

NEWSLETTER

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Editorial Note

Bilthoven, 1 October 2019

Dear colleague,

I hope you had a nice and relaxing summer time. Currently, fall has started with wind and rain here in the Netherlands. In fact quite normal circumstances, but we need to get used to it again after some very hot periods during summer.

In the previous Newsletter we informed you that the DIS voting of Amendment 1 to EN ISO 6579-1:2017 (**ISO 6579-1:2017/DAmD 1:2019**) started on 7 July. The balloting closed on 30 September, and I was just informed that the voting was 100% positive (no negative votes), with only 4 pages of editorial comments. This is a very good result! Quite likely, we can then go to the final publication immediately after introducing the (relevant) editorial comments. We will keep you posted about the progress with the document.

Also in the previous Newsletter we informed you about the timetables of the **Proficiency Tests** organised in fall of this year. The PT for detection of *Salmonella* in samples from the primary production stage is currently running and the PT on typing of *Salmonella* will be organised in November. The timetable of this latter study is included again in this Newsletter. Please be reminded that the PT on typing of *Salmonella* includes a new, voluntary part for molecular sub-typing to determine whether some strains concern common types (cluster analysis). The (molecular) method used is free of choice (e.g. PFGE, MLVA, WGS). However, we will only organise this part of the study when we receive a sufficient number of applications (>7). Therefore, make sure that you subscribe to this part of the study when you are interested. As said, this part of the study is voluntary and the performance of the NRLs-*Salmonella* will not be judged on basis of the outcome of this part of the study.

In July we have sent you, for information, the second version of the Guidance Document for the organisation of Proficiency Tests by NRLs for national networks, including partial outsourcing. The document was drafted by 5 EURLs and updated compared to Version 1, taking into account the comments of the 5 NRL networks. The document gives guidance on the choice of technical criteria for NRLs to organise PTs on the following analyses, depending on the NRL mandate:

- Detection of *Campylobacter*, *Listeria monocytogenes*, *Salmonella* and STEC in the food chain;
- Enumeration of *Campylobacter*, coagulase positive staphylococci and *Listeria monocytogenes* in the food chain;
- (Sero)Typing of *Campylobacter*, *Salmonella*, *Listeria monocytogenes*, coagulase positive staphylococci and STEC.

The content of the guidance document is based upon EN ISO 22117.

For your information, the following article was published in July of this year:

Roan Pijnacker, Timothy J Dallman, Aloys S L Tijsma, Gillian Hawkins, Lesley Larkin, Saara M Kotila, Giusi Amore, Ettore Amato, Pamina M Suzuki, Sarah Denayer, Sofieke Klamer, Judit Pászti, Jacquelyn McCormick, Hassan Hartman, Gareth J Hughes, Lin C T Brandal, Derek Brown, Joël Mossong, Cecilia Jernberg, Luise Müller, Daniel Palm, Ettore Severi, Joanna Gołębiowska, Blaženka Hunjak, Slawomir Owczarek, Simon Le Hello, Patricia Garvey, Kirsten Mooijman, Ingrid H M Friesema, Coen van der Weijden, Menno van der Voort,

Valentina Rizzi, Eelco Franz, on behalf of the International Outbreak Investigation Team. An international outbreak of *Salmonella enterica* serotype Enteritidis linked to eggs from Poland: a microbiological and epidemiological study. The Lancet Infectious Diseases, Vol 19, Issue, pp 778-786, July 2019.

Best wishes,
Kirsten Mooijman
Coordinator EURL-*Salmonella*

Contribution of the EURL-*Salmonella*

Timetable EURL-*Salmonella* Proficiency Test Typing 2019

Week	Date	Topic
39	23 September	Emailing of the link to the registration form for the typing study. Please register by 18 October at the latest.
43	21-25 October	Emailing of the protocol 2019.
45	4-8 November	Shipment of the parcels to the participants as Biological Substance Category B (UN 3373). If you did not receive the parcel by 8 November , please contact the EURL- <i>Salmonella</i> .
45	4-8 November	Sending the link for the web based test report on serotyping to the participants.
45	4-8 November	Sending the link for the web based test report on PFGE and/or MLVA and/or WGS cluster analysis to the participants in a separate email.
45	4-8 November	<i>Upon receipt</i> : Starting the identification of the strains, according to the usual practice of the laboratory.
50	13 December 2019 at the latest	Deadline for completing the electronic submission of serotyping results: 13 December 2019 After this deadline, the electronic submission form for serotyping results will be closed.
	31 January 2020 at the latest	Deadline for completing the electronic submission of PFGE/MLVA/WGS cluster analysis results: 31 January 2020
	February 2020	Serotyping: Reporting of individual laboratory results and Interim Summary Report.
	April/May 2020	Pilot PFGE/MLVA/WGS cluster analysis: Reporting of individual laboratory results and Summary Report.
	Summer 2020	Final report.

From the Literature

Salmonella-related Literature from Scopus: July – September 2019

McWhorter, A.R., Chousalkar, K.K.

From hatch to egg grading: Monitoring of Salmonella shedding in free-range egg production systems

(2019) *Veterinary Research*, 50 (1), art. no. 58, .

ABSTRACT: Human cases of salmonellosis are frequently linked with the consumption of contaminated table eggs. Recently, there has been an increase in consumer demand for cage-free eggs precipitating the need for a greater understanding of Salmonella dynamics in free-range production systems. A longitudinal study was conducted to determine the points in production where birds are most likely to be exposed to Salmonella and where the risk of egg contamination is highest. In this study, two free-range flocks were sampled from hatch to the end of production. At hatch, all chicks were Salmonella negative and remained negative during rearing. During production, the proportion of positive samples was low on both farms. Salmonella positive samples were detected intermittently for Flock A. Dust, nest box, and egg belt swabs had the highest proportion of positive samples and highest overall loads of Salmonella. The egg grading floor was swabbed at different points following the processing of eggs from Flock A. Only the suction cups that handle eggs prior to egg washing tested positive for Salmonella. Swabs collected from machinery handling eggs after washing were Salmonella negative. During production, positive samples from Flock B were observed at only single time point. Dust has been implicated as a source of Salmonella that can lead to flock to flock contamination. Bulk dust samples were collected and tested for Salmonella. The proportion of positive dust samples was low and is likely due to physical parameters which are not likely to support the survival of Salmonella in the environment. ISSN: 09284249

Kaldalu, N., Tenson, T.

Slow growth causes bacterial persistence

(2019) *Science Signaling*, 12 (592), art. no. eaay1167, .

ABSTRACT: Bacterial persisters survive antibiotic treatment and can cause chronic infections. In this issue of Science Signaling, Pontes and Groisman suggest that there is no specific molecular pathway responsible for persister formation in Salmonella and that slow growth is the decisive factor. ISSN: 19450877

Ferrari, R.G., Rosario, D.K.A., Cunha-Neto, A., Mano, S.B., Figueiredo, E.E.S., Conte-Junior, C.A.

Worldwide Epidemiology of Salmonella Serovars in Animal-Based Foods: a Meta-analysis

(2019) *Applied and environmental microbiology*, 85 (14), .

ABSTRACT: Salmonella spp. are among the most important foodborne pathogens and the third leading cause of human death among diarrheal diseases worldwide. Animals are the primary source of this pathogen, and animal-based foods are the main transmission route to humans. Thus, understanding the global epidemiology of Salmonella serovars is key to controlling and monitoring this bacterium. In this context, this study aimed to evaluate the prevalence and diversity of Salmonella enterica serovars in animal-based foods (beef, pork, poultry, and seafood) throughout the five continents (Africa, the Americas [North and Latin America], Asia, Europe, and Oceania). The meta-analysis consisted of a chemometric assessment (hierarchical cluster analysis and principal component analysis) to identify the main epidemiological findings, including the prevalence and diversity of the Salmonella serovars in each matrix. Regarding the serovar distribution, S Typhimurium presented a cosmopolitan distribution, reported in all four assessed matrices and continents; poultry continues to play a central role in the dissemination of the Enteritidis serovar to humans, and Anatum and Weltevreden were the most frequently found in beef and seafood, respectively. Additionally, we recommended careful monitoring of certain serovars, such as Derby, Agona, Infantis, and Kentucky. Finally, given the scientific data regarding the most frequently reported serovars and which matrices constitute the main vehicles for the transmission of this pathogen, control programs may be improved, and specific interventions may be implemented in an attempt to reduce the risk of this pathogen reaching humans. IMPORTANCE Salmonellosis is caused by Salmonella spp. and is the third leading cause of death among food-transmitted diseases. This pathogen is commonly disseminated in domestic and wild animals, and the infection's symptoms are characterized by acute fever, nausea, abdominal pain, and diarrhea. The animals are the

primary source of salmonellae, and animal-based foods are the main transmission route to humans. Therefore, data collected from these sources could contribute to future global interventions for effective control and surveillance of *Salmonella* along the food chain. In light of this, the importance of our research is in identifying the prevalence of *Salmonella* serovars in four animal-based food matrices (pork, poultry, beef, and seafood) and to evaluate the importance that each matrix has as the primary source of this pathogen to humans. Copyright ISSN: 10985336

Crabb, H.K., Allen, J.L., Devlin, J.M., Wilks, C.R., Gilkerson, J.R.

Spatial Distribution of Salmonella enterica in Poultry Shed Environments Observed by Intensive Longitudinal Environmental Sampling
(2019) *Applied and environmental microbiology*, 85 (14), .

ABSTRACT: Detection of salmonellae within poultry environments is an important component of many food safety programs, but sampling approaches vary greatly and may not enable the detection of salmonellae when bacteria are present at a low prevalence or concentration. Intensive longitudinal sampling within caged sheds enabled us to undertake a longitudinal analysis of the spatial distribution of salmonellae in caged shed environments. Both the number of samples collected and location of sample collection within a poultry shed were important to ensure the best chance of detecting *Salmonella* spp. Differences in the within-shed spatial distribution of *Salmonella enterica* subspecies *enterica* serovar Typhimurium [$\chi^2(27, 1,538) = 54.4; P < 0.001$] and *Salmonella enterica* subspecies *enterica* serovar Infantis [$\chi^2(27, 1,538) = 79.8; P < 0.0001$] were identified. More than one *Salmonella enterica* serovar was detected in each shed on the same sampling occasion; 5% of all samples contained more than one serovar. Samples collected on the north side of the shed (odds ratio [OR], 1.77; 95% confidence interval [CI], 1.17-2.68), on the sheltered side of the shed (OR, 1.90; 95% CI, 1.26-2.89), and during winter (OR, 48.41; 95% CI, 23.56-104.19) were more likely to be positive for salmonellae. The within-shed differences observed in the both the sample prevalence and spatial location of the serovar detected indicate that there are important shed microenvironmental factors that influence the survival and/or distribution of salmonellae. These factors should be taken into consideration when environmental surveillance is undertaken for salmonellae in flocks housed in cage sheds. IMPORTANCE Routine epidemiological surveillance for salmonellae in poultry relies initially on environmental sampling. Intensive, spatially homogenous sampling, as conducted within this study, confirmed that the sampling methodology conducted within a poultry environment is a nontrivial part of sampling design. The frequency of sampling is especially important when the prevalence of *Salmonella* spp. is low. These factors must be taken into consideration in the design of studies for the detection of salmonellae in poultry sheds. ISSN: 10985336

Said, M.B., Saad, M.B., Achouri, F., Bousselmi, L., Ghrabi, A.

Detection of active pathogenic bacteria under stress conditions using lytic and specific phage
(2019) *Water Science and Technology*, 80 (2), pp. 282-289.

ABSTRACT: In this study, we have monitored the potential activity of a foodborne and waterborne pathogenic bacterium, *Salmonella typhi*, under starvation conditions. The interaction between lytic phage and starved-VBNC pathogenic bacteria was studied to establish reliable methods for the detection of active cells before resuscitation. The analysis of phage kinetic parameters has demonstrated the flexibility of lytic with the quantity and mainly the quality of host cells. After 2 h of phage-starved-VBNC bacteria interaction, the reduction of phage amplification rate can reveal the ability of specific-lytic phage to recognize and to attach to their host cells with a probability of burst and release of infectious phages by active bacteria. After an extension of the latent period, the boost of the phage amplification rate was directly related to the positive interaction between potential intracellular 'engaged' phages and potential active bacteria. Furthermore, the modeling of the *Salmonella*-specific phage growth cycle in relationship with starved host cells can highlight the impact of the viability and the activity state of the host cells on the phage's growth cycle. ISSN: 02731223

Niemi, J.K., Heinola, K., Simola, M., Tuominen, P.

Salmonella control programme of pig feeds is financially beneficial in Finland
(2019) *Frontiers in Veterinary Science*, 6 (JUL), art. no. 200, .

ABSTRACT: To promote public health, Finland has adopted a stringent *Salmonella* control policy. However, the rationale of *Salmonella* control in pig feeds has been debated after a European Union (EU)-wide cost-benefit analysis, which provided mixed, country-specific results on whether control measures are economically beneficial. The aim of this study was to analyze the costs and benefits of current pig feed *Salmonella* control in Finland

compared to a reduced control scenario. In addition, this study contributes to the literature by looking at the costs across stakeholder groups. The costs of preventive and monitoring measures were assessed, and a Monte Carlo model was developed to simulate costs caused by *Salmonella* contaminations along the pork supply chain (including feed importation, commercial feed manufacturing, feed transportation, mobile feedmixers, pig farms, slaughterhouses) and because of human salmonellosis originating from contaminated feed. The data were collected from official records and feed sector operators by surveys and interviews. The prevalence of *Salmonella* was obtained from a previously conducted risk assessment study. The total costs of pig feed *Salmonella* control were estimated on average to be €4.2–5.4 million per year (95% of simulated years between €2.1 and €9.1 million) for the current control scenario, and €33.8–34.8 million per year (95% €2.2 to €26.0 million) for the reduced control scenario. In the reduced control scenario, the monitoring and prevention costs were decreased down to €1.1–2.1 million, and the costs of *Salmonella* contaminations and human salmonellosis were up by €32.7 million when compared to the current control scenario. The results suggest that the current pig feed *Salmonella* control policy of Finland is economically profitable. It can reduce the costs caused by feed-related *Salmonella* contaminations on average by €29.4 million per year and provides public health benefits. Pig feed *Salmonella* control can support the effectiveness of the Finnish *Salmonella* Control Programme. The current pig feed *Salmonella* control policy benefits the consumers, while a substantial part of the costs are covered by feed operators. In order to increase the acceptability of current policy, greater attention to the allocation of financial responsibilities regarding the control measures may be required. ISSN: 22971769

Souza, M.N., Lehmann, F.K.M., De Carli, S., Kipper, D., Fonseca, A.S.K., Ikuta, N., Lunge, V.R.

Molecular detection of Salmonella serovars Enteritidis, Heidelberg and Typhimurium directly from pre-enriched poultry samples
(2019) *British Poultry Science*, 60 (4), pp. 388-394.

ABSTRACT: 1. *Salmonella* is one of the most important pathogens in public health and it is usually associated with food-borne diseases. *Salmonella* serovars Enteritidis and Typhimurium are widespread in the world with outbreaks frequently associated with consumption of poultry products; furthermore, there is an increasing public health concern with the wide dissemination of the serovar Heidelberg in poultry flocks. 2. The aim of the experiment was to develop and to validate rapid methods to detect *Salmonella* serovars Enteritidis, Typhimurium, and Heidelberg by real-time PCRs and test isolates from pre-enriched poultry samples. 3. Three real-time PCRs were developed and used in combination to detect the serovars Enteritidis, Typhimurium and Heidelberg. These assays were validated by the analysis of 126 *Salmonella* isolates, eight other enteric bacterial species and 34 naturally contaminated poultry samples after pre-enrichment with buffered peptone water (BPW). 4. Real-time PCRs detected the isolates of the most important poultry serovars (Enteritidis, Typhimurium and Heidelberg) with 100% inclusivity and exclusivity in each assay. The PCR identified monophasic variants of the serovars Typhimurium and Heidelberg. All PCRs were validated in detecting these specific serovars directly from pre-enriched poultry samples. The whole analytical procedure was performed in less than 24 h in a veterinary diagnostic laboratory. ISSN: 00071668

Mensah, N., Tang, Y., Cawthraw, S., Abuoun, M., Fenner, J., Thomson, N.R., Mather, A.E., Petrovska-Holmes, L.

Determining antimicrobial susceptibility in Salmonella enterica serovar Typhimurium through whole genome sequencing: A comparison against multiple phenotypic susceptibility testing methods
(2019) *BMC Microbiology*, 19 (1), art. no. 148, .

ABSTRACT: Background: UK public health organisations perform routine antimicrobial susceptibility tests (ASTs) to characterise the potential for antimicrobial resistance in *Salmonella enterica* serovars. Genetic determinants of these resistance mechanisms are detectable by whole genome sequencing (WGS), however the viability of WGS-based genotyping as an alternative resistance screening tool remains uncertain. We compared WGS-based genotyping, disk diffusion and agar dilution to the broth microdilution reference AST for 102 *Salmonella enterica* serovar Typhimurium (*S. Typhimurium*) isolates across 11 antimicrobial compounds. Results: Genotyping concordance, interpreted using epidemiological cut-offs (ECOFFs), was 89.8% (1007/1122) with 0.83 sensitivity and 0.96 specificity. For seven antimicrobials interpreted using *Salmonella* clinical breakpoints, genotyping produced 0.84 sensitivity and 0.88 specificity. Although less accurate than disk diffusion (0.94 sensitivity, 0.93 specificity) and agar dilution (0.83 sensitivity, 0.98 specificity), genotyping performance improved to 0.89 sensitivity and 0.97 specificity when

two antimicrobials with relatively high very major error rates were excluded (streptomycin and sulfamethoxazole). Conclusions: An 89.8% concordance from WGS-based AST predictions using ECOFF interpretations suggest that WGS would serve as an effective screening tool for the tracking of antimicrobial resistance mechanisms in *S. Typhimurium*. For use as a standalone clinical diagnostic screen, further work is required to reduce the error rates for specific antimicrobials. ISSN: 14712180

Peeters, L., Dewulf, J., Boyen, F., Brossé, C., Vandersmissen, T., Rasschaert, G., Heyndrickx, M., Cargnel, M., Pasmans, F., Maes, D.

Effects of attenuated vaccine protocols against Salmonella Typhimurium on Salmonella serology in subclinically infected pig herds

ABSTRACT: Vaccination of pigs against *Salmonella Typhimurium* (*S. Typhimurium*) can be effective for the control of *Salmonella* infections at the farm level and reduce the risk of *Salmonella* contamination in the food chain. However, vaccination may interfere with herd serological status in serology-based *Salmonella* monitoring programs. The present study investigated the effects of an attenuated *S. Typhimurium* vaccine (Salmoporc, IDT Biologika) on *Salmonella* serology in sows, neonatal piglets and slaughter pigs from three subclinically infected herds. Within each herd, five different vaccination protocols were tested as follows: group 1, vaccination of sows; group 2, vaccination of sows and piglets; group 3, vaccination of sows and fattening pigs; group 4, vaccination of piglets; and group 5 vaccination of fattening pigs. Each group was compared to a non-vaccinated control group (group 6). Sera were analyzed by ELISA (HerdChek Swine *Salmonella*, IDEXX Laboratories) and sample-to-positive (S/P) ratios were calculated. At day 3 after farrowing, but not before vaccination, S/P ratios in vaccinated sows (mean: 2.21) were significantly higher than S/P ratios in non-vaccinated sows (mean: 0.87, $P < 0.001$). S/P ratios in 3-day old piglets from vaccinated sows (mean: 2.46) were significantly higher than S/P ratios in similar piglets from non-vaccinated sows (mean: 0.73, $P < 0.001$). At slaughter, S/P ratios in pigs from groups 2, 3, 4 and 5 were significantly higher than those in the non-vaccinated control group ($P < 0.001$). Therefore, vaccination of piglets and fattening pigs could have implications for current serology-based *Salmonella* monitoring programs in slaughter pigs. ISSN: 10900233

Gil, M., Duma-Kocan, P., Rudy, M., Stanistawczyk, R.

Salmonella serovars in food according to rasff system notifications in 2000-2017 [Serowary Salmonella w zywosci wg powiadomien systemu rasff w latach 2000-2017] (2019) Medycyna Weterynaryjna, 75 (7), pp. 394-397.

ABSTRACT: The article concerns the occurrence of *Salmonella* bacilli in various food groups in the years 2000-2017 and an analysis of the incidence of its serovars. The analysis was based on data collected in the RASFF system regarding notifications submitted as a result of the presence of salmonella in food. In the analyzed period, the most frequent serovars were *S. Typhimurium* (503 notifications), *S. Enteritidis* (401 notifications) and *S. Infantis* (106 notifications). A disturbing phenomenon is a marked increase in the frequency of occurrence of *Salmonella* in the analyzed food. To a large extent this situation resulted from the increase in the frequency of *Salmonella* detection in poultry and poultry meat products, as well as more frequent occurrence in fruits and vegetables, herbs and spices as well as in nuts and their products, and in seeds. ISSN: 00258628

Prestes, F.S., da Silva, A.C.M., Pereira, A.A.M., Nascimento, M.D.S.D.

Impact of peanut roasting on Salmonella spp. survival (2019) LWT, 108, pp. 168-173.

ABSTRACT: This study evaluated the efficiency of the dry and oil roasting processes in the inactivation of a pool of five serotypes of *Salmonella* isolated from the peanut supply chain (Miami, Muenster, Yoruba, Javiana and Glostrup). The Weibull model was fitted to the data to describe the thermal inactivation of *Salmonella* in peanut matrices. The time to achieve 5-log reduction of the pathogen during dry roasting at 125 °C was significantly different between the matrices ($p < 0.05$), being 1.5-fold more on the blanched peanuts than on the in-shell peanuts. Only the 145 and 160 °C protocols resulted in reductions > 5 -log MPN/g of *Salmonella* for both matrices, with T_{5d} of 56 and 39 min for blanched peanuts and 46 and 44 min for in-shell peanuts, respectively. For the oil roasting, the Weibull model predicted the first decimal reduction after 7.2 s and 0.012 s at 115 °C and 145 °C, respectively. To achieve reduction of 5 log *Salmonella* it would take 3.0 min at 115 °C, and 0.40 min at 145 °C. The results demonstrated that temperature, heat process and in some cases the type of matrix influence the thermal resistance of *Salmonella* on peanuts. ISSN: 00236438

Arruda, B.L., Burrough, E.R., Schwartz, K.J.

Salmonella enterica I 4,[5],12:I:- associated with lesions typical of swine enteric salmonellosis

(2019) *Emerging Infectious Diseases*, 25 (7), pp. 1377-1379.

ABSTRACT: *Salmonella enterica* serotype I 4,[5],12:i:- has been increasingly isolated from swine. However, its pathogenic potential is not well characterized. Analysis of swine cases confirmed a strong positive association between isolation of I 4,[5],12:i:- and lesions of enteric salmonellosis and suggested a similar pathogenic potential as that for *Salmonella* Typhimurium. ISSN: 10806040

Lacharme-Lora, L., Owen, S.V., Blundell, R., Canals, R., Wenner, N., Perez-Sepulveda, B., Fong, W.Y., Gilroy, R., Wigley, P., Hinton, J.C.D.

The use of chicken and insect infection models to assess the virulence of African Salmonella Typhimurium ST313

(2019) *PLoS Neglected Tropical Diseases*, 13 (7), art. no. e0007540, .

ABSTRACT: Over recent decades, *Salmonella* infection research has predominantly relied on murine infection models. However, in many cases the infection phenotypes of *Salmonella* pathovars in mice do not recapitulate human disease. For example, *Salmonella* Typhimurium ST313 is associated with enhanced invasive infection of immunocompromised people in Africa, but infection of mice and other animal models with ST313 have not consistently reproduced this invasive phenotype. The introduction of alternative infection models could help to improve the quality and reproducibility of pathogenesis research by facilitating larger-scale experiments. To investigate the virulence of *S. Typhimurium* ST313 in comparison with ST19, a combination of avian and insect disease models were used. We performed experimental infections in five lines of inbred and one line of outbred chickens, as well as in the alternative chick embryo and *Galleria mellonella* wax moth larvae models. This extensive set of experiments identified broadly similar patterns of disease caused by the African and global pathovariants of *Salmonella* Typhimurium in the chicken, the chicken embryo and insect models. A comprehensive analysis of all the chicken infection experiments revealed that the African ST313 isolate D23580 had a subtle phenotype of reduced levels of organ colonisation in inbred chickens, relative to ST19 strain 4/74. ST313 isolate D23580 also caused reduced mortality in chicken embryos and insect larvae, when compared with ST19 4/74. We conclude that these three infection models do not reproduce the characteristics of the systemic disease caused by *S. Typhimurium* ST313 in humans. ISSN: 19352727

Sahu, B., Singh, S.D., Behera, B.K., Panda, S.K., Das, A., Parida, P.K.

Rapid detection of Salmonella contamination in seafoods using multiplex PCR

(2019) *Brazilian Journal of Microbiology*, 50 (3), pp. 807-816.

ABSTRACT: Effective monitoring of *Salmonella* contamination in seafood processing to conform the requirements of HACCP is a great challenge today. Such challenges can be effectively addressed, if the conventional detection methods are replaced with DNA-based molecular methods. Accordingly, it was aimed to develop a robust PCR protocol for specific detection of *Salmonella* spp. Out of the different primers screened, one pair of primers developed in this study targeting *invA* gene demonstrated 100% inclusivity for a wide range of *Salmonella* serotypes and 100% exclusivity for wide range of non-target species. The *in silico* analysis of the nucleotide sequence obtained from the PCR product suggests its potential as a hybridization probe for genus specific detection of *Salmonella* spp. contamination. The PCR protocol was sensitive enough to detect 15 cells per reaction using crude DNA prepared within a short time directly from artificially contaminated shrimp tissue. The study demonstrated that the result of PCR reaction can come out on the same day of sample arrival. Incorporation of this pair of primers in a multiplex PCR designed for simultaneous detection of four common seafood-borne human pathogens yielded 147 bp, 302 bp, 403 bp, and 450 bp distinct DNA bands specifically targeting *E. coli*, toxigenic *Vibrio cholerae*, *Salmonella* spp., and *V. parahaemolyticus*, respectively in a single PCR tube. The PCR methods developed in this study has the potential to be used in the seafood processing plants for effective monitoring of CCPs required for implementation of HACCP-based quality assurance system. ISSN: 15178382

Ukuku, D.O., Mukhopadhyay, S., Olanya, O.M., Niemira, B.A.

Effect of cold storage on survivors and recovery of injured Salmonella bacteria on fresh-cut pieces prepared from whole melons treated with heat and hydrogen peroxide

(2019) *Journal of Food Processing and Preservation*, 43 (7), art. no. e13943, .

ABSTRACT: Cantaloupes and honeydew melons inoculated with 10⁷ CFU/ml *Salmonella* bacteria were treated with 1.5% of hydrogen peroxide (H₂O₂) at 20°C and minimally treated at 70°C for 3 min before storage at 5, 10, and 20°C. *Salmonella* bacteria recovered

from the cantaloupe and honeydew melon rind surfaces averaged 4.5 and 3.9 log CFU/cm², respectively, before antimicrobial treatments. Fresh-cut pieces prepared at each day of storage were immediately placed inside the refrigerator (5°C) while some pieces were left at room temperature (20°C) for 30 and 60 min before refrigeration. After minimal thermal hydrogen peroxide treatments, injured *Salmonella* on whole cantaloupes was significantly ($p > 0.05$) different than honeydew melons. Cold storage of treated melons at 5°C led to significant ($p > 0.05$) reduction of the injured bacteria and below detection in fresh-cut pieces even after enrichment process suggesting that minimal thermal antimicrobial processing will reduce transfer of bacteria to fresh-cut pieces during fresh-cut preparation. Practical applications: The results of this study indicate that minimal thermal treatment with 1.5% of hydrogen peroxide at 70°C for 3 min and immediate cold storage of treated whole melons designated for fresh-cut preparation will reduce transfer of microbial populations from melons rind surfaces to fresh-cut pieces during fresh-cut preparation. ISSN: 01458892

Pijnacker, R., Dallman, T.J., Tijmsa, A.S.L., Hawkins, G., Larkin, L., Kotila, S.M., Amore, G., Amato, E., Suzuki, P.M., Denayer, S., Klamer, S., Pászti, J., McCormick, J., Hartman, H., Hughes, G.J., Brandal, L.C.T., Brown, D., Mossong, J., Jernberg, C., Müller, L., Palm, D., Severi, E., Gołębiowska, J., Hunjak, B., Owczarek, S., Le Hello, S., Garvey, P., Mooijman, K., Friesema, I.H.M., van der Weijden, C., van der Voort, M., Rizzi, V., Franz, E., Bertrand, S., Brennan, M., Browning, L., Bruce, R., Cantaert, V., Chattaway, M., Coia, J., Couper, S., Žohar Čretnik, T., Daniel, O., Dionisi, A.M., Fabre, L., Fitz-James, I., Florek, K., Florianová, M., Fox, E., Frelih, T., Grilc, E., Katalinic Jankovic, V., Jourdan, N., Karpíšková, R., van den Kerkhof, H., Kuiling, S., Kurečić Filipović, S., Laisnez, V., Lange, H., deLappes, N., Leblanc, J., Luzzi, I., Mandilara, G., Mather, H., Mattheus, W., Mellou, K., Morgan, D., de Pinna, E., Ragimbeau, C., Røed, M.H., Salmenlinna, S., Smith, R., Smith-Palmer, A., Špačková, M., Torpdahl, M., Trkov, M., Trönnberg, L., Tzani, M., Utsi, L., Wasyl, D., Weicherding, P.

An international outbreak of Salmonella enterica serotype Enteritidis linked to eggs from Poland: a microbiological and epidemiological study
(2019) *The Lancet Infectious Diseases*, 19 (7), pp. 778-786.

ABSTRACT: Background: *Salmonella* spp are a major cause of food-borne outbreaks in Europe. We investigated a large multi-country outbreak of *Salmonella enterica* serotype Enteritidis in the EU and European Economic Area (EEA). Methods: A confirmed case was defined as a laboratory-confirmed infection with the outbreak strains of S Enteritidis based on whole-genome sequencing (WGS), occurring between May 1, 2015, and Oct 31, 2018. A probable case was defined as laboratory-confirmed infection with S Enteritidis with the multiple-locus variable-number tandem repeat analysis outbreak profile. Multi-country epidemiological, trace-back, trace-forward, and environmental investigations were done. We did a case-control study including confirmed and probable cases and controls randomly sampled from the population registry (frequency matched by age, sex, and postal code). Odds ratios (ORs) for exposure rates between cases and controls were calculated with unmatched univariable and multivariable logistic regression. Findings: 18 EU and EEA countries reported 838 confirmed and 371 probable cases. 509 (42%) cases were reported in 2016, after which the number of cases steadily increased. The case-control study results showed that cases more often ate in food establishments than did controls (OR 3.4 [95% CI 1.6–7.3]), but no specific food item was identified. Recipe-based food trace-back investigations among cases who ate in food establishments identified eggs from Poland as the vehicle of infection in October, 2016. Phylogenetic analysis identified two strains of S Enteritidis in human cases that were subsequently identified in salmonella-positive eggs and primary production premises in Poland, confirming the source of the outbreak. After control measures were implemented, the number of cases decreased, but increased again in March, 2017, and the increase continued into 2018. Interpretation: This outbreak highlights the public health value of multi-country sharing of epidemiological, trace-back, and microbiological data. The re-emergence of cases suggests that outbreak strains have continued to enter the food chain, although changes in strain population dynamics and fewer cases indicate that control measures had some effect. Routine use of WGS in salmonella surveillance and outbreak response promises to identify and stop outbreaks in the future. Funding: European Centre for Disease Prevention and Control; Directorate General for Health and Food Safety, European Commission; and National Public Health and Food Safety Institutes of the authors' countries (see Acknowledgments for full list). ISSN: 14733099

Sabeeh, R.A., Neamah, A.J., Jawad, A.A.

The cytotoxic effects of lipopolysaccharide extracted from a local isolate of Salmonella enteritidis on breast and ovarian cancer cell lines

(2019) *Indian Journal of Public Health Research and Development*, 10 (6), pp. 925-930.

ABSTRACT: The current in vitro study was performed to evaluate the anti-tumor cytotoxic effects of lipopolysaccharide (LPS) extracted from a local isolate of Salmonella enteritidis on breast and ovarian cancer cell lines, MCF-7, SKOV-3 respectively. Here, a local isolate of S. Enteritidis was obtained from Al-Diwaniyah Teaching Hospital, Al-Diwaniyah City, Iraq. The isolate was exposed to a series of morphological examinations and API 20E kit-based test to confirm the identity of the bacterium. Then, the bacterium was used in a polymerase chain reaction (PCR), and partial sequencing is targeting the RNA polymerase subunit beta (rpoB) gene at pieces of 1090bp and 897bp respectively to confirm the bacterium identity. After that, the bacterium LPS extracted to study its cytotoxic effects against MCF-7 and SKOV-3 cancer cell lines, in comparison with a commercial LPS using different concentrations (100µg/ml, 200µg/ml, 300µg/ml, 400µg/ml, and 500µg/ml). The results of the cytotoxicity effects showed that both LPSs had significant ($p < 0.05$) cytotoxicity against both cell lines. This study reveals that LPS isolated from Salmonella enteritidis might provide a promising substance for controlling breast and ovarian cancers. ISSN: 09760245

Snyder, T.R., Boktor, S.W., M'ikanatha, N.M.

Salmonellosis outbreaks by food vehicle, serotype, season, and geographical location, United States, 1998 to 2015

(2019) *Journal of Food Protection*, 82 (7), pp. 1191-1199.

ABSTRACT: Salmonella is a major cause of foodborne illness in the United States. Although salmonellosis outbreaks are relatively common, food vehicles and other characteristics are not well understood. We obtained data for salmonellosis outbreaks from 1998 to 2015 that were submitted by public health jurisdictions to the Centers for Disease Control and Prevention's Foodborne Disease Outbreak Surveillance System. In total, 2,447 outbreaks (yearly average, 136) with a confirmed or suspected etiology of nontyphoidal Salmonella were identified. The outbreaks included 65,916 individual cases (mean, 27 cases per outbreak). Food vehicles were identified in 49% of the outbreaks. Frequently implicated foods included eggs (12.5%), chicken (12.4%), and pork (6.5%). Fifty-five (2.2%) outbreaks had fatalities; 87 (0.1%) individuals died. Of those outbreaks with a reported serotype, the most commonly identified were Enteritidis (29.1%), Typhimurium (12.6%), and Newport (7.6%). Serotypes with a statistically significant increase over time included Braenderup and I 4,[5],12:i:-. Some serotypes were commonly associated with outbreaks due to certain food vehicles; 81% of outbreaks due to eggs were associated with serotype Enteritidis. Food commodities that were most commonly associated with multistate outbreaks were nuts and seeds, sprouts, and fruits. Outbreaks occurred most frequently in summer. States with the highest number of salmonellosis outbreaks per 100,000 population were Alaska (0.137) and Minnesota (0.121); states with the lowest were Delaware (< 0.001) and Wyoming (< 0.001). The highest number of salmonellosis cases per 100,000 population were in Washington, DC (4.786) and Arkansas (3.857). Geographic variations in outbreaks may reflect differences in outbreak detection, investigation, reporting, or risk. In addition to collaboration, data-driven public health interventions are needed to decrease infection rates and to prevent complications related to salmonellosis. ISSN: 0362028X

Bogdanovicová, K., Kameník, J., Dorotíková, K., Strejcek, J., Krepelová, S., Dušková, M., Haruštíaková, D.

Occurrence of foodborne agents at food service facilities in the Czech republic

(2019) *Journal of Food Protection*, 82 (7), pp. 1096-1103.

ABSTRACT: The aim of this study was to investigate the occurrence of foodborne agents at food service facilities in the Czech Republic. The sampling, performed from April 2016 to November 2017, focused on the microbiological monitoring of the environment at the establishment (EFS; $n = 298$) and the hands of staff (HFS; $n = 159$). The analysis targeted the presence of the following bacteria: Escherichia coli (focusing on the presence of Shiga toxinogenic E. coli), Bacillus cereus, Staphylococcus aureus, Listeria monocytogenes, Salmonella spp., and Campylobacter spp. A swab method using sterile abrasive sponges was used to detect bacteria in EFS; a glove-juice method was used to monitor microbial contamination on HFS. The presence of E. coli was confirmed in 11.8% of samples (12.4%, EFS; 10.7%, HFS; $P = 0.650$). The presence of Shiga toxinogenic E. coli was not confirmed in the samples. B. cereus was detected most frequently, in 39.6% of all samples taken (44.6%, EFS; 30.2%, HFS; $P = 0.003$). S. aureus was detected in 17.9% of samples (17.4%, EFS; 18.9%, HFS; $P = 0.703$). Of S. aureus isolates, 58.5% were found to be

positive for the presence of genes producing staphylococcal enterotoxins (70%, HFS; 52.0%, EFS). *L. monocytogenes* was detected in only one sample (0.2%; EFS). The presence of *Salmonella* spp. and *Campylobacter* spp. was not confirmed. The occurrence of *B. cereus*, *S. aureus*, and *E. coli* was dependent on the season of the year. *B. cereus* and *S. aureus* occurred less frequently in the summer months, although *E. coli* was recorded more frequently. *B. cereus*, *S. aureus*, and *E. coli* were detected in almost half of the tested samples. The relatively high percentage of *B. cereus* and *S. aureus* isolates from EFS corresponded with the model in the final European Food Safety Authority reports on the occurrence of foodborne disease outbreaks in the European Union. Managers of food service facilities should focus on reducing the occurrence of *B. cereus* and *S. aureus*.
ISSN: 0362028X

Kukleci, E., Smulders, F.J.M., Hamidi, A., Bauer, S., Paulsen, P.

Prevalence of foodborne pathogenic bacteria, microbial levels of hygiene indicator bacteria, and concentrations of biogenic amines in ready-to-eat meat products at retail in the republic of Kosovo

(2019) *Journal of Food Protection*, 82 (7), pp. 1135-1140.

ABSTRACT: The aim of this study was to investigate the microbiological safety and quality and biogenic amine concentrations of ready-to-eat meat products at retail in and around the capital of the Republic of Kosovo. A total of 128 ready-to-eat meat products from 49 retail shops were sampled in November 2017 and March 2018. Pathogenic *Escherichia coli* and *Salmonella* were not detected in enrichment cultures from 25-g samples. Levels of lecithinase-positive *Staphylococcus aureus* were consistently <2 log CFU/g. *Listeria monocytogenes* was detected in 4 of 88 cooked-cured products (25-g samples). Cooked-cured meats had significantly higher water activity (*aw*) and pH ($P < 0.001$) than did dried or fermented meats. All samples of dried or fermented meats had either low pH and *aw* or had a shelf life <5 days. Thus, by definition these products would be classified as not able to support growth of *L. monocytogenes* according to European Union food safety microbiological criteria. Among the cooked-cured products, total bacteria counts and lactic acid bacteria counts were >2 -log higher in sliced than in nonsliced items. In the dried or fermented samples, *E. coli* and Enterobacteriaceae (at ≥ 1 and ≥ 2 log CFU/g, respectively) and *L. monocytogenes* (25 g) were detected in the 11 samples that had been wrapped in cling plastic when handed over to the consumer but not in the 29 vacuum-packed samples. The maximum histamine concentration was 263.9 mg/kg. Putrescine concentrations of health concern (based on 100-g portions and 60 kg of body weight) were found in 1 of 88 cooked-cured and 8 of 40 dried or fermented samples. Median concentrations of cadaverine, histamine, putrescine, and tyramine were higher in dried or fermented than in cooked-cured samples. Results suggest that in the Republic of Kosovo non-vacuum-packed dried or fermented meats are more prone to contamination under retail conditions and these meats become contaminated during transport, handling, and retail sale.

ISSN: 0362028X

Tan, Z., Chekabab, S.M., Yu, H., Yin, X., Diarra, M.S., Yang, C., Gong, J.

Growth and virulence of salmonella typhimurium mutants deficient in iron uptake
(2019) *ACS Omega*, 4 (8), pp. 13218-13230.

ABSTRACT: The present study investigated the effects of iron, iron chelators, and mutations of *tonB* or *iroN* *fepA* genes on the growth and virulence of *Salmonella* Typhimurium. Results indicated that organic iron (ferric citrate and ferrous-l-ascorbate) supported better growth of *Salmonella* compared to inorganic iron. Among tested chelators, 2,2'-bipyridyl at 500 μ M showed the highest inhibition of *Salmonella* growth with 5 μ M ferrous sulfate. Deletion of genes (*tonB*- and *iroN*- *fepA*-) in the iron uptake system attenuated *Salmonella* invasion of Caco-2 cells and its ability to damage the epithelial monolayer. The expression of all tested host genes in Caco-2 was not affected under the iron-poor condition. However, claudin 3, tight junction protein 1, tumor necrosis factor α (TNF- α), and interleukin-8 (IL-8) were altered under the iron-rich condition depending on individual mutations. In *Caenorhabditis elegans*, a significant down-regulation of ferritin 1 expression was observed when the nematode was infected by the wild-type (WT) strain.

ISSN: 24701343

Sapkota, S., Adhikari, S., Khadka, S., Adhikari, M., Kandel, H., Pathak, S., Pandey, A., Pandey, A.

Multi-drug resistant extended-spectrum beta-lactamase producing E. coli and Salmonella on raw vegetable salads served at hotels and restaurants in Bharatpur, Nepal
(2019) *BMC Research Notes*, 12 (1), art. no. 516, .

ABSTRACT: Objective: Antimicrobial resistance among the bacteria present in ready-to-eat foods like vegetable salads is an emerging concern today. The current study was

undertaken to investigate the presence of multi-drug resistant extended-spectrum β -lactamase (ESBL) producing *E. coli* and *Salmonella* spp. in raw vegetable salads served at hotels and restaurants in Bharatpur. A total of 216 salad samples were collected from three different grades of hotels and restaurants and examined for the presence of *E. coli* and *Salmonella* spp. in Microbiology laboratory of Birendra Multiple Campus by conventional microbiological techniques. Results: Out of 216 samples, 66 samples (35.2%) showed the presence of *Salmonella* spp. whereas *E. coli* was recovered from 29 (13.4%) samples of which 3 samples harbored *E. coli* O157: H7. Antibiotic susceptibility testing revealed that 9 (13.6%) *Salmonella* and 4 (13.8%) *E. coli* isolates were detected as multi-drug resistant. Total ESBL producers reported were 5 (7.57%) *Salmonella* and 4 (13.8%) *E. coli*. The study also assessed a significant association between occurrence of *E. coli* and *Salmonella* with different grades of hotels and restaurants, personal hygiene and literacy rate of chefs and with the type of cleaning materials used to wash knives and chopping boards ($p < 0.05$). The findings suggest an immediate need of attention by the concerned authorities to prevent the emergence and transmission of food-borne pathogens and infections antimicrobial resistance among them. ISSN: 17560500

Iñiguez-Moreno, M., Gutiérrez-Lomelí, M., Avila-Novoa, M.G.

Kinetics of biofilm formation by pathogenic and spoilage microorganisms under conditions that mimic the poultry, meat, and egg processing industries

(2019) *International Journal of Food Microbiology*, 303, pp. 32-41.

ABSTRACT: Pathogens and spoilage microorganisms can develop multispecies biofilms on food contact surfaces; however, few studies have been focused on evaluated mixed biofilms of these microorganisms. Therefore this study investigated the biofilm development by pathogenic (*Bacillus cereus*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enterica* Enteritidis and Typhimurium serotypes) and spoilage (*Bacillus cereus* and *Pseudomonas aeruginosa*) microorganisms onto stainless-steel (SS) and polypropylene B (PP) coupons; under conditions that mimic the dairy, meat, and egg processing industry. Biofilms were developed in TSB with 10% chicken egg yolk (TSB + EY), TSB with 10% meat extract (TSB + ME) and whole milk (WM) onto SS and PP. Each tube was inoculated with 25 μ L of each bacteria and then incubated at 9 or 25 $^{\circ}$ C, with enumeration at 1, 48, 120, 180 and 240 h. Biofilms were visualized by epifluorescence and scanning electron microscopy (SEM). Biofilm development occurred at different phases, depending on the incubation conditions. In the reversible adhesion, the cell density of each bacteria was between 1.43 and 6.08 Log₁₀ CFU/cm² ($p < 0.05$). Moreover, significant reductions in bacteria appeared at 9 $^{\circ}$ C between 1 and 48 h of incubation. Additionally, the constant multiplication of bacteria in the biofilm occurred at 25 $^{\circ}$ C between 48 and 180 h of incubation, with increments of 2.08 Log₁₀ CFU/cm² to *S. Typhimurium*. Population establishment was observed between 48 and 180 h and 180–240 h incubation, depending on the environmental conditions (25 and 9 $^{\circ}$ C, respectively). For example, in TSB + ME at 25 $^{\circ}$ C, *S. Typhimurium*, *P. aeruginosa*, and *L. monocytogenes* showed no statistical differences in the amounts between 48 and 180 h incubation. The dispersion phase was identified for *L. monocytogenes* and *B. cereus* at 25 $^{\circ}$ C. Epifluorescence microscopy and SEM allowed visualizing the bacteria and extracellular polymeric substances at the different biofilm stages. In conclusion, pathogens and spoilage microorganisms developed monospecies with higher cellular densities than multispecies biofilms. In multispecies biofilms, the time to reach each biofilm phase varied is depending on environmental factors. Cell count decrements of 1.12–2.44 Log₁₀ CFU/cm² occurred at 48 and 240 h and were most notable in the biofilms developed at 9 $^{\circ}$ C. Additionally, cell density reached by each microorganism was different, *P. aeruginosa* and *Salmonella* were the dominant microorganisms in the biofilms while *B. cereus* showed the lower densities until undetectable levels. ISSN: 01681605

Aviv, G., Cornelius, A., Davidovich, M., Cohen, H., Suwandi, A., Galeev, A., Steck, N., Azriel, S., Rokney, A., Valinsky, L., Rahav, G., Grassl, G.A., Gal-Mor, O.

Differences in the expression of SPI-1 genes pathogenicity and epidemiology between the emerging Salmonella enterica serovar Infantis and the model Salmonella enterica serovar Typhimurium

(2019) *The Journal of infectious diseases*, 220 (6), pp. 1071-1081.

ABSTRACT: BACKGROUND: *Salmonella enterica* serovar *Infantis* (*S. Infantis*) is one of the ubiquitous serovars of the bacterial pathogen *S. enterica* and recently has been emerging in many countries worldwide. Nonetheless, not much is known about its epidemiology, host adaptation, and virulence. METHODS: Epidemiological and molecular approaches were used together with tissue-culture and mouse models to conduct phenotypic comparison with the model *S. enterica* serovar *Typhimurium*. RESULTS: We show that *S. Infantis* is more frequently associated with infections in infants <2 years old and prone to cause

significantly less invasive infections than serovar Typhimurium. Moreover, although *S. Infantis* adheres better to host cells and highly colonizes mouse intestines soon after infection, it is significantly less invasive and induces much lower inflammation and disease in vivo than *S. Typhimurium*. These differences were associated with lower expression of *Salmonella* pathogenicity island (SPI) 1 genes in *S. Infantis* than in *S. Typhimurium*.
CONCLUSIONS: Our results demonstrate previously unknown differences in the epidemiology, virulence pathway expression, and pathogenicity between two highly abundant *Salmonella* serovars and suggest that native variation in the expression of the SPI-1 regulon is likely to contribute to epidemiological and virulence variation between genetically similar nontyphoidal *Salmonella* serovars. ISSN: 15376613

Samphaongern, C., Pipatpanukul, C., Sriksirin, T.

Development of rapid qualitative detection DNA microarray platform for Salmonella typhimurium for food safety

(2019) *IOP Conference Series: Materials Science and Engineering*, 526 (1), art. no. 012003, .

ABSTRACT: Screening of possible bacteria contamination in food products is one of the measures implemented to avoid potential health hazards. In food industry, standard cell culture technique is widely used to monitor bacterial contamination. However, the main drawback of this technique is its inherited time consumed during the culturing step, which requires about 72 hours. In this research, an alternative DNA microarray technique was developed for a qualitative screening of a possible *Salmonella typhimurium* contamination in chicken, with the aim of reducing the detection time down to 3 hrs. The identification of *S. typhimurium* in food samples was carried out by hybridization of the possible contaminant with specific probe immobilized on a biochip. Novel DNA probes were designed as 16-50 base pairs of nucleotides to recognize with specific parts in *S. typhimurium* genomic. At least two DNA probes were identified as the candidate probes which had the potential to promote the best hybridization. DNA microarray was fabricated by mixing candidate DNA probes with photoactive polymer network, (poly(DMAA-mABP-SSNa)), and was printed onto a plastic substrate by non-contact microspotter. DNA probe was covalently immobilized onto the surface by 254 nm of UV lamp with 1.25 J/cm². PCR product functionalized with biotin was hybridized with DNA probe and labelled with streptavidin-cy5. Specific binding yielded fluorescence signal. The intensity image signal was read-out by a fluorescent microarray reader. Two genes specific to *S. typhimurium* (*fimC* and *invA*), were investigated by using specially designed DNA primers and DNA probes. From the assay optimization, it was found that 1 mg/ml of polymer hydrogel concentration, 10 μM of DNA probe concentration, 10 μg/ml of labelling concentration, and 2 nL of array volume yielded the highest signal intensity. The results were calibrated into CFU/ml (cell forming unit). The system was applied successfully for the detection of *S. typhimurium* without any contamination. The biochip validation with spiked sample (DNA standard) is currently underway. ISSN: 17578981

Tasmin, R., Gulig, P.A., Parveen, S.

Detection of virulence plasmid-encoded genes in salmonella typhimurium and salmonella kentucky isolates recovered from commercially processed chicken carcasses

(2019) *Journal of Food Protection*, 82 (8), pp. 1364-1368.

ABSTRACT: *Salmonella enterica* serovar Typhimurium is one of the leading causes of nontyphoidal gastroenteritis of humans in the United States. Commercially processed poultry carcasses are frequently contaminated with *Salmonella* serovar Kentucky in the United States. The aim of the study was to detect the *Salmonella* virulence plasmid containing the *spv* genes from *Salmonella* isolates recovered from commercially processed chicken carcasses. A total of 144 *Salmonella* isolates (*Salmonella* Typhimurium, n = 72 and *Salmonella* Kentucky, n = 72) were used for isolation of plasmids and detection of corresponding virulence genes (*spvA*, *spvB*, and *spvC*). Only four (5.5%) *Salmonella* Typhimurium isolates tested positive for all three virulence genes and hence were classified as possessing the virulence plasmid. All isolates of *Salmonella* Kentucky were negative for the virulence plasmid and genes. These results indicate that the virulence plasmid, which is very common among clinical isolates of Typhimurium and other *Salmonella* serovars (e.g., Enteritidis, Dublin, Choleraesuis, Gallinarum, Pullorum, and Abortusovis), may not be present in a significant portion of commercially processed chicken carcass isolates. ISSN: 0362028X

Shushe, O., Wroblewski, D., MacGowan, C.E., Passaretti, T., Musser, K., Mingle, L.

Detection of Salmonella spp. in retail meat products: a comparison between a discontinued commercial kit and a laboratory-developed screening method

(2019) *Letters in Applied Microbiology*, 69 (2), pp. 116-120.

ABSTRACT: The National Antimicrobial Resistance Monitoring System for Enteric Bacteria retail food surveillance programme screens retail meat samples for the presence of *Salmonella* spp. to track antimicrobial resistance in food. In this study, a laboratory developed real-time PCR assay that detects *Salmonella* spp. was evaluated as a screening method to replace the discontinued 3M TECRA kit. The 3M TECRA kit was a commercially available, visual immunoassay used to screen food samples for the presence of *Salmonella* spp. This kit was discontinued in September 2016 by the manufacturer and an alternative screening method was needed to replace the discontinued TECRA kit. *Salmonella* spp. is detected by the real-time PCR assay earlier in the screening process than by the TECRA kit. *Salmonella* spp. can also be reliably isolated from the enrichment broth earlier in the protocol. Additionally, cost analysis shows that the real-time PCR assay saves \$2.50 per sample. New York State Department of Health currently uses this real-time PCR assay as a screening method for the presence of *Salmonella* spp. in retail meat samples. The assay allows for continued monitoring of antimicrobial resistance in *Salmonella* spp., while providing a cost savings and a decrease in turnaround time. Significance and Impact of the Study: The National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS) tracks antimicrobial susceptibility of enteric bacteria in people, food and animals (<https://www.fda.gov/animalveterinary/safetyhealth/antimicrobialresistance/nationalantimicrobialresistancemonitoringsystem/>). The New York State Department of Health (NYSDOH) became a NARMS retail food surveillance (RFS) site in 2003. The NARMS-RFS programme screens retail meat samples from grocery stores in the United States for the presence of *Salmonella* spp. and other enteric pathogens to monitor the prevalence of antimicrobial resistance among these pathogens. The NYSDOH developed a rapid and cost-effective real-time PCR assay to screen for *Salmonella* spp. in retail meat products. ISSN: 02668254

Paudel, S.K., Bhargava, K., Kotturi, H.

Antimicrobial activity of cinnamon oil nanoemulsion against Listeria monocytogenes and Salmonella spp. on melons
(2019) *LWT*, 111, pp. 682-687.

ABSTRACT: *Listeria* and *Salmonella* related recalls and outbreaks are of major concern to the melon industry. Cinnamon oil has shown its usefulness in antimicrobial food treatment. However, its applications are limited due to poor solubility of cinnamon oil in water. Utilization of Cinnamon oil nanoemulsion may offer effective antimicrobial treatment to the melon industry. Different formulations of cinnamon oil nanoemulsion were made by ultrasonication using Tween 80 as an emulsifier. The particle size of emulsion was determined by dynamic light scattering. Microbroth dilution assay was performed on *Listeria monocytogenes* and *Salmonella* spp. to find out the antimicrobial efficacy of cinnamon oil nanoemulsion. Honeydew and cantaloupe were artificially inoculated with the bacterial strains mentioned above followed by treatment in nanoemulsion (control, 0.1%, 0.25%, and 0.5%) for 1 min. The average diameter of our nanoemulsion was 9.63 ± 0.3 nm. Minimum inhibitory concentration (MIC) of cinnamon oil nanoemulsion for *Listeria monocytogenes* and *Salmonella* spp. strains was 0.078% v/v and 0.039% v/v, respectively. Compared to the water control, 0.5% nanoemulsion showed up to 7.7 and 5.5 log reductions in *Listeria monocytogenes* and *Salmonella* spp., respectively. The data suggests that cinnamon oil nanoemulsion can be used as an effective natural microbial control agent for melons. ISSN: 00236438

Rossi, C., Chaves-López, C., Možina, S.S., Di Mattia, C., Scuota, S., Luzzi, I., Jenič, T., Paparella, A., Serio, A.

Salmonella enterica adhesion: Effect of *Cinnamomum zeylanicum* essential oil on lettuce
(2019) *LWT*, 111, pp. 16-22.

ABSTRACT: *Salmonella* is a foodborne pathogen able to adhere and persist on biotic and abiotic surfaces, including vegetables, which are even more linked to foodborne outbreaks. In this work, first we investigated the capability of *Salmonella* to adhere on different surfaces (stainless steel, polypropylene and lettuce), then we evaluated the potential effect of essential oils in reducing the adhesion and persistence of the pathogen on lettuce. Eight essential oils (EO) were tested on five *Salmonella enterica* strains (serovars Derby, Thompson, Napoli, Kasenyi and Veneziana). *Cinnamomum zeylanicum* EO (CEO) was the most effective, according to Minimal Inhibitory Concentration (MIC 1.25–1.87 $\mu\text{L}/\text{mL}$) and to growth/inhibition dynamics. To simulate real conditions, a cocktail of *Salmonella* Kasenyi, S. Veneziana and S. Napoli was applied on the three surfaces, but it adhered only on lettuce, starting from 1 h of contact (4.59 ± 0.34 Log UFC/cm²). Five $\mu\text{L}/\text{mL}$ CEO, applied on lettuce, immediately reduced the loosely and strongly attached cells (reduction of 0.78 Log and 0.63 Log CFU/cm², respectively), with significant effect up to 120 h. CEO also inhibited the Polyphenol Oxidase activity, thus preserving lettuce colour during

storage. Cinnamon EO could therefore help to improve safety and appearance of fresh-cut lettuce during storage. ISSN: 00236438

Etter, A.J., West, A.M., Burnett, J.L., Wu, S.T., Veenhuizen, D.R., Ogas, R.A., Oliver, H.F.

Salmonella enterica subsp. enterica serovar Heidelberg food isolates associated with a salmonellosis outbreak have enhanced stress tolerance capabilities

(2019) *Applied and Environmental Microbiology*, 85 (16), art. no. e01065-19, .

ABSTRACT: *Salmonella enterica* serovar Heidelberg is currently the 12th most common serovar of *Salmonella enterica* causing salmonellosis in the United States and results in twice the average incidence of blood infections caused by nontyphoidal salmonellae. Multiple outbreaks of salmonellosis caused by *Salmonella Heidelberg* resulted from the same poultry processor, which infected 634 people during 2013 and 2014. The hospitalization and invasive illness rates were 38% and 15%, respectively. We hypothesized that the outbreak strains of *Salmonella Heidelberg* had enhanced stress tolerance and virulence capabilities. We sourced nine food isolates collected during the outbreak investigation and three reference isolates to assess their tolerance to heat and sanitizers, ability to attach to abiotic surfaces, and invasiveness in vitro. We performed RNA sequencing on three isolates (two outbreak-associated isolates and a reference *Salmonella Heidelberg* strain) with various levels of heat tolerance to gain insight into the mechanism behind the isolates' enhanced heat tolerance. We also performed genomic analyses to determine the genetic relationships among the outbreak isolates. Ultimately, we determined that (i) six *Salmonella Heidelberg* isolates associated with the foodborne outbreak had enhanced heat tolerance, (ii) one outbreak isolate with enhanced heat tolerance also had an enhanced biofilm-forming ability under stressful conditions, (iii) exposure to heat stress increased the expression of *Salmonella Heidelberg* multidrug efflux and virulence genes, and (iv) outbreak-associated isolates were likely transcriptionally primed to better survive processing stresses and, potentially, to cause illness. ISSN: 00992240

Petrin, S., Longo, A., Barco, L., Cortini, E., Peruzzo, A., Antonelli, P., Ramon, E., Cibin, V., Lettini, A.A., Ricci, A., Losasso, C.

Different Resolution Power of Multilocus Variable-Number Tandem Repeat Analysis and Whole-Genome Sequencing in the Characterization of S. 1,4,[5],12i:- Isolates
(2019) *Foodborne Pathogens and Disease*, 16 (8), pp. 558-561.

ABSTRACT: *Salmonella enterica* serovar 1,4,[5],12i:- has emerged over the last two decades as one of the most common serovars causing human salmonellosis in Europe. It is supposed to originate from *Salmonella enterica* serovar Typhimurium due to antigenic and genotypic similarities between the two serovars. Due to the high level of similarity, the multilocus variable-number tandem repeat analysis (MLVA) protocol designed for *Salmonella Typhimurium* routine typing is commonly used also for the characterization of *S. 1,4,[5],12i:-*. Nevertheless, the *Salmonella Typhimurium*-based MLVA protocol often shows poor discriminatory power for *S. 1,4,[5],12i:-*. Indeed, only a limited number of MLVA profiles have been described for *S. 1,4,[5],12i:-*. Moreover, based on the MLVA clustering, *S. 1,4,[5],12i:-* is supposed to display high clonality. The aim of the present work was to assess whether the five loci of *Salmonella Typhimurium* investigated by MLVA are sufficiently accurate to correctly assign *S. 1,4,[5],12i:-* isolates. For this purpose, 38 epidemiologically unrelated *S. 1,4,[5],12i:-* were subjected to whole-genome sequencing. Isolates were selected among a collection of monophasic strains isolated in Italy from different sources over the period 2014-2016 and belonging to the five most commonly detected MLVA profiles. Results confirmed the possible clonality for *S. 1,4,[5],12i:-* serovar in the light of the scarce difference observed in terms of single-nucleotide polymorphisms (SNPs) among investigated isolates. Nevertheless, unrelated isolates on the basis of the difference of SNP number were characterized as indistinguishable by MLVA profile, thus suggesting an insufficient resolution of MLVA. Hence, we can conclude that MLVA-based approach does not seem a valuable proxy to deepen into the epidemiological relationship among *S. 1,4,[5],12i:-* isolates. These evidences can be useful to avoid incorrect assignment especially when surveillance data are used for outbreak investigations. ISSN: 15353141

Lambertini, E., Ruzante, J.M., Chew, R., Apodaca, V.L., Kowalczyk, B.B.

The public health impact of different microbiological criteria approaches for Salmonella in chicken parts

(2019) *Microbial Risk Analysis*, 12, pp. 44-59.

ABSTRACT: Non-typhoidal *Salmonella* is a significant foodborne pathogen causing over a million illnesses each year in the United States. Poultry is one of the food commodities

most frequently associated with *Salmonella* infections. While government, research, and industry efforts have reduced *Salmonella* contamination in poultry to some extent, the incidence of salmonellosis has not changed significantly and is still above the public health goals of Healthy People 2020, and novel and more comprehensive approaches are needed. In this paper, the public health impact of implementing different microbiological criteria (MC) for *Salmonella* in chicken parts was evaluated using a quantitative risk assessment approach. Four hypothetical scenarios, including a no-action baseline and three alternative scenarios, were considered. Scenario 1 modeled a prevalence-based microbiological criterion based on the proportion of positive samples in an establishment, Scenario 2 modeled a microbiological criterion based on the concentration of *Salmonella* in samples, and Scenario 3 modeled a combination of the two. With exception of the baseline, all three scenarios assumed that different interventions would be adopted for non-compliant establishments (Scenario 1) or lots (Scenario 2), with Scenario 3 combining establishment-level and lot-level interventions. The product was assumed to be sold to consumers as raw, and contamination via undercooked product as well as cross contamination in consumer kitchens were considered as potential exposure routes. Risk was characterized by the probability of illness and the preventable fraction of risk, which was calculated for each scenario in comparison with the baseline. Simulation results show that, depending on the parameters of specific sampling strategies, both prevalence-based and concentration-based MC coupled with interventions could significantly lower risk (range of 60–88% in mean preventable fraction of risk). Overall, while the model is preliminary and subject to the stated limitations, it is likely that a combination approach including establishment-level and lot-level interventions would be highly effective in reducing risk and, therefore, benefit public health. The effectiveness of all MC was impacted by several assumptions and model parameters. In particular, the prevalence MC threshold and the concentration reduction associated with the establishment-level intervention impacted the preventable fraction of risk for Scenario 1, and the concentration MC threshold and the variability across lots impacted the risk outcomes for Scenario 2. Overall, high variance in risk outputs was observed, mainly associated with a high variance in concentration inputs. This model provides a risk-based approach to test different MC approaches for chicken parts at both lot and establishment levels, and over a wide range of scenarios of input contamination distributions, interventions, and consumer behaviors. Model estimates, as well as the ability to distinguish between variability and uncertainty, could be improved by additional data on the distribution of *Salmonella* concentrations across and within establishments. ISSN: 23523522

Banerjee, A., Bardhan, R., Chowdhury, M., Joardar, S.N., Isore, D.P., Batabyal, K., Dey, S., Sar, T.K., Bandyopadhyay, S., Dutta, T.K., Samanta, I.

Characterization of beta-lactamase and biofilm producing Enterobacteriaceae isolated from organized and backyard farm ducks

(2019) *Letters in Applied Microbiology*, 69 (2), pp. 110-115.

ABSTRACT: This study was undertaken to detect the occurrence of beta-lactamase and biofilm producing Enterobacteriaceae in healthy ducks. A total 202 cloacal swabs were collected from ducks kept in organized (n = 92) and backyard (n = 110) farms in West Bengal (India). The ducks had no history of antibiotic intake. Among the 87 phenotypically beta-lactamase producing *Escherichia coli*, 19 (17.43%), 6 (5.05%) and 15 (13.76%) isolates possessed blaTEM, blaSHV and blaCTX-M respectively. Whereas, 5 (38.46%) *Salmonella* isolates were found to harbour blaCTX-M. In *K. pneumoniae* 10 (33.33%), 3 (13.33%), 4 (13.33%) isolates possessed blaTEM, blaSHV and blaCTX-M respectively. The sequences of selected PCR products were found 98% cognate with blaCTX-M-9, blaSHV-12 and blaTEM-1. Beta-lactamase producing *E. coli* isolates belonged to 14 different serogroups such as O1, O2, O3, O5, O7, O8, O35, O83, O84, O88, O119, O128, O145 and O157. Moreover, 87 *E. coli* (79.82%), six *Salmonella* (46.15%) and 13 *K. pneumoniae* (43.33%) isolates were detected as AmpC producers possessing blaAmpC. Majority of *E. coli* (46.79%), *Salmonella* (46.15%) and *K. pneumoniae* (70%) isolates were detected as biofilm producers and possessed the associated genes (*csgA*, *sdiA*, *rcsA*, *rpoS*). Significantly higher occurrence of beta-lactamase and biofilm producing Enterobacteriaceae isolates was detected in backyard ducks than organized farms. Significance and Impact of the Study: Consumption of antibiotic through feed or during therapy is considered as potential reason for generation of antimicrobial resistant bacteria in birds. This study provides valuable evidence that exposure to contaminated environment may be an additional source for generation of antimicrobial resistant bacteria in backyard ducks. The backyard ducks are reared by marginal farmers in India who cannot offer antibiotics to them either through feed or during therapy due to high cost. The study also reveals a significant correlation between biofilm formation and possession of antimicrobial resistance genes in the bacterial isolates from the ducks. ISSN: 02668254

Criscuolo, A., Issenhuth-Jeanjean, S., Didelot, X., Thorell, K., Hale, J., Parkhill, J., Thomson, N.R., Weill, F.-X., Falush, D., Brisse, S.

The speciation and hybridization history of the genus Salmonella (2019) Microbial genomics, 5 (8), .

ABSTRACT: Bacteria and archaea make up most of natural diversity, but the mechanisms that underlie the origin and maintenance of prokaryotic species are poorly understood. We investigated the speciation history of the genus *Salmonella*, an ecologically diverse bacterial lineage, within which *S. enterica* subsp. *enterica* is responsible for important human food-borne infections. We performed a survey of diversity across a large reference collection using multilocus sequence typing, followed by genome sequencing of distinct lineages. We identified 11 distinct phylogroups, 3 of which were previously undescribed. Strains assigned to *S. enterica* subsp. *salamae* are polyphyletic, with two distinct lineages that we designate *Salamae* A and B. Strains of the subspecies *houtenae* are subdivided into two groups, *Houtenae* A and B, and are both related to Selander's group VII. A phylogroup we designate VIII was previously unknown. A simple binary fission model of speciation cannot explain observed patterns of sequence diversity. In the recent past, there have been large-scale hybridization events involving an unsampled ancestral lineage and three distantly related lineages of the genus that have given rise to *Houtenae* A, *Houtenae* B and VII. We found no evidence for ongoing hybridization in the other eight lineages, but detected subtler signals of ancient recombination events. We are unable to fully resolve the speciation history of the genus, which might have involved additional speciation-by-hybridization or multi-way speciation events. Our results imply that traditional models of speciation by binary fission and divergence are not sufficient to account for *Salmonella* evolution. ISSN: 20575858

Söderlund, R., Jernberg, C., Trönnberg, L., Pääjärvi, A., Ågren, E., Lahti, E.

Linked seasonal outbreaks of Salmonella Typhimurium among passerine birds, domestic cats and humans, Sweden, 2009 to 2016

(2019) Euro surveillance : bulletin European sur les maladies transmissibles = European communicable disease bulletin, 24 (34), .

ABSTRACT: In 2016, an outbreak of *Salmonella* Typhimurium (STm) with multilocus variable-number tandem repeat analysis (MLVA) profiles historically associated with passerine birds (2-[11-15]-[3-4]-NA-212) occurred among passerines, cats and humans in Sweden. Our retrospective observational study investigated the outbreak and revisited historical data from 2009-16 to identify seasonality, phylogeography and other characteristics of this STm variant. Outbreak isolates were analysed by whole-genome single nucleotide polymorphism (SNP) typing. The number of notified cases of passerine-associated STm among passerines, cats and humans per month and county, and their MLVA profiles, were compared to birdwatchers' counts of passerines. Seasonal trend decomposition and correlation analysis was performed. Outbreak isolates did not cluster by host on SNP level. Passerine-associated STm was seasonal for birds, cats and humans, with a peak in March. Cases and counts of passerines at bird feeders varied between years. The incidence of passerine-associated STm infections in humans was higher in the boreal north compared with the southern and capital regions, consistent with passerine population densities. Seasonal mass migration of passerines appears to cause STm outbreaks among cats certain years in Sweden, most likely via predation on weakened birds. Outbreaks among humans can follow, presumably caused by contact with cats or environmental contamination. ISSN: 15607917

Siira, L., MacDonald, E., Holmbakken, G.M., Sundar, T., Meyer-Myklestad, L., Lange, H., Brandal, L.T., Naseer, U., Johannessen, G.S., Bergsjø, B., Espenhain, L., Vold, L., Nygård, K.

Increasing incubation periods during a prolonged monophasic Salmonella Typhimurium outbreak with environmental contamination of a commercial kitchen at Oslo Airport, Norway, 2017

(2019) Euro surveillance : bulletin European sur les maladies transmissibles = European communicable disease bulletin, 24 (34), .

ABSTRACT: In September 2017, a cluster of monophasic *Salmonella* Typhimurium isolates was identified at the National Reference Laboratory for Enteropathogenic Bacteria in Norway. We investigated the cluster to identify the source and implement control measures. We defined a case as a person with laboratory-confirmed salmonellosis with the outbreak strain multiple locus variable-number tandem repeat analysis type. We conducted descriptive epidemiological and environmental investigations and performed whole genome sequencing (WGS) with core and accessory genome multilocus sequence typing of all isolates from cases or the environment connected with this outbreak. We identified 21

cases, residing in 10 geographically dispersed counties, all of whom had consumed food or drinks from a café at Oslo Airport. Case distribution by date of symptom onset suggested that a point source was introduced in mid-August followed by continued environmental contamination. The incubation periods ranged 0-16 days and increased as the outbreak progressed, likely due to increasingly low-dose exposure as control measures were implemented. WGS confirmed an identical cluster type-944 in all cases and six environmental specimens from the café. Control measures, including temporary closure and kitchen refurbishment, failed to eliminate the environmental source. We recommend strengthened hygiene measures for established environmental contamination during an outbreak. ISSN: 15607917

Foster, D., Jacob, M., Stowe, D., Smith, G.

Exploratory cohort study to determine if dry cow vaccination with a Salmonella Newport bacterin can protect dairy calves against oral Salmonella challenge
(2019) *Journal of Veterinary Internal Medicine*, 33 (4), pp. 1796-1806.

ABSTRACT: Background: Salmonellosis is a major cause of morbidity and mortality in neonatal calves, often occurring before preventative vaccines can be administered. Hypothesis/Objective: To evaluate the protective effect on calves of colostrum from cows vaccinated with a commercially available Salmonella Newport bacterin against a Salmonella Typhimurium challenge. Animals: Twenty Holstein bull calves from a university dairy farm. Methods: Nonrandomized placebo-controlled trial in which colostrum was harvested from 30 cows that received 2 doses of either Salmonella bacterin or saline before calving. Colostrum collected from each group was pooled and fed to 2 groups of 10 calves at birth. At approximately 2 weeks of age, calves were challenged with Salmonella Typhimurium. Clinical, hematologic, microbiological, and postmortem findings were compared between the 2 groups. Results: No differences in mortality, clinical findings, hematology results, blood and fecal cultures, or necropsy findings between the 2 groups were observed. Vaccinated cows had higher colostrum titers, and calves fed this colostrum had higher serum titers (mean difference, 0.429; mean [SE], 0.852 [0.02] for vaccinated versus 0.423 [0.02] for control calves). Conclusions and Clinical Importance: Transfer of colostrum immunoglobulins from Salmonella enterica serotype Newport bacterin to neonatal calves was not sufficient to decrease mortality, clinical signs, sepsis, intestinal damage, or fecal shedding when exposed to a highly pathogenic Salmonella isolate. A large-scale randomized controlled clinical trial is needed to evaluate the efficacy of this bacterin when administered in the dry period for prevention of salmonellosis in neonatal calves. ISSN: 08916640

McWhorter, A.R., Tearle, R., Moyle, T.S., Chousalkar, K.K.

In vivo passage of Salmonella Typhimurium results in minor mutations in the bacterial genome and increases in vitro invasiveness
(2019) *Veterinary Research*, 50 (1), art. no. 71, .

ABSTRACT: Eggs and raw or undercooked egg-containing food items are frequently identified as the bacterial source during epidemiological investigation of Salmonella outbreaks. Multi-locus variable number of tandem repeats analysis (MLVA) is a widely used Salmonella typing method enabling the study of diversity within populations of the same serotype. In vivo passage, however, has been linked with changes in MLVA type and more broadly the Salmonella genome. We sought to investigate whether in vivo passage through layer hens had an effect on MLVA type as well as the bacterial genome and whether any mutations affected bacterial virulence. Layer hens were infected with either Salmonella Typhimurium DT9 (03-24-11-11-523) as part of a single infection or were co-infected with an equal amount of Salmonella Mbandaka. Salmonella shedding in both single and co-infected birds was variable over the course of the 16-week experiment. Salmonella Typhimurium and Salmonella Mbandaka were identified in feces of co-infected birds. Salmonella colonies isolated from fecal samples were subtyped using MLVA. A single change in SSTR-6 was observed in Salmonella Typhimurium strains isolated from co-infected birds. Isolates of Salmonella Typhimurium of both the parent (03-24-11-11-523) and modified (03-24-12-11-523) MLVA type were sequenced and compared with the genome of the parent strain. Sequence analysis revealed that in vivo passaging resulted in minor mutation events. Passaged isolates exhibited significantly higher invasiveness in cultured human intestinal epithelial cells than the parent strain. The microevolution observed in this study suggests that changes in MLVA may arise more commonly and may have clinical significance. ISSN: 09284249

Kyakuwair, M., Olupot, G., Amoding, A., Nkedi-Kizza, P., Basamba, T.A.

How Safe is Chicken Litter for Land Application as an Organic Fertilizer? A Review
(2019) *International journal of environmental research and public health*, 16 (19), .

ABSTRACT: Chicken litter application on land as an organic fertilizer is the cheapest and most environmentally safe method of disposing of the volume generated from the rapidly expanding poultry industry worldwide. However, little is known about the safety of chicken litter for land application and general release into the environment. Bridging this knowledge gap is crucial for maximizing the benefits of chicken litter as an organic fertilizer and mitigating negative impacts on human and environmental health. The key safety concerns of chicken litter are its contamination with pathogens, including bacteria, fungi, helminthes, parasitic protozoa, and viruses; antibiotics and antibiotic-resistant genes; growth hormones such as egg and meat boosters; heavy metals; and pesticides. Despite the paucity of literature about chicken litter safety for land application, the existing information was scattered and disjointed in various sources, thus making them not easily accessible and difficult to interpret. We consolidated scattered pieces of information about known contaminants found in chicken litter that are of potential risk to human, animal, and environmental health and how they are spread. This review tested the hypothesis that in its current form, chicken litter does not meet the minimum standards for application as organic fertilizer. The review entails a meta-analysis of technical reports, conference proceedings, peer-reviewed journal articles, and internet texts. Our findings indicate that direct land application of chicken litter could be harming animal, human, and environmental health. For example, counts of pathogenic strains of *Escherichiacoli* (105-1010 CFU g⁻¹) and Coliform bacteria (106-108 CFU g⁻¹) exceeded the maximum permissible limits (MPLs) for land application. In Australia, 100% of broiler litter tested was contaminated with *Actinobacillus* and re-used broiler litter was more contaminated with *Salmonella* than non-re-used broiler litter. Similarly, in the US, all (100%) broiler litter was contaminated with *Escherichiacoli* containing genes resistant to over seven antibiotics, particularly amoxicillin, ceftiofur, tetracycline, and sulfonamide. Chicken litter is also contaminated with a vast array of antibiotics and heavy metals. There are no standards set specifically for chicken litter for most of its known contaminants. Even where standards exist for related products such as compost, there is wide variation across countries and bodies mandated to set standards for safe disposal of organic wastes. More rigorous studies are needed to ascertain the level of contamination in chicken litter from both broilers and layers, especially in developing countries where there is hardly any data; set standards for all the contaminants; and standardize these standards across all agencies, for safe disposal of chicken litter on land. ISSN: 16604601

Ocaña-de Jesús, R.L., Gutiérrez-Ibáñez, A.T., Sánchez-Pale, J.R., Mariezcurrena-Berasain, M.D., Laguna-Cerda, A., Hernández-Chiñas, U., Rojas-Puebla, I.

Motility and Survival of Salmonella Enterica Subspecies Enterica Serovar Enteritidis in Tomato Plants (Solanum lycopersicum L)

(2019) *International Microbiology*, 22 (3), pp. 363-368.

ABSTRACT: The presence of enteropathogens such as *Salmonella* affects the quality and safety of vegetables that are consumed in a minimally processed state. Worldwide, tomatoes are one of the main vegetables whose raw consumption has caused health alerts. As such, the aim of this study was to determine the motility and survival of *Salmonella enterica* subspecies *enterica* serovar *Enteritidis* on greenhouse-grown tomato plants. A completely randomized experimental design was used, and bacteria were inoculated into the substrate at the time of transplanting as well as by puncturing the plant stem, petiole, and peduncle during the vegetative, flowering, and fruiting stages. Survival was monitored throughout the production cycle; motility was evaluated separately in plant organs separated from the point of inoculation. *Salmonella enteritidis* survived the 120 days of the experiment both at the point of inoculation and in other organs of the tomato plant. For all treatments, there was a significant difference ($P < 0.05$) between bacterial counts in the root (12.45 ± 2.52 to 160 ± 4.01 CFU/g), stem (16.10 ± 2.31 to 90.55 ± 3.62 CFU/g), flower (7.0 ± 2.15 to 51.10 ± 3.80 CFU/g), and fruit (8.75 ± 2.38 to 28.2 ± 3.29 CFU/g). The results of the study indicate that *Salmonella enteritidis* in contact with tomato plants is a latent danger because its ability to enter, survive, and move within tomato plants until reaching the fruit, limits the effectiveness of commonly used disinfection methods, it would potentiate the risk to human health. ISSN: 11396709

Islam, M.S., Zhou, Y., Liang, L., Nime, I., Liu, K., Yan, T., Wang, X., Li, J.

Application of a Phage Cocktail for Control of Salmonella in Foods and Reducing Biofilms (2019) *Viruses*, 11 (9), .

ABSTRACT: *Salmonella* contamination in foods and their formation of biofilms in food processing facility are the primary bacterial cause of a significant number of foodborne outbreaks and infections. Broad lytic phages are promising alternatives to conventional technologies for pathogen biocontrol in food matrices and reducing biofilms. In this study, 42 *Salmonella* phages were isolated from environmentally-sourced water samples. We

characterized the host range and lytic capacity of phages LPSTLL, LPST94 and LPST153 against *Salmonella* spp., and all showed a wide host range and broad lytic activity. Electron microscopy analysis indicated that LPSTLL, LPST94, and LPST153 belonged to the family of Siphoviridae, Ackermannviridae and Podoviridae, respectively. We established a phage cocktail containing three phages (LPSTLL, LPST94 and LPST153) that had broad spectrum to lyse diverse *Salmonella* serovars. A significant decrease was observed in *Salmonella* with a viable count of 3 log₁₀ CFU in milk and chicken breast at either 25 °C or 4 °C. It was found that treatment with phage cocktail was able to significantly reduced biofilm on a 96-well microplate (44-63%) and on a stainless steel surface (5.23 to 6.42 log₁₀). These findings demonstrated that the phage cocktail described in this study can be potentially used as a biological control agent against *Salmonella* in food products and also has the effect to reduce *Salmonella* formed biofilms. ISSN: 19994915

Bridier, A., Le Grandois, P., Moreau, M.-H., Prénom, C., Le Roux, A., Feurer, C., Soumet, C.

Impact of cleaning and disinfection procedures on microbial ecology and Salmonella antimicrobial resistance in a pig slaughterhouse
(2019) *Scientific reports*, 9 (1), p. 12947.

ABSTRACT: To guarantee food safety, a better deciphering of ecology and adaptation strategies of bacterial pathogens such as *Salmonella* in food environments is crucial. The role of food processing conditions such as cleaning and disinfection procedures on antimicrobial resistance emergence should especially be investigated. In this work, the prevalence and antimicrobial resistance of *Salmonella* and the microbial ecology of associated surfaces communities were investigated in a pig slaughterhouse before and after cleaning and disinfection procedures. *Salmonella* were detected in 67% of samples and isolates characterization revealed the presence of 15 PFGE-patterns belonging to five serotypes: S.4,5,12:i:-, Rissen, Typhimurium, Infantis and Derby. Resistance to ampicillin, sulfamethoxazole, tetracycline and/or chloramphenicol was detected depending on serotypes. 16S rRNA-based bacterial diversity analyses showed that *Salmonella* surface associated communities were highly dominated by the Moraxellaceae family with a clear site-specific composition suggesting a persistent colonization of the pig slaughterhouse. Cleaning and disinfection procedures did not lead to a modification of *Salmonella* susceptibility to antimicrobials in this short-term study but they tended to significantly reduce bacterial diversity and favored some genera such as *Rothia* and *Psychrobacter*. Such data participate to the construction of a comprehensive view of *Salmonella* ecology and antimicrobial resistance emergence in food environments in relation with cleaning and disinfection procedures. ISSN: 20452322

Prasertsee, T., Chuammitri, P., Deeudom, M., Chokesajjawatee, N., Santiyanont, P., Tadee, P., Nuangmek, A., Tadee, P., Sheppard, S., Pascoe, B., Patchanee, P.
Core genome sequence analysis to characterize Salmonella enterica serovar Rissen ST469 from a swine production chain

(2019) *International Journal of Food Microbiology*, 304, pp. 68-74.

ABSTRACT: *Salmonella enterica* subsp. *enterica* serotype Rissen is the predominant serotype found in Thai pork production and can be transmitted to humans through contamination of the food chain. This study was conducted to investigate the genetic relationships between serovar Rissen isolates from all levels of the pork production chain and evaluate the ability of the in silico antimicrobial resistance (AMR) genotypes to predict the phenotype of serovar Rissen. A total of 38 serovar Rissen isolates were tested against eight antibiotic agents by a disk diffusion method and the whole genomes of all isolates were sequenced to detect AMR genetic elements using the ResFinder database. A total of 86.84% of the isolates were resistant to tetracycline, followed by ampicillin (78.96%) and sulfonamide-trimethoprim (71.05%). Resistance to more than one antimicrobial agent was observed in 78.95% of the isolates, with the most common pattern showing resistance to ampicillin, chloramphenicol, streptomycin, sulfonamide-trimethoprim, and tetracycline. The results of genotypic AMR indicated that 89.47% of the isolates carried tet(A), 84.22% carried bla_{TEM}-1B, 78.95% carried sul3, and 78.95% carried dfrA12. The genotypic prediction of phenotypic resistance resulted in a mean sensitivity of 97.45% and specificity of 75.48%. Analysis by core genome multilocus sequence typing (cgMLST) demonstrated that the *Salmonella* isolates from various sources and different locations shared many of the same core genome loci. This implies that serovar Rissen has infected every stage of the pork production process and that contamination can occur in every part of the production chain. ISSN: 01681605

Sarjit, A., Ravensdale, J.T., Coorey, R., Fegan, N., Dykes, G.A.

Salmonella response to physical interventions employed in red meat processing facilities

(2019) *Food Control*, 103, pp. 91-102.

ABSTRACT: Salmonella is a foodborne bacterial pathogen associated with red meat production worldwide. Survival of, and cross contamination with, this pathogen at any stage from the farm to the processing facility may result in human disease. Cattle, goat and sheep are common animal reservoirs of Salmonella. Various interventions to control the spread of this pathogen during red meat processing have been applied. Physical interventions, such as chilling, thermal treatments, ionizing radiation, ultraviolet radiation, as well as novel interventions such as high pressure treatment and ultrasound, have been used with varying success to reduce Salmonella during processing. This review examines the use of physical interventions to control Salmonella during processing and the stress response of Salmonella towards these interventions. Most existing studies do not provide a very strong insight into the molecular mechanisms of survival of Salmonella after exposure to these physical interventions. Attachment of Salmonella onto red meat species may be strain specific and the genetic profile of these strains may contribute to the survival of Salmonella during red meat processing. Bacterial sigma factors which may act as DNA repair proteins may induce the expression of virulence genes. Salmonella may develop a tolerance mechanism to these physical interventions by enhancing the expression of genes which may provide protection to cells during processing. Developing effective approaches for reduction and elimination of this pathogen on red meat still remains a challenge due to a lack of understanding of the stress response of different serovars of Salmonella. Genome wide stress responses and validation of the gene expression of Salmonella transcriptomes and proteomes need to be intensified to determine phenotypic and genotypic profiles of Salmonella on different red meat species under these different stressors. ISSN: 09567135

Channaiah, L.H., Michael, M., Acuff, J.C., Phebus, R.K., Thippareddi, H., Milliken, G.
Evaluation of thermal inactivation parameters of Salmonella in whole wheat multigrain bread

(2019) *Food Microbiology*, 82, pp. 334-341.

ABSTRACT: This study was conducted to validate a simulated commercial whole wheat multigrain bread baking process at 375 °F (190.6 °C) oven temperature for 35 min to inactivate Salmonella, and to determine the thermal inactivation parameters of a 7-serovar Salmonella cocktail in whole wheat multigrain bread dough. A ≥ 5 -log CFU/g reduction in Salmonella population was achieved by 15 min, and no viable Salmonella was detected after enrichment plating by 16 min. The a w of the bread crumb (0.96) after baking and 60 min of cooling was similar to that of pre-baked bread dough, whereas the a w of bread crust decreased to 0.81 at the end of baking and cooling. The D-values of the Salmonella cocktail in bread dough were 59.6, 20.0 and 9.7 min at 50, 52 and 55 °C, respectively; and the z-value was 6.5 °C. ISSN: 07400020

Harris, C.E., Josselson, L.N.B., Bourassa, D.V., Fairchild, B.D., Kiepper, B.H., Buhr, R.J.

Evaluation of Drinking Water Antimicrobial Interventions on Water Usage, Feed Consumption, and Salmonella Retention in Broilers Following Feed and Water Withdrawal
(2019) *Journal of Applied Poultry Research*, 28 (3), pp. 699-711.

ABSTRACT: A series of experiments were conducted to measure the effects of adding antimicrobials for 2 d: cetylpyridinium chloride (CPC), hydrogen peroxide (HP), and/or sodium bisulfate (SB) to water drinker lines of market-age broilers on water usage, feed consumption, and inoculated Salmonella retention during feed and water withdrawal. The following experiments were evaluated: Salmonella retention with CPC (Experiment 1), water and feed consumption with CPC (Experiment 2) or with CPC and HP (Experiment 3), Salmonella retention with HP (Experiment 4) or with HP and SB (Experiment 5). For Experiment 1, water usage in the CPC treatment pens was only 14% of control pens and had no impact on Salmonella retention. For Experiments 2 and 3, the water usage by broilers was depressed 40 to 97% compared to controls. For Experiment 4, the number of Salmonella-positive enriched crop samples was significantly lower for 50 ppm HP+ citric acid (CA) pens (17%) compared to control (100%). For Experiment 5, water antimicrobial treatments did not differ significantly in Salmonella recovery from the control for both enriched crops (65%) or ceca (82%). Neither CPC nor SB was determined to be effective intervention against Salmonella when added to drinking water during feed/water withdrawal. In Experiment 4, 50 ppm HP+CA was an effective Salmonella intervention in crops while broilers remain on water during feed withdrawal. ISSN: 10566171

Chaves, R.D., Aspridou, Z., Sant'Ana, A.S., Koutsoumanis, K.P.

Effect of chlorine stress on the subsequent growth behavior of individual Salmonella cells
(2019) *Food Research International*, 123, pp. 311-316.

ABSTRACT: The present work investigates the effect of chlorine stress on the subsequent growth behavior of individual *Salmonella* cells. A time-lapse microscopy method was used which allowed to evaluate the effect of chlorine on the division times of *Salmonella* individual cells and the percentage of cells able to divide after the treatment. The results showed that the percentage of cells able to divide after the chlorine treatment decreased from 92.7% for untreated cells to 43.12% and 22% for cell exposed to 127 and 150 mg/l chlorine for 3 min, respectively. The first division time of *Salmonella* cells was not affected by the chlorine stress at the lower tested concentration of 11 mg/l. Exposure at higher chlorine concentrations however, resulted in significantly longer and more variable division times. The mean first division times were 1.46 ± 0.61 , 1.41 ± 0.53 , 1.69 ± 0.59 , 5.34 ± 4.03 and 19.2 ± 8.71 h after 3 min treatments with 0, 11, 61, 127 and 150 mg/l chlorine, respectively. The effect of chlorine on the second division time of the cells was milder compared to the first division. Exposure of cells to chlorine concentrations up to 61 mg/l did not affect the second division. These results indicate that the daughter cells have no "memory" of the chlorine treatment at these concentrations. For cells exposed to the highest tested chlorine concentration of 150 mg/l the mean second division time was almost 3.5 times longer compared to untreated cells indicating that potential damages of the cells caused by the chlorine treatment are not fully repaired in the second generation. The quantitative data provided by this study at the level of individual cell may lead to a better understanding of microbial resistance to chlorine and improve sanitation and decontamination procedures in the food industry. ISSN: 09639969

Crucello, A., Furtado, M.M., Chaves, M.D.R., Sant'Ana, A.S.

Transcriptome sequencing reveals genes and adaptation pathways in Salmonella Typhimurium inoculated in four low water activity foods
(2019) *Food Microbiology*, 82, pp. 426-435.

ABSTRACT: *Salmonella enterica* serotypes have been reported as the agent of various outbreaks occurred after the consumption of low water activity (a_w) foods. When the pathogen encounters harsh conditions, several regulatory networks are activated through dynamic differential gene expression that lead to cell survival for prolonged periods. In this work, the transcriptome of *S. enterica* serovar Typhimurium using RNA-Seq, after cells' inoculation in four distinct types of low a_w foods (milk chocolate, powdered milk, black pepper, and dried pet food), following storage at 25 °C per 24 and 72 h was studied. The findings of this study suggest that gene regulation is influenced by the food composition mainly in the first 24 h post-inoculum, proceeded by the induction of similar genes shared among all samples. It was possible to evaluate the differences on each type of food matrix regarding the bacteria adaptation, as well as the similarities provoked by low a_w . The results reveal genes that may play key roles in response to desiccation in *Salmonella*, as well as the pathways in which they are involved. ISSN: 07400020

Iacumin, L., Comi, G.

Microbial quality of raw and ready-to-eat mung bean sprouts produced in Italy
(2019) *Food Microbiology*, 82, pp. 371-377.

ABSTRACT: The aim of this study was to determine the microbial quality of mung bean sprouts produced in Italy. The presence of pathogenic microorganisms (Shiga toxin-producing *Escherichia coli* (STEC), *Salmonella* spp. and *Listeria monocytogenes*), total coliforms, and total viable counts (TVCs) were determined. The study covered five years of sprout production. The results demonstrated that no pathogenic microorganisms were present, and the microbial load was less than 6 log CFU/g. The mung bean sprouts currently produced in Italy were found to be acceptable for consumption. An additional aim was to determine the fate of different strains of STEC, *L. monocytogenes* and *Salmonella* spp. by intentionally inoculating mung bean seeds during sprouting and by using chlorinated water to reduce the concentration of these strains in seeds and sprouts. The data demonstrated that these strains increased over 5–6 log CFU/g within 3 days from inocula. The chlorinated washing solution reduced the concentration of the investigated strains in seeds and sprouts by approximately 3 and 7 log CFU/g, respectively. However, it was not possible to completely eliminate the pathogens from either the mung bean seeds or sprouts. Despite these encouraging results, the producer's attention to hygienic quality should not be reduced when attempting to produce safe-to-consume mung bean sprouts. ISSN: 07400020

Meinen, A., Simon, S., Banerji, S., Szabo, I., Malorny, B., Borowiak, M., Hadziabdic, S., Becker, N., Lubber, P., Lohr, D., Harms, C., Plenge-Bönig, A., Mellou, K., Mandilara, G., Mossong, J., Ragimbeau, C., Weicherding, P., Hau, P., Dědičová, D., Šafaříková, L., Nair, S., Dallman, T.J., Larkin, L., McCormick, J., De Pinna, E., Severi, E., Kotila, S., Niskanen, T., Rizzi, V., Deserio, D., Flieger, A., Stark, K.

Salmonellosis outbreak with novel Salmonella enterica subspecies enterica serotype (11:z41:e,n,z15) attributable to sesame products in five European countries, 2016 to 2017 (2019) Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin, 24 (36), .

ABSTRACT: In spring 2016, Greece reported an outbreak caused by a previously undescribed *Salmonella* enterica subsp. enterica serotype (antigenic formula 11:z41:e,n,z15) via the Epidemic Intelligence Information System for Food- and Waterborne Diseases and Zoonoses (EPIS-FWD), with epidemiological evidence for sesame products as presumptive vehicle. Subsequently, Germany, Czech Republic, Luxembourg and the United Kingdom (UK) reported infections with this novel serotype via EPIS-FWD. Concerned countries in collaboration with the European Centre for Disease Prevention and Control (ECDC) and European Food Safety Authority (EFSA) adopted a common outbreak case definition. An outbreak case was defined as a laboratory-confirmed notification of the novel *Salmonella* serotype. Between March 2016 and April 2017, 47 outbreak cases were notified (Greece: n = 22; Germany: n = 13; Czech Republic: n = 5; Luxembourg: n = 4; UK: n = 3). Whole genome sequencing revealed the very close genetic relatedness of isolates from all affected countries. Interviews focusing on sesame product consumption, suspicious food item testing and trace-back analysis following *Salmonella* spp. detection in food products identified a company in Greece where sesame seeds from different countries were processed. Through European collaboration, it was possible to identify and recall sesame spread as one contaminated food item serving as vehicle of infection and trace it back to its origin. ISSN: 15607917

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Occupational risk of salmonellosis and campylobacteriosis: A nationwide population-based registry study

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ABSTRACT: Objectives Occupational exposure to animals and foods thereof is a poorly characterised risk factor for salmonellosis and campylobacteriosis, the main causes of bacterial gastroenteritis in the Western world. We performed a population-based registry study in the Netherlands to assess whether differences exist in the incidence of reported salmonellosis and campylobacteriosis cases among occupational groups, and whether they can be explained by differences in the magnitude of exposure to these pathogens, as defined by serology. Methods Person-level occupational data for all Dutch residents were linked to lab-confirmed salmonellosis and campylobacteriosis data, and to serological data from a previous national serosurvey. SIRs for salmonellosis and campylobacteriosis among occupational sectors and specific high-risk occupations were calculated based on the total employed population. Moreover, *Salmonella* and *Campylobacter* seroincidence rates were compared among sectors and high-risk occupations. Results Occupational exposure to live animals or manure and working in the sale of animal-derived food products were associated with significantly increased risks of salmonellosis (SIR 1.55-1.82) and campylobacteriosis (SIR 1.36-1.65). Moreover, incidences were significantly higher in specific industrial sectors, as well as healthcare and social work sectors. Mean seroincidence rates ranged from 1.28 to 2.30 infections/person-year for *Campylobacter*, and from 0.36 to 0.99 for *Salmonella*, with only slightly higher rates for people in high-risk occupations. Conclusions Significant differences in reported salmonellosis and campylobacteriosis incidence exist among occupational sectors, with the highest incidence in those persons occupationally exposed to live animals. These differences are only partially reflected in the serology. ISSN: 13510711

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Facultative anaerobes shape multispecies biofilms composed of meat processing surface bacteria and Escherichia coli O157:H7 or Salmonella enterica serovar Typhimurium (2019) Applied and Environmental Microbiology, 85 (17), art. no. e01123, .

ABSTRACT: This study investigated the microbial dynamics in multispecies biofilms of *Escherichia coli* O157:H7 strain 1934 (O157) or *Salmonella enterica* serovar Typhimurium ATCC 14028 (ST) and 40 strains of meat processing surface bacteria (MPB). Biofilms of O157 or ST with/without MPB were developed on stainless steel coupons at 15°C for up to 6 days. Bacteria in suspensions (inoculum, days 2 and 6) and biofilms (days 2 and 6) were enumerated by plating. The composition of multispecies cultures was determined by 16S rRNA gene sequencing. In suspensions, levels of O157 and ST were ~2 log higher in single-species than in multispecies cultures on both sampling days. ST was 3 log higher in single-species than in multispecies biofilms. A similar trend, though to a lesser extent, was observed for O157 in biofilms on day 2 but not on day 6. No difference (P > 0.05) in bacterial counts was noted for the two MPB-pathogen cocultures at any time during incubation. Bacterial diversity in multispecies cultures decreased with incubation time,

irrespective of the pathogen or culture type. The changes in the relative abundance of MPB were similar for the two MPB-pathogen cocultures, though different interbacterial interactions were noted. Respective fractions of ST and O157 were 2.1% and 0.97% initially and then 0.10% and 0.07% on day 2, and 0.60% and 0.04% on day 6. The relative proportions of facultative anaerobes in both multispecies cultures were greater in both suspensions and biofilms than in the inoculum. *Citrobacter*, *Hafnia*, *Aeromonas*, and *Carnobacterium* predominated in biofilms but not always in the planktonic cultures.
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