined for non-typhoidal *Salmonella* using standard bacteriologic culture and biochemical tests. The disk diffusion method (Kirby-Bauer test) was employed to evaluate the isolates for their susceptibility against five antimicrobials. Results: Non-typhoidal *Salmonella* were isolated from 26 (6.3%) of the rectal swab samples, with significantly higher occurrence in diarrheic (15.2%) than non-diarrheic (5.5%) dogs. The risk of *Salmonella* harboring was significantly higher in female dogs than in male dogs (OR = 2.5, $p = 0.027$). Dogs fecal shedding of *Salmonella* was relatively higher in households who used offal as a main feed type for their dogs (23.1%; 95% CI = 5–53.8) than those who used leftover food (10.1%; 95% CI = 5.7–16.1) and practiced mixed feeding system (17%; 95% CI = 7.6–30.8). *Salmonella* isolates showed higher resistance to ampicillin (41.7%), while all isolates were fully susceptible to gentamicin. Moreover, 58.3% of *Salmonella* isolates showed resistance to at least one of the tested antimicrobials. Majorities (72.7%) of the dog owners had no awareness on the risk of zoonotic salmonellosis from dog and all of the respondents use bare hand to clean dog kennel. Conclusion: Our study reveals the importance of both diarrheic and apparently healthy housed dogs in the harboring and shedding of antimicrobial resistant non-typhoidal *Salmonella*. The risk of non-typhoidal *Salmonella* spread among pet owners is not negligible, especially in households who use offal as main feed type. Therefore, an integrated approach such as: proper dog handling practices; continuous evaluation of antimicrobial resistance; and rational use of antimicrobials in the field of veterinary sector are necessary to tackle the problem. ISSN: 17466148