

NEWSLETTER

European Union Reference Laboratory for *Salmonella*

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European Union Reference Laboratory for *Salmonella*

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Editorial Note

Bilthoven, 1 October 2021

Dear colleague,

Hopefully you had a nice summer and was able to relax despite limitations due to the **SARS-CoV-2 virus**. Luckily the number of vaccinated people is steadily increasing, and resulting, in general, in a decrease in the number of COVID-19 cases. In our country, several measures are no longer into force and we are slowly going back to the 'old normal' situation. Even the colleagues not doing laboratory work are allowed to work again at the institute for 1 or 2 days a week. This is even something we need to get used to again after working at home for such a long time.

Despite all limitations due to the SARS-CoV-2 virus we were still able to organise the Proficiency Tests (PTs) during the crisis.

Currently the NRLs-*Salmonella* are performing the **PT on detection of *Salmonella* in samples from the primary production stage (PPS)**. The samples under investigation are boot socks with chicken faeces. The deadline for reporting the results of this PT is 29 October 2021.

In November the **PT on typing of *Salmonella*** will be organised, containing an obligatory part on serotyping of *Salmonella*, and a voluntary part on cluster analysis (using MLVA and/or WGS). The time table for this PT was included in the previous Newsletter, as well as in the current one.

Currently we are drafting replies to the comments received on **draft ISO/CD TS 6579-4 on identification of monophasic *Salmonella* Typhimurium** and are preparing the next (draft) version of this ISO document (draft ISO/DTS 6579-4). Recently we have also started with the preparations for the organisation of an interlaboratory study (ILS) for determination of the performance characteristics of the 3 PCR protocols described in this draft ISO/TS 6579-4. For the set-up of this ILS the information described in EN ISO 16140-6 has to be followed, indicating that at least 10 laboratories shall test 16 target strains and 8 non-target strains with the PCR protocols described in the (final) draft version of ISO/TS 6579-4. The set-up of the ILS, including the choice of the strains and the timing of the ILS (probably May/June 2022) will be discussed with the members of ISO-WG10 in the coming months. Next, an invitation for participation in this ILS will be sent to the members of ISO/TC34/SC9 as well as to the NRLs-*Salmonella*. We will keep you informed when more details about the ILS are available, as well as about the next draft version of ISO/TS 6579-4.

In August 2021, we were informed that the 'Single Market Programme' (Reg (EU) 2021/690) and the Commission Implementing Decision C(2021) 3046 'on the financing of the Programme for single market, competitiveness of enterprises, including small and medium-sized enterprises, and European statistics and the adoption of the work programme for 2021-2024' have been adopted. Hence, finally the call for the **European Union reference laboratories (EURLs) work programmes 2021-2022** could be launched. In January of this year, the EURL-*Salmonella* work programme 2021 was already informally agreed with the technical desk officer, but a formal agreement was not yet possible. By mid-September 2021 we have now formally submitted our work programme for 2021-2022 to DG SANTE. Before the end of this year we hope to be informed

about the formal agreement of the work programme of the current year and of 2022. When the work programme 2021-2022 is formally adopted we will publish it in a Newsletter for your information.

Best wishes,
Kirsten Mooijman
Coordinator EURL-*Salmonella*

Contribution of the EURL-*Salmonella*

Timetable EURL- *Salmonella* Proficiency Test Typing 2021 Serotyping and optional part MLVA and/or WGS Cluster Analysis

Week	Date	Subject
39	Week of 27 September	Emailing of the link to the registration form for the typing study. Please register by 15 October 2021 at the latest.
43	Week of 25 October	Emailing of the protocol 2021.
45	Monday 8 November	Shipment of the parcels to the participants as Biological Substance Category B (UN 3373).
45	Week of 8 November	<i>Upon receipt:</i> Starting the identification of the strains, according to the usual practice of the laboratory. Sending the link for the result form on serotyping to the participants. Sending the link for the result form on MLVA and/or WGS Cluster Analysis to the participants in a separate email.
50	17 December 2021 at the latest	Deadline for completing the electronic submission of Serotyping results: 17 December 2021. After this deadline, the result form for serotyping will be closed.
	28 January 2022 at the latest	Deadline for completing the electronic submission of MLVA/WGS Cluster Analysis results: 28 January 2022.
	February 2022	Serotyping: Evaluation of individual laboratory results and Interim summary report.
	April/May 2022	MLVA/WGS Cluster Analysis: Evaluation of individual laboratory results and Interim summary report.

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

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From the Literature

Salmonella-related Literature from Scopus: July – September 2021

Hernandez, S.M., Maurer, J.J., Yabsley, M.J., Peters, V.E., Presotto, A., Murray, M.H., Curry, S., Sanchez, S., Gerner-Smidt, P., Hise, K., Huang, J., Johnson, K., Kwan, T., Lipp, E.K.

Free-Living Aquatic Turtles as Sentinels of Salmonella spp. for Water Bodies
(2021) *Frontiers in Veterinary Science*, 8, art. no. 674973, .

ABSTRACT: Reptile-associated human salmonellosis cases have increased recently in the United States. It is not uncommon to find healthy chelonians shedding *Salmonella enterica*. The rate and frequency of bacterial shedding are not fully understood, and most studies have focused on captive vs. free-living chelonians and often in relation to an outbreak. Their ecology and significance as sentinels are important for understanding *Salmonella* transmission. In 2012–2013, *Salmonella* prevalence was determined for free-living aquatic turtles in man-made ponds in Clarke and Oconee Counties, in northern Georgia (USA) and the correlation between species, basking ecology, demographics (age/sex), season, or landcover with prevalence was assessed. The genetic relatedness between turtle and archived, human isolates, as well as, other archived animal and water isolates reported from this study area was examined. *Salmonella* was isolated from 45 of 194 turtles (23.2%, range 14–100%) across six species. Prevalence was higher in juveniles (36%) than adults (20%), higher in females (33%) than males (18%), and higher in bottom-dwelling species (31%; common and loggerhead musk turtles, common snapping turtles) than basking species (15%; sliders, painted turtles). *Salmonella* prevalence decreased as forest cover, canopy cover, and distance from roads increased. Prevalence was also higher in low-density, residential areas that have 20–49% impervious surface. A total of 9 different serovars of two subspecies were isolated including 3 *S. enterica* subsp. *arizonae* and 44 *S. enterica* subsp. *enterica* (two turtles had two serotypes isolated from each). Among the *S. enterica* serovars, Montevideo (n = 13) and Rubislaw (n = 11) were predominant. *Salmonella* serovars Muenchen, Newport, Mississippi, Inverness, Brazil, and Paratyphi B. var L(+) tartrate positive (Java) were also isolated. Importantly, 85% of the turtle isolates matched pulsed-field gel electrophoresis patterns of human isolates, including those reported from Georgia. Collectively, these results suggest that turtles accumulate *Salmonella* present in water bodies, and they may be effective sentinels of environmental contamination. Ultimately, the *Salmonella* prevalence rates in wild aquatic turtles, especially those strains shared with humans, highlight a significant public health concern. ISSN: 22971769

Vasicek, E.M., O'Neal, L., Parsek, M.R., Fitch, J., White, P., Gunn, J.S.

L-Arabinose Transport and Metabolism in Salmonella Influences Biofilm Formation
(2021) *Frontiers in Cellular and Infection Microbiology*, 11, art. no. 698146, .

ABSTRACT: L-arabinose inducible promoters are commonly used in gene expression analysis. However, nutrient source and availability also play a role in biofilm formation; therefore, L-arabinose metabolism could impact biofilm development. In this study we examined the impact of L-arabinose on *Salmonella enterica* serovar Typhimurium (*S. Typhimurium*) biofilm formation. Using mutants impaired for the transport and metabolism of L-arabinose, we showed that L-arabinose metabolism negatively impacts *S. Typhimurium* biofilm formation in vitro. When L-arabinose metabolism is abrogated, biofilm formation returned to baseline levels. However, without the ability to import extracellular L-arabinose, biofilm formation significantly increased. Using RNA-Seq we identified several gene families involved in these different phenotypes including curli expression, amino acid synthesis, and L-arabinose metabolism. Several individual candidate genes were tested for their involvement in the L-arabinose-mediated biofilm phenotypes, but most played no significant role. Interestingly, in the presence of L-arabinose the diguanylate cyclase gene *adrA* was downregulated in wild type *S. Typhimurium*. Meanwhile *cyaA*, encoding an adenylate cyclase, was downregulated in an L-arabinose transport mutant. Using an IPTG-inducible plasmid to deplete c-di-GMP via *viaA* expression, we were able to abolish the increased biofilm phenotype seen in the transport mutant. However, the mechanism by which the L-arabinose import mutant forms significantly larger biofilms remains to be determined. Regardless, these data suggest that L-arabinose metabolism influences intracellular c-di-GMP levels and therefore biofilm formation. These findings are important when considering the use of an L-arabinose inducible promoter in biofilm conditions. ISSN: 22352988

Diaconu, E.L., Alba, P., Feltrin, F., Di Matteo, P., Iurescia, M., Chelli, E., Donati, V., Marani, I., Giacomi, A., Franco, A., Carfora, V.

Emergence of IncHI2 Plasmids With Mobilized Colistin Resistance (mcr)-9 Gene in ESBL-Producing, Multidrug-Resistant Salmonella Typhimurium and Its Monophasic Variant ST34 From Food-Producing Animals in Italy

(2021) *Frontiers in Microbiology*, 12, art. no. 705230, .

ABSTRACT: A collection of 177 genomes of *Salmonella* Typhimurium and its monophasic variant isolated in 2014–2019 from Italian poultry/livestock (n = 165) and foodstuff (n = 12), previously screened for antimicrobial susceptibility and assigned to ST34 and single-locus variants, were studied in-depth to check the presence of the novel mcr-9 gene and to investigate their genetic relatedness by whole genome sequencing (WGS). The study of accessory resistance genes revealed the presence of mcr-9.1 in 11 ST34 isolates, displaying elevated colistin minimum inhibitory concentration values up to 2 mg/L and also a multidrug-resistant (MDR) profile toward up to seven antimicrobial classes. Five of them were also extended-spectrum beta-lactamases producers (blaSHV-12 type), mediated by the corresponding antimicrobial resistance (AMR) accessory genes. All mcr-9-positive isolates harbored IncHI2-ST1 plasmids. From the results of the Mash analysis performed on all 177 genomes, the 11 mcr-9-positive isolates fell together in the same subcluster and were all closely related. This subcluster included also two mcr-9-negative isolates, and other eight mcr-9-negative ST34 isolates were present within the same parental branch. All the 21 isolates within this branch presented an IncHI2/2A plasmid and a similar MDR gene pattern. In three representative mcr-9-positive isolates, mcr-9 was demonstrated to be located on different IncHI2/IncHI2A large-size (~277–297 kb) plasmids, using a combined Illumina–Oxford Nanopore WGS approach. These plasmids were also compared by BLAST analysis with publicly available IncHI2 plasmid sequences harboring mcr-9. In our plasmids, mcr-9 was located in a ~30-kb region lacking different genetic elements of the typical core structure of mcr-9 cassettes. In this region were also identified different genes involved in heavy metal metabolism. Our results underline how genomics and WGS-based surveillance are increasingly indispensable to achieve better insights into the genetic environment and features of plasmid-mediated AMR, as in the case of such IncHI2 plasmids harboring other MDR genes beside mcr-9, that can be transferred horizontally also to other major *Salmonella* serovars spreading along the food chain. ISSN: 1664302X

Sodagari, H.R., Sahibzada, S., Robertson, I., Habib, I., Wang, P.

Whole-Genome Comparative Analysis Reveals Association Between Salmonella Genomic Variation and Egg Production Systems

(2021) *Frontiers in Veterinary Science*, 8, art. no. 666767, .

ABSTRACT: Non-typhoidal *Salmonella*, particularly *Salmonella enterica* serovar Typhimurium (*S. Typhimurium*), is the predominant endemic serovar in the Australian egg production industry and is one of the most frequently reported serovars in foodborne infections in Australia. This study was conducted to investigate the genomic characteristics of *Salmonella* isolated from retail table eggs in Western Australia and to identify the impact of production systems on genomic characteristics of *Salmonella* such as virulence and antimicrobial resistance. A total of 40 non-typhoidal *Salmonella* isolates [*S. Typhimurium* isolates (n = 28) and *Salmonella Infantis* isolates (n = 12)] sourced from retail eggs produced by different production systems (barn-laid, cage, and free-range) in Western Australia were sequenced by whole-genome sequencing. The isolates were de novo assembled, annotated, and analyzed. The results indicated an association between *Salmonella* genomic variation and the system used to raise poultry for egg production (p-value < 0.05). All but one of the *S. Infantis* isolates were recovered from eggs collected from poultry raised under barn and cage production systems. A higher proportion (83.3%) of *S. Typhimurium* isolates were recovered from the eggs produced by free-range production system as compared with those produced under barn (76.9%) and cage production systems (53.3%). Our analysis indicated that *Salmonella* isolated from the eggs produced by barn and cage production systems had more virulence genes than the isolates of the free-range produced eggs. A low carriage of antimicrobial-resistant gene was detected in the isolates of this study. We have built a *Salmonella* genomics database and characteristics-linked gene pools to facilitate future study, characterization, and tracing of *Salmonella* outbreaks. ISSN: 22971769

ABSTRACT: The widespread use of antibiotics, especially those with a broad spectrum of activity, has resulted in the development of multidrug resistance in many strains of bacteria, including *Salmonella*. *Salmonella* is among the most prevalent causes of intoxication due to the consumption of contaminated food and water. Salmonellosis caused by this pathogen is pharmacologically treated using antibiotics such as fluoroquinolones, ceftriaxone, and azithromycin. This foodborne pathogen developed several molecular mechanisms of resistance both on the level of global and local transcription modulators. The increasing rate of antibiotic resistance in *Salmonella* poses a significant global concern, and an improved understanding of the multidrug resistance mechanisms in *Salmonella* is essential for choosing the suitable antibiotic for the treatment of infections. In this review, we summarized the current knowledge of molecular mechanisms that control gene expression related to antibiotic resistance of *Salmonella* strains. We characterized regulators acting as transcription activators and repressors, as well as two-component signal transduction systems. We also discuss the background of the molecular mechanisms of the resistance to metals, regulators of multidrug resistance to antibiotics, global regulators of the LysR family, as well as regulators of histonelike proteins.
ISSN: 20760817

Guillén, S., Marcén, M., Álvarez, I., Mañas, P., Cebrián, G.

Influence of the initial cell number on the growth fitness of salmonella enteritidis in raw and pasteurized liquid whole egg, egg white, and egg yolk
(2021) *Foods*, 10 (7), art. no. 1621, .

ABSTRACT: *Salmonella* growth in egg and egg products has been widely studied, but there are still some aspects that are not fully known. The objective of this work was to study the influence of the initial cell number on the growth fitness of *Salmonella* Enteritidis in raw and pasteurized egg products. Growth curves of five *Salmonella* Enteritidis strains in raw and pasteurized egg products, starting from different initial numbers, were obtained and fitted to the Baranyi and Roberts model. The results revealed that lower initial numbers led to longer lag phases () and lower maximum specific growth rates (μ_{max}) in raw liquid whole egg. Similar results were observed in raw egg white (except for one strain). Conversely, no influence ($p > 0.05$) of the initial concentration on *Salmonella* growth parameters in raw egg yolk was observed. On the other hand, no influence of the initial number of cells on *Salmonella* growth fitness in commercial pasteurized liquid whole egg was observed. The results obtained demonstrate that the disappearance of this initial-dose dependency phenomenon was dependent on the intensity of the thermal treatment applied. Finally, the influence of the initial number was, in general, lower in pasteurized than in raw egg white, but large differences among strains were observed.
ISSN: 23048158

Fenske, G.J., Scaria, J.

Analysis of 56,348 genomes identifies the relationship between antibiotic and metal resistance and the spread of multidrug-resistant non-typhoidal salmonella
(2021) *Microorganisms*, 9 (7), art. no. 1468, .

ABSTRACT: *Salmonella* enterica is common foodborne pathogen that generates both enteric and systemic infections in hosts. Antibiotic resistance is common in certain serovars of the pathogen and of great concern to public health. Recent reports have documented the co-occurrence of metal resistance with antibiotic resistance in one serovar of *S. enterica*. Therefore, we sought to identify possible co-occurrence in a large genomic dataset. Genome assemblies of 56,348 strains of *S. enterica* comprising 20 major serovars were downloaded from NCBI. The downloaded assemblies were quality controlled and in silico serotyped to ensure consistency and avoid improper annotation from public databases. Metal and antibiotic resistance genes were identified in the genomes as well as plasmid replicons. Co-occurrent genes were identified by constructing a co-occurrence matrix and grouping said matrix using k-means clustering. Three groups of co-occurrent genes were identified using k-means clustering. Group 1 was comprised of the *pco* and *sil* operons that confer resistance to copper and silver, respectively. Group 1 was distributed across four serovars. Group 2 contained the majority of the genes and little to no co-occurrence was observed. Metal and antibiotic co-occurrence was identified in group 3 that contained genes conferring resistance to: arsenic, mercury, beta-lactams, sulfonamides, and tetracyclines. Group 3 genes were also associated with an IncQ1 class plasmid replicon. Metal and antibiotic co-occurrence from group 3 genes is mostly isolated to one clade of *S. enterica* I 4,[5],12:i:-. ISSN: 20762607

Horlbog, J.A., Stephan, R., Stevens, M.J.A., Overesch, G., Kittl, S., Napoleoni, M., Silenzi, V., Nüesch-inderbinen, M., Albini, S.

Feedborne salmonella enterica serovar jerusalem outbreak in different organic poultry flocks in switzerland and italy linked to soya expeller

(2021) *Microorganisms*, 9 (7), art. no. 1367, .

ABSTRACT: Poultry feed is a leading source of *Salmonella* infection in poultry. In Switzerland, heat-treated feed is used to reduce *Salmonella* incursions into flocks in conventional poultry production. By contrast, organic feed is only treated with organic acids. In 2019, the Swiss National Reference Center for Enteropathogenic Bacteria identified the rare serovar *S. Jerusalem* from samples of organic soya feed. Further, in July 2020, the European Union's Rapid Alert System for Food and Feed published a notification of the detection of *S. Jerusalem* in soya expeller from Italy. During 2020, seven *S. Jerusalem* isolates from seven different poultry productions distributed over six cantons in Switzerland were reported, providing further evidence of a possible outbreak. Using whole-genome sequencing (WGS), *S. Jerusalem* isolates from feed and from animals in Switzerland were further characterized and compared to *S. Jerusalem* from organic poultry farm environments in Italy. WGS results showed that feed isolates and isolates from Swiss and Italian poultry flocks belonged to the sequence type (ST)1028, grouped in a very tight cluster, and were closely related. This outbreak highlights the risk of spreading *Salmonella* by feed and emphasizes the need for a heat-treatment process for feed, also in organic poultry production. ISSN: 20762607

Kent, E., Okafor, C., Caldwell, M., Walker, T., Whitlock, B., Lear, A.

Control of Salmonella Dublin in a bovine dairy herd

(2021) *Journal of Veterinary Internal Medicine*, 35 (4), pp. 2075-2080.

ABSTRACT: *Salmonella enterica* serovar Dublin (*S. Dublin*) was diagnosed in a dairy herd after signs of acute gastroenteritis and sepsis. Two hundred eighty-three Holstein cattle were sampled resulting in 700 observations, and serology for *S. Dublin* was performed. Holstein cattle sampled were divided by origin and arrival date to determine on-farm exposure. Prevalence estimates were calculated and compared with control measures implemented on the dairy during the outbreak. One group of cows, presumed to be the original carrier animals, had the highest overall seroprevalence (76.5%). Seroprevalence decreased throughout the study, coinciding with testing and management changes. This report documents biosecurity measures that identified *S. Dublin* after the purchase of subclinical carrier cattle and the steps taken to successfully control herd transmission. ISSN: 08916640

Roy, P.K., Ha, A.J.-W., Mizan, M.F.R., Hossain, M.I., Ashrafudoulla, M., Toushik, S.H., Nahar, S., Kim, Y.K., Ha, S.-D.

Effects of environmental conditions (temperature, pH, and glucose) on biofilm formation of Salmonella enterica serotype Kentucky and virulence gene expression

(2021) *Poultry Science*, 100 (7), art. no. 101209, .

ABSTRACT: *Salmonella* is a foodborne pathogen and an emerging zoonotic bacterial threat in the food industry. The aim of this study was to evaluate the biofilm formation by a cocktail culture of 3 wild isolates of *Salmonella enterica* serotype Kentucky on plastic (PLA), silicon rubber (SR), and chicken skin surfaces under various temperatures (4, 10, 25, 37, and 42°C) and pH values (4.0, 5.0, 6.0, 7.0, and 8.0). Then, at the optimum temperature and pH, the effects of supplementation with glucose (0, 0.025, 0.05, and 0.4% w/v) on biofilm formation were assessed on each of the surfaces. The results indicated that higher temperatures (25 to 42°C) and pH values (7.0 and 8.0) led to more robust biofilm formation than lower temperatures (4 and 10°C) and lower pH levels (4.0 to 6.0). Moreover, biofilm formation was induced by 0.025% glucose during incubation at the optimum temperature (37°C) and pH (7.0) but inhibited by 0.4% glucose. Consistent with this finding, virulence related gene (*rpoS*, *rpoH*, *hilA*, and *avrA*) expression was increased at 0.025% glucose and significantly reduced at 0.4% glucose. This results also confirmed by field emission scanning electron microscope, confocal laser scanning microscopy, and autoinducer-2 determination. This study concluded that optimum environmental conditions (temperature 37°C, pH 7.0, and 0.25% glucose) exhibited strong biofilm formation on food and food contact surfaces as well as increased the virulence gene expression levels, indicating that these environmental conditions might be threatening conditions for food safety. ISSN: 00325791

Mughini-Gras, L., van Hoek, A.H.A.M., Cuperus, T., Dam-Deisz, C., van Overbeek, W., van den Beld, M., Wit, B., Rapallini, M., Wullings, B., Franz, E., van der Giessen, J., Dierikx, C., Opsteegh, M.

Prevalence, risk factors and genetic traits of Salmonella Infantis in Dutch broiler flocks
(2021) *Veterinary Microbiology*, 258, art. no. 109120, .

ABSTRACT: *Salmonella* Infantis is a poultry-adapted *Salmonella enterica* serovar that is increasingly reported in broilers and is also regularly identified among human salmonellosis cases. An emerging *S. Infantis* mega-plasmid (pESI), carrying fitness, virulence and antimicrobial resistance genes, is also increasingly found. We investigated the prevalence, genetic characteristics and risk factors for (pESI-carrying) *S. Infantis* in broilers. Faecal

tested. A questionnaire about farm characteristics was also administered. Sampling was performed in July 2018-May 2019, three weeks before slaughter. Fourteen flocks (in 10 farms) were *S. Infantis*-positive, resulting in a 3.7 % flock-level and 5.1 % farm-level prevalence. Based on multi-locus sequence typing (MLST), all isolates belonged to sequence type 32. All but one isolate carried a pESI-like mega-plasmid. Core-genome MLST showed considerable heterogeneity among the isolates, even within the same farm, with a few small clusters detected. The typical pESI-borne multi-resistance pattern to aminoglycosides, sulphonamide and tetracycline (93 %), as well as trimethoprim (71 %), was found. Additionally, resistance to (fluoro)quinolones based on *gyrA* gene mutations was detected. *S. Infantis* was found more often in flocks using salinomycin as coccidiostat, where flock thinning was applied or litter quality was poor, whereas employing external cleaning companies, wheat in feed, and vaccination against infectious bronchitis, were protective. Suggestive evidence for vertical transmission from hatcheries was found. A heterogeneous (pESI-carrying) *S. Infantis* population has established itself in Dutch broiler flocks, calling for further monitoring of its spread and a comprehensive appraisal of control options. ISSN: 03781135

Lin, Q., Chousalkar, K.K., McWhorter, A.R., Khan, S.

Salmonella Hessarek: An emerging food borne pathogen and its role in egg safety (2021) *Food Control*, 125, art. no. 107996, .

ABSTRACT: In Australia and other parts of the world, contaminated eggs or egg-containing food products are common vehicles for human *Salmonella* outbreaks. Recently, an uncommon serotype, *Salmonella* Hessarek, has emerged in foodborne salmonellosis due to the consumption of contaminated eggs and egg products. Limited research is available on the behaviour of *Salmonella* Hessarek in eggs. Therefore, this study was performed to understand the penetration ability and transcriptional behavior of *Salmonella* Hessarek in table eggs stored at different temperatures. The assay revealed that the penetration ability of *Salmonella* Hessarek was significantly ($P < 0.05$) affected by the egg storage temperature. *Salmonella* penetration into egg contents was significantly higher in cold (collected 3 h post-oviposition) compared with warm (collected immediately post-oviposition) eggs stored at 25 °C. There was 4 and 2.39 log increase in *Salmonella* cells in yolk and albumen, respectively, of the eggs stored at ambient temperature. The gene expression data indicated that genes regulate the pathways involved in stress and metabolism, such as *yafD*, *proP*, *rpoS*, *phoP*, *adkF*, and *purG* were significantly upregulated in yolk and on the eggshell surface, at refrigerated temperature. The gene expression data suggested that at refrigerated temperature, *Salmonella* Hessarek maintained its cell viability through upregulating the key genes necessary for survival. The findings further showed that not all genes involved in the vital functions of *Salmonella* were consistently regulated at 5 °C and 25 °C in different egg contents. This study revealed that *Salmonella* Hessarek has the capacity to penetrate and survive in eggshell pores. Storage of eggs at refrigerated temperature can reduce the penetration and replication risk of *Salmonella* Hessarek in eggs. ISSN: 09567135

Kaczorek-

, P., Franaszek, A., Dziewulska, D.,

Can domestic pigeon be a potential carrier of zoonotic Salmonella? (2021) *Transboundary and Emerging Diseases*, 68 (4), pp. 2321-2333.

ABSTRACT: *Salmonellosis* is one of the most important bacterial diseases in pigeons. This study aimed to estimate the prevalence of *Salmonella* spp. in domestic pigeons (*Columba livia* f. *domestica*) in Poland, its antimicrobial susceptibility (both phenotypic and genotypic), and its capability for biofilm formation. The presence of selected virulence genes, nucleotide homology of selected genes, and susceptibility to bacteriophages were investigated as well. From the 585 pigeons tested, 5.47% turned out positive. All isolated strains were recognized as *Salmonella enterica* ser. Typhimurium. The asymptomatic pigeons were carriers of 37.5% of the isolates. The dominant variants were as follows: 1,4,[5],12,:1,2 (53.13%) and 1,4,[5],12,:- (31.25%). Most of the strains analysed showed the ability to produce biofilm after 24 and 48 hr of incubation (59.38% and 53.13%, respectively). Over 90% of the strains were confirmed for *lpfA*, *agafA*, *invA*, *sivH*, and *avrA* virulence genes. Also, of the thirteen antimicrobial susceptibility genes, the following were confirmed: *sul1*, *tet(A)*, *blaTEM-1*, *floR*, *strA*, and *strB*. The most common

were the *strB* (18%) and *tet(A)* (12%) genes that are responsible for coding resistance to aminoglycosides and tetracyclines, respectively. Most of the strains were phenotypically resistant to oxytetracycline (46.88%), neomycin (53.13%) and tylosin (100%). The susceptibility of the investigated *Salmonella* strains to the bacteriophages was between 33% and 100%. MLST, PCR MP and ERIC PCR analyses indicated a very high genetic similarity of the investigated strains (over 99%). Results of our study indicate that *Salmonella enterica* ser. Typhimurium is still an important agent in domestic pigeons and that its antimicrobial resistance increases. Alarming is also the confirmation of a single-phase variant 1,4,[5],12:i,-, which could have increased virulence and multi-drug resistance encoded on the plasmid. Most importantly, however, such strains have been isolated from humans with clinical symptoms of *Salmonella* infection. ISSN: 18651674

Meijerink, N., van den Biggelaar, R.H.G.A., van Haarlem, D.A., Stegeman, J.A., Rutten, V.P.M.G., Jansen, C.A.

A detailed analysis of innate and adaptive immune responsiveness upon infection with Salmonella enterica serotype Enteritidis in young broiler chickens (2021) Veterinary research, 52 (1), p. 109.

ABSTRACT: *Salmonella enterica* serotype Enteritidis (SE) is a zoonotic pathogen which causes foodborne diseases in humans as well as severe disease symptoms in young chickens. More insight in innate and adaptive immune responses of chickens to SE infection is needed to understand elimination of SE. Seven-day-old broiler chickens were experimentally challenged with SE and numbers and responsiveness of innate and adaptive immune cells as well as antibody titers were assessed. SE was observed in the ileum and spleen of SE-infected chickens at 7 days post-infection (dpi). At 1 dpi numbers of intraepithelial cytotoxic CD8+ T cells were significantly increased alongside numerically increased intraepithelial IL-2R⁺ and 20E5+ natural killer (NK) cells at 1 and 3 dpi. At both time points, activation of intraepithelial and splenic NK cells was significantly enhanced. At 7 dpi in the spleen, presence of macrophages and expression of activation markers on dendritic cells were significantly increased. At 21 dpi, SE-induced proliferation of splenic CD4+ and CD8+ T cells was observed and SE-specific antibodies were detected in sera of all SE-infected chickens. In conclusion, SE results in enhanced numbers and activation of innate cells and we hypothesized that in concert with subsequent specific T cell and antibody responses, reduction of SE is achieved. A better understanding of innate and adaptive immune responses important in the elimination of SE will aid in developing immune-modulation strategies, which may increase resistance to SE in young broiler chickens. ISSN: 12979716

Mustafa, G.R., Zhao, K., He, X., Chen, S., Liu, S., Mustafa, A., He, L., Yang, Y., Yu, X., Penttinen, P., Ao, X., Liu, A., Shabbir, M.Z., Xu, X., Zou, L.

Heavy Metal Resistance in Salmonella Typhimurium and Its Association With Disinfectant and Antibiotic Resistance (2021) Frontiers in Microbiology, 12, art. no. 702725, .

ABSTRACT: Metals are widely used in animal feed for their growth-stimulating and antimicrobial effects, yet their use may potentially promote the proliferation of antibiotic resistance through co-selection. We studied the prevalence and associations of metal, antibiotic, and disinfectant resistances of 300 *Salmonella Typhimurium* isolates from pig meat, pig manure, chicken meat, poultry manure, and human stool from Sichuan, China. Seventy four percent of the 300 *Salmonella Typhimurium* isolates were considered resistant to Cu, almost 50% to Zn and Cr, over 25% to Mn and Cd, and almost 10% to Co. Most of the isolates carried at least one heavy metal resistance gene (HMRG). The Cr-Zn-Cd-resistance gene *czcD* was carried by 254 isolates and the Cu-resistance genes *pcoR* and *pcoC* by 196 and 179 isolates, respectively. Most of the isolates were resistant to at least one antibiotic and almost 80% were multidrug-resistant. The prevalence of resistance to six antibiotics was higher among the pig meat and manure isolates than among other isolates, and that of streptomycin and ampicillin were highest among the pig meat isolates and that of ciprofloxacin and ofloxacin among the pig manure isolates. From 55 to 79% of the isolates were considered resistant to disinfectants triclosan, trichloroisocyanuric acid, or benzalkonium chloride. The metal resistances and HMRGs were associated with resistance to antibiotics and disinfectants. Especially, Cu-resistance genes were associated with resistance to several antibiotics and disinfectants. The transfer of the Cr-Zn-Cd-resistance gene *czcD*, Cu-resistance gene *pcoC*, and Co-Ni-resistance gene *cnrA* into *Escherichia coli* and the increased Cu-resistance of the transconjugants implied that the resistance genes were located on conjugative plasmids. Thus, the excessive use of metals and disinfectants as feed additives and in animal care may have the potential to promote antibiotic resistance through co-selection and maintain and promote antibiotic resistance even in the absence of antibiotics. ISSN: 1664302X

Primavilla, S., Roila, R., Zicavo, A., Ortenzi, R., Branciaro, R., Kika, T.S., Valiani, A., Ranucci, D.

Salmonella spp. In pigs slaughtered in small and medium-sized abattoirs in central Italy: Preliminary results on occurrence and control strategies

(2021) *Applied Sciences (Switzerland)*, 11 (16), art. no. 7600, .

ABSTRACT: Salmonella in pork is still a relevant safety issue in the EU, and specific regulations are in force to control this hazard in the meat chain, in a from farm to fork perspective. In Italy, the control is mainly based on official sampling at the slaughterhouse level. The prevalence of Salmonella, and isolated serovars, was investigated during a three-year survey in small and medium slaughterhouses in central Italy. A total of 400 pig carcasses samples were collected by official authorities during the observation period. Data were also analyzed according to the year and season of sampling. The overall Salmonella prevalence in the five selected abattoirs was 13%, with no differences due to the slaughterhouses size and seasons of sampling. An increasing trend in Salmonella prevalence was registered over the years. The main serovars detected were Salmonella enterica 4, [5], 12:i:- and Derby. The data emphasized that the number of contaminated carcasses is relatively high with respect to the level set by EU legislation, and equally distributed in the area, and therefore, appropriate monitoring and control strategies also need to be developed at the farm level. ISSN: 20763417

Hu, X., Sun, X., Luo, S., Wu, S., Chu, Z., Zhang, X., Liu, Z., Wu, J., Wang, X., Liu, C., Wang, X.

Inactivation of salmonella enterica serovar enteritidis on chicken eggshells using blue light (2021) *Agriculture (Switzerland)*, 11 (8), art. no. 762, .

ABSTRACT: Salmonella enterica serovar Enteritidis (S. Enteritidis) is a pathogen that poses a health risk. Blue light (BL), an emerging sanitization technology, was employed for the first time in the present study to inactivate S. Enteritidis on eggshell surfaces and its influence on maintaining eggshell freshness was investigated systematically. The results showed that 415 nm-BL irradiation at a dose of 360 J/cm² reduced 5.19 log CFU/mL of S. Enteritidis in vitro. The test on eggshells inoculated with S. Enteritidis showed that a BL dose at 54.6 J/cm² caused a 3.73 log CFU reduction per eggshell surface and the impact of BL inactivation could be sustained in post-5-week storage. The quality of the tested eggs (weight loss, yolk index, Haugh unit (HU) and albumen pH) demonstrated that BL treatments had negligible effects on the albumen pH of eggs. However, compared to the control, BL-treated eggs showed lower weight loss and higher HU after 5 weeks of storage p could not be determined after 5 weeks of storage. Besides, the total amino acid content of the BL-treated egg was higher than the control, exhibiting an advantage of BL irradiation in maintaining the nutrient quality of whole eggs. The current study determined the efficacy of BL against S. Enteritidis on eggshell and suggested that BL could be an effective application in maintaining the freshness and quality of eggs. ISSN: 20770472

Mellou, K., Gkova, M., Panagiotidou, E., Tzani, M., Sideroglou, T., Mandilara, G.

Diversity and resistance profiles of human non-typhoidal salmonella spp. In Greece, 2003-2020

(2021) *Antibiotics*, 10 (8), art. no. 983, .

ABSTRACT: Salmonella spp. is one of the most common foodborne pathogens in humans. Here, we summarize the laboratory surveillance data of human non-typhoidal salmonellosis in Greece for 2003-2020. The total number of samples declined over the study period ($p < 0.001$). Of the 193 identified serotypes, S. Enteritidis was the most common (52.8%), followed by S. Typhimurium (11.5%), monophasic S. Typhimurium 1,4,[5],12:i:- (4.4%), S. Bovismorbificans (3.4%) and S. Oranienburg (2.4%). The isolation rate of S. Enteritidis declined ($p < 0.001$), followed by an increase of the less common serotypes. Monophasic S. Typhimurium has been among the five most frequently identified serotypes every year since it was first identified in 2007. Overall, Salmonella isolates were resistant to penicillins (11%); aminoglycosides (15%); tetracyclines (12%); miscellaneous agents (sulphonamides, trimethoprim, chloramphenicol and streptomycin) (12%) and third-generation cephalosporins (2%). No isolate was resistant to carbapenems. In total, 2070 isolates (24%) were resistant to one or two antimicrobial classes and 903 (10%) to three and more. Out of the 1166 isolates resistant to fluoroquinolones (13%), 845 (72%) were S. Enteritidis. S. Enteritidis was also the most frequently identified serotype with a resistance to third-generation cephalosporins (37%, 62/166), followed by S. Typhimurium (12%, 20/166). MDR was most frequently identified for S. Typhimurium and its monophasic variant (resistant phenotype of ampicillin, streptomycin, tetracycline and sulphamethoxazole with or without chloramphenicol or trimethoprim). ISSN: 20796382

Bella, C.D., Costa, A., Sciortino, S., Oliveri, G., Cammilleri, G., Geraci, F., Monaco, D.L., Carpintieri, D., Bue, G.L., Bongiorno, C., Altomare, A., Cipri, V., Pitti, R., Lanzillo, C., Arcoleo, G., Allegro, R.

Validation of a commercial loop-mediated isothermal amplification (Lamp)-based kit for the detection of salmonella spp. according to iso 16140:2016

(2021) Applied Sciences (Switzerland), 11 (15), art. no. 6669, .

ABSTRACT: The traditional cultural method (PCR and Real-Time PCR) for *Salmonella* spp. detection and identification is laborious and time-consuming. A qualitative LAMP method detecting *Salmonella* spp. was validated in compliance with ISO 16140:2016. The results show a relative accuracy, sensitivity, and specificity of 100% in comparison with the reference method ISO 6579-1:2017; the LOD₅₀ was set as 0.4 CFU/g. Additionally, a field study was carried out comparing the LAMP kit, a commercially available Real-Time PCR kit (FoodProof *Salmonella*, Biotecon Diagnostics), and the reference cultural method. The *Salmonella* spp. LAMP kit was suitable for reliable detection of *Salmonella* spp., simplifying and reducing the extent and the steps of the analytical process. A total of 105 samples of raw poultry meat were screened for the presence of *Salmonella* spp. according to three methods: The LAMP kit *Salmonella* spp. (Enbitech), the Real-Time PCR kit FoodProof *Salmonella* (Biotecon), and the reference cultural method. Using these three methods, only one sample out of the 105 (0.95%) tested was positive for *Salmonella* spp. This sample was further investigated using the reference method described in ISO 6579-3:2014, in order to characterise the *Salmonella* strain. Following this further biochemical identification and serological typing, the isolate was characterised as *Salmonella* Infantis. ISSN: 20763417

Esteban-cuesta, I., Labrador, M., Hunt, K., Reese, S., Fischer, J., Schwaiger, K., Gareis, M.

Phenotypic and genetic comparison of a plant-internalized and an animal-isolated salmonella choleraesuis strain

(2021) Microorganisms, 9 (8), art. no. 1554, .

ABSTRACT: Contamination of fresh produce with human pathogens poses an important risk for consumers, especially after raw consumption. Moreover, if microorganisms are internalized, no re-moval by means of further hygienic measures would be possible. Human pathogenic bacteria identified in these food items are mostly of human or animal origin and an adaptation to this new niche and particularly for internalization would be presumed. This study compares a plant-internalized and an animal-borne *Salmonella* enterica subsp. enterica serovar Choleraesuis aiming at the identification of adaptation of the plant-internalized strain to its original environment. For this purpose, a phenotypical characterization by means of growth curves under conditions resembling the indigenous environment from the plant-internalized strain and further analyses using Pulsed-field gel electrophoresis and Matrix-assisted laser desorption ionization time of flight spectrometry were assessed. Furthermore, comparative genomic analyses by means of single nucleotide polymorphisms and identification of present/absent genes were performed. Although some phenotypical and genetic differences could be found, no signs of a specific adaptation for colonization and internalization in plants could be clearly identified. This could suggest that any *Salmonella* strain could directly settle in this niche without any evolutionary process being necessary. Further comparative analysis including internalized strains would be necessary to assess this question. However, these kinds of strains are not easily available. ISSN: 20762607

Koprinarova, M.

Methods to improve molecular detection of salmonella in complex herbal matrices containing inhibitors

(2021) Journal of Food Protection, 84 (8), pp. 1309-1314.

ABSTRACT: *Salmonella* is one of the main causes of foodborne diseases worldwide. Molecular tests such as the PCR assay are rapid and sensitive and are increasingly becoming the preferred method for pathogen detection. However, the presence in the analyzed samples of substances that reduce the sensitivity of the assay or totally inhibit PCR amplification might result in failure of pathogen detection. Using a multiplex real-time PCR assay, I investigated the detection of *Salmonella* enterica serovar Typhimurium in three herbal matrices containing inhibiting substances: (i) chamomile (*Matricaria recutita*), (ii) sage (*Salvia officinalis*), and (iii) mint (*Menthae piperitae*). Internal positive controls in the multiplex PCR reactions indicated the degree of inhibition. All three herbs inhibited PCR amplification at the standard matrix concentration (10% suspension). I applied and compared four approaches for overcoming the negative effect of the matrices on the PCR

detection of *Salmonella*. The efficiency strongly depended on the matrix and the method used for removing the inhibitory substances. By using a series of centrifugation steps combined with a direct PCR, I removed the PCR inhibitors and successfully detected the pathogen in each of the tested matrices. This approach did not significantly decrease the sensitivity of the PCR assay, and the detection of the pathogen was with a quantification cycle delay of only 1.48 ± 1.05 cycles compared with the control. Thus, the proposed simple, efficient, reliable, quick, and cost-effective method allowed for removal of PCR inhibitors and subsequent detection of foodborne bacterial pathogens in complex matrices containing PCR inhibitors. ISSN: 0362028X

Pal, A., Bailey, M.A., Talorico, A.A., Krehling, J.T., Macklin, K.S., Price, S.B., Buhr, R.J., Bourassa, D.V.

Impact of poultry litter Salmonella levels and moisture on transfer of Salmonella through associated in vitro generated dust
(2021) *Poultry Science*, 100 (8), art. no. 101236, .

ABSTRACT: Dust present in poultry houses can contain high concentrations of microorganisms and has the potential to include pathogens from the litter. The objective of this study was to examine in vitro the potential for litter to dust transfer of aerobic bacteria, *Salmonella*, *E. coli*, and coliforms, and the role of the litter moisture on this process. Poultry litter was inoculated with 102 to 109 CFU/mL of *Salmonella* Typhimurium to evaluate litter to dust transfer of bacteria (Experiment 1). To evaluate the effect of litter moisture on litter to dust microbial transfer (Experiment 2), litter was inoculated with 109 *S. Typhimurium* with increasing amounts of sterilized water added for moisture adjustment. Dust was generated by blowing air in a direct stream onto inoculated litter while simultaneously collecting dust through impingement. Following litter and dust sample collection, microbial analyses for aerobic plate counts (APC), *Salmonella*, *E. coli*, and coliforms were conducted. Both experiments were repeated 5 times and their data analyzed by one-way ANOVA and simple logistic regression. In Experiment 1, APC of litter (\log_{10} CFU/g) and dust samples (\log_{10} CFU/L) were 10.55 and 4.92, respectively. *Salmonella* ranged from 1.70 to 6.16 \log_{10} CFU/g in litter and only one dust sample had 1.10 \log_{10} CFU/L of *Salmonella*. As *Salmonella* levels in litter increased, the probability of obtaining a dust *Salmonella* positive result also increased. In Experiment 2, attained moisture percentages were 13.0, 18.2, 23.0, 28.2, and 33.3%. Litter recovery for APC, *Salmonella*, *E. coli*, and coliforms counts did not differ ($P > 0.05$) with increasing moisture levels. Dust sample bacterial counts significantly decreased with increasing moisture levels ($P < 0.0001$). Results from this in vitro study indicate that there is potential for *Salmonella* to be present in generated dust and the higher levels of *Salmonella* in litter increase the likelihood of detecting *Salmonella* in dust. Additionally, with higher litter moisture percentage, prevalence of *Salmonella* in generated dust was decreased. ISSN: 00325791

Pérez-Lavalle, L., Carrasco, E., Vallesquino-Laguna, P., Cejudo-Gómez, M., Posada-Izquierdo, G.D., Valero, A.

Internalization capacity of Salmonella enterica sv Thompson in strawberry plants via root
(2021) *Food Control*, 126, art. no. 108080, .

ABSTRACT: Strawberry production represents an agriculture sector of high relevance for the Spanish economy, due to their strawberries, highly appreciated for their organoleptic characteristics and health benefits. However, the reported outbreaks in different countries by enteric pathogens associated with this product have increased the safety concerns among different stakeholders. A number of factors and pathways for introducing pathogens in the strawberry production chain has been investigated. However, information on the potential internalization of enteric pathogens in strawberries is still scarce. The present study aimed to evaluate the potential internalization of *Salmonella enterica* sv Thompson via root in strawberry plants under the application of successive contamination events through capillary irrigation. Strawberry plants were organized in three groups (16 plants per group), and they were submitted to different contamination scenarios; groups 1, 2 and 3 were irrigated 1, 2 and 3 times respectively, with 100 mL of water containing *Salmonella* Thompson (8.8 \log_{10} CFU/mL) every other day for 9 days. Fruits, calyces, and leaves were analyzed during the experiment, while roots were analyzed on the 9th day. The results showed that all calyces analyzed were negative, while one strawberry sample (group 3; day 8), four leaves samples (groups 2 and 3; day 8) and one root sample (group 3; day 9) were positive for *Salmonella* Thompson, representing the 0.85% (95% C.I.: [0.02–4.63%]), 8.88% (95% C.I.: [2.48–21.22%]), and 2.22% (95% C.I.: [0.06–11.77%]), respectively, of the overall samples analyzed. A significant association between the increased frequency of contamination (three irrigations) and the presence of the pathogen in the samples was elucidated. Our findings, in comparison with other studies, also point

out the importance of watering by drip irrigation as a more hygienic and safer agronomic practice than systems like sprinkling irrigation. ISSN: 09567135

Wilson, A., Chandry, P.S., Turner, M.S., Courtice, J.M., Fegan, N.

Comparison between cage and free-range egg production on microbial composition, diversity and the presence of Salmonella enterica (2021) Food Microbiology, 97, art. no. 103754, .

ABSTRACT: The microbial composition of the food production environment plays an important role in food safety and quality. This study employed both 16 S rRNA gene sequencing technology and culture-based techniques to investigate the bacterial microbiota of an egg production facility comprising of both free-range and conventional cage housing systems. The study also aimed to detect the presence of *Salmonella enterica* and determine whether its presence was positively or negatively associated with other taxa. Our findings revealed that microbiota profiles of free-range and cage houses differ considerably in relation to the relative abundance and diversity with a number of taxa unique to each system and to individual sampling sites within sheds. Core to each housing system were known inhabitants of the poultry gastrointestinal tracts, *Romboutsia* and *Turicibacter*, as well as common spoilage bacteria. Generally, free-range samples contained fewer taxa and were dominated by *Staphylococcus equorum*, differentiating them from the cage samples. *Salmonella enterica* was significantly associated with the presence of a taxa belonging to the *Carnobacteriaceae* family. The results of this study demonstrate that the diversity and composition of the microbiota is highly variable across egg layer housing systems, which could have implications for productivity, food safety and spoilage. ISSN: 07400020

Kent, E., Okafor, C., Caldwell, M., Walker, T., Whitlock, B., Lear, A.

Control of Salmonella Dublin in a bovine dairy herd (2021) Journal of Veterinary Internal Medicine, 35 (4), pp. 2075-2080.

ABSTRACT: *Salmonella enterica* serovar Dublin (S. Dublin) was diagnosed in a dairy herd after signs of acute gastroenteritis and sepsis. Two hundred eighty-three Holstein cattle were sampled resulting in 700 observations, and serology for S. Dublin was performed. Holstein cattle sampled were divided by origin and arrival date to determine on-farm exposure. Prevalence estimates were calculated and compared with control measures implemented on the dairy during the outbreak. One group of cows, presumed to be the original carrier animals, had the highest overall seroprevalence (76.5%). Seroprevalence decreased throughout the study, coinciding with testing and management changes. This report documents biosecurity measures that identified S. Dublin after the purchase of subclinical carrier cattle and the steps taken to successfully control herd transmission. ISSN: 08916640

-Czarnak, J., Wódz, K., Kizerwetter-

Citrobacter braakii yield false-positive identification as salmonella, a note of caution (2021) *Foods*, 10 (9), art. no. 2177, .

ABSTRACT: Background: Globally, *Salmonella enterica* is one of the leading causes of foodborne illness in humans. Food of animal origin is obligatorily tested for the presence of this pathogen. Unfortunately, in meat and meat products, this is often hampered by the presence of background microbiota, which may present as false-positive *Salmonella*. Methods: For the identification of *Salmonella* spp. from meat samples of beef, pork, and poultry, the authorized detection method is PN-EN ISO 6579-1:2017-04 with the White-Kauffmann-Le Minor scheme, two biochemical tests: API 20E and VITEK II, and a real-time PCR-based technique. Results: Out of 42 presumptive strains of *Salmonella*, 83.3% *Salmonella enterica* spp. *enterica*, 14.3% *Citrobacter braakii*, and 12.4% *Proteus mirabilis* were detected from 180 meat samples. Conclusions: Presumptive strains of *Salmonella* should be identified based on genotypic properties such as DNA-based methods. The aim of this study was the isolation and identification of *Salmonella* spp. from miscellaneous meat sorts: beef, pork, and poultry. ISSN: 23048158

Uelze, L., Bloch, A., Borowiak, M., Grobbel, M., Deneke, C., Fischer, M., Malorny, B., Pietsch, M., Simon, S., Szabó, I., Tausch, S.H., Fischer, J.

What wgs reveals about salmonella enterica subsp. Enterica in wildlife in germany (2021) Microorganisms, 9 (9), art. no. 1911, .

ABSTRACT: The aim of this study was to gain an overview of the genetic diversity of *Salmonella* found in wildlife in Germany. We were particularly interested in exploring whether wildlife acts as a reservoir of certain serovars/subtypes or antimicrobial resistance

(AMR) genes. Moreover, we wanted to explore the potential of *Salmonella* in spreading from wildlife to livestock and humans. To answer these questions, we sequenced 260 *Salmonella enterica* subsp. *enterica* isolates sampled between 2002 and 2020 from wildlife across Germany, using short-read whole genome sequencing. We found, consistent with previous findings, that some *Salmonella* sequence types are associated with certain animal species, such as *S. Choleraesuis* ST145 with wild boar and *S. Enteritidis* ST183 with hedgehogs. Antibiotic resistance was detected in 14.2% of all isolates, with resistance against important WATCH group antibiotics present in a small number of isolates. We further found that wildlife isolates do not form separate phylogenetic clusters distant to isolates from domestic animals and foodstuff, thus indicating frequent transmission events between these reservoirs. Overall, our study shows that *Salmonella* in German wildlife are diverse, with a low AMR burden and close links to *Salmonella* populations of farm and food-production environments. ISSN: 20762607

da Costa, M.R., Pessoa, J., Meemken, D., Nesbakken, T.

A systematic review on the effectiveness of pre-harvest meat safety interventions in pig herds to control salmonella and other foodborne pathogens (2021) Microorganisms, 9 (9), art. no. 1825, .

ABSTRACT: This systematic review aimed to assess the effectiveness of pre-harvest interventions to control the main foodborne pathogens in pork in the European Union. A total of 1180 studies were retrieved from PubMed® and Web of Science for 15 pathogens identified as relevant in EFSA's scientific opinion on the public health hazards related to pork (2011). The study selection focused on controlled studies where a cause-effect could be attributed to the interventions tested, and their effectiveness could be inferred. Altogether, 52 studies published from 1983 to 2020 regarding *Campylobacter* spp., *Clostridium perfringens*, Methicillin-resistant *Staphylococcus aureus*, *Mycobacterium avium*, and *Salmonella* spp. were retained and analysed. Research was mostly focused on *Salmonella* (n = 43 studies). In-feed and/or water treatments, and vaccination were the most tested interventions and were, overall, successful. However, the previously agreed criteria for this systematic review excluded other effective interventions to control *Salmonella* and other pathogens, like *Yersinia enterocolitica*, which is one of the most relevant biological hazards in pork. Examples of such successful interventions are the Specific Pathogen Free herd principle, stamping out and repopulating with disease-free animals. Research on other pathogens (i.e., Hepatitis E, *Trichinella spiralis* and *Toxoplasma gondii*) was scarce, with publications focusing on epidemiology, risk factors and/or observational studies. Overall, high herd health coupled with good management and biosecurity were effective to control or prevent most foodborne pathogens in pork at the pre-harvest level. ISSN: 20762607

Savic, B., Zdravkovic, N., Radanovic, O., Jezdimirovic, N., Kureljusic, B., Stevancevic, O.

A Salmonella enterica subspecies enterica serovar Choleraesuis outbreak in weaned piglets in Serbia: clinical signs, pathologic changes, and microbiologic features (2021) Journal of Veterinary Diagnostic Investigation, 33 (5), pp. 993-996.

ABSTRACT: *Salmonella enterica* subspecies *enterica* serovar *Choleraesuis* is rarely detected in Europe, but the clinical disease has been reported in wild boars. We describe here the clinical findings, pathologic changes, and microbiologic features of swine salmonellosis caused by *S. enterica* serovar *Choleraesuis* in weaned piglets in Serbia. In April 2019, on a large farrow-to-finish pig farm, increased mortality was reported in weaned piglets, marked by lethargy, anorexia, pyrexia, and respiratory distress. Gross pathology revealed dermal cyanosis, mesenteric lymphadenopathy, splenomegaly, hepatomegaly, interstitial pneumonia, and colitis. By direct culturing of lung, liver, spleen, and lymph nodes, *S. enterica* ser. *Choleraesuis* variant Kunzendorf was isolated after years of absence of the disease in pig farms in Europe. The source of this salmonellosis outbreak caused by *S. enterica* ser. *Choleraesuis* remains unknown. ISSN: 10406387

Badie, F., Saffari, M., Moniri, R., Alani, B., Atoof, F., Khorshidi, A., Shayestehpour, M.

The combined effect of stressful factors (temperature and pH) on the expression of biofilm, stress, and virulence genes in Salmonella enterica ser. Enteritidis and Typhimurium (2021) Archives of Microbiology, 203 (7), pp. 4475-4484.

ABSTRACT: *Salmonella enterica* is a major food borne pathogen that creates biofilm. *Salmonella* biofilm formation under different environmental conditions is a public health problem. The present study was aimed to evaluate the combined effects of stressful factors (temperature and pH) on the expression of biofilm, stress, and virulence genes in *Salmonella Enteritidis* and *Salmonella Typhimurium*. In this study, the effect of

temperature (2, 8, 22.5, 37, 43 °C) and pH (2.4, 3, 4.5, 6, 6.6) on the expression of biofilm production genes (*adr A*, *bap A*), virulence genes (*hil A*, *inv A*) and the stress gene (*RpoS*) of *S. Enteritidis* and *S. Typhimurium* was evaluated. The response surface methodology (RSM) approach was used to evaluate the combined effect of the above factors. The highest expression of *adr A*, *bap A*, *hil A*, and *RpoS* gene for *S. Typhimurium* was at 22 °C–pH 4.5 (6.39-fold increase), 37 °C–pH 6 (3.92-fold increase), 37 °C–pH 6 (183-fold increase), and 37 °C–pH 3 (43.8-fold increase), respectively. The *inv A* gene of *S. Typhimurium* was decreased in all conditions. The *adr A*, *bap A*, *hil A*, *inv A*, and *RpoS* gene of *S. Enteritidis* had the highest expression level at 8 °C–pH 3 (4.09-fold increase), 22 °C–pH 6 (2.71-fold increase), 8 °C pH 3 (190-fold increase), 22 °C–pH 4.5 (9.21-fold increase), and 8 °C–pH 3 (16.6-fold), respectively. Response surface methodology (RSM) indicated that the temperature and pH had no significant effect on the expression level of *adr A*, *bap A*, *hil A*, *Inv A*, and *RpoS* gene in *S. Enteritidis* and *S. Typhimurium*. The expression of biofilm production genes (*adr A*, *bap A*), virulence genes (*hil A*, *inv A*) and the stress gene (*RpoS*) of *S. Enteritidis* and *S. Typhimurium* is not directly and exclusively associated with temperature and pH conditions. ISSN: 03028933

Talorico, A.A., Bailey, M.A., Munoz, L.R., Chasteen, K.S., Pal, A., Krehling, J.T., Bourassa, D.V., Buhr, R.J., Macklin, K.S.

The use of roller swabs for Salmonella detection in poultry litter
(2021) *Journal of Applied Poultry Research*, 30 (3), art. no. 100163, .

ABSTRACT: Litter sampling is utilized as a noninvasive and practical method to determine broiler flock *Salmonella* status. The common methods include boot cover/sock (BC), drag swab (DS), or litter grab sampling (LG). Roller swabs are a new research method that can be used to sample litter without entering research pens. This study aimed to assess the use of roller swabs (RS) for *Salmonella* (S) detection in comparison to BC, DS, and LG. For Experiment 1, litter was sampled for two weeks following a broiler flock that was challenged at 6 days of age with 1×10^7 cfu of a nalidixic acid resistant strain of *Salmonella Enteritidis* to establish a high litter prevalence of S. In Experiment 2, sampling occurred after a subsequent flock was raised on the same litter. In Experiment 1, S was detected by RS less frequently (81%) than DS (95%), BC (97%), and LG (98%; $P < 0.0001$). In Experiment 2, S detection using RS (23%) was similar to LG (17%), higher than DS (6%, $P = 0.022$), and lower than BC (55%, $P < 0.0001$). Although RS were a less sensitive sampling method when the litter prevalence of S was high, RS were equivalent to or better than LG and DS methods when S prevalence was low. The use of roller swabs allows for sampling of litter without entering each pen and has the potential to be utilized for *Salmonella* detection in research pen trials. ISSN: 10566171