

NEWSLETTER

European Union Reference Laboratory for *Salmonella*

Vol. 26 No. 4
December 2020

ISSN 2211-6877



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

Produced by

European Union Reference Laboratory for *Salmonella*

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Editorial Note

Bilthoven, 7 January 2021

Dear colleague,

Hopefully you have been able to enjoy the Christmas break, despite the limitations which are in force in many countries due to the COVID-19 pandemic. I do hope that you are all still healthy and I would like to **wish you all the best and very good health in 2021**. In all EU countries the vaccinations against the SARS-CoV-2 virus have started and I sincerely hope that this year we can go back to the 'old normal'.

Despite all limitations due to the SARS-CoV-2 virus, we were still able to organise two Proficiency Tests (PTs) in the last quarter of 2020 and we are currently also preparing the next PT to be organised in March this year.

In October 2020 the **PT on detection of *Salmonella* in samples from the Primary Production Stage (PPS) and in Food 2020** was organised. The samples concerned artificially contaminated hygiene sponges, and NRLs-*Salmonella* investigating PPS samples as well as NRLs-*Salmonella* investigating Food samples could participate. In total 65 NRLs for *Salmonella* participated in this study: 38 NRLs PPS and 27 NRLs Food, originating from 34 countries. The results of this PT (interim summary and NRLs' own results) were reported to the participants shortly before the Christmas break and will also become available at our EURL-*Salmonella* website soon.

In November 2020 the **PT on typing of *Salmonella*** was organised. This study contained an obligatory part on serotyping of *Salmonella*, as well as a voluntary part on cluster analysis. For this PT we have used extended deadlines for reporting the results so that the NRLs have more time to perform the analysis in this difficult time with lack of staff due to illnesses or being busy for other activities (often related to investigations to SARS-CoV-2) or lack of materials. The deadline for submission of the serotyping results will be in the coming week and the deadline for the cluster analysis is by the end of January 2021.

In March 2021 we are planning to organise the **PT on detection of *Salmonella* in a food sample**. The matrix of choice will be liquid whole egg. The time table for this PT was sent to the NRLs shortly before the Christmas break and is included in this Newsletter.

On 25 September 2020 the inter-EURLs WG on NGS organised an online conference with the support of the Med-Vet-Net association, entitled: '**Science meets Policy' conference: Modern technologies to enable response to crises. Next Generation Sequencing to tackle food-borne diseases**. This conference was well received and counted over 500 participants. The presentations given at this conference can be found at the website of the EURL-*E. coli*, through this link: [link to presentations 25 September 2020](#)

You may have noticed that we are currently revising our **website** (www.eurlsalmonella.eu), by introducing new headings and updating the information. For example, we have introduced a special **page on Next Generation Sequencing (NGS)**. At this page you can find links to guidance documents in the area of application of NGS, produced by the inter-EURLs Working Group on NGS. Several guidance documents are already available, and more will follow soon. Please have a look at it through the following [link](#).

Due to late adoption of the Multiannual Financial Framework (MFF) 2021-2027 and of the new Single Market Programme Regulation (SMP) by the Council

and the European Parliament, the grant applications for EURLs work programs can be submitted only in the course of 2021. In order to ensure the continuity of the activities, the EURLs had to informally submit to the relevant DG SANTE technical desk officer the annual work program for the year 2021 by the end of December 2020/early January 2021. The work program should be informally agreed with the technical desk officer in January 2021. Shortly before the Christmas break we have sent the EURL-*Salmonella* work program 2021 to the desk officer at DG SANTE. As soon as this work program is informally agreed, we will also inform the NRLs-*Salmonella* about the details by publication of the work program in the (next) Newsletter.

Best wishes,
Kirsten Mooijman
Coordinator EURL-*Salmonella*

Contribution of the EURL-*Salmonella*

Timetable PT FOOD 2021

EURL- *Salmonella* Proficiency Test Food 2021
Detection of *Salmonella* in liquid whole egg



Week	Date	Subject
7	20 February 2021	E-mailing of the link to the registration form for the Proficiency Test. Please register by 20 February 2021 at the latest.
8	22 – 27 February 2021	E-mailing of the protocol and instructions for the result form to the NRLs. E-mailing the link for the result form to the participants.
9	1 March 2021	Shipment of the parcels to the participants as Biological Substance Category B (UN 3373). Preparation of media by the NRLs.
10	8 March 2021	Performance of the Proficiency Test.
12	31 March 2021 at the latest	Deadline for completing the result form: 31 March 2021 (23:59h CET) After this deadline the result form will be closed

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: October – December 2020

Kim, J.-H., Oh, S.-W.

Rapid and sensitive detection of E. coli O157:H7 and S. Typhimurium in iceberg lettuce and cabbage using filtration, DNA concentration, and qPCR without enrichment (2020) Food Chemistry, 327, art. no. 127036, .

ABSTRACT: The outbreaks due to the low number of foodborne pathogens present in ready-to-eat products can be prevented by rapid and sensitive detection method. However, as a conventional detection method, it is impossible to monitor foodborne bacteria existing which is less than 50 cfu/25 g in a food. This study was designed to investigate the possibility of detecting 1 cfu in the short-term through filtration, DNA concentration, and qPCR. As a result of the filtration + DNA concentration method, the recovery concentrations of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium was not significantly different from initial inoculation (>7 cfu/25 g). In iceberg lettuce and cabbage, this method was able to detect 7 and 7 cfu/25 g of *E. coli* and 68 and 5 cfu/25 g of *S. Typhimurium*. We demonstrated the potential of the filtration + DNA concentration method as a shorter time alternative to conventional enrichment-based rapid detection in vegetables. ISSN: 03088146

Garrido-Maestu, A., Azinheiro, S., Roumani, F., Carvalho, J., Prado, M.

Application of Short Pre-enrichment, and Double Chemistry Real-Time PCR, Combining Fluorescent Probes and an Intercalating Dye, for Same-Day Detection and Confirmation of Salmonella spp. and Escherichia coli O157 in Ground Beef and Chicken Samples (2020) Frontiers in Microbiology, 11, art. no. 591041, .

ABSTRACT: Molecular methods, particularly those based on real-time PCR (qPCR), have become a popular approach to detect pathogens in food samples. This technique may take advantage of hydrolysis fluorescent probes for increased specificity. Even though suitable, this approach loses the capacity of performing result confirmation by melt curve analysis. In the current study, we developed an alternative approach, combining fluorescent probes along with an intercalating dye (SYBR Green) in order to simultaneously detect, and confirm the result, of two foodborne pathogens (*Salmonella* spp. and *Escherichia coli* O157). This new approach named double chemistry qPCR was combined with a short pre-enrichment in order to obtain a multiplex "same-day" detection method for the selected pathogens. The evaluation of the novel method in spiked food samples (ground beef and chicken breast) obtained values of relative sensitivity, specificity, and accuracy higher than 95%, and Cohen's kappa of 0.92, with a Limit of Detection₉₅ below 5 cfu/25 g, demonstrating its reliability. In addition to this, the method was challenged by inoculating heat-stressed bacteria as well as dead ones. It was observed that it was also possible to detect stressed bacteria with an initial inoculation level below 10 cfu/25 g. Also, it was noticed that high initial concentration of either pathogen (higher than 10⁴ cfu/25 g) was needed in order to generate false positive results due to the presence of dead bacteria, thus the method presents potential for its application in the specific detection of live microorganisms. ISSN: 1664302X

Donoso, A., Paredes, N., Retamal, P.

Detection of Antimicrobial Resistant Salmonella enterica Strains in Larval and Adult Forms of Lesser Mealworm (Alphitobius diaperinus) From Industrial Poultry Farms (2020) Frontiers in Veterinary Science, 7, art. no. 577848, .

ABSTRACT: The lesser mealworms (*Alphitobius diaperinus*) constitute a common cosmopolitan pest in poultry flocks and may colonize the litter in adult and larval forms. Previous studies have documented their potential as carriers of enteric pathogens. In this context, *S. enterica* constitutes a prioritized zoonotic agent in the poultry industry due to the sanitary risks and economic losses associated with its presence. The aim of this study is to describe the presence of *S. enterica* strains in larval and adult forms of *A. diaperinus* collected from poultry litter belonging to industrial farms located in the central zone of Chile. A total of 403 specimens (203 adults and 200 larvae) were sampled from three farms and 25 flocks. For bacteriological isolation, beetles were processed to differentiate external and internal contamination. Then, isolates were serotyped according to the

Kauffman-White scheme and antimicrobial resistance phenotypes were determined using the disk diffusion method. Gene sequences from the megaplasmid pESI were identified through a PCR based test. These procedures led to the detection of 15 *S. enterica* isolates, belonging to serotypes Infantis (14) and Livingstone (1), from both adults (6) and larval (9) specimens, with a similar external (7) and internal (8) distribution. Furthermore, all *S. Infantis* isolates showed antimicrobial resistance and evidence of megaplasmid pESI carriage, with all possessing multidrug-resistant phenotypes. Our results confirm that *A. diaperinus* constitutes a potential reservoir of zoonotic *Salmonella* strains of sanitary and economic concern for the industry and for public health. ISSN: 22971769

Baron, F., Cochet, M.-F., Alabdeh, M., Guérin-Dubiard, C., Gautier, M., Nau, F., Andrews, S.C., Bonnassie, S., Jan, S.

Egg-White Proteins Have a Minor Impact on the Bactericidal Action of Egg White Toward Salmonella Enteritidis at 45°C

(2020) *Frontiers in Microbiology*, 11, art. no. 584986, .

ABSTRACT: *Salmonella enterica* serovar Enteritidis is noted for its ability to survive the harsh antibacterial activity of egg white which is presumed to explain its occurrence as the major food-borne pathogen associated with the consumption of eggs and egg products. Liquid egg white is a major ingredient for the food industry but, because of its thermal fragility, pasteurization is performed at the modest temperature of 57°C (for 2–6 min). Unfortunately, such treatment does not lead to sufficient reduction in *S. Enteritidis* contamination, which is a clear health concern when the product is consumed without cooking. However, egg white is able to limit *S. Enteritidis* growth due to its alkaline pH, iron deficiency and multiple antimicrobial proteins. This anti-*Salmonella* activity of egg white is temperature dependent and becomes bactericidal once the incubation temperature exceeds 42°C. This property is exploited in the highly promising pasteurization treatment (42–45°C for 1–5 days) which achieves complete killing of *S. Enteritidis*. However, the precise mechanism and the role of the egg-white proteins are not fully understood. Here, the impact of exposure of *S. Enteritidis* to egg white-based media, with or without egg-white proteins (>10 kDa), under bactericidal conditions (45°C) was explored by measuring survival and global expression. Surprisingly, the bactericidal activity of egg white at 45°C was only slightly affected by egg-white proteins indicating that they play a minor role in the bactericidal activity observed. Moreover, egg-white proteins had minimal impact on the global-gene-expression response to egg white such that very similar, major regulatory responses (20% genes affected) were observed both with and without egg-white proteins following incubation for 45 min at 45°C. Egg-white proteins caused a significant change in expression for just 64 genes, including the *psp* and lysozyme-inhibitor responses genes which is suggestive of an early membrane perturbation effect. Such damage was supported by disruption of the proton motive force by egg-white proteins. In summary, the results suggest that low-mass components of egg white are largely responsible for the bactericidal activity of egg white at 45°C. ISSN: 1664302X

Crouch, C.F., Nell, T., Reijnders, M., Donkers, T., Pugh, C., Patel, A., Davis, P., van Hulten, M.C.W., de Vries, S.P.W.

Safety and efficacy of a novel inactivated trivalent Salmonella enterica vaccine in chickens (2020) *Vaccine*, 38 (43), pp. 6741-6750.

ABSTRACT: Food poisoning in humans caused by *Salmonella enterica* remains a significant global public health concern, with the majority of infections associated with the consumption of contaminated eggs or poultry products. The safety and efficacy of a novel inactivated trivalent *Salmonella enterica* vaccine containing in addition to *Salmonella* serovars Enteritidis (O:9, serogroup D) and Typhimurium (O:4, serogroup B) also serovar Infantis (O:7, serogroup C1) formulated with an aluminium hydroxide-gel adjuvant was evaluated under field conditions. A total of 10,229 broiler breeder pullets, housed under commercial conditions, were vaccinated at 10 and 17 weeks of age by the intramuscular route in the breast muscle. The vaccine was safe with no local or systemic reactions or adverse effects on bird performance related to the vaccine detected. Vaccination resulted in notable increases in serovar specific antibodies that were maintained until at least 56 weeks of age. Vaccinated birds subjected to homologous challenges around onset of lay showed significantly reduced faecal shedding and organ invasion. Following heterologous challenge with *S. Hadar* (O:8, serogroup C2) faecal shedding was significantly reduced. These results demonstrate that this novel vaccine could play a significant role in a comprehensive *Salmonella* control programme intended to reduce both the incidence of food poisoning in humans and the use of antibiotics during poultry production. ISSN: 0264410X

Bottari, B., Bancalari, E., Barera, A., Ghidini, S., Gatti, M.

Evaluating the presence of human pathogens in commercially frozen, biologically appropriate raw pet food sold in Italy
(2020) *Veterinary Record*, 187 (7), p. 50.

ABSTRACT: Background Biologically appropriate raw food (BARF) diet is becoming more and more popular among pet owners in Europe. However, there are documented microbiological risks associated with raw feeding, and this study aimed to determine the presence of human pathogens in commercially frozen BARF products sold in Italy. Methods *Salmonella* species, *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Campylobacter* species were identified. The general microbiological quality of BARF products and hygiene were also evaluated. Sample size was limited and therefore the study may not be representative of a larger sample. Results None of the tested samples showed total bacterial count (TBC) higher than the limit set to consider a sample unacceptable. However, 14 out of 21 samples showed TBC higher than the limit set to consider a sample marginally acceptable. A high percentage of samples were contaminated by the aforementioned pathogens, highlighting the need for pet owners to be aware of the risks of this feeding strategy both to themselves and to their pets. Conclusions Considering that BARF diet meals can be prepared at home using the hands, as well as tools and spaces that could be shared, guidelines on safer handling of these pet food products should be recommended by veterinarians and nutritionists. ISSN: 00424900

Payen, M., de la Gándara, M.P., Cointe, A., Massiot, A., Bidet, P., Weill, F.-X., Bonacorsi, S.

Diagnostic challenge of gastrointestinal infection due to lactose-fermenting Salmonella enterica subsp. enterica serovar 4,5:I:-
(2020) *Diagnostic Microbiology and Infectious Disease*, 98 (2), art. no. 115105, .

ABSTRACT: Here, we describe a case of a nontyphoidal *Salmonella* disease caused by a *Salmonella enterica* serovar 4,5:i:- (monophasic *Salmonella typhimurium*) which acquired a Lac operon. This lactose-fermenting bacterium presents a major challenge for phenotypical detection of *Salmonella*. Only specific agar plates or molecular techniques allow reliable detection. ISSN: 07328893

Don, S., Ammini, P., Nayak, B.B., Kumar, S.H.

Survival behaviour of Salmonella enterica in fish and shrimp at different conditions of storage
(2020) *LWT*, 132, art. no. 109795, .

ABSTRACT: Seafood contamination with *Salmonella enterica* is a public health concern. The objective of this study was to investigate the survivability of *Salmonella enterica* serotype Typhimurium in fish and shrimp subjected to chilled (1 ± 2 °C) and frozen storage (-18 ± 2 °C). Bombay duck fish (*Harpadon nehereus*) and shrimp (*Parapenaeopsis stylifera*) were surface inoculated with 8 and 4 log CFU/g levels of *S. Typhimurium*. Survivability was studied by surface plating method on *Salmonella*-specific selective agar at regular intervals. In fish and shrimp inoculated with 8 log CFU/g and stored at -18 °C, viable counts could be detected for up to 90 days. At lower inoculation level of 4 log CFU/g, *Salmonella* was viable up to 60 days in frozen fish, while in frozen shrimp the bacterium could survive for only 15 days. In fish and shrimp stored in ice for 15 days, *Salmonella* showed 1–2 log CFU/g reduction in counts irrespective of initial inoculation levels. The results of this study suggest that the survival behaviour of *Salmonella* is influenced by the seafood matrix, temperature of storage and the population density of the bacterium itself. Therefore, strict monitoring of handling and storage procedures is necessary to control the processes that foster the survival and multiplication of *Salmonella* in the seafood chain. ISSN: 00236438

Gand, M., Mattheus, W., Roosens, N., Dierick, K., Marchal, K., Bertrand, S., De Keersmaecker, S.C.J.

A genosero-typing system for a fast and objective identification of Salmonella serotypes commonly isolated from poultry and pork food sectors in Belgium
(2020) *Food Microbiology*, 91, art. no. 103534, .

ABSTRACT: Humans are mostly contaminated by *Salmonella* through the consumption of pork- and poultry-derived food products. Therefore, a strict monitoring of *Salmonella* serotypes in food-producing animals is needed to limit the transmission of the pathogen to humans. Additionally, *Salmonella* can lead to economic loss in the food sector. Previously, a genosero-typing method using the MOL-PCR and Luminex technology was developed for the identification of the 6 *Salmonella* serotypes, and their variants, subjected to an official control in the Belgian food sector. In this study, 3 additional assays using the same technology were developed for the rapid and cost-effective detection of 13 dangerous

highly invasive serotypes or other serotypes frequently isolated from the Belgian poultry and pork sector, i.e. Agona, Anatum, Brandenburg, Choleraesuis, Derby, Enteritidis vaccine strains, Gallinarum var. Gallinarum/Pullorum, Livingstone, Mbandaka, Minnesota, Ohio, Rissen and Senftenberg. Moreover, the previously developed first MOL-PCR assay was improved for *S. Paratyphi B* and serogroup O:3 detection. Finally, a Decision Support System hosted by a web application was created for an automatic and objective interpretation of the Luminex raw data. The 3 new assays and the modifications of the first assay were validated with a 100% accuracy, using 553 *Salmonella* and non-*Salmonella* strains in total. ISSN: 07400020

Martín-Maldonado, B., Vega, S., Mencía-Gutiérrez, A., Lorenzo-Rebenaque, L., de Frutos, C., González, F., Revuelta, L., Marin, C.

Urban birds: An important source of antimicrobial resistant Salmonella strains in Central Spain

(2020) *Comparative Immunology, Microbiology and Infectious Diseases*, 72, art. no. 101519, .

ABSTRACT: Antimicrobial resistance (AMR) is one of the most important threats of the 21st century. Wild birds have been described as reservoirs of AMR in different bacterial species, such as *Salmonella* spp. Privation of food, climate change and overpopulation have forced many wild species to modify their feeding habits, attending urban areas. In this context, the aim of this study was to study *Salmonella* presence, as well as related AMR in urban birds that inhabit the city and its surroundings. A total of 300 urban birds were sampled for *Salmonella* detection according to the ISO 6579-1:2017 (Annex D) recommendations, and serotyping was carried out according to the White-Kauffman-Le Minor scheme. Antimicrobial susceptibility was tested following 2013/652/EU Decision guides. Wild birds analysed were positive for *Salmonella* in 12.3 % of cases, with white storks fed in landfills as the most *Salmonella* prevalent species ($p < 0.05$). The most common serovars isolated were zoonotic (*S. Enteritidis*, *S. Typhimurium* and *S. Typhimurium* monophasic variant). From *Salmonella* isolated strains, 40.5 % were resistant to the most prevalent AMRs found in urban birds were ciprofloxacin (36.4 %), nalidixic acid (36.4 %) and colistin (27.3 %). The scientific community, public administration and population in general should work together to control antimicrobial administration and drug waste management in order to decrease the development and spread of AMR. ISSN: 01479571

Castro-Vargas, R.E., Herrera-Sánchez, M.P., Rodríguez-Hernández, R., Rondón-Barragán, I.S.

Antibiotic resistance in Salmonella spp. isolated from poultry: A global overview

(2020) *Veterinary World*, 13 (10), pp. 2070-2084.

ABSTRACT: *Salmonella enterica* is the most important foodborne pathogen, and it is often associated with the contamination of poultry products. Annually, *Salmonella* causes around 93 million cases of gastroenteritis and 155,000 deaths worldwide. Antimicrobial therapy is the first choice of treatment for this bacterial infection; however, antimicrobial resistance has become a problem due to the misuse of antibiotics both in human medicine and animal production. It has been predicted that by 2050, antibiotic-resistant pathogens will cause around 10 million deaths worldwide, and the WHO has suggested the need to usher in the post-antibiotic era. The purpose of this review is to discuss and update the status of *Salmonella* antibiotic resistance, in particular, its prevalence, serotypes, and antibiotic resistance patterns in response to critical antimicrobials used in human medicine and the poultry industry. Based on our review, the median prevalence values of *Salmonella* in broiler chickens, raw chicken meat, and in eggs and egg-laying hens were 40.5% (interquartile range [IQR] 11.5-58.2%), 30% (IQR 20-43.5%), and 40% (IQR 14.2-51.5%), respectively. The most common serotype was *Salmonella Enteritidis*, followed by *Salmonella Typhimurium*. The highest antibiotic resistance levels within the poultry production chain were found for nalidixic acid and ampicillin. These findings highlight the need for government entities, poultry researchers, and producers to find ways to reduce the impact of antibiotic use in poultry, focusing especially on active surveillance and finding alternatives to antibiotics. ISSN: 09728988

da Silva, D.C., Lopes, S.M., de Aquino, N.S.M., Elias, S.D.O., Duda, H.A., Tondo, E.C.

Mathematical modelling and validation of Salmonella enterica growth in sushi exposed to different time-temperature scenarios found in real sushi establishments

(2020) *Food Research International*, 136, art. no. 109609, .

ABSTRACT: In this study mathematical models to predict *Salmonella enterica* growth in sushi at different temperatures were developed considering data obtained in 26

restaurants in Southern Brazil. The sushi type chosen to develop the models was the one that presented the highest total aerobic mesophilic counts among sushis collected in the establishments. *Salmonella* was inoculated (2–3 log UFC/g) in this sushi type prepared in the laboratory (pH 4.8; aw 0.98) and incubated under isothermal conditions at 7, 15, 20, 25 and 37 °C. Baranyi and Roberts model was used to describe *Salmonella* growth curves, generating R2 values of ≥ 0.98 and RMSE values of ≤ 0.24 log CFU/g/h for primary models. Ratkowsky's equation was used in secondary model, generating R2 of 0.99 and RMSE of 0.02 log CFU/g/h. The model validation was simulated under non-isothermal conditions, using the worst-case scenario that was built through data from the environmental conditions and data obtained from the restaurants. The non-isothermal conditions were performed at 36.3 °C for 6 h, 10 °C for 24 h and 29.5 °C for 6 h sequentially, reaching 6.7 log CFU/g of *Salmonella* and generating RMSE of 0.06 log CFU/g/h, Bias factor of 0.97 and Accuracy factor of 1.03. The negligible growth time (ζ) for *Salmonella*, considering the average of higher distribution temperatures of chosen sushi type (approximately 18 °C), was 8.9 h. However, growth rates of total aerobic mesophilic demonstrated that at 15 °C and 20 °C, the lag phases were approximately 11 and 5 h respectively. Based on these results, we suggest for sushi distribution the use of temperatures of ≤ 15 °C for 6 h (maximum time of distribution allowed in Brazil) considering the *Salmonella* growth. ISSN: 09639969

Malekmohammadi, S., Shah, M.K., Townsend Ramsett, M.K., Bergholz, T.M.

Survival and thermal resistance among four Salmonella serovars inoculated onto flaxseeds (2020) Food Microbiology, 91, art. no. 103516, .

ABSTRACT: Thermal resistance among *Salmonella* serovars has been shown to vary, however, such data are minimal for *Salmonella* inoculated onto low moisture foods. We evaluated survival and subsequent thermal resistance for 32 strains of *Salmonella* from four serovars (Agona, Enteritidis, Montevideo, and Tennessee) on flaxseed over 24 weeks. After inoculation, flaxseeds were adjusted to aw = 0.5 and stored at 22 °C. After 24 weeks at 22 °C, strains of serovar Agona had a significantly slower rate of reduction compared to those of Enteritidis and Montevideo (adj. p ≤ 0.05). Inoculated flaxseeds were processed at 71 °C with vacuum steam pasteurization at 4 time points during storage. Average initial D71°C values ranging from 1.0 to 1.5 min were similar across serovars. Over 24 weeks, D71°C varied in a serovar-dependent manner. D71°C at 8, 16, and 24 weeks did not change significantly for Enteritidis and Montevideo but did for Tennessee and Agona. While significant, the differences in D71°C over time were less than 1 min, indicating that storage time prior to heat treatment would have a minimal effect on the processing time required to inactivate *Salmonella* on flaxseed. ISSN: 07400020

Ortega, N., Fanelli, A., Serrano, A., Martínez-Carrasco, C., Escribano, F., Tizzani, P., Candela, M.G.

Salmonella seroprevalence in wild boar from Southeast Spain depends on host population density

(2020) Research in Veterinary Science, 132, pp. 400-403.

ABSTRACT: Salmonellosis is the second most prevalent zoonosis in Europe and it has considerable economic and health implications for its monitoring and control as well as being among the most prevalent pathogens on livestock farms. The wild boar (*Sus scrofa*) has been identified as a competent host and spreader of *Salmonella* spp. There has been a significant increase in wild boar population in Europe in recent decades, and it is even present in urban areas. This study evaluates the spatial distribution of the seroprevalence of *Salmonella* spp. in wild boar from Murcia (Southeast Spain) and its relationship with host-related risk factors (sex, age, location and density). The presence of antibodies against *S. Typhimurium* and *Choleraesuis* in 269 serum of wild boars hunted in Murcia between 2015 and 2019 were analyzed using a commercial ELISA test (PrioCHECK porcine *Salmonella* kit). The seroprevalence were spatially distributed using Kernel function, and wild boar density using Gaussian kernel estimates (spatialEco version 1.1.1). The risk function was estimated as the ratio between the intensity of positive samples and the wild boar density. The overall seroprevalence was 19.3% (IC95% 16.9–21.8), showing a significant spatial aggregation. The highest seroprevalence detected was 51.8% (IC95% 42.2–61.5) in a specific area with high risk of infection (76–100%) and was related to the wild boar density. Only marginal differences were detected for sex and age. The use of ELISA combined with QGIS (version 3.6.0) has allowed the identification of areas of *Salmonella* occurrence associated with high density as risk factor. ISSN: 00345288

Jacob, J.J., Rachel, T., Shankar, B.A., Gunasekaran, K., Iyadurai, R., Anandan, S., Veeraraghavan, B.

MLST based serotype prediction for the accurate identification of non typhoidal Salmonella serovars

(2020) *Molecular Biology Reports*, 47 (10), pp. 7797-7803.

ABSTRACT: Traditional serotyping based on the phenotypic variation of O- and H-antigen remains as the gold-standard for the identification and classification of Salmonella isolates for last 70 years. Although this classification is a globally recognized nomenclature, huge diversity of Salmonella serotypes have made the serovar identification to be very complex. Seven gene multilocus sequence typing (MLST) on the other hand can provide serovar prediction as well as the evolutionary origin between the serovars. In this study non typhoidal Salmonella (NTS) strains (n = 45) isolated from clinical samples (blood, faeces and pus) were identified by traditional phenotypic serotyping and biochemical testing. All the tested Salmonella isolates were designated as serovar Typhimurium based on phenotyping. However, by MLST 60% (27/45) of the isolates were S. Typhimurium, 35.5% (16/45) were S. Agona (ST13), 2.2% (1/45) were S. Kentucky (ST198) and 2.2% (1/45) were S. Saintpaul (ST27). MLST analysis assigned S. Typhimurium isolates as ST36 (18/127), ST19 (7/27) and ST313 (2/27). Mismatches in serovar designation between MLST database and phenotypic serotyping can be due to the misinterpretation of phenotypic serotyping as the antigenic structures of S. Typhimurium, S. Agona differs by a surface antigen. MLST based phylogeny of study isolates showed clustering according to sequence types. Concordance between MLST based sequence type and phenotypic serotype is important to provide insights into genetic population structure of Salmonella. ISSN: 03014851

Williams, M.S., Ebel, E.D., Saini, G., Nyirabahizi, E.

Changes in salmonella contamination in meat and poultry since the introduction of the pathogen reduction and hazard analysis and critical control point rule

(2020) *Journal of Food Protection*, 83 (10), pp. 1707-1717.

ABSTRACT: In 1996, the Food Safety and Inspection Service (FSIS) published its pathogen reduction and hazard analysis and critical control point (PR-HACCP) rule. The intention of this program was to reduce microbial contamination on meat, poultry, and egg products. The program was implemented in stages between January 1998 and January 2000, with sampling for Escherichia coli O157:H7 and/or Salmonella in large production establishments beginning in 1998. As the PR-HACCP program begins its third decade, it is reasonable to question whether there have been reductions in the frequency of pathogen-contaminated meat and poultry products reaching consumers. This study summarizes the results for over 650,000 samples collected by FSIS between 2000 and 2018 in slaughter and processing establishments across the United States and compares these results to the roughly 100,000 retail samples collected by the U.S. Food and Drug Administration between 2002 and 2017. The data demonstrate that there has been an overall reduction in the occurrence of Salmonella on meat and poultry products, but the direction and magnitude of change has not been consistent over time or across commodities. Although the available data do not support the identification of causal factors for the observed changes, a historical review of the timing of various factors and policy decisions generates potential hypotheses for the observed changes. ISSN: 0362028X

Hill, A., Muñoz, V., Downes, J., Schuppers, M., Buncic, S., O'Brien, S., Stärk, K.D.C.

To Sample or Not to Sample? An Analysis of the Need for Salmonella Sampling of Smaller Poultry Processors

(2020) *Risk Analysis*, 40 (10), pp. 2093-2111.

ABSTRACT: Within the European Union (EU), microbiological criteria (MC) sampling for Salmonella in poultry was introduced in 2005. In particular, processors had to meet a target of fewer than seven positive samples out of 50. However, processors producing small amounts of poultry meat did not have to sample if national authorities determined this was an acceptable risk. The U.K. Food Standards Agency (FSA) thus has a sampling regime based on throughput that allows smaller processors not to sample. In 2011, the limit of 7/50 was reduced to 5/50. Given the current uncertainty regarding U.K. trade relations with the EU, the U.K. FSA decided to conduct a new risk assessment of the risks of Salmonella produced by smaller processors, to determine whether sampling was now necessary. Current evidence suggests that an MC sampling regime in smaller slaughterhouses is not warranted from a national public health perspective. Because of the insensitivities of the MC sampling scheme, the introduction of MC sampling into smaller slaughterhouses would only be necessary if the suspected carcass prevalence was 15% or more. While our analysis is prone to uncertainty, we estimated that the carcass prevalence in smaller processors is below this. Thus, we recommended that the current sampling framework, allowing smaller processors not to sample, was still applicable. ISSN: 02724332

Arienzo, A., Murgia, L., Fraudentali, I., Gallo, V., Angelini, R., Antonini, G.

Microbiological quality of ready-to-eat leafy green salads during shelf-life and home-refrigeration

(2020) *Foods*, 9 (10), art. no. 1421, .

ABSTRACT: The market of ready-to-eat leafy green salads is experiencing a noticeable growth in Europe. Since they are intended to be consumed without additional treatments, these ready-to-eat products are associated with a high microbiological risk. The aim of this work was to evaluate the microbiological quality and safety of ready-to-eat leafy green salads sold in widespread supermarket chains in Lazio, Italy, on the packaging date during shelf-life and during home-refrigeration. The study also aimed to determine the differences between low-, medium-, and high-cost products. *Salmonella* spp. and *L. monocytogenes* were chosen as safety indicators as specified by European regulations while total aerobic mesophilic bacteria and *Escherichia coli* were chosen as quality indicators as suggested by national guidelines. Analyses were performed following the ISO standards and in parallel for the evaluation of total aerobic mesophilic bacteria, with an alternative colorimetric system, the Micro Biological Survey method, in order to propose a simple, affordable and accurate alternative for testing the microbiological quality of products, especially suitable for small and medium enterprises and on-site analyses. The study revealed high, unsatisfactory, total bacterial loads in all analyzed samples on the packaging date and expiry date and a very high prevalence of *Salmonella* spp. (67%) regardless of the selected varieties and cost categories; *L. monocytogenes* was not recovered aligning with the results obtained in other studies. ISSN: 23048158

Ashour, E.A., Abd El-Hack, M.E., Shafi, M.E., Alghamdi, W.Y., Taha, A.E., Swelum, A.A., Tufarelli, V., Mulla, Z.S., El-Ghareeb, W.R., El-Saadony, M.T.

Impacts of green coffee powder supplementation on growth performance, carcass characteristics, blood indices, meat quality and gut microbial load in broilers

(2020) *Agriculture (Switzerland)*, 10 (10), art. no. 457, pp. 1-19.

ABSTRACT: This work investigated the effect of dietary green coffee powder (GCP) on the growth, body characteristics, antioxidant status, serum metabolites, and meat quality of 308 Ross broiler chickens. A total of 180 non-sexed seven-day old chicks were randomly distributed into three experimental groups. The untreated group received a basal diet (control), while the second and third groups were fed the original diet fortified with 1.25 and 2.5 g GCP/kg, respectively, for four weeks. Results revealed an improvement ($p < 0.05$) in live body weight in the group fed 2.5 g/kg GCP at five weeks of age compared to the other groups. Broiler Chicks fed the 1.25 g/kg GCP diet showed a more significant decrease in live body weight than the control group at five weeks of age. The GCP supplementation at level 2.5 g/kg GCP enhanced feed conversion ratio at 3–5 and 1–5 weeks of age. Dietary treatments did not affect ($p > 0.05$) carcass, dressing, and giblets percentages. Different levels of GCP significantly affected the liver, abdominal fat, intestinal length, and lymphoid organs percentages. The changes in serum blood traits and serum oxidative stress markers were not significant among the control and the treated groups. With the increment levels of GCP, results showed elevated values of meat pH, lightness (L^*), and redness (a^*), as well as a significant decrease in its yellowness (b^*). Broilers fed 2.5 g/kg GCP diet showed a significant reduction in the total bacterial count, total yeasts and molds count, *Escherichia coli*, *Enterococcus* spp., and *Salmonella* spp., as well as increasing counts of lactic acid bacteria. The dietary GCP 2.5 g/kg diet supplementation could be used to decrease the pathogenic microorganisms' populations in the broiler's gut. The present experiment has shown that in unsexed broiler chickens, green coffee powder at level of 2.5 g/kg improved the live body weight and feed conversion ratio and decreased the pathogenic bacterial count in the gut. ISSN: 20770472

Mohammed, A.N., Mohamed, D.A., Mohamed, M.B.E., El Bably, M.A.

Assessment of drinking water quality and new disinfectants for water treatment in a small commercial poultry farm

(2020) *Journal of Advanced Veterinary Research*, 10 (4), pp. 206-212.

ABSTRACT: This work designed to monitor the hygienic quality of drinking water supply in a small commercial poultry farm and to assess the efficiency of different new disinfectant compounds against some pathogenic bacterial isolates. A total of 60 water samples was collected from both the main source and drinkers for physico-chemical and bacteriological examination. The sensitivity pattern of 40 strains of bacterial isolates to commonly used disinfectants in poultry facilities for water supply treatment was evaluated using the broth macro-dilution method. Results, the mean values of both alkalinity and total hardness were found in the highest rate of 183.0 ± 17.6 and 345.6 ± 7.6 mg/l compared to their values in the main water source. Furthermore, the mean value of ammonia in drinkers besides nitrite, and phos-phate discovered at the highest level compared to the main water supply

(1.36±0.31, 3.4±0.46, 26.3±0.78 mg/l, respectively). Both *E. coli* and *Shigella* spp. in drinkers were detected at the highest isolation rate (22.6%). *Salmonella kentucky* (*S. kentucky*: O20, 8 H I) isolates were found at the highest rate of 57.1%. Whilst the pathogenic *E. coli* serotyping Poly3 (O157) recorded at 66.67%, followed by *E. coli* O114 33.33%. Biocidal efficiency of Klorsept 25 disinfectant against investigated pathogenic bacterial isolates was 100% at a concentration of 2.0 mg/l after 180 min of exposure. Whilst the efficiency of calcium hypochlorite Ca (Ocl)₂ against *E. coli* and *S. kentucky* was 100% at a concentration of 0.5 mg/l and exposure time 120 min. The susceptibility of all bacterial isolates to H₂O₂ disinfectant at a concentration of 5.0 % was 100% within 60 min contact time. In conclusion, the investigation of hygienic quality of water supply should be occurred periodically to ensure the safety of water source for poultry chick's health. The sensitivity of the studied pathogenic bacterial isolates is 100% to Klorsept 25 disinfectant at a concentration of 2.0 mg/l, calcium hypochlorite (Ca (Ocl)₂) at 0.5 mg/l, and H₂O₂ at 5.0 % at exposure time does not exceed 180 min. ISSN: 20906269

Awasthi, M.K., Liu, T., Awasthi, S.K., Duan, Y., Pandey, A., Zhang, Z.

Manure pretreatments with black soldier fly Hermetia illucens L. (Diptera: Stratiomyidae): A study to reduce pathogen content

(2020) *Science of the Total Environment*, 737, art. no. 139842, .

ABSTRACT: This study aim was to investigate the influence of black soldier fly larvae (BSFL) *Hermetia illucens* L. (Diptera: Stratiomyidae) on pathogenic bacteria (PB) survival in the chicken manure (CM), pig manure (PM), cow manure (COM) and sewage sludge (SS) compost. Three kinds of manure [chicken (T2), pig (T4) and cow (T6)] and SS (T8) were inoculated with BSFL (1.2:7 ratio on fresh weight basis) and without BSFL (T1, T3, T5 and T7) was used as control and experiment lasted for 9 days. The results indicated that BSFL amendment 90–93% of PB abundances (RAs) was significantly mitigate in CM and COM (T2 and T6), and 86–88% in PM and SS compost. However, relatively greater abundance of PB was recorded in the T4 and T8 treatments. Most of the PB belong to Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes phylum and their community composition varied from phylum to species levels among the all treatments. The PB composition was significantly altered by BSFL amendment and also important role play to enhance in compost quality. Interestingly, *Bacillus* and *Clostridium* were significantly very less abundant present in BSFL applied treatments, but considerably higher population of these bacterial genus and its associated species were identifies from control or without BSFL applied treatments. Overall, without BSFL blended-all three kinds of manure-composts have comparatively greater PB abundance than with BSFL applied treatments, as the PB species *Listeria monocytogenes*_FSL_R2-503, *Staphylococcus aureus*_M0406, *Bacillus anthracis*, *Listeria ivanovii*, *Staphylococcus aureus*_C0673, *Salmonella Bacillus cereus*_VD115, *Mycobacterium tuberculosis*_FJ05194 and *Pseudomonas aeruginosa* has relatively greater RAs, followed by *Bartonella bacilliformis*_Ver075; *Bordetella pertussis*_2356847; *Brucella melitensis*_ADMAS-G1; *Klebsiella pneumoniae*_LCT-KP182 and *Corynebacterium jeikeium*_K411 respectively. Thus, chicken manure composting with BSFL addition is efficient technology for the organic waste recycling and conversion of sanitized matured compost with significantly less abundance of pathogenic bacteria. ISSN: 00489697

Rojas, F., Ibacache-Quiroga, C.

A forecast model for prevention of foodborne outbreaks of non-typhoidal salmonellosis (2020) *PeerJ*, 8, art. no. e10009, .

ABSTRACT: Background. This work presents a forecast model for non-typhoidal salmonellosis outbreaks. Method. This forecast model is based on fitted values of multivariate regression time series that consider diagnosis and estimation of different parameters, through a very flexible statistical treatment called generalized auto-regressive and moving average models (GSARIMA). Results. The forecast model was validated by analyzing the cases of *Salmonella enterica* serovar Enteritidis in Sydney Australia (2014–2016), the environmental conditions and the consumption of high-risk food as predictive variables. Conclusions. The prediction of cases of *Salmonella enterica* serovar Enteritidis infections are included in a forecast model based on fitted values of time series modeled by GSARIMA, for an early alert of future outbreaks caused by this pathogen, and associated to high-risk food. In this context, the decision makers in the epidemiology field can led to preventive actions using the proposed model. ISSN: 21678359

Tawyabur, M., Islam, M.S., Sobur, M.A., Hossain, M.J., Mahmud, M.M., Paul, S., Hossain, M.T., Ashour, H.M., Rahman, M.T.

Isolation and characterization of multidrug-resistant escherichia coli and salmonella spp. From healthy and diseased turkeys
(2020) *Antibiotics*, 9 (11), art. no. 770, pp. 1-14.

ABSTRACT: Diseases caused by *Escherichia coli* (*E. coli*) and *Salmonella* spp. can negatively impact turkey farming. The aim of this study was to isolate and characterize multidrug-resistant (MDR) *E. coli* and *Salmonella* spp. in healthy and diseased turkeys. A total of 30 fecal samples from healthy turkeys and 25 intestinal samples from diseased turkeys that died of enteritis were collected. Bacterial isolation and identification were based on biochemical properties and polymerase chain reaction (PCR). Antibiogram profiles were determined by disk diffusion. The tetracycline-resistance gene *tetA* was detected by PCR. All samples were positive for *E. coli*. Only 11 samples (11/30; 36.67%) were positive for *Salmonella* spp. from healthy turkeys, whereas 16 (16/25; 64%) samples were positive for *Salmonella* spp. from diseased turkeys. *E. coli* isolated from diseased turkeys showed higher resistance to levofloxacin, gentamicin, chloramphenicol, ciprofloxacin, streptomycin, and tetracycline. *Salmonella* spp. isolated from healthy turkeys exhibited higher resistance to gentamicin, chloramphenicol, ciprofloxacin, streptomycin, imipenem, and meropenem. All *E. coli* and *Salmonella* spp. from both healthy and diseased turkeys were resistant to erythromycin. *Salmonella* spp. from both healthy and diseased turkeys were resistant to tetracycline. Multidrug resistance was observed in both *E. coli* and *Salmonella* spp. from diseased turkeys. Finally, the *tetA* gene was detected in 93.1% of the *E. coli* isolates and in 92.59% of the *Salmonella* spp. isolates. To the best of our knowledge, this is the first study to isolate and characterize *tetA*-gene-containing MDR *E. coli* and *Salmonella* spp. from healthy and diseased turkeys in Bangladesh. Both microorganisms are of zoonotic significance and represent a significant public health challenge. ISSN: 20796382

Chuppava, B., Wilke, V., Hartung, C.B., El-Wahab, A.A., Grone, R., von Felde, A., Kamphues, J., Visscher, C.

Effect of a high proportion of rye in compound feed for reduction of salmonella typhimurium in experimentally infected young pigs
(2020) *Microorganisms*, 8 (11), art. no. 1629, pp. 1-14.

ABSTRACT: Public health concerns and the potential for food-borne zoonotic transmission have made *Salmonella* a subject of surveillance programs in food-producing animals. Forty-two piglets (25 d of age and initially 7.48 kg) were used in a 28 d infection period to evaluate the effects of a high proportion of rye on reducing *Salmonella* Typhimurium. Piglets were divided into two diet groups: control diet (wheat 69%) and experimental diet (rye 69%). After a one-week adaptation period, all piglets were orally infected with *Salmonella* Typhimurium (107 log CFU/mL; 2mL/pig). *Salmonella* in fecal shedding were evaluated at day 1, 3, 5, 7 and then weekly after infection. At the end of the experimental period (at day 28 after infection), the piglets were euthanized to sample feces, cecal digesta contents and ileocecal lymph nodes to determine the bacterial counts of *Salmonella*. The results suggest that the bacterial counts in the experimental group fed rye diets showed evidence of reducing *Salmonella* fecal shedding from day 14 onwards and decreasing the number of *Salmonella* in cecal digesta. However, the translocation of *Salmonella* in ileocecal lymph nodes was not affected. Furthermore, feed intake, weight gain and feed conversion did not differ between the groups ($p > 0.05$). ISSN: 20762607

Jia, Z., Peng, Y., Yan, X., Zhang, Z., Fang, T., Li, C.

One-step kinetic analysis of competitive growth of Salmonella spp. and background flora in ground chicken
(2020) *Food Control*, 117, art. no. 107103, .

ABSTRACT: The present study was aimed at developing a mathematical model to predict the growth of *Salmonella* in ground chicken in the presence of the background microflora. Ground chicken was inoculated with a cocktail of *S. Typhimurium* and *S. Enteritidis* and incubated at various isothermal temperatures (8-33 °C). *Salmonella* grew at all temperatures, except at 8 °C, while the background microflora could grow under all conditions. The observed growth data of both *Salmonella* and background microflora at temperatures between 12 and 33 °C were analyzed simultaneously to develop the predictive growth models. The Huang-Jameson effect (HJE) model and Huang-Lotka-Volterra (HLV) model were used to describe the growth and interaction between *Salmonella* and background microflora. The Huang square-root model was used to evaluate the effect of temperature on the growth rates and lag times of *Salmonella* and background microflora. A one-step analysis method was used to directly build the tertiary models and to determine the kinetic parameters from the growth curves. The minimum growth temperature (T_{min}) for *Salmonella* estimated by both the HJE and HLV models was 7.2 °C. The T_{min} for background flora determined by HJE and HLV model was 1.3 and 1.8 °C, respectively. Under competition, the growth rate of *Salmonella* could be lower than

that of the background microflora at temperatures below 16.8 °C, while Salmonella would grow faster than the background microflora at temperatures above 16.8 °C. With relatively low value of RMSE (0.3 log CFU/g), the HJE and HLV models could both successfully describe the growth of Salmonella and native microflora and the interaction between the two. Although the Akaike Information Criterion (AIC) value of the HJE model (-349.1) was slightly smaller than that of the HLV model (-339.4), both models were practically equal in accuracy for predicting the competitive growth of Salmonella and background flora in ground chicken. The HJE model and kinetic parameters were validated using separate isothermal and dynamic growth data. The validation results indicated the competition model was accurate, with the RMSE of the predictions was 0.3 log CFU/g. Overall, the residual errors of predictions followed a normal distribution, with approximately 86.3% of them were within ± 0.5 log CFU/g. The results from this study may be useful for microbial risk assessments of Salmonella and shelf-life prediction of ground chicken.
ISSN: 09567135

Bearson, B.L., Trachsel, J.M., Shippy, D.C., Sivasankaran, S.K., Kerr, B.J., Loving, C.L., Brunelle, B.W., Curry, S.M., Gabler, N.K., Bearson, S.M.D.

The role of salmonella genomic island 4 in metal tolerance of salmonella enterica serovar i 4,[5],12:i:-pork outbreak isolate USDA15WA-1
(2020) *Genes*, 11 (11), art. no. 1291, pp. 1-19.

ABSTRACT: Multidrug-resistant (MDR; resistance to >3 antimicrobial classes) Salmonella enterica serovar I 4,[5],12:i:-strains were linked to a 2015 foodborne outbreak from pork. Strain USDA15WA-1, associated with the outbreak, harbors an MDR module and the metal tolerance element Salmonella Genomic Island 4 (SGI-4). Characterization of SGI-4 revealed that conjugational transfer of SGI-4 resulted in the mobile genetic element (MGE) replicating as a plasmid or integrating into the chromosome. Tolerance to copper, arsenic, and antimony compounds was increased in Salmonella strains containing SGI-4 compared to strains lacking the MGE. Following Salmonella exposure to copper, RNA-seq transcriptional analysis demonstrated significant differential expression of diverse genes and pathways, including induction of at least 38 metal tolerance genes (copper, arsenic, silver, and mercury). Evaluation of swine administered elevated concentrations of zinc oxide (2000 mg/kg) and copper sulfate (200 mg/kg) as an antimicrobial feed additive (Zn+Cu) in their diet for four weeks prior to and three weeks post-inoculation with serovar I 4,[5],12:i:-indicated that Salmonella shedding levels declined at a slower rate in pigs receiving in-feed Zn+Cu compared to control pigs (no Zn+Cu). The presence of metal tolerance genes in MDR Salmonella serovar I 4,[5],12:i:-may provide benefits for environmental survival or swine colonization in metal-containing settings. ISSN: 20734425

Machado Lopes, S., Carmo da Silva, D., César Tondo, E.

Effect of curing and heat treatments on the Salmonella survival and physicochemical properties of chicken egg yolk
(2020) *Food Research International*, 137, art. no. 109680, .

ABSTRACT: Cured egg yolk is a novel gastronomic preparation, which a salt and sugar mixture gradually diffuses into the egg yolk, promoting its solidification from the exterior to the inner parts and greatly concentrates fat and flavor. This study was undertaken to analyze the effect of curing and its association with heat treatments on the Salmonella survival and physicochemical properties of chicken egg yolks. Contaminated egg yolks (8.4 log₁₀ CFU/g) were covered by a mixture of sugar and salt and stored at 4.5 °C for curing, for 2, 24, 72 and 144 h. The cured yolks were subjected to the heat treatments: 62 °C for 30 min in temperature-controlled water circulator or at 80 °C for 3 h in oven. None of the treatments promoted the complete inactivation of Salmonella (detection limit of 100 CFU/g). However, Salmonella populations were significantly reduced ($p \leq 0.05$) when heat processes were applied, reaching a maximum reduction of 5.6 log₁₀ CFU/g when the yolks were cured for 2 h and subsequently treated in temperature-controlled water circulator (62 °C for 30 min). This treatment kept the physicochemical properties similar to the ones without heat treatment, while the oven treatment showed considerable changes on texture, water activity and visual color. In conclusion, the use of curing time of 2 h followed by temperature-controlled water circulator process can improve the safety of cured egg yolks made from high quality eggs. However, other methods should be considered and analyzed to promote a complete inactivation of Salmonella.
ISSN: 09639969

Parker, A.M., Mohler, V.L., Gunn, A.A., House, J.K.

Development of a qPCR for the detection and quantification of Salmonella spp. in sheep feces and tissues
(2020) *Journal of Veterinary Diagnostic Investigation*, 32 (6), pp. 835-843.

ABSTRACT: *Salmonella* spp. are common causes of disease in intensive livestock production systems, and contamination of foodstuffs is of significant concern for public health. Therefore, the identification and quantification of *Salmonella* spp. is important for monitoring the level of fecal shedding or tissue colonization in infected animals and animal products. We developed and evaluated a quantitative PCR (qPCR) method on spiked sheep tissue and fecal samples for the detection and quantification of *Salmonella* spp. Without the use of a pre-enrichment step, the qPCR limit of detection (LOD) results for sheep fecal (4×10^4 – 6×10^3 cfu/g) and tissue (4×10^5 – 4×10^3 cfu/g) samples were not adequate for detection purposes. With the inclusion of a 6-h pre-enrichment step in buffered peptone water (BPW), the LOD was 9 cfu/g (2.57×10^1 copies/g) in sheep feces, and 5.4 cfu/g (3.22 copies/g) sheep tissue. Comparison of the 6-h BPW qPCR method with a 24-h mannitol–selenite–cystine broth enrichment culture method using spiked samples revealed a sensitivity of 91% and 92%, respectively, and a specificity of 100% for both methods. The correlation was significant between the quantity (copies/mL) of *Salmonella* spp. in BPW at 6 h and at 0 h, allowing semiquantitative analysis. Our results demonstrate that, following inclusion of a 6-h pre-enrichment step in BPW, qPCR is semiquantitative with improved LODs of *Salmonella* spp. in sheep fecal and tissue samples. ISSN: 10406387

Gast, R.K., Jones, D.R., Guraya, R., Anderson, K.E., Karcher, D.M.

Research Note: Horizontal transmission and internal organ colonization by Salmonella Enteritidis and Salmonella Kentucky in experimentally infected laying hens in indoor cage-free housing

(2020) *Poultry Science*, 99 (11), pp. 6071-6074.

ABSTRACT: The transmission of *Salmonella* to humans via contaminated eggs is an international public health concern. *S. Enteritidis* is deposited inside eggs after colonizing reproductive tissues of infected hens. Diverse housing facility characteristics and flock management practices influence *Salmonella* persistence and transmission in poultry, but the food safety consequences of different housing systems for laying hens remain unresolved. The present study compared the horizontal transmission of infection and invasion of internal organs during the first 2 wk after experimental *S. Enteritidis* and *S. Kentucky* infection of laying hens in indoor cage-free housing. Groups of 72 hens were housed in isolation rooms simulating commercial cage-free barns, and 1/3 of the hens in each room were orally inoculated with either *S. Enteritidis* (2 rooms) or *S. Kentucky* (2 rooms). At 6 d and 12 d postinoculation, 12 inoculated and 24 contact-exposed hens in each room were euthanized, and samples of liver, spleen, ovary, oviduct, and intestinal tract were removed for bacteriologic culturing. All orally inoculated hens were positive for intestinal colonization by *S. Enteritidis* at 6 d postinfection, and 70.8% of contact-exposed hens had become colonized by 12 d. *S. Enteritidis* was isolated from 100% of livers and 50.0% of ovaries from inoculated birds at 6 d and from 41.7% of livers and 10.4% of ovaries from contact-exposed birds at 12 d. The majority of both orally inoculated and contact-exposed hens were positive for intestinal colonization by *S. Kentucky* at 6 d, but *S. Kentucky* was found in other internal organs of both inoculated and contact-exposed hens significantly ($P < 0.05$) less often than *S. Enteritidis* at both sampling intervals. These results indicate that *Salmonella* infection can spread rapidly and extensively among hens in cage-free indoor housing, including a high frequency of internal organ involvement for invasive *S. Enteritidis*. ISSN: 00325791

Cargnel, M., Maes, D., Peeters, L., Dispas, M.

Combining quantitative and qualitative approaches to determine viability of a potential Salmonella Typhimurium vaccination program in pigs in Belgium

(2020) *Preventive Veterinary Medicine*, 184, art. no. 105132, .

ABSTRACT: Vaccinating pigs against *Salmonella Typhimurium* (ST) might be a way to control ST infections at farm level and reduce human infections. Two main issues have to be addressed before such a mandatory vaccination program can be implemented: the effective reduction of attributable human incidence has to be demonstrated and all socio-economic barriers impacting the attitude and motivation of the pig sector have to be lifted. The present research used a quantitative microbial risk assessment model to estimate the effect of different hypothetical *Salmonella* spp. and ST mitigation strategies on the annual prevalence of human salmonellosis along the minced pork production chain. In addition, a qualitative study aimed to list the potential concerns of the pig sector about the implementation of a hypothetical future vaccination program. The following themes were the most often mentioned: awareness, vaccine cost-benefit/effectiveness, legislation, monovalent vaccine, time and labour required to vaccinate, vaccine registration and trade restriction. Cost-effectiveness and cost-benefit of vaccination were cited by all the key interviewees ($n = 12$). However, based on the quantitative microbial risk assessment model, vaccination alone may not be sufficiently effective to reduce the annual human

salmonellosis prevalence. A combination of different control measures along the food chain, with a special focus on interventions at the slaughterhouse, might be more effective in achieving the desired goal than vaccination alone. ISSN: 01675877

Rodrigues Marques Ferreira, Í.H., de Souza Pedrosa, G.T., Jung, J., Ferreira de Melo, A.N., Campagnollo, F.B., Schaffner, D.W., Magnani, M.

Modeling Salmonella enterica fate in fresh-cut pepper (Capsicum annuum L.) during storage as a function of temperature and relative humidity
(2020) LWT, 133, art. no. 109849, .

ABSTRACT: This study assessed the fate of Salmonella enterica (S. Enteritidis and S. Typhimurium) on cut peppers (Capsicum annuum L.) at two initial levels (4.5 or 2.5 log CFU/g) over six days (0–144 h) at nine conditions, combining temperatures (7, 14 and 21 °C) and relative humidity (RH; 15, 50, and 100%). S. enterica showed a maximum growth rate (μ_{max}) ranging from -0.0083 to 0.0681 1/h, and the highest μ_{max} was achieved at 14 and 21 °C and 100% RH. S. enterica growth did not occur at the lowest temperature and lower RH, at either inoculum level. S. enterica had a μ_{max} of 0.0123 ± 0.0016 1/h when inoculated at 2.5 log CFU/g at 7 °C and 100% RH, but only survived inoculated at 4.5 log CFU/g. For a range of temperature and RH combinations, growth was observed only in cut peppers inoculated at 2.5 log CFU/g. Primary model fits were generally good (R^2 0.61–0.99) as were secondary models ($R^2 \sim 0.97$) for μ_{max} as a function of temperature and RH and their interaction. S. enterica behavior on cut peppers is dependent on starting concentration as well as temperature and RH. The generated models may be useful tools to manage the risk of Salmonella growth in cut peppers.

ISSN: 00236438

Thomas, M., Feng, Y.

Risk of Foodborne Illness from Pet Food: Assessing Pet Owners' Knowledge, Behavior, and Risk Perception

(2020) Journal of Food Protection, 83 (11), pp. 1998-2007.

ABSTRACT: Pet food has been identified as a source of pathogenic bacteria, including Salmonella and Escherichia coli. A recent outbreak linked to Salmonella-contaminated pet treats infected .150 people in the United States. The mechanism by which contaminated pet food leads to human illness has not been explicated, and pet owners' food safety knowledge and their pet food handling practices have not been reported. This study was conducted to evaluate pet owners' food safety knowledge and pet food handling practices through an online consumer survey. The survey consisted of 62 questions and assessed (i) owners' food safety knowledge and pet food handling practices; (ii) owners' interaction with pets; and (iii) owners' risk perception related to their own health, their children's health, and their pets' health. The survey was pilot tested among 59 pet owners before distribution to a national consumer panel managed by Qualtrics XM (Provo, UT). All participants (n ¼ 1,040) were dog and/or cat owners in the United States. Almost all pet owners (93%) interacted with their pets, and most cuddled, allowed their pets to lick them, and slept with their pets. Less than one-third of pet owners washed their hands with soap after interacting with their pets. Over half (58%) of the owners reported washing their hands after feeding their pets. Most pet owners fed their pets dry pet food and dry pet treats. Some fed their pets raw meat or raw animal product diets because they believed these diets to be beneficial to the pet's overall health. Many owners (78%) were unaware of pet food recalls or outbreaks associated with foodborne pathogens. Less than 25% considered dry pet foods and treats as a potential source of foodborne pathogens. Pet owners were more concerned when seeing news about pets becoming ill from pet food and less concerned when seeing news about people becoming ill from pet food. The findings of this study indicated the need for consumer education about handling pet food. These results can help researchers develop more accurate risk assessment models and consumer education messages related to pet food handling. ISSN: 0362028X

Gil-Molino, M., García, A., Zurita, S.G., Martín-Cano, F.E., García-Jiménez, W., Risco, D., Rey, J., Fernández-Llario, P., Quesada, A.

Spread of antimicrobial resistance by salmonella enterica serovar choleraesuis between close domestic and wild environments

(2020) Antibiotics, 9 (11), art. no. 750, pp. 1-13.

ABSTRACT: The Salmonella enterica serovar Choleraesuis affects domestic pig and wild boar (WB), causing clinical salmonellosis. Iberian swine production is based on a free-range production system where WB and Iberian pig (IP) share ecosystems. This study focuses on the negative impact on the pork industry of infections due to this serotype, its role in the spread of antibiotic resistance, and its zoonotic potential. Antibiotic resistance (AR) and genetic relationships were analyzed among 20 strains of S. Choleraesuis isolated

from diseased WB and IP sampled in the southwest region of the Iberian Peninsula. AR was studied using the Kirby–Bauer method with the exception of colistin resistance, which was measured using the broth microdilution reference method. Resistance and Class 1 integrase genes were measured using PCR, and the genetic relationship between isolates and plasmid content by pulsed field gel electrophoresis. The results show a higher incidence of AR in isolates from IP. Phylogenetic analysis revealed seven profiles with two groups containing isolates from IP and WB, which indicates circulation of the same clone between species. Most pulsotypes presented with one plasmid of the same size, indicating vertical transmission. AR determinants blaTEM and tetA were routinely found in IP and WB, respectively. One isolate from IP expressed colistin resistance and presented the mcr-1 gene carried by a plasmid. This study suggests that *S. Choleraesuis* circulates between WB and IP living in proximity, and also that the mobilization of AR genes by plasmids is low. Furthermore, the detection of plasmid-mediated colistin resistance in bacteria from IP is alarming and should be monitored. ISSN: 20796382

McLauchlin, J., Aird, H., Charlett, A., Elviss, N., Jørgensen, F., Willis, C.

Microbiological Quality of Cooked Chicken: Results of Monitoring in England (2013 to 2017) (2020) Journal of Food Protection, 83 (11), pp. 1989-1997.

ABSTRACT: Results from monitoring of the microbiological quality of 2,721 samples of ready-to-eat cooked chicken collected between 2013 to 2017 in England were reviewed: 70% of samples were from retail, catering, or manufacture and 30% were imported and collected at English ports. Samples were tested for a range of bacterial pathogens and indicator organisms. Six samples (,1%) had unsatisfactory levels of pathogens that were potentially injurious to health. Neither *Salmonella* nor *Campylobacter* were recovered from any samples. Two samples from catering settings contained either an unsatisfactory level of *Bacillus cereus* (5 3 10⁶ CFU/g) or an unsatisfactory level of coagulase-positive staphylococci (1.6 3 10⁴ CFU/g). *Listeria monocytogenes* was recovered from 36 samples (1 at manufacture, 26 at catering, and 9 at retail) and in 4 samples, unsatisfactory levels (>10² CFU/ g) were detected (3 samples collected at catering and 1 sample at retail). For *L. monocytogenes*, there were no significant differences between the rates of contamination for the samples collected from ports, manufacture, retail supermarkets, and other retailers (P ¼ 0.288). There were no differences between the rates of contamination for other potential pathogens detected between samples from different settings. The prevalence of hygiene indicators (*Escherichia coli*, Enterobacteriaceae, and aerobic colony counts) at import was significantly lower than in samples collected from manufacturers, retail, or catering (P, 0.01). Samples collected from catering gave poorer results than those from all other settings. Regardless of the stage in the food chain, samples from Thailand and from other non-European Union countries were of significantly better microbiological quality with respect to indicator organisms than those from the United Kingdom or from other European Union countries (P ¼,0.001). ISSN: 0362028X

Kokkinakis, M.N., Fragkiadakis, G.A., Lapidakis, N.E., Kokkinaki, A.N.

Assessing microbiological quality of ready-to-eat prepacked sandwiches, in Crete, Greece (2020) Journal of Food Science and Technology, 57 (11), pp. 4220-4227.

ABSTRACT: The microbiological quality of pre-packed sandwiches, prepared by a company which had implemented the Hazard Analysis Critical Control Points system, was assessed at retail level, in Crete, Greece. Totally, we analyzed 225 sandwiches (S1: ham, cheese; S2: ham, cheese, tomato; S3: tuna salad), for specific pathogens (*Listeria monogytenes*, *Salmonella* spp, *Staphylococcus aureus*) and hygiene indicators (*Escherichia coli*, Enterobacteriaceae, Aerobic Colony Count-ACC). Pathogens were not detected. The *E. coli* numbers enumerated in day 0 (factory level) were found within acceptable levels ' 100 cfu/g; limited samples had unsatisfactory values at the 3rd day of retailing storage (7%, 7%, and 27% ' 100 cfu/g for S1, S2, and S3, respectively), which were further increased at the 7th day (20%, 33% and 53% ' 100 cfu/g for S1, S2, and S3, respectively). The Enterobacteriaceae numbers mean log CFU/g were in the satisfactory or acceptable category with an increase in the range of 19.5–49.5% at the 7th day, nevertheless never exceeded the borderline of 4 log CFU/g. All ACC values were satisfactory or acceptable as no value higher than 7 log CFU/g was recorded. Overall there was a difference between the three sandwiches types, with S2 and S3, exhibiting higher levels than S1, possibly due to the extra ingredients. A number of corrective actions can be applied, as i.e. revision of cooking-chilling times, sanitizing procedures, staff hygiene practices and training etc. ISSN: 00221155

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

Stress resistance of emerging poultry-associated Salmonella serovars (2020) International Journal of Food Microbiology, 335, art. no. 108884, .

ABSTRACT: In recent years, the on-farm prevalence of some poultry-related *Salmonella* serovars such as *S. Kentucky*, *S. Heidelberg*, *S. Livingstone* and *S. Mbandaka* has increased significantly, even replacing *S. Enteritidis* and *S. Typhimurium* as the most frequently isolated serovars in some production settings and countries. For this reason, the aim of this work was to determine the resistance to several stressing agents and food preservation technologies, in laboratory media and in egg products, of 4 strains of these emerging *Salmonella* serovars associated to poultry and poultry products and to make comparisons with 4 *S. Enteritidis* strains. First, the resistance to acid pH, hydrogen peroxide, NaCl, heat, HHP, PEF and UV of the 8 *Salmonella* strains studied was determined and compared in laboratory media. From this part of the study, it was concluded that variability in resistance to stress among the 8 studied strains varied depending on the investigated agent/technology. However, differences in resistance (2D-values) were always lower than 3.3-fold. Results obtained also indicated that the strains of the emerging serovars studied would display lower acid and NaCl resistance, higher heat resistance and similar oxidative, HHP, PEF and UV resistance than *S. Enteritidis*. Then, the resistance of these 8 strains was evaluated and compared in egg, egg products and poultry manure. For some agents -including osmotic stresses, UV and PEF- there was a very good correspondence between the results obtained in laboratory media and in real food matrices and poultry manure ($r > 0.85$; $p < 0.01$). A significant relationship was also found for acid and HHP resistance ($p < 0.05$) and a trend for heat resistance ($p < 0.10$). Therefore, in general terms, conclusions drawn from the study carried out in laboratory media - regarding intraspecific variability and the relative resistance of the different strains - might be extrapolated, although with caution, to real food scenarios. Results obtained in this investigation would help to better understand the physiology and ecology of *Salmonella* and to design better egg preservation strategies. ISSN: 01681605

Gautam, B., Govindan, B.N., Gänzle, M., Roopesh, M.S.

Influence of water activity on the heat resistance of Salmonella enterica in selected low-moisture foods

(2020) *International Journal of Food Microbiology*, 334, art. no. 108813, .

ABSTRACT: Low-moisture foods (LMF with water activity, $a_w < 0.85$) including pet foods and black pepper powder have consistently been associated with foodborne disease caused by *Salmonella enterica*. Increased heat resistance and prolonged survival at low-moisture conditions, however, remain major challenges to achieve effective inactivation of *Salmonella* in low-moisture foods. At low water activity (a_w) conditions, heat resistance of *Salmonella* is greatly enhanced when compared to high a_w conditions. This study aimed to quantify the effect of a_w on the heat resistance of *Salmonella enterica* in pet food pellets and black pepper powder. Pet food pellets were inoculated with two strains of heat resistant *S. enterica* and black pepper powder was inoculated with a 5-strain cocktail of *Salmonella*. Both inoculated food samples were equilibrated at 0.33, 0.54, and 0.75 a_w in controlled humidity chambers. Inoculated pet food pellets and black pepper powder in closed aluminum cells were heat treated at specific temperatures for selected times. The results showed that the Weibull model fitted well the inactivation data. At a specific temperature, the rate of inactivation increased with the increase in the a_w from 0.33 to 0.75, and the 3-log reduction times decreased for *Salmonella* in both food samples with the increase in a_w . Water adsorption isotherms of pet food pellets and black pepper powder at initial and treatment temperatures were developed to understand the change in a_w during heat treatments. The change in a_w during heat treatment was dependent on the type of food matrix, which possibly influenced the thermal inactivation of *Salmonella* in pet food pellets and black pepper powder. The quantitative analysis of heat reduction of *Salmonella* with respect to a_w aids in selection of the appropriate initial a_w to develop effective heat treatment protocols for adequate reduction of *Salmonella* in pet foods and black pepper powder. ISSN: 01681605

Bao, H., Wang, S., Zhao, J.-H., Liu, S.-L.

Salmonella secretion systems: Differential roles in pathogen-host interactions

(2020) *Microbiological Research*, 241, art. no. 126591, .

ABSTRACT: The bacterial genus *Salmonella* includes a large group of food-borne pathogens that cause a variety of gastrointestinal or systemic diseases in hosts. *Salmonella* use several secretion devices to inject various effectors targeting eukaryotic hosts, or bacteria. In the past few years, considerable progress has been made towards understanding the structural features and molecular mechanisms of the secretion systems of *Salmonella*, particularly regarding their roles in host-pathogen interactions. In this review, we summarize the current advances about the main characteristics of the *Salmonella* secretion systems. Clarifying the roles of the secretion systems in the process

of infecting various hosts will broaden our understanding of the importance of microbial interactions in maintaining human health and will provide information for developing novel therapeutic approaches. ISSN: 09445013

McWhorter, A.R., Sexton, M., Chousalkar, K.K.

The effects of varied food acid ratios and egg components on Salmonella Typhimurium culturability from raw egg-based sauces
(2020) *Food Microbiology*, 92, art. no. 103555, .

ABSTRACT: Raw egg-based sauces, such as mayonnaise and aioli, are frequently identified as sources of *Salmonella* during outbreaks of human cases of foodborne gastrointestinal disease. In this study, we surveyed aioli and mayonnaise recipes from different popular food websites to identify potential risk factors that may lead to the survival of *Salmonella* Typhimurium. In laboratory experiments, different ratios of food acids were used to determine if lemon juice, vinegar, or a combination of both restricted *Salmonella* Typhimurium culturability. We found that as long as the pH was below 4.2, bacterial culturability was limited. The use of whole egg alone or in combination with egg yolk was also investigated. Sauce preparations containing whole egg exhibited higher pH and supported *Salmonella* Typhimurium culturability longer than those containing yolk only. Ten restaurant prepared sauces were also obtained to further characterize the effect of preparation variability. Sauce preparations with a pH \leq 3.8 did not support bacterial culturability after 4 h incubation at any temperature. The higher the pH the longer *Salmonella* Typhimurium remained culturable. Based on this study, it is recommended that raw egg-based foods are acidified, then stored at room temperature for at least 4 h prior to consumption. ISSN: 07400020

Lee, A., Bortoluzzi, C., Pilla, R., Kogut, M.H.

A role for the microbiota in the immune phenotype alteration associated with the induction of disease tolerance and persistent asymptomatic infection of Salmonella in the chicken
(2020) *Microorganisms*, 8 (12), art. no. 1879, pp. 1-15.

ABSTRACT: Previous studies have shown a tissue immune phenotype-altering event occurring on days 2 and 4 in the ceca post-*Salmonella* challenge. To evaluate the involvement of the cecal microbiota in the phenotype reprogramming, we hypothesized that the addition of subtherapeutic bacitracin (BMD) will affect the cecal microbiota. Therefore, the objective of this study was to determine if the antibiotic-mediated changes in the microbiota composition influenced the immune phenotype induced by *Salmonella* enteritidis infection of the chicken cecum. A total of 112 fertile eggs were obtained for each experiment, repeated for a total of three separate times. The ceca and cecal contents were collected on days 2 and 4 post-infection for mRNA expression TaqMan assay and 16S rRNA gene microbiota sequencing. The results demonstrate the effects of bacitracin on cecal composition and its interaction with *Salmonella* enteritidis in young chicks. There is a preliminary indication of phenotype change in the *Salmonella*-challenged group provided subtherapeutic BMD due to the shifting cecal microbiota and cecal immune response, indicating the addition of bacitracin during infection altered the cecal phenotype. These data demonstrate the potential involvement of the microbiota in reprogramming immune phenotype (disease resistance to disease tolerance) induced by *Salmonella* in the chicken cecum. ISSN: 20762607

Magossi, G., Lambertini, E., Noll, L., Bai, J., Jones, C., Nagaraja, T.G., Phebus, R., Woodworth, J., Trinetta, V.

Potential risk-factors affecting Salmonella sp. and Escherichia coli occurrence and distribution in Midwestern United States swine feed mills
(2020) *Journal of Applied Microbiology*, 129 (6), pp. 1744-1750.

ABSTRACT: Aim: This study aimed to evaluate the patterns and potential risk factors associated with the occurrence of *Salmonella* sp. and *Escherichia coli* in selected United States swine feed mills. Methods and Results: A total of 405 samples were collected during fall 2018, spring and summer 2019 from selected sites including floors, equipment, shoes and feed in six feed mills in the US Midwest region. Each sample was analysed for the presence of *Salmonella* and *E. coli* with culture methods and confirmed by PCR. A survey regarding production volumes, hygiene practices and microbial testing capabilities was conducted in each facility. All mills had at least one sampling site positive for either *Salmonella* or *E. coli*. Of the 405 samples, 4.7, and 14.1% were positive for *Salmonella* sp., and *E. coli* respectively. Sites with higher percentages of positive samples were the receiving, manufacturing, and control area floors. The survey responses indicated that the age of the mill might be a risk factor for bacterial contamination: the older the facility, the higher the number of positive samples. Other risk factors evaluated, such as the production capacity, did not appear to relate to bacterial prevalence. Conclusion: The data

documents the presence of E. coli and Salmonella in selected US swine feed mills, and an association between E. coli occurrence and number of ingredient suppliers to feed mill. Significance and Impact of the Study: This information could be used to understand risk factors affecting the occurrence of Salmonella sp. and E. coli in feed mills and help implement monitoring and mitigation strategies for public health. ISSN: 13645072

Jia, S., McWhorter, A.R., Andrews, D.M., Underwood, G.J., Chousalkar, K.K.

Challenges in vaccinating layer hens against salmonella typhimurium
(2020) *Vaccines*, 8 (4), art. no. 696, pp. 1-12.

ABSTRACT: Salmonella Typhimurium is among the most common causes of bacterial foodborne gastrointestinal disease in humans. Food items containing raw or undercooked eggs are frequently identified during traceback investigation as the source of the bacteria. Layer hens can become persistently infected with Salmonella Typhimurium and intermittently shed the bacteria over the course of their productive lifetime. Eggs laid in a contaminated environment are at risk of potential exposure to bacteria. Thus, mitigating the bacterial load on farms aids in the protection of the food supply chain. Layer hen producers use a multifaceted approach for reducing Salmonella on farms, including the all-in-all-out management strategy, strict biosecurity, sanitization, and vaccination. The use of live attenuated Salmonella vaccines is favored because they elicit a broader host immune response than killed or inactivated vaccines that have been demonstrated to provide cross-protection against multiple serovars. Depending on the vaccine, two to three doses of Salmonella Typhimurium vaccines are generally administered to layer hens within the first few weeks. The productive life of a layer hen, however, can exceed 70 weeks and it is unclear whether current vaccination regimens are effective for that extended period. The objective of this review is to highlight layer hen specific challenges that may affect vaccine efficacy. ISSN: 2076393X

Apenteng, O.O., Arnold, M.E., Vigre, H.

Using stochastic dynamic modelling to estimate the sensitivity of current and alternative surveillance program of Salmonella in conventional broiler production
(2020) *Scientific Reports*, 10 (1), art. no. 19441, .

ABSTRACT: Since 2018, the EU commission has declared the Danish broiler industry to be Salmonella free. However, there is continuous Salmonella pressure from the environment, and a number of parent flocks and broiler flocks become infected annually. When a parent flock becomes infected, the infection can be transmitted vertically to the broiler flocks, before the parent flock is detected and destroyed, including the eggs at the hatchery. To address this issue, we developed stochastic dynamic modelling of transmission of Salmonella in parent flocks and combined that with the relation between flock prevalence and test sensitivity for environmental samples in the flock. Results suggested that after 10 and 100 infected hens were seeded, the likelihood of detecting an infected parent flock within the three first weeks after the infection was strongly influenced by the taking of five boot swabs (95% CI 70–100) instead of two (95% CI 40–100) or the supplementing of the two boot swabs by a dust sample (95% CI 43–100). Results suggest that the likelihood of detecting the broiler flock as infected in the program was estimated to at least 99% in broiler flock even if only one chicken was initially infected. These findings are of relevance for managing parent flocks and eggs at the hatchery in case of Salmonella infection in parent flocks in the Danish poultry. ISSN: 20452322

Jovčić, B., Novović, K., Filipić, B., Velhner, M., Todorović, D., Matović, K., Rašić, Z., Nikolić, S., Kiškarolj, F., Kojić, M.

Genomic characteristics of colistin-resistant salmonella enterica subsp. Enterica serovar infantis from poultry farms in the Republic of Serbia
(2020) *Antibiotics*, 9 (12), art. no. 886, pp. 1-13.

ABSTRACT: The antimicrobial susceptibility testing was conducted on 174 single isolates from poultry farms in Serbia and it was determined that seven Salmonella spp. were multidrug resistant. Sixteen serotypes were detected, but only serotype Infantis confirmed reduced susceptibility to colistin. Seven colistin resistant Salmonella Infantis were studied in detail using the WGS approach. Three sequence types were identified corresponding to different epizootiology region. The isolate from the Province of Vojvodina 3842 and isolates from Jagodina (92 and 821) are represented by the sequence type ST413 and ST11, respectively. Four isolates from Kraljevo are ST32, a common S. Infantis sequence type in humans, poultry and food. The fosfomycin resistance gene fosA7 in isolate 3842 and the vgaA gene in isolate 8418/2948 encoding resistance to pleuromutilins were reported for the first time in serovar Infantis. The changes in relative expression of the phoP/Q, mgrB and pmrA/B genes were detected. Single nucleotide polymorphisms of the pmrB gene, including transitions Val164Gly or Val164Met, and Arg92Pro are described. Analyses of

quinolone resistance determining region revealed substitutions Ser83Tyr in GyrA protein and Thr57Ser and Ser80Arg in ParC protein. Based on WGS data, there are two major clusters among analyzed Salmonella Infantis isolates from central Serbia. ISSN: 20796382

Kushwaha, S.K., Bhavesh, N.L.S., Abdella, B., Lahiri, C., Marathe, S.A.

The phylogenomics of CRISPR-Cas system and revelation of its features in Salmonella (2020) Scientific Reports, 10 (1), art. no. 21156, .

ABSTRACT: Salmonellae display intricate evolutionary patterns comprising over 2500 serovars having diverse pathogenic profiles. The acquisition and/or exchange of various virulence factors influences the evolutionary framework. To gain insights into evolution of Salmonella in association with the CRISPR-Cas genes we performed phylogenetic surveillance across strains of 22 Salmonella serovars. The strains differed in their CRISPR1-leader and cas operon features assorting into two main clades, CRISPR1-STY/cas-STY and CRISPR1-STM/cas-STM, comprising majorly typhoidal and non-typhoidal Salmonella serovars respectively. Serovars of these two clades displayed better relatedness, concerning CRISPR1-leader and cas operon, across genera than between themselves. This signifies the acquisition of CRISPR1/Cas region could be through a horizontal gene transfer event owing to the presence of mobile genetic elements flanking CRISPR1 array. Comparison of CRISPR and cas phenograms with that of multilocus sequence typing (MLST) suggests differential evolution of CRISPR/Cas system. As opposed to broad-host-range, the host-specific serovars harbor fewer spacers. Mapping of protospacer sources suggested a partial correlation of spacer content with habitat diversity of the serovars. Some serovars like serovar Enteritidis and Typhimurium that inhabit similar environment/infect similar hosts hardly shared their protospacer sources. ISSN: 20452322

Munck, N., Leekitchaenphon, P., Litrup, E., Kaas, R., Meinen, A., Guillier, L., Tang, Y., Malorny, B., Palma, F., Borowiak, M., Gourmelon, M., Simon, S., Banerji, S., Petrovska, L., Dallman, T.J., Hald, T.

Four European Salmonella Typhimurium datasets collected to develop WGS-based source attribution methods (2020) Scientific Data, 7 (1), art. no. 75, .

ABSTRACT: Zoonotic Salmonella causes millions of human salmonellosis infections worldwide each year. Information about the source of the bacteria guides risk managers on control and preventive strategies. Source attribution is the effort to quantify the number of sporadic human cases of a specific illness to specific sources and animal reservoirs. Source attribution methods for Salmonella have so far been based on traditional wet-lab typing methods. With the change to whole genome sequencing there is a need to develop new methods for source attribution based on sequencing data. Four European datasets collected in Denmark (DK), Germany (DE), the United Kingdom (UK) and France (FR) are presented in this descriptor. The datasets contain sequenced samples of Salmonella Typhimurium and its monophasic variants isolated from human, food, animal and the environment. The objective of the datasets was either to attribute the human salmonellosis cases to animal reservoirs or to investigate contamination of the environment by attributing the environmental isolates to different animal reservoirs. ISSN: 20524463

European Food Safety Authority (EFSA)

Technical specifications on a randomisation of sampling for the purpose of antimicrobial resistance monitoring from food-producing animals and food as from 2021 (2020) EFSA Journal, 18 (12), art. no. e06364, .

ABSTRACT: To monitor antimicrobial resistance in zoonotic and indicator bacteria from food-producing animal populations and meat thereof under Decision 2020/1729, a guidance for randomised sampling procedures is provided. Prospective and retrospective sampling plans for samples and isolates are addressed. The former involves collecting sufficient numbers of representative animal and food samples from which recovered isolates are tested for antimicrobial susceptibility; the latter involves selecting randomly Salmonella isolates from collections constituted within the framework of the national control programmes in poultry flocks. A generic proportionate stratified sampling process and numerical illustrations of proportional allocation are provided. Stratified sampling of Salmonella isolates from poultry primary productions is performed with proportional allocation to the size of the isolate collections available in official laboratories. An alternative approach would be a simple random sampling within the sampling frame of flocks positive for Salmonella. Stratified sampling of caecal samples, accounting for at least 60% of the domestic production of food-producing animal populations monitored, with proportionate allocation to the slaughterhouse production, allows for the collection of representative isolates of Campylobacter and indicator E. coli and enterococci in various

animal populations. Sampling of different chilled fresh meat categories is performed at retail outlets serving the final consumer, with proportional allocation of the number of samples to the population of geographical areas accounting for at least 80% of the national population, to test for the presence of ESBL-/AmpC-/carbapenemase-producing *E. coli*. Stratified sampling of imported fresh meat is performed at border control posts, with proportional allocation to the number of consignments and origin to test *Salmonella* and indicator *E. coli* for antimicrobial susceptibility, and to test for the presence of ESBL-/AmpC-/carbapenemase-producing *E. coli*. The corresponding sampling design is based on the reliable existing TRACES statistics, and the effect of the UK leaving the EU cannot be considered at this stage because of the major uncertainties still associated with it. These technical specifications should be updated as needed based on the first monitoring campaigns and trends in AMR. ISSN: 18314732

Banerji, S., Simon, S., Tille, A., Fruth, A., Flieger, A.

Genome-based Salmonella serotyping as the new gold standard (2020) Scientific Reports, 10 (1), art. no. 4333, .

ABSTRACT: *Salmonella enterica* is the second most reported bacterial cause of food-borne infections in Europe. Therefore molecular surveillance activities based on pathogen subtyping are an important measure of controlling Salmonellosis by public health agencies. In Germany, at the federal level, this work is carried out by the National Reference Center for *Salmonella* and other Bacterial Enteric Pathogens (NRC). With rise of next generation sequencing techniques, the NRC has introduced whole-genome-based typing methods for *S. enterica* in 2016. In this study we report on the feasibility of genome-based in silico serotyping in the German setting using raw sequence reads. We found that SeqSero and seven gene MLST showed 98% and 95% concordance, respectively, with classical serotyping for the here evaluated serotypes, including the most common German serotypes *S. Enteritidis* and *S. Typhimurium* as well as less frequently found serotypes. The level of concordance increased to >99% when the results of both in silico methods were combined. However, both tools exhibited misidentification of monophasic variants, in particular monophasic *S. Typhimurium* and therefore need to be fine-tuned for reliable detection of this epidemiologically important variant. We conclude that with adjustments *Salmonella* genome-based serotyping might become the new gold standard. ISSN: 20452322

Fonseca, J.M., Ravishankar, S., Sanchez, C.A., Park, E., Nolte, K.D.

Assessing the food safety risk posed by birds entering leafy greens fields in the US southwest

(2020) International Journal of Environmental Research and Public Health, 17 (23), art. no. 8711, pp. 1-16.

ABSTRACT: In the US Southwest, it is common to observe birds in leafy green fields, though the risk they contribute to foodborne outbreaks remains unclear. In this study, we investigated and recorded the relationship between birds near leafy green fields and the risk for contaminated irrigation water or leafy green plants. We monitored the presence of birds for over two years and performed cloacal swab analysis for non-pathogenic *Escherichia coli*, *E. coli* O157:H7 and *Salmonella enterica*, while also monitoring the incidence of other microbial indicators. We also assessed the risks from bird feces by performing observations in a commercial field reported with *Salmonella* positive samples and by analyzing the survival of foodborne pathogens in bird feces. Our results showed that most of the birds near the crop fields were resident small birds. We did not observe a correlation between the number of birds in sites and the incidence of indicator bacteria (e.g., coliforms, *E. coli*) in irrigation canal water, with the exception of one out of four sites where water flow was low or stagnant. Using walk-in-traps, 305 birds were captured and placed in short-term captivity to determine the presence of various bacteria. None of the birds tested positive for *E. coli* O157:H7 or *Salmonella*. However, nearly 40% of the birds captured were confirmed positive for non-pathogenic *E. coli*. We found no correlation between age (young, adult, unknown), gender (male, female, unknown) and the incidence of *E. coli* positive birds, but we observed significantly higher probability of incidence during October–December. The role of relative humidity and temperature on bacterial survival appeared to play a key role in the survival of *Salmonella* on the leaves of spinach plants in a commercial field. This was also confirmed in laboratory conditions where *Salmonella* inoculated in bird feces and exposed to 15° C and 80% RH (Relative humidity) survived beyond 133 days, while at 26° C and 40% RH, the organism was undetectable after 63 days. Our results suggest that local birds associated with leafy green fields likely pose a minimal impact of risk for food contamination, but also points out the need for increased analysis specifically for *E. coli* O157:H7. Furthermore, our study suggests the need for expanding research that addresses risks associated with large migratory birds, especially

in areas where stagnated water sources would be used for overhead sprinkle irrigation.
ISSN: 16617827

Chadwick, E.V., MacKay, L., Krehling, J.T., MacKlin, K.S.

Inoculation Route and Serotype Alter Salmonella Recovery in the Broiler's Digestive Tract (2020) Avian Diseases, 64 (4), pp. 467-470.

ABSTRACT: *Salmonella enterica* serotypes Enteritidis (SE) and Heidelberg (SH) are consistently linked to poultry-related foodborne outbreaks and can be isolated from broiler parts in processing facilities. In order to control this pathogen's establishment in the broiler, entryways at the farm that lead to colonization must be considered. The objective of these trials was to determine if the inoculation route of either SE or SH altered its recovery in a market-age broiler's digestive tract if chicks were dosed on day of hatch. Chicks were given a 10⁴ colony-forming units inoculation of SE or SH on day 0 via one of five inoculation routes (oral, intratracheal, subcutaneous, ocular, or cloacal) and then placed in pens (60-100 chicks/treatment). Broilers were reared for 32-36 days, then euthanized, and samples of trachea, crop, liver and spleen (pooled), cecum, and a cloacal swab were collected. Samples were enriched and then analyzed on yes/no criteria based on *Salmonella* growth. Data were analyzed in JMP Pro 14.1 using the GLM procedure with the Student t-test to separate serotype means and a Tukey honestly significant difference test to separate inoculation means ($P \leq 0.05$). All samples collected and all inoculation routes resulted in recovery of either serotype. The intratracheal inoculation, mimicking inhaled fomites, resulted in significantly higher recovery of *Salmonella* serotypes than did the other inoculation routes ($P < 0.0001$), indicating the importance of controlling respiratory contamination. When comparing serotypes, there was a significantly greater recovery of SH compared to SE based on samples collected ($P = 0.001$). SH also had significantly greater recovery from the cecum ($P < 0.001$) and the cloacal swab ($P = 0.02$). These trials indicate the need for further investigation of the intratracheal route, as well as reinforcing that the potential of systemic infection through grow out with either serotype is highly probable preharvest. ISSN: 00052086

Maes, S., De Reu, K., Van Weyenberg, S., Lories, B., Heyndrickx, M., Steenackers, H.

Pseudomonas putida as a potential biocontrol agent against *Salmonella* Java biofilm formation in the drinking water system of broiler houses (2020) *BMC Microbiology*, 20 (1), art. no. 373, .

ABSTRACT: Background: Environmental biofilms can induce attachment and protection of other microorganisms including pathogens, but can also prevent them from invasion and colonization. This opens the possibility for so-called biocontrol strategies, wherein microorganisms are applied to control the presence of other microbes. The potential for both positive and negative interactions between microbes, however, raises the need for in depth characterization of the sociobiology of candidate biocontrol agents (BCAs). The inside of the drinking water system (DWS) of broiler houses is an interesting niche to apply BCAs, because contamination of these systems with pathogens plays an important role in the infection of broiler chickens and consequently humans. In this study, *Pseudomonas putida*, which is part of the natural microbiota in the DWS of broiler houses, was evaluated as BCA against the broiler pathogen *Salmonella* Java. Results: To study the interaction between these species, an in vitro model was developed simulating biofilm formation in the drinking water system of broilers. Dual-species biofilms of *P. putida* strains P1, P2, and P3 with *S. Java* were characterized by competitive interactions, independent of *P. putida* strain, *S. Java* inoculum density and application order. When equal inocula of *S. Java* and *P. putida* strains P1 or P3 were simultaneously applied, the interaction was characterized by mutual inhibition, whereas *P. putida* strain P2 showed an exploitation of *S. Java*. Lowering the inoculum density of *S. Java* changed the interaction with *P. putida* strain P3 also into an exploitation of *S. Java*. A further increase in *S. Java* inhibition was established by *P. putida* strain P3 forming a mature biofilm before applying *S. Java*. Conclusions: This study provides the first results showing the potential of *P. putida* as BCA against *S. Java* in the broiler environment. Future work should include more complex microbial communities residing in the DWS, additional *Salmonella* strains as well as chemicals typically used to clean and disinfect the system. ISSN: 14712180

Cocciolo, G., Circella, E., Pugliese, N., Lupini, C., Mescolini, G., Catelli, E., Borchert-Stuhlträger, M., Zoller, H., Thomas, E., Camarda, A.

Evidence of vector borne transmission of Salmonella enterica enterica serovar Gallinarum and fowl typhoid disease mediated by the poultry red mite, Dermanyssus gallinae (De Geer, 1778) (2020) Parasites and Vectors, 13 (1), art. no. 513, .

ABSTRACT: Background: The poultry red mite *Dermanyssus gallinae* (De Geer, 1778) is a major ectoparasite of poultry. Infestations are found in most laying hen farms in Europe, and breeder flocks have also been reported to be affected. Mite infestation has detrimental effects on animal welfare, it causes significant economic losses, and, additionally, *D. gallinae* is often considered as a vector for pathogens. Despite suspicion of a close relationship between the poultry red mite and *Salmonella enterica enterica* serovar Gallinarum biovar Gallinarum (serovar Gallinarum), the causative agent of fowl typhoid disease (FT), there has been no definitive proof of mite-mediated transmission. Therefore, an investigation was conducted to determine if *D. gallinae*-mediated transmission of serovar Gallinarum could be demonstrated among four different hen groups. Methods: Two groups of 8 hens (A and B) were experimentally infected with serovar Gallinarum in two isolators. After 7 days, when birds showed signs of FT, about 25,000 mites were introduced. After 3 days, mites were harvested and used to infest two other hen groups of 8 (C and D), in two separate isolators. The health status of hens was constantly monitored; detection and quantification of serovar Gallinarum were performed by PCR and qPCR from mites and organs of dead hens. The maximum likelihood estimation of the infection rate and mite vectorial capacity were calculated. Results: Clinical disease was observed in groups infected with serovar Gallinarum (A and B) and in hens of groups C and D infested with mites harvested from the isolators containing groups A and B. In all four groups, serovar Gallinarum was detected from liver, spleen, ovary, and cecum of hens, thus confirming the diagnosis of FT. Mite analysis demonstrated the presence of the pathogen, with an estimated infection rate ranging between 13.72 and 55.21 infected per thousand mites. Vectorial capacity was estimated to be 73.79. Conclusions: Mites harvested from birds infected with serovar Gallinarum were shown to carry the mite, and then to transfer serovar Gallinarum to isolated groups of pathogen-free birds that subsequently showed signs of FT. Mite vectorial capacity was high, demonstrating that *D. gallinae* should be considered an effective vector of FT. [Figure not available: see fulltext.]
ISSN: 17563305

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Field investigations of multidrug-resistant Salmonella Infantis epidemic strain incursions into broiler flocks in England and Wales
(2020) *Avian Pathology*, 49 (6), pp. 631-641.

ABSTRACT: *Salmonella Infantis* is a major public health concern and has become established in the broiler sector in some European countries, as well as globally, and is frequently multidrug resistant (MDR). Three broiler farms in England and Wales, which had incursions of MDR *S. Infantis* between 2013 and 2017, were investigated longitudinally. The company feed mill and two associated hatcheries were intensively sampled. Following each visit, advice on cleaning, disinfection and other control measures for *Salmonella* was given to help eliminate *S. Infantis* from the premises. Four samples collected from inside the broiler houses after cleaning and disinfection were *Salmonella*-positive, indicating cleaning and disinfection within houses was generally effective. However, the exterior of persistently infected houses remained substantially contaminated and feeding systems could not be sampled. Clearance of *S. Infantis* from affected houses requires additional attention to decontamination of these aspects. Sixty *S. Infantis* isolates were tested for antimicrobial susceptibility by disk diffusion tests. All isolates were MDR, with resistance to at least nalidixic acid (Na), tetracycline (T), compound sulphonamide (Su), streptomycin (S) and furazolidone. This is a similar resistance pattern to the previously identified MDR (NaSSuT) clone in some European countries. The study shows that to remove *S. Infantis* from premises effectively, a combined approach to poultry houses and the surrounding farm environment is necessary. A revised cleaning and disinfection programme was developed that was associated with the clearance of MDR *S. Infantis* from persistently infected and newly infected broiler flocks, and UK livestock remains free of MDR *S. Infantis*. **RESEARCH HIGHLIGHTS** Standard cleaning and disinfection protocols did not completely eliminate infection. A revised cleaning and disinfection programme was developed. Disinfecting feeder lines and external areas was key to eliminating *S. Infantis*. Identified similar antimicrobial resistance pattern to MDR epidemic *S. Infantis*.
ISSN: 03079457