

NEWSLETTER

European Union Reference Laboratory for *Salmonella*

Vol. 23 No. 4
December 2017

ISSN 2211-6877



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

Produced by

European Union Reference Laboratory for *Salmonella*

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

phone: +31 30 274 3537 (Kirsten Mooijman)
+31 30 274 4290 (Wilma Jacobs)

e-mail: kirsten.mooijman@rivm.nl
wilma.jacobs@rivm.nl

Contents

Editorial Note.....	4
Contribution of the EURL- <i>Salmonella</i>	6
From the Literature.....	7

Editorial Note

Bilthoven, 5 January 2018

Dear colleagues,

First of all I would like to wish you **a very good and healthy 2018!**

Hopefully you have had a joyful Christmas break and a good start of the New Year. We look forward to cooperate with you all again in this New Year!

I would like to start with summarising the EURL-*Salmonella* interlaboratory studies organized in the last quarter of 2017, and planned for the first quarter of 2018.

In October 2017, the **combined interlaboratory comparison study on detection of *Salmonella* in Food and samples from the Primary Production Stage (PPS)** was organised. As you may remember, we organised this combined study to be able to change the order of the interlaboratory studies for detection of *Salmonella* in samples from the primary production stage (PPS) and for detection of *Salmonella* in food/feed samples, and not to lose one type of study within one year. Up to 2017, the interlaboratory study for detection of *Salmonella* in PPS was organised in February/March of each year. However, for several years we have been facing problems with the choice of the matrix due to Avian Influenza outbreaks. Problems with Avian Influenza are generally related to migration of wild birds in fall and winter and may affect the interlaboratory study in February/March. Also this winter, there has been an outbreak of Avian Influenza in some areas in the Netherlands. Such an outbreak results in several measures, amongst others in a ban of transport of poultry faeces in some areas. Luckily we were prepared this year, as the interlaboratory study of February/March 2018 does not contain faeces samples, but animal feed.

In the combined study of October 2017 hygiene swabs had to be analysed, and a larger group of NRLs participated than usual as NRLs-*Salmonella* for the analysis of food as well as for the analysis of PPS could participate. This resulted in a total of 56 participants (33 NRLs for food, 23 NRLs for PPS), of which 54 fulfilled the criteria of good performance. The interim summary and the NRLs' own results were sent to all participants in December 2017 and the interim summary is also available at the EURL-*Salmonella* website:

<http://www.eurlsalmonella.eu/dsresource?type=pdf&disposition=inline&objectid=rivmp:337240&versionid=&subobjectname=>

In November, the **22nd interlaboratory comparison study on typing of *Salmonella*** was organised. The deadline for reporting the serotyping results was shortly before Christmas, meaning that the analysis of the data will soon be started. Deadline for reporting the PFGE typing results is extended to Friday 26 January 2018.

In the last quarter of 2017, we also started with the preparation of the **4th interlaboratory comparison study on the detection of *Salmonella* in animal feed**. This study is scheduled for February/March 2018 and the timetable is included in this newsletter.

Later than in other years and for the first time in a new template from EC DG-Sante, we have submitted the **work program and budget forecast for the activities of the EURL-*Salmonella* for 2018** in November 2017. The work programs and planned budgets of all (46) EURLs are currently under review and the decision on all plans is expected in February 2018. The final work program of the EURL-*Salmonella* for 2018 will therefore not be published in this newsletter, but in the one of March 2018.

Part of the work under the new work program is the establishment of a **new working group of 8 EURLs on Whole Genome Sequencing (WGS)**. The main aims of this working group are to promote the use of WGS across the EURLs' networks, build WGS capacity within the EU and to harmonise as much as possible the activities for WGS (e.g. interlaboratory studies, trainings) amongst the EURLs. The EURLs which are part of this working group are: EURL-VTEC (coordinator), EURL-*Listeria monocytogenes*, EURL-Coagulase Positive Staphylococci, EURL-*Salmonella*, EURL-*Campylobacter*, EURL-Parasites, EURL-Antimicrobial Resistance, and EURL-Foodborne viruses. The kick-off meeting of this working group was in November 2017 and a first activity which is foreseen is an inventory amongst all 8 EURLs' networks to have a better idea on the specific needs of the EURLs in relation to WGS. This inventory is planned to be performed in the first months of 2018. As soon as more information is available we will inform you.

In the last quarter of 2017, we started with the preparation of the EURL-*Salmonella* **workshop of 2018**. We gladly have accepted the kind invitation of Lennart Melin of the NRL-*Salmonella* in Sweden to organise the workshop in Uppsala, Sweden. By mid-December we have sent you the first announcement of the workshop and informed you about the planned dates. However, when sending you this first announcement the hotel rooms were not yet arranged and it turned out that on the originally chosen dates (31 May and 1 June) several hotels were already fully booked. We are still negotiating with a hotel, but at the same time we are also looking for options earlier in the same week. Therefore, it may be the case that we need to move the workshop to dates earlier in the week of 31 May. As soon as we have more information, we will let you know.

Best wishes,
Kirsten Mooijman
Coordinator EURL-*Salmonella*

Contribution of the EURL-*Salmonella*

Time table interlaboratory comparison study animal feed (2018) Detection of *Salmonella* in chicken feed

Week (2018)	Dates	Subject
7	12 – 16 February	Mailing of the protocol and instructions for the web based test report to the NRLs by email. Sending the link and the password for the electronic results form to the participants by email.
8	19 February	Mailing of parcels to the NRLs as Biological Substance Cat. B (UN3373) by door-to-door courier service Preparation of media by the NRLs
9	26 February	Performance of the study
12	<u>Before 22 March</u>	Deadline for completing the electronic submission of results: 21 March 2018 (23:59h CET) After this deadline the electronic submission form will be closed.

From the Literature

Salmonella-related Literature from Scopus: October – December 2017

Methner, U., Berndt, A., Locke, M.

Salmonella Enteritidis with double deletion in phoP flhC and a competitive exclusion culture elicit substantial additive protective effects against Salmonella exposure in newly hatched chicks

(2017) *Vaccine*, 35 (45), pp. 6076-6082.

ABSTRACT: A live *Salmonella Enteritidis* vaccine (SE 147N Δ phoP flhC), able to express both a homologous intestinal colonisation-inhibition effect and a systemic invasion-inhibition effect, was tested for its potential to generate a postulated additive protective effect in case of combined application with a competitive exclusion (CE) culture against *Salmonella* exposure in very young chicks. Both, SE 147N Δ phoP flhC and the CE culture alone were highly protective against systemic and intestinal colonisation of the challenge strain in case of moderate *Salmonella* exposure, consequently, additive protective effects in combined use could not be detected. However, in case of high *Salmonella Enteritidis* challenge with 10⁶ cfu/bird at day 3 of life the combination of the Δ phoP flhC vaccine and the CE culture resulted in a protective effect much more pronounced than either of the single preparations and most substantial compared to untreated control birds. The term additive protective effects reflects the recognition that exclusion effects by gut flora cultures and inhibition effects by *Salmonella* vaccines are caused by different mechanisms. ISSN: 0264410X

Hsu, C.-K., Micallef, S.A.

Plant-mediated restriction of Salmonella enterica on tomato and spinach leaves colonized with Pseudomonas plant growth-promoting rhizobacteria

(2017) *International Journal of Food Microbiology*, 259, pp. 1-6.

ABSTRACT: Reducing *Salmonella enterica* association with plants during crop production could reduce risks of fresh produce-borne salmonellosis. Plant growth-promoting rhizobacteria (PGPR) colonizing plant roots are capable of promoting plant growth and boosting resistance to disease, but the effects of PGPR on human pathogen-plant associations are not known. Two root-colonizing *Pseudomonas* strains S2 and S4 were investigated in spinach, lettuce and tomato for their plant growth-promoting properties and their influence on leaf populations of *S. enterica* serovar Newport. Plant roots were inoculated with *Pseudomonas* in the seedling stage. At four (tomato) and six (spinach and lettuce) weeks post-germination, plant growth promotion was assessed by shoot dry weight (SDW) and leaf chlorophyll content measurements. Leaf populations of *S. Newport* were measured after 24 h of leaf inoculation with this pathogen by direct plate counts on Tryptic Soy Agar. Root inoculation of spinach cv. 'Tyee', with *Pseudomonas* strain S2 or S4 resulted in a 69% and 63% increase in SDW compared to non-inoculated controls ($p < 0.005$ and $p < 0.01$, respectively). Similarly, Romaine lettuce cv. 'Parris Island Cos' responded positively to S2 and S4 inoculation (53% and 48% SDW increase, respectively; $p < 0.05$), and an increase in leaf chlorophyll content ($p < 0.001$), compared to controls. Tomato cv. 'Nygous' yielded significantly greater SDW (74%, $p < 0.01$ and 54%, $p < 0.05$ for S2 and S4, respectively), and also higher leaf chlorophyll content (19% and 29%, $p < 0.001$, respectively) relative to controls. Leaf chlorophyll content only increased in S4-inoculated tomato cv. 'Moneymaker' plants (27%, $p < 0.001$), although both S2 and S4 promoted plant growth by over 40% compared to controls ($p < 0.01$ and $p < 0.05$, respectively). No significant growth promotion was detected in tomato cv. 'BHN602', but S2-inoculated plants had elevated leaf chlorophyll content (13%, $p < 0.01$). Root inoculation with *Pseudomonas* S4 restricted *S. Newport* populations inoculated on leaves of spinach ($p < 0.001$) and all three tomato cultivars ($p < 0.05$), compared to controls, 24 h post *Salmonella* inoculation. Impairment of *S. Newport* leaf populations was also observed on spinach when plant roots were inoculated with S2 ($p < 0.01$). With an initial leaf inoculum of approximately 6.0 log CFU of *S. Newport*/plant, the significantly greater reduction of *S. Newport* populations on *Pseudomonas*-treated plants than those on non-inoculated control plants after 24 h was modest with differences of one log or less. By contrast, the survival of *S. Newport* on the leaves of Romaine lettuce was not influenced by *Pseudomonas* root colonization. These findings provide evidence that root inoculation of certain specialty crops with beneficial *Pseudomonas* strains exhibiting PGPR properties may not only promote plant growth, but also reduce the fitness of epiphytic *S. enterica* in the

phyllosphere. Plant-mediated effects induced by PGPR may be an effective strategy to minimize contamination of crops with *S. enterica* during cultivation. ISSN: 01681605

Boskovic, M., Djordjevic, J., Ivanovic, J., Janjic, J., Zdravkovic, N., Glisic, M., Glamoclija, N., Baltic, B., Djordjevic, V., Baltic, M.

Inhibition of Salmonella by thyme essential oil and its effect on microbiological and sensory properties of minced pork meat packaged under vacuum and modified atmosphere (2017) International Journal of Food Microbiology, 258, pp. 58-67.

ABSTRACT: The antibacterial activity of thyme essential oil (TEO) was evaluated against four serovars of *Salmonella* (*S. Enteritidis*, *S. Typhimurium*, *S. Montevideo* and *S. Infantis*), experimentally inoculated (106 CFU/g) in minced pork, which was treated with different concentrations of the TEO (0.3%, 0.6% and 0.9%) packaged under vacuum or MAP (30%O₂/50%CO₂/20% N₂) and stored at 3 ± 1 °C for 15 days. GC-MS analysis of the TEO was performed in order to determine composition, and the predominant constituent was thymol (50.48%), followed by p-cymene and linalool. The minimum inhibitory concentration was determined for each *Salmonella* serovar studied. Among the tested active compounds, thymol and carvacrol exhibited the greatest inhibitory effect followed by TEO, with minimum inhibitory concentrations of 320 to 640 µg/ml. *S. Enteritidis* was the most sensitive serovar. During the storage period, *Salmonella* counts in pork were reduced by 1.69–4.05 log CFU/g. The influence of TEO on Enterobacteriaceae, lactic acid bacteria and total viable count was determined in control mince with no added *Salmonella*. The most pronounced antibacterial effect was achieved by the combination MAP and 0.9% TEO. Although the antibacterial activities of all studied concentrations of TEO in pork were evident and significant (P < 0.05), sensory analysis showed that 0.3% TEO was the most acceptable to trained panellists. ISSN: 01681605

Muvhali, M., Smith, A.M., Rakgantso, A.M., Keddy, K.H.

Investigation of Salmonella Enteritidis outbreaks in South Africa using multi-locus variable-number tandem-repeats analysis, 2013-2015

(2017) BMC Infectious Diseases, 17 (1), art. no. 661, .

ABSTRACT: Background: *Salmonella enterica* serovar *Enteritidis* (*Salmonella Enteritidis*) has become a significant pathogen in South Africa, and the need for improved molecular surveillance of this pathogen has become important. Over the years, multi-locus variable-number tandem-repeats analysis (MLVA) has become a valuable molecular subtyping technique for *Salmonella*, particularly for highly homogenic serotypes such as *Salmonella Enteritidis*. This study describes the use of MLVA in the molecular epidemiological investigation of outbreak isolates in South Africa. Methods: Between the years 2013 and 2015, the Centre for Enteric Diseases (CED) received 39 *Salmonella Enteritidis* isolates from seven foodborne illness outbreaks, which occurred in six provinces. MLVA was performed on all isolates. Results: Three MLVA profiles (MLVA profiles 21, 22 and 28) were identified among the 39 isolates. MLVA profile 28 accounted for 77% (30/39) of the isolates. Isolates from a single outbreak were grouped into a single MLVA profile. A minimum spanning tree (MST) created from the MLVA data showed a close relationship between MLVA profiles 21, 22 and 28, with a single VNTR locus difference between them. Conclusions: MLVA has proven to be a reliable method for the molecular epidemiological investigation of *Salmonella Enteritidis* outbreaks in South Africa. These foodborne outbreaks emphasize the importance of the One Health approach as an essential component for combating the spread of zoonotic pathogens such as *Salmonella Enteritidis*. ISSN: 14712334

Elbehiry, A., Marzouk, E., Hamada, M., Al-Dubaib, M., Alyamani, E., Moussa, I.M., AlRowaidhan, A., Hemeg, H.A.

Application of MALDI-TOF MS fingerprinting as a quick tool for identification and clustering of foodborne pathogens isolated from food products

(2017) New Microbiologica, 40 (4), pp. 269-278.

ABSTRACT: Foodborne pathogens can be associated with a wide variety of food products and it is very important to identify them to supply safe food and prevent foodborne infections. Since traditional techniques are timeconsuming and laborious, this study was designed for rapid identification and clustering of foodborne pathogens isolated from various restaurants in Al-Qassim region, Kingdom of Saudi Arabia (KSA) using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). Sixty-nine bacterial and thirty-two fungal isolates isolated from 80 food samples were used in this study. Preliminary identification was carried out through culture and BD Phoenix™ methods. A confirmatory identification technique was then performed using MALDI-TOF MS. The BD Phoenix results revealed that 97% (67/69 isolates) of bacteria were correctly identified as 75% *Enterobacter cloacae*, 95.45% *Campylobacter jejuni* and 100% for

Escherichia coli, *Salmonella enterica*, *Staphylococcus aureus*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae*. While 94.44% (29/32 isolates) of fungi were correctly identified as 77.77% *Alternaria alternate*, 88.88% *Aspergillus Niger* and 100% for *Aspergillus flavus*, *Penicillium digitatum*, *Candida albicans* and *Debaryomyces hansenii*. However, all bacterial and fungal isolates were 100% properly identified by MALDI-TOF MS fingerprinting with a score value ≥ 2.00 . A gel view illustrated that the spectral peaks for the identified isolates fluctuate between 3, 000 and 10, 000 Da. The results of main spectra library (MSP) dendrogram showed that the bacterial and fungal isolates matched with 19 and 9 reference strains stored in the Bruker taxonomy, respectively. Our results indicated that MALDI-TOF MS is a promising technique for fast and accurate identification of foodborne pathogens. ISSN: 11217138

Arunima, A., Yelamanchi, S.D., Padhi, C., Jaiswal, S., Ryan, D., Gupta, B., Sathe, G., Advani, J., Gowda, H., Keshava Prasad, T.S., Suar, M.

Omics of Food-Borne Gastroenteritis: Global Proteomic and Mutagenic Analysis of Salmonella enterica Serovar Enteritidis

(2017) *OMICS A Journal of Integrative Biology*, 21 (10), pp. 571-583.

ABSTRACT: *Salmonella* Enteritidis causes food-borne gastroenteritis by the two type three secretion systems (TTSS). TTSS-1 mediates invasion through intestinal lining, and TTSS-2 facilitates phagocytic survival. The pathogens' ability to infect effectively under TTSS-1-deficient background in host's phagocytes is poorly understood. Therefore, pathobiological understanding of TTSS-1-defective nontyphoidal Salmonellosis is highly important. We performed a comparative global proteomic analysis of the isogenic TTSS-1 mutant of *Salmonella* Enteritidis (M1511) and its wild-type isolate P125109. Our results showed 43 proteins were differentially expressed. Functional annotation further revealed that differentially expressed proteins belong to pathogenesis, tRNA and ncRNA metabolic processes. Three proteins, tryptophan subunit alpha chain, citrate lyase subunit alpha, and hypothetical protein 3202, were selected for in vitro analysis based on their functional annotations. Deletion mutants generated for the above proteins in the M1511 strain showed reduced intracellular survival inside macrophages in vitro. In sum, this study provides mass spectrometry-based evidence for seven hypothetical proteins, which will be subject of future investigations. Our study identifies proteins influencing virulence of *Salmonella* in the host. The study complements and further strengthens previously published research on proteins involved in enteropathogenesis of *Salmonella* and extends their role in noninvasive Salmonellosis. ISSN: 15362310

Kuan, C.H., Lim, L.W.K., Ting, T.W., Rukayadi, Y., Ahmad, S.H., Wan Mohamed Radzi, C.W.J., Thung, T.Y., Ramzi, O.B., Chang, W.S., Loo, Y.Y., Kuan, C.S., Yeo, S.-K., Radu, S.

Simulation of decontamination and transmission of Escherichia coli O157:H7, Salmonella Enteritidis, and Listeria monocytogenes during handling of raw vegetables in domestic kitchens

(2017) *Food Control*, 80, pp. 395-400.

ABSTRACT: Epidemiological data indicates that a large number of foodborne illnesses are attributed to cross-contamination during food preparation in the domestic kitchen. The objectives of this study were to evaluate the efficiency of household washing practices in removing *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* Enteritidis on artificially contaminated lettuce and to determine the transfer rate of these three foodborne pathogens from contaminated lettuce to wash water, tomato, cabbage, and cutting boards during washing and cutting processes. Washing under the running tap water with scrubbing for 60 s was the most effective method in reducing pathogen populations by 1.86–2.60 log₁₀ CFU/g. Also, final rinsing and scrubbing practices were found to enhance the efficiency of washing treatment. In this study, the transfer rates of *S. Enteritidis*, *E. coli* O157:H7, and *L. monocytogenes* from cutting board to cabbage and tomato via cutting process (17.5–31.7%) were higher ($P < 0.05$) than from wash water to cabbage and tomato (0.8–23.0%) during washing treatment. Overall, our findings suggest that wash water and cutting board can be potential vehicles in the dissemination of foodborne pathogens. Therefore, there is a need to promote consumer awareness for proper handling practices in the kitchen to minimise the risk of foodborne infection. ISSN: 09567135

Barba-Vidal, E., Buttow Roll, V.F., Garcia Manzanilla, E., Torrente, C., Moreno Muñoz, J.A., Pérez, J.F., Martín-Orúe, S.M.

Blood parameters as biomarkers in a Salmonella spp. disease model of weaning piglets (2017) *PLoS ONE*, 12 (10), art. no. e0186781, .

ABSTRACT: Background: The weaning pig is used as an experimental model to assess the impact of diet on intestinal health. Blood parameters (BP) are considered a useful tool in humans, but there is very scarce information of such indicators in the weaning pig. The objective of the present study is to evaluate the use of different BP as indicators in an experimental model of salmonellosis. Methodology: Seventy-two 28-day-old piglets were divided into four groups in a 2x2 factorial arrangement, with animals receiving or not a probiotic combination based on *B. infantis* IM1® and *B. lactis* BPL6 (10⁹ colony forming units (cfu)/d) and orally challenged or not a week later with *Salmonella* Typhimurium (5x10⁸ cfu). Blood samples of one animal per pen (N = 24) were taken four days post-inoculation for the evaluation of different BP using an I-stat® System and of plasmatic concentrations of zinc, iron and copper. Principal findings: Results reported marginal deficiencies of zinc in piglets at weaning. Moreover, plasmatic zinc, copper and iron presented good correlations with weight gain (r 0.57, r -0.67, r 0.54 respectively; P < 0.01). Blood electrolytes (Na⁺, Cl⁻ and K⁺) decreased (P < 0.01) only when the performance of the animals was seriously compromised and clinical symptoms were more apparent. Acid-base balance parameters such as HCO₃⁻, TCO₂ and BE_{ecf} significantly correlated with weight gain, but only in the challenged animals (r -0.54, r -0.55, and r -0.51, respectively; P < 0.05), suggesting metabolic acidosis depending on *Salmonella* infection. Glucose was affected by the challenge (P = 0.040), while Htc and Hgb increased with the challenge and decreased with the probiotic (P < 0.05). Furthermore, correlations of Glu, Htc and Hgb with weight gain were observed (P < 0.05). Overall, BP could be regarded as simple, useful indexes to assess performance and health of weaning piglets. ISSN: 19326203

Liu, H., Dong, H., Chen, Z., Lin, L., Chen, H., Li, S., Deng, Y.

Magnetic nanoparticles enhanced microarray detection of multiple foodborne pathogens (2017) Journal of Biomedical Nanotechnology, 13 (10), pp. 1333-1343.

ABSTRACT: In order to ensure food safety and minimize the occurrence of foodborne illness, it is critical to monitor food production for the presence of foodborne pathogens. This highlights the need for rapid, sensitive, and selective methods to detect pathogens in food products. In this study, a magnetic nanoparticles enhanced oligonucleotide microarray assay was developed for rapid and sensitive identification of *Escherichia coli* O157:H7, *Salmonella enterica*, *Vibrio cholerae* and *Campylobacter jejuni* in food. Biotin labeled forward primer and Cy3 labeled reverse primer were used for exponential PCR amplification. Magnetic nanoparticles with covalently attached streptavidin were then used to capture the PCR products. After denaturation at 95 °C and magnetic separation, the Cy3-labeled target strand was collected and concentrated in the supernatant for hybridization. By using streptavidin-modified magnetic nanoparticles (SA-MNPs) for rapid generation of purified single stranded DNA target, we successfully increased hybridization signal and improved sensitivity for pathogen detection on DNA microarray. In comparison with the conventional single stranded target preparation methods, this magnetic nanoparticles based method yielded up to 15-fold increase in the hybridization signal and achieved 1~2 orders of magnitude enhancement on limit of detection. The assay sensitivity for identification of *Salmonella enterica* in ground chicken sample was 200 cells/g of food without a pre-enrichment, and the sensitivity was increased 100-fold (~2 cells/g) following 5 hr pre-enrichment at 37°C. The results indicate that the magnetic nanoparticles enhanced microarray method has great potential for detection of food-borne pathogens in food samples with both high specificity and high sensitivity. ISSN: 15507033

Moré, E., Ayats, T., Ryan, P.G., Naicker, P.R., Keddy, K.H., Gaglio, D., Witteveen, M., Cerdà-Cuellar, M.

Seabirds (Laridae) as a source of Campylobacter spp., Salmonella spp. and antimicrobial resistance in South Africa

(2017) Environmental Microbiology, 19 (10), pp. 4164-4176.

ABSTRACT: Zoonotic thermophilic *Campylobacter* and nontyphoidal *Salmonella enterica* are a major cause of foodborne human gastroenteritis worldwide. There is little information about reservoirs of these zoonotic agents in Africa. Thus, chicks of kelp gulls (*Larus dominicanus*, n = 129) and greater crested terns (*Thalasseus bergii*, n = 100) were studied at five colonies on the Western Cape coast (South Africa) during summer 2013/2014. *Campylobacter* spp. occurrence was 14.0% (CI_{95%}: 9.9–19.3), with *C. jejuni* the most frequently isolated species, whilst that of *Salmonella* was 27.5% (CI_{95%}: 21.9–33.9) overall, with a higher prevalence in gulls (43.0%, CI_{95%}: 34.8–52.4) than terns (7.0%, CI_{95%}: 3.1–14.4). Among the 16 different *S. enterica* serovars found, Anatum, Enteritidis and Hadar were the most frequent. The same or highly similar pulsed-field gel electrophoresis genotype was found in some *Salmonella* isolates from seabirds and humans presenting with salmonellosis in Cape Town hospitals. Both *Campylobacter* and

Salmonella isolates exhibited antimicrobial resistance to several agents, including critically important antimicrobials (quinolones, tetracyclines and β -lactams) and multidrug resistance in *Salmonella* serovars from kelp gulls. Our results highlight the importance of seabirds as reservoirs of *Campylobacter* and *Salmonella* resistant strains and their role in the maintenance and transmission of these bacteria in the environment, with implications for public health. ISSN: 14622912

Abdullah, W.Z.W., Mackey, B.M., Karatzas, K.A.G.

Determination of the relative effects of temperature, pH and water activity in food systems: A meta-analysis study

(2017) *Malaysian Applied Biology*, 46 (3), pp. 67-70.

ABSTRACT: The aim of this study is to use ComBase to determine the relative effects of temperature, pH, and water activity in the inactivation rates of *Salmonella enterica* in a range of foods. This is performed to determine whether any of the above factors have a dominant effect on survival. The inactivation rates of *Salmonella* were obtained from original raw data in the ComBase browser and from complete ComBase data for *Salmonella*. A total of 972 data of different types of food systems and data of individual types of food from ComBase were analysed. Over the range of 0–90°C, the z values calculated for the food data is 14°C. At 0–46°C relevant to intermediate moisture foods (IMF), the z values for the food data was 22°C, indicating a moderate effect of temperature. The z value for inactivation at 47–90°C was 11°C, indicating that temperature has an important effect on survival. This study shows that the effect of temperature is clearer at high temperatures than in the low temperature region. It suggests that the inactivation of *Salmonella* in food systems is slightly dominated by temperature and that the pH and aw levels appear to be less influential. ISSN: 01268643

de la Cruz, M.L., Conrado, I., Nault, A., Perez, A., Dominguez, L., Alvarez, J.

Vaccination as a control strategy against Salmonella infection in pigs: A systematic review and meta-analysis of the literature

(2017) *Research in Veterinary Science*, 114, pp. 86-94.

ABSTRACT: Consumption or handling of improperly processed or cooked pork is considered one of the top sources for foodborne salmonellosis, a common cause of intestinal disease worldwide. Asymptomatic carrier pigs may contaminate pork at slaughtering; therefore, pre-harvest reduction of *Salmonella* load can contribute to reduce public health risk. Multiple studies have evaluated the impact of vaccination on controlling *Salmonella* in swine farms, but results are highly variable due to the heterogeneity in vaccines and vaccination protocols. Here, we report the results of an inclusive systematic review and a meta-analysis of the peer-reviewed scientific literature to provide updated knowledge on the potential effectiveness of *Salmonella* vaccination. A total of 126 articles describing the use of *Salmonella* vaccines in swine were identified, of which 44 fulfilled the inclusion criteria. Most of the studies (36/44) used live vaccines, and *S. Typhimurium* and *S. Choleraesuis* were the predominant serotypes evaluated. Vaccine efficacy was most often measured through bacteriological isolation, and pooled estimates of vaccine efficacy were obtained as the difference in the percentage of positive animals when available. Attenuated and inactivated vaccines had similar efficacy [Risk Difference = - 26.8% (- 33.8, - 19.71) and - 29.5% (- 44.4, - 14.5), respectively]. No serotype effect was observed on the efficacy recorded for attenuated vaccines; however, a higher efficacy of inactivated vaccines against *S. Choleraesuis* was observed, though in a reduced sample. Results from the meta-analysis here demonstrate the impact that vaccination may have on the control of *Salmonella* in swine farms and could help in the design of programs to minimize the risk of transmission of certain serotypes through the food chain. ISSN: 00345288

Sloan, A., Wang, G., Cheng, K.

Traditional approaches versus mass spectrometry in bacterial identification and typing

(2017) *Clinica Chimica Acta*, 473, pp. 180-185.

ABSTRACT: Biochemical methods such as metabolite testing and serotyping are traditionally used in clinical microbiology laboratories to identify and categorize microorganisms. Due to the large variety of bacteria, identifying representative metabolites is tedious, while raising high-quality antisera or antibodies unique to specific biomarkers used in serotyping is very challenging, sometimes even impossible. Although serotyping is a certified approach for differentiating bacteria such as *E. coli* and *Salmonella* at the subspecies level, the method is tedious, laborious, and not practical during an infectious disease outbreak. Mass spectrometry (MS) platforms, especially matrix assisted laser desorption and ionization-time of flight mass spectrometry (MALDI-TOF-MS), have recently become popular in the field of bacterial identification due to their fast speed and low cost. In the past few years, we have used liquid chromatography-tandem mass

spectrometry (LC-MS/MS)-based approaches to solve various problems hindering serotyping and have overcome some insufficiencies of the MALDI-TOF-MS platform. The current article aims to review the characteristics, advantages, and disadvantages of MS-based platforms over traditional approaches in bacterial identification and categorization. ISSN: 00098981

Zankari, E., Allesøe, R., Joensen, K.G., Cavaco, L.M., Lund, O., Aarestrup, F.M.

PointFinder: A novel web tool for WGS-based detection of antimicrobial resistance associated with chromosomal point mutations in bacterial pathogens
(2017) *Journal of Antimicrobial Chemotherapy*, 72 (10), pp. 2764-2768.

ABSTRACT: Background: Antibiotic resistance is a major health problem, as drugs that were once highly effective no longer cure bacterial infections. WGS has previously been shown to be an alternative method for detecting horizontally acquired antimicrobial resistance genes. However, suitable bioinformatics methods that can provide easily interpretable, accurate and fast results for antimicrobial resistance associated with chromosomal point mutations are still lacking. Methods: Phenotypic antimicrobial susceptibility tests were performed on 150 isolates covering three different bacterial species: *Salmonella enterica*, *Escherichia coli* and *Campylobacter jejuni*. The web-server ResFinder-2.1 was used to identify acquired antimicrobial resistance genes and two methods, the novel PointFinder (using BLAST) and an in-house method (mapping of raw WGS reads), were used to identify chromosomal point mutations. Results were compared with phenotypic antimicrobial susceptibility testing results. Results: A total of 685 different phenotypic tests associated with chromosomal resistance to quinolones, polymyxin, rifampicin, macrolides and tetracyclines resulted in 98.4% concordance. Eleven cases of disagreement between tested and predicted susceptibility were observed: two *C. jejuni* isolates with phenotypic fluoroquinolone resistance and two with phenotypic erythromycin resistance and five colistin-susceptible *E. coli* isolates with a detected *pmrB* V161G mutation when assembled with Velvet, but not when using SPAdes or when mapping the reads. Conclusions: PointFinder proved, with high concordance between phenotypic and predicted antimicrobial susceptibility, to be a user-friendly web tool for detection of chromosomal point mutations associated with antimicrobial resistance. ISSN: 03057453

Jensen, M.B.F., Schjørring, S., Björkman, J.T., Torpdahl, M., Litrup, E., Nielsen, E.M., Niskanen, T.

External quality assessment for molecular typing of Salmonella 2013–2015: performance of the European national public health reference laboratories
(2017) *European Journal of Clinical Microbiology and Infectious Diseases*, 36 (10), pp. 1923-1932.

ABSTRACT: We report the results of three consecutive External Quality Assessments (EQAs) for molecular subtyping of *Salmonella* to assess the performance of the European national public health reference laboratories (NPHRLs). The EQA included the molecular typing methods used for European enhanced surveillance of human *Salmonella* infections: pulsed field gel electrophoresis (PFGE), including gel analysis by the use of the software BioNumerics, and 5-locus multiple locus variable number of tandem repeat analysis (MLVA) for serovar Typhimurium. The participation in the PFGE laboratory part was higher (27/35) than in the gel analysis (19/35) and MLVA (15/35), suggestive of the need for capacity building in methods requiring specialized equipment (MLVA) or software (gel analysis). The majority (25/27) of the participating NPHRLs produced inter-laboratory comparable PFGE gel(s). Two laboratories continued to produce low-quality gels and should have additional technical assistance in the future. In particular, two gel quality evaluation parameters, measuring "image acquisition and running conditions" and "bands", were identified to cause gel quality problems throughout the EQAs. Despite the high number of laboratories participating in the PFGE laboratory part, the participation in gel analysis was low, although increasing. In the MLVA part, the NPHRLs correctly assigned 96% (405/420) allelic profiles according to the nomenclature. In conclusion, the EQAs identified critical parameters for unsuccessful performance and helped to offer assistance to those laboratories that needed it most. The assessments supported the development of quality in molecular typing and promoted the harmonization of subtyping methods used for EU/EEA-wide surveillance of human *Salmonella* infections. ISSN: 09349723

Gymoese, P., Sørensen, G., Litrup, E., Olsen, J.E., Nielsen, E.M., Torpdahl, M.

Investigation of outbreaks of Salmonella enterica serovar typhimurium and its monophasic variants using whole-genome sequencing, Denmark
(2017) *Emerging Infectious Diseases*, 23 (10), pp. 1631-1639.

ABSTRACT: Whole-genome sequencing is rapidly replacing current molecular typing methods for surveillance purposes. Our study evaluates core-genome single-nucleotide

polymorphism analysis for outbreak detection and linking of sources of *Salmonella enterica* serovar Typhimurium and its monophasic variants during a 7-month surveillance period in Denmark. We reanalyzed and defined 8 previously characterized outbreaks from the phylogenetic relatedness of the isolates, epidemiologic data, and food traceback investigations. All outbreaks were identified, and we were able to exclude unrelated and include additional related human cases. We were furthermore able to link possible food and veterinary sources to the outbreaks. Isolates clustered according to sequence types (STs) 19, 34, and 36. Our study shows that core-genome single-nucleotide polymorphism analysis is suitable for surveillance and outbreak investigation for *Salmonella* Typhimurium (ST19 and ST36), but whole genome-wide analysis may be required for the tight genetic clone of monophasic variants (ST34). ISSN: 10806040

Corrente, M., Sangiorgio, G., Grandolfo, E., Bodnar, L., Catella, C., Trotta, A., Martella, V., Buonavoglia, D.

Risk for zoonotic Salmonella transmission from pet reptiles: A survey on knowledge, attitudes and practices of reptile-owners related to reptile husbandry (2017) Preventive Veterinary Medicine, 146, pp. 73-78.

ABSTRACT: Reptiles are becoming increasingly popular as pets. Those animals are reservoirs of a wide variety of *Salmonella* serotypes, that may be transmitted to warm-blooded animals, including humans. Accordingly, good hygiene practices related to husbandry are important for prevention of Reptile-associated salmonellosis (RAS). A cross-sectional study was conducted among reptile owners, by administration of a detailed questionnaire. In addition, the cloacal swabs of the sampled reptiles were screened for *Salmonella* spp. and the husbandry management practices were evaluated in order to assess any possible link between the presence of *Salmonella* spp. and the hygiene practices. The response rate to the questionnaire was 66.6% (100 out of 150 contacted owners). In 26 out of 100 families, members at risk of RAS (children and elderly) were present. One hundred animals were screened for the presence of *Salmonella* spp. The prevalence of *Salmonella* spp. carriers was 57% (Confidence interval 47–66%). Co-habitation of the animals with other reptiles in the same terrarium was associated with a 2-fold increase in the risk of infection by *Salmonella* spp. (Odds ratio = 2.3, CI 1.2; 13, p = 0.02). Animals handled by owners that did not report washing their hands after the cleaning procedures or the handling were exposed to a 3-fold increase in the risk of infection (OR = 3.1, CI 1.1; 16, p = 0.019). When drinking water was not replaced regularly, the animals were 7 times more exposed to infection (OR = 6.8, CI 1.8; 25, p = 0.005). When the diet was constituted by rodents, 27 out of 48 reptiles (56.3%) were fed with live animals. In the present survey the typical reptile owner was a person, aware of ethological aspects of reptile husbandry but ignorant of some ethical recommendations and poorly informed about the health risks for himself and for the other family members. Prevention of RAS must rely mainly on information and education, with the veterinarian health bodies primarily involved in this difficult task. ISSN: 01675877

Hahn, J., Kim, E., You, Y.S., Gunasekaran, S., Lim, S., Choi, Y.J.

A Switchable Linker-Based Immunoassay for Ultrasensitive Visible Detection of Salmonella in Tomatoes (2017) Journal of Food Science, 82 (10), pp. 2321-2328.

ABSTRACT: Abstract: On-site detection for sensitive identification of foodborne pathogens on fresh produce with minimal use of specialized instrumentation is crucial to the food industry. A switchable linker (SL)-based immunoassay was designed for ultrasensitive on-site detection of *Salmonella* in tomato samples. The assay is based on large-scale aggregation of gold nanoparticles (GNPs), induced by a quantitative relationship among the biotinylated *Salmonella* polyclonal antibody (b-Ab) used as the SL, the functionalized GNPs, and *Salmonella*. Important factors such as the concentration of SLs, time required for large-scale aggregation, and selectivity of b-Ab were optimized to minimize the detection time (within 45 min with gentle agitation) and achieve the lowest limit of detection (LOD; 10 CFU/g in tomato samples) possible. This SL-based immunoassay with its relatively low LOD and short detection time may meet the need for rapid, simple, on-site analysis of pathogens in fresh produce. Practical Application: The novel switchable linker-based immunoassay is a rapid, specific, and sensitive method that has potential applications for routine diagnostics of *Salmonella* in tomato products. These advantages make it a practical approach for general use in the processing industry to detect *Salmonella* rapidly and to implement appropriate regulatory procedures. Furthermore, it could be applied to other fresh products including cantaloupe, strawberry, and cucumbers. ISSN: 00221147

Huang, J.Y., Patrick, M.E., Manners, J., Sapkota, A.R., Scherzinger, K.J., Tobin-D'Angelo, M., Henao, O.L., Cole, D.J., Vieira, A.R.

Association between wetland presence and incidence of Salmonella enterica serotype Javiana infections in selected US sites, 2005-2011

(2017) *Epidemiology and Infection*, 145 (14), pp. 2991-2997.

ABSTRACT: Salmonella causes an estimated 1·2 million illnesses annually in the USA. Salmonella enterica serotype Javiana (serotype Javiana) is the fourth most common serotype isolated from humans, with the majority of illnesses occurring in southeastern states. The percentage of wetland cover by wetland type and the average incidence rates of serotype Javiana infection in selected counties of the Foodborne Disease Active Surveillance Network (FoodNet) were examined. This analysis explored the relationship between wetland environments and incidence in order to assess whether regional differences in environmental habitats may be associated with observed variations in incidence. Findings suggest that environmental habitats may support reservoirs or contribute to the persistence of serotype Javiana, and may frequently contribute to the transmission of infection compared with other Salmonella serotypes. ISSN: 14694409

Grygorcewicz, B., Grudziński, M., Wasak, A., Augustyniak, A., Pietruszka, A., Nawrotek, P.

Bacteriophage-mediated reduction of Salmonella Enteritidis in swine slurry
(2017) *Applied Soil Ecology*, 119, pp. 179-182.

ABSTRACT: Slurry is considered one of the best fertilizers. Nevertheless, it may contain harmful bacteria. Spreading it on the soil may contribute to transfer of these microorganisms in the environment. This could be avoided with effective biocontrol methods Bacteriophage sall_v01 specific to several Salmonella enterica serovars was isolated from wastewater and characterized. The lytic activity of bacteriophage sall_v01 showed its effectiveness for the growth reduction of Salmonella Enteritidis at the multiplicity of infection = 1, with latent periods = 25 min, and the burst size approx. 107 ± 8 new virions per cell after 45 min at 37 °C. Bacteriophage-mediated treatment of experimentally contaminated swine slurry resulted in 3.8 log CFU/mL reduction in quantity of Salmonella Enteritidis. Based on our results, phage sall_v01 could be considered a potential biocontrol agent against Salmonella Enteritidis contamination in agriculture and animal production. ISSN: 09291393

Barbosa, F.D.O., Freitas Neto, O.C.D., Batista, D.F.A., Almeida, A.M.D., Rubio, M.D.S., Alves, L.B.R., Vasconcelos, R.D.O., Barrow, P.A., Berchieri Junior, A.

Contribution of flagella and motility to gut colonisation and pathogenicity of Salmonella Enteritidis in the chicken

(2017) *Brazilian Journal of Microbiology*, 48 (4), pp. 754-759.

ABSTRACT: Salmonella Enteritidis causes fowl paratyphoid in poultry and is frequently associated to outbreaks of food-borne diseases in humans. The role of flagella and flagella-mediated motility into host-pathogen interplay is not fully understood and requires further investigation. In this study, one-day-old chickens were challenged orally with a wild-type strain Salmonella Enteritidis, a non-motile but fully flagellated (SE Δ motB) or non-flagellated (SE Δ fliC) strain to evaluate their ability to colonise the intestine and spread systemically and also of eliciting gross and histopathological changes. SE Δ motB and SE Δ fliC were recovered in significantly lower numbers from caecal contents in comparison with Salmonella Enteritidis at early stages of infection (3 and 5 dpi). The SE Δ motB strain, which synthesises paralysed flagella, showed poorer intestinal colonisation ability than the non-flagellated SE Δ fliC. Histopathological analyses demonstrated that the flagellated strains induced more intense lymphoid reactivity in liver, ileum and caeca. Thus, in the present study the flagellar structure and motility seemed to play a role in the early stages of the intestinal colonisation by Salmonella Enteritidis in the chicken. ISSN: 15178382

Xu, C., Ren, X., Feng, Z., Fu, Y., Hong, Y., Shen, Z., Zhang, L., Liao, M., Xu, X., Zhang, J.

Phenotypic characteristics and genetic diversity of salmonella enterica serotype derby isolated from human patients and foods of animal origin

(2017) *Foodborne Pathogens and Disease*, 14 (10), pp. 593-599.

ABSTRACT: Salmonella enterica serotype Derby is among the three most common serotypes of nontyphoidal Salmonella isolated from patients with diarrhea in China. In this study, 133 Salmonella Derby isolates from human patients (n = 74) and foods of animal origin (n = 59) in Shanghai, China, between September 2013 and December 2014, were selected to study its phenotypic characteristics and genetic diversity. The isolates were subjected to antimicrobial susceptibility testing, plasmid replicon typing, virulence profile determination, and molecular subtyping by pulsed-field gel electrophoresis (PFGE).

Isolates were frequently resistant to tetracycline (87.22%), sulfisoxazole (74.44%), and streptomycin (62.41%), and a low frequency of resistance was found toward ofloxacin (3.01%), ceftazidime (2.26%), and cefepime (1.50%); in addition, 93 (69.92%) isolates were multidrug resistant. The most common plasmid incompatibility replicon types were the IncF family (FIA, 51.31%; FIC, 27.82%; and FIB, 21.80%) and IncP types (35.34%): these plasmid types may be associated with the spread of antibiotic resistance and virulence genes. All isolates were positive for the *Salmonella* pathogenicity island (SPI) gene *avrA* and the fimbrial gene *bcfC* from among the 10 virulence genes detected, and most of them carried *ssaQ* (99.25%), *mgtC* (97.74%), *siiD* (98.50%), *sopB* (97.74%), and *sopE* (96.99%). PFGE showed 68 patterns in nine main clusters at an 85% similarity threshold. Most of the isolates from different sources possessed the same fingerprints or molecular profiles in each cluster, which strongly suggests the possibility that foods of animal origin, especially pork, serve as an important source for human infection. Moreover, this diversity may suggest strains originating from multiple clones. Therefore, surveillance on this serotype should be strengthened to prevent transmission of *Salmonella* Derby from foods of animal origin, especially pork, to humans. ISSN: 15353141

Lambertini, E., Barouei, J., Schaffner, D.W., Danyluk, M.D., Harris, L.J.

Modeling the risk of salmonellosis from consumption of pistachios produced and consumed in the United States

(2017) *Food Microbiology*, 67, pp. 85-96.

ABSTRACT: The risk of salmonellosis from consumption of pistachios produced and consumed in the U.S. was assessed through quantitative microbial risk assessment. Data on *Salmonella* prevalence and concentration on pistachios, nut crop volume, storage times and temperatures during processing and handling, and reductions during storage or from roasting were derived from laboratory experiments, published literature, and industry expert opinion. Uncertainty was analyzed via what-if scenarios for *Salmonella* prevalence, concentration, storage reduction, treatment variability, portion of crop treated, and increased consumption. The estimated U.S. incidence of salmonellosis when 100% of pistachios were exposed to a 4 ± 0 log reduction treatment averaged 1.4 cases per billion servings, or <1 case/year, without considering *Salmonella* decline during storage. Including *Salmonella* decline during storage reduced the salmonellosis estimates approximately 10-fold. The predicted arithmetic mean number of cases associated with individual 500,000-kg storage silos, contaminated at the highest observed levels, ranged from 5 to 530 when the product was consumed untreated, but was reduced to below 1 case per silo when a 4 ± 0 log reduction treatment was applied. Assuming a uniform 4-log reduction treatment is applied to 100% of the crop and there is no decline of *Salmonella* during storage, the assessment indicates the following: 10-fold increases in either *Salmonella* prevalence or concentration, 2-fold increases in both prevalence and concentration, or consumption of >0.05% of untreated product volume yield an arithmetic mean risk of >1 case/year. ISSN: 07400020

Chatt, C., Nicholds-Trainor, D., Scrivener, A., Suleman, S., Harvey, M., Dallman, T., Hawker, J., Sibal, B.

Outbreak of Salmonella enteritidis PT14b gastroenteritis at a restaurant in England: the use of molecular typing to achieve a successful prosecution

(2017) *Public Health*, 151, pp. 51-58.

ABSTRACT: Objectives To describe an outbreak of *Salmonella* enteritidis phage type (PT) 14b in people who had eaten at a restaurant, and the investigation and subsequent prosecution of the food business operator (FBO). Study design The local health protection team and environmental health department formed an outbreak control team to investigate the outbreak. Methods Epidemiological, microbiological, and environmental investigations were undertaken. Epidemiological investigations involved case finding and interviews. Microbiological investigation: stool samples from the suspected cases and environmental samples from the implicated food business were investigated. *Salmonella* isolates obtained were subjected to multiple locus variable-number tandem repeat analysis (MLVA) profiling and whole genome sequencing. In addition, adenosine triphosphate (ATP) hygiene swab tests were used to verify the quality of cleaning procedures and data loggers were used to determine the water temperature of the mechanical dishwasher. Results Fifteen cases of illness where the causative agent was shown to be *S. enteritidis* PT14b were identified, all of whom had eaten at the same restaurant. *S. enteritidis* PT14b was also identified from three of the 11 food and environmental samples taken at the restaurant and found to have the same MLVA profile as the cases. A case for prosecution was built and the FBO was successfully prosecuted in July 2015. Conclusions This investigation highlighted that the use of molecular typing as part of thorough

epidemiological, microbiological, and environmental investigations can present a robust case for prosecution against restaurants which pose a risk to public health.
ISSN: 00333506

Garrido-Maestu, A., Fuciños, P., Azinheiro, S., Carvalho, J., Prado, M.

Systematic loop-mediated isothermal amplification assays for rapid detection and characterization of Salmonella spp., Enteritidis and Typhimurium in food samples (2017) Food Control, 80, pp. 297-306.

ABSTRACT: European Authorities have made a great effort to decrease the incidence of salmonellosis, but yearly thousands of cases are still reported, being most of them associated with serovars Enteritidis and Typhimurium. In the current study a set of methods for fast detection of these pathogens was developed and evaluated. The methods were based on loop-mediated isothermal amplification due to its advantages. The methods targeted three genes, *invA*, *safA* and *STM4497*, and each one of them was evaluated independently so that they can be targeted directly or in a sequential mode: first screening for the genus *Salmonella* and secondly on typing those positive samples. In this process, the results were compared against qPCR. The methods were able to detect $\leq 10\text{ cfu}/25\text{ g}$, making them suitable for official analyses, and food industry self-monitoring. Of most importance, the limit of detection, relative sensitivity, specificity and accuracy, positive and negative predictive values and the index kappa of concordance, were determined, being all higher than 97%. This demonstrates the reliability of the methods described in this study, which may be comparable with classical culture/serotyping of *Salmonella* but allowing a much faster response in case of positive results. Finally, a mathematical model was implemented to fit the data recorded by the qPCR thermocycler, allowing a more consistent determination of the parameters describing the qLAMP process, which may be easily implemented in other assays where accurate determination of T_t is needed for quantification purposes. ISSN: 09567135

Elhariri, M., Aleslamboly, Y.S., Elshater, M.A., Elhelw, R., Refai, M.K.

Rapid salmonella detection in different food samples by direct-PCR (2017) Bioscience Research, 14 (4), pp. 1005-1010.

ABSTRACT: The aim of the present work was to compare Direct-PCR on food samples or combined with pre-enrichment and / or selective enrichment media with the standard conventional microbiological methods and to investigate the most rapid & sensitive technique for the detection & identification of *Salmonella* species. Two hundred samples of retail chicken meat & byproducts in addition to 200 samples of beef meat & byproducts were tested by conventional isolation method & Direct-PCR techniques. Direct-PCR was performed on food samples, pre-enrichment and selective enrichment broth without DNA extraction. The less sensitive detection method was Direct-PCR on food samples 5% for both chicken & meat samples followed by conventional isolation method 7% for chicken & 5% for meat. The most optimized technique was Direct-PCR on selective 9.5 & 11.5 % for chicken & meat samples, respectively followed by the Direct-PCR on pre-enrichment. ISSN: 18119506

de Freitas Costa, E., Corbellini, L.G., da Silva, A.P.S.P., Nauta, M.

A Stochastic Model to Assess the Effect of Meat Inspection Practices on the Contamination of the Pig Carcasses (2017) Risk Analysis, 37 (10), pp. 1849-1864.

ABSTRACT: The objective of meat inspection is to promote animal and public health by preventing, detecting, and controlling hazards originating from animals. With the improvements of sanitary level in pig herds, the hazards profile has shifted and the inspection procedures no longer target major foodborne pathogens (i.e., not risk based). Additionally, carcass manipulations performed when searching for macroscopic lesions can lead to cross-contamination. We therefore developed a stochastic model to quantitatively describe cross-contamination when consecutive carcasses are submitted to classic inspection procedures. The microbial hazard used to illustrate the model was *Salmonella*, the data set was obtained from Brazilian slaughterhouses, and some simplifying assumptions were made. The model predicted that due to cross-contamination during inspection, the prevalence of contaminated carcass surfaces increased from 1.2% to 95.7%, whereas the mean contamination on contaminated surfaces decreased from 1 $\log\text{CFU}/\text{cm}^2$ to $-0.87 \log\text{CFU}/\text{cm}^2$, and the standard deviations decreased from 0.65 to 0.19. These results are explained by the fact that, due to carcass manipulations with hands, knives, and hooks, including the cutting of contaminated lymph nodes, *Salmonella* is transferred to previously uncontaminated carcasses, but in small quantities. These small quantities can easily go undetected during sampling. Sensitivity analyses gave insight into the model performance and showed that the touching and cutting of lymph nodes during

inspection can be an important source of carcass contamination. The model can serve as a tool to support discussions on the modernization of pig carcass inspection.
ISSN: 02724332

Huang, L., Hwang, C.-A.

Dynamic analysis of growth of Salmonella Enteritidis in liquid egg whites
(2017) *Food Control*, 80, pp. 125-130.

ABSTRACT: Salmonella Enteritidis (SE) is a common foodborne pathogen associated with eggs and egg products. This research was conducted to study the kinetics of growth and survival of SE in liquid egg whites (LEW). A dynamic temperature profile that exposed SE to suboptimal temperatures and below the minimum growth temperature (T_{min}) was used with two isothermal conditions to develop kinetic models. One-step dynamic analysis was used to directly construct a tertiary model for describing the growth and survival of SE and determine the kinetic parameters. The results of kinetic analysis showed that the T_{min} was 7.7 °C and SE may die off at a rate of 2.78×10^{-3} log CFU/ml per h per °C below the T_{min}. The root mean square error (RMSE) of the model was 0.5 log CFU/ml, with 76.6% of the residual errors within ± 0.5 log CFU/ml of the experimental observations. The model was validated under both dynamic temperature and isothermal conditions. Both growth and survival of SE was accurately predicted, with the RMSE of validation at ≤ 0.5 log CFU/ml. For all the validation tests, nearly 75% of the residual errors were within ± 0.5 log CFU/ml of the experimental observations. This study clearly demonstrated that the one-step dynamic analysis method is an accurate and efficient method for direct construction of predictive models and estimation of the associated kinetic parameters that govern the growth and survival of microorganisms in food. Since the mathematical model has been validated, it can be used to predict the growth and survival of SE in LEW during storage and distribution and for conducting risk assessment of this microorganism.
ISSN: 09567135

Puri, M.A.A., Joelsson, A.C., Terkhorn, S.P., Brown, A.S., Gaudio, Z.E., Siciliano, N.A.

Comparative evaluation of veriflow® salmonella species to USDA and FDA culture-based methods for the detection of salmonella spp. in food and environmental samples
(2017) *Journal of AOAC International*, 100 (5), pp. 1445-1457.

ABSTRACT: Veriflow® Salmonella species (Veriflow SS) is a molecular-based assay for the presumptive detection of Salmonella spp. from environmental surfaces (stainless steel, sealed concrete, plastic, and ceramic tile), dairy (2% milk), raw meat (20% fat ground beef), chicken carcasses, and ready-to-eat (RTE) food (hot dogs). The assay utilizes a PCR detection method coupled with a rapid, visual, flow-based assay that develops in 3 min post-PCR amplification and requires only an 18 h enrichment for maximum sensitivity. The Veriflow SS system eliminates the need for sample purification, gel electrophoresis, or fluorophore-based detection of target amplification and does not require complex data analysis. This Performance Tested Method SM validation study demonstrated the ability of the Veriflow SS method to detect low levels of artificially inoculated or naturally occurring Salmonella spp. in eight distinct environmental and food matrixes. In each reference comparison study, probability of detection analysis indicated that there was no significant difference between the Veriflow SS method and the U.S. Department of Agriculture Food Safety and Inspection Service Microbiology Laboratory Guidebook Chapter 4.06 and the U.S. Food and Drug Administration Bacteriological Analytical Manual Chapter 5 reference methods. A total of 104 Salmonella strains were detected in the inclusivity study, and 35 nonspecific organisms went undetected in the exclusivity study. The study results show that the Veriflow SS method is a sensitive, selective, and robust assay for the presumptive detection of Salmonella spp. sampled from environmental surfaces (stainless steel, sealed concrete, plastic, and ceramic tile), dairy (2% milk), raw meat (20% fat ground beef), chicken carcasses, and RTE food (hot dogs). ISSN: 10603271

Dash, L., Khaparde, A., Vivek, K., Shastri, J.

Gastrointestinal carriage of Salmonella species and intestinal parasites, and nasal and hand carriage of Staphylococcus aureus among asymptomatic food handlers
(2017) *Annals of Tropical Medicine and Public Health*, 10 (5), pp. 1195-1198.

ABSTRACT: Background: Food borne diseases continue to be a public health problem globally. Food handlers (FHs) have been implicated in food borne outbreaks. Asymptomatic carriers go unnoticed and are thus an important source of pathogens. Aims and Objectives: This study was conducted to detect intestinal carriage of Salmonella species and parasites as well as nasal and hand carriage of Staphylococcus aureus (S. aureus) among asymptomatic FHs. Personal hygiene practices followed by them were recorded. Materials and Methods: A total of 300 asymptomatic FHs were studied. A semi-structured

questionnaire was filled. Nasal swabs and finger impressions were taken on mannitol salt agar plates which were incubated overnight at 37°C; colonies suggestive of *S. aureus* were identified and confirmed by standard biochemical tests. Stool culture for *Salmonella* species was done on MacConkey agar, Xylose Lysine Deoxycholate agar and simultaneously inoculated in Selenite F broth for further processing; colonies suggestive of *Salmonella* species were identified by standard biochemical tests and *Salmonella* antisera (Denka Seiken, Japan). Stool-routine/microscopy for parasites was done by gross examination, direct saline, and iodine mount followed by concentration method (saturated salt solution). An arbitrary 10-point scale used in earlier studies was utilized for classifying the level of personal hygiene of FHs. Results: *Salmonella* Typhi was detected in stool culture of two FHs. Intestinal parasites detected in 10 (3.3%) subjects, included *Ascaris lumbricoides* (5; 1.7%), *Entamoeba histolytica* (3; 1.0%), and *Giardia intestinalis* (2; 0.66%). *S. aureus* carriage was noted in anterior nares (116; 38.7%) and hand (83; 27.7%). A total of 149 (50%) FHs were *S. aureus* carriers. Conclusion: This study indicates that FHs may be a potential source of food borne pathogens. ISSN: 17556783

Odoch, T., Wasteson, Y., L'Abée-Lund, T., Muwonge, A., Kankya, C., Nyakarahuka, L., Tegule, S., Skjerve, E.

Prevalence, antimicrobial susceptibility and risk factors associated with non-typhoidal Salmonella on Ugandan laying hen farms

(2017) *BMC Veterinary Research*, 13 (1), art. no. 365, .

ABSTRACT: Background: Non-typhoidal *Salmonella* (NTS) are among the leading global foodborne pathogens and a significant public health threat. Their occurrence in animal reservoirs and their susceptibilities to commonly used antimicrobials are poorly understood in developing countries. The aim of this study was to estimate the prevalence, determine antimicrobial susceptibility and identify risk factors associated with NTS presence in laying hen farms in Uganda through a cross-sectional study. Results: Pooled faecal samples were collected from 237 laying hen farms and these were analysed for NTS following standard laboratory procedures. In total, 49 farms (20.7%; 95% Confidence interval (CI): 15.6-25.6%) were positive for NTS presence. Altogether, ten *Salmonella* serotypes were identified among the confirmed 78 isolates, and the predominant serotypes were *Salmonella* Newport (30.8%), *S. Hadar* (14.1%), *S. Aberdeen* (12.8%), *S. Heidelberg* (12.8%), and *S. Bolton* (12.8%). Phenotypic antimicrobial resistance was detected in 45(57.7%) of the isolates and the highest resistance was against ciprofloxacin (50.0%) followed by sulphonamides (26.9%) and sulphamethoxazole/trimethoprim (7.7%). Resistance was significantly associated with sampled districts ($p=0.034$). Resistance to three or more drugs, multi-drug resistance (MDR) was detected in 12 (15.4%) of the isolates, 9 (75%) of these were from Wakiso district. A multivariable logistic model identified large farm size (OR=7.0; 95% CI: 2.5-19.8) and the presence of other animal species on the farm (OR=5.9; 95% CI: 2.1-16.1) as risk factors for NTS prevalence on farms. Having a separate house for birds newly brought to the farms was found to be protective (OR=0.4; 95% CI: 0.2-0.8). Conclusion: This study has highlighted a high prevalence and diversity of NTS species in laying hen farms in Uganda and identified associated risk factors. In addition, it has demonstrated high levels of antimicrobial resistance in isolates of NTS. This could be because of overuse or misuse of antimicrobials in poultry production. Also importantly, the insights provided in this study justifies a strong case for strengthening One Health practices and this will contribute to the development of NTS control strategies at local, national and international levels. ISSN: 17466148

Nair, D.V.T., Johny, A.K.

Food grade pimenta leaf essential oil reduces the attachment of Salmonella enterica Heidelberg (2011 Ground Turkey Outbreak Isolate) on to Turkey Skin

(2017) *Frontiers in Microbiology*, 8 (NOV), art. no. 2328, .

ABSTRACT: *Salmonella* attached to the poultry skin is a major source of carcass contamination during processing. Once attached to the poultry skin, it is difficult to detach and inactivate *Salmonella* by commonly used antimicrobial agents since the pathogen is entrapped deeply in the feather follicles and the crevices on the skin. Essential oils could be natural, safe, and effective alternatives to synthetic antimicrobial agents during commercial and organic processing setup. The present study evaluated the efficacy of pimenta (*Pimenta officinalis* Lindl.) leaf essential oil (PEO), and its nanoemulsion in reducing *Salmonella* Heidelberg attachment on to turkey (*Meleagris gallopavo*) skin during simulated scalding (65°C) and chilling (4°C) steps in poultry processing. A multidrug resistant *S. Heidelberg* isolate from the 2011 ground turkey outbreak in the United States was used in the study. Results showed that PEO and the nanoemulsion resulted in significant reduction of *S. Heidelberg* attachment on turkey skin. Turkey skin samples treated with 1.0% PEO for 5 min resulted in > 2 log₁₀ CFU/sq. inch reduction of *S.*

Heidelberg at 65 and 4°C, respectively (n = 6; P < 0.05). Similarly, skin samples treated with 1.0% pimenta nanoemulsion (PNE) for 5 min resulted in 1.5- and 1.8-log₁₀ CFU/sq. inch reduction of *S. Heidelberg* at 65 and 4°C, respectively (n = 6; P < 0.05). In addition, PEO and PNE were effective in reducing *S. Heidelberg* on skin during short-term storage at 4 and 10°C (temperature abuse) (n = 6; P < 0.05). No *Salmonella* was detected in the dipping solution containing 0.5 or 1.0% PEO or PNE, whereas a substantial population of the pathogen survived in the control dipping solution. The results were validated using scanning electron -, and confocal - microscopy techniques. PEO or PNE could be utilized as an effective antimicrobial agent to reduce *S. Heidelberg* attachment to turkey skin during poultry processing. ISSN: 1664302X

Felten, A., Vila Nova, M., Durimel, K., Guillier, L., Mistou, M.-Y., Radomski, N.

First gene-ontology enrichment analysis based on bacterial coregenome variants: Insights into adaptations of Salmonella serovars to mammalian- and avian-hosts (2017) BMC Microbiology, 17 (1), art. no. 222, .

ABSTRACT: Background: Many of the bacterial genomic studies exploring evolution processes of the host adaptation focus on the accessory genome describing how the gains and losses of genes can explain the colonization of new habitats. Consequently, we developed a new approach focusing on the coregenome in order to describe the host adaptation of *Salmonella* serovars. Methods: In the present work, we propose bioinformatic tools allowing (i) robust phylogenetic inference based on SNPs and recombination events, (ii) identification of fixed SNPs and InDels distinguishing homoplastic and non-homoplastic coregenome variants, and (iii) gene-ontology enrichment analyses to describe metabolic processes involved in adaptation of *Salmonella enterica* subsp. *enterica* to mammalian- (*S. Dublin*), multi- (*S. Enteritidis*), and avian- (*S. Pullorum* and *S. Gallinarum*) hosts. Results: The 'VARCall' workflow produced a robust phylogenetic inference confirming that the monophyletic clade *S. Dublin* diverged from the polyphyletic clade *S. Enteritidis* which includes the divergent clades *S. Pullorum* and *S. Gallinarum* (i). The scripts 'phyloFixedVar' and 'FixedVar' detected non-synonymous and non-homoplastic fixed variants supporting the phylogenetic reconstruction (ii). The scripts 'GetGOxML' and 'EveryGO' identified representative metabolic pathways related to host adaptation using the first gene-ontology enrichment analysis based on bacterial coregenome variants (iii). Conclusions: We propose in the present manuscript a new coregenome approach coupling identification of fixed SNPs and InDels with regards to inferred phylogenetic clades, and gene-ontology enrichment analysis in order to describe the adaptation of *Salmonella* serovars *Dublin* (i.e. mammalian-hosts), *Enteritidis* (i.e. multi-hosts), *Pullorum* (i.e. avian-hosts) and *Gallinarum* (i.e. avian-hosts) at the coregenome scale. All these polyvalent Bioinformatic tools can be applied on other bacterial genus without additional developments. ISSN: 14712180

Thomas, M., Fenske, G.J., Antony, L., Ghimire, S., Welsh, R., Ramachandran, A., Scaria, J.

Whole genome sequencing-based detection of antimicrobial resistance and virulence in non-typhoidal Salmonella enterica isolated from wildlife (2017) Gut Pathogens, 9 (1), art. no. 66, .

ABSTRACT: The aim of this study was to generate a reference set of *Salmonella enterica* genomes isolated from wildlife from the United States and to determine the antimicrobial resistance and virulence gene profile of the isolates from the genome sequence data. We sequenced the whole genomes of 103 *Salmonella* isolates sampled between 1988 and 2003 from wildlife and exotic pet cases that were submitted to the Oklahoma Animal Disease Diagnostic Laboratory, Stillwater, Oklahoma. Among 103 isolates, 50.48% were from wild birds, 0.9% was from fish, 24.27% each were from reptiles and mammals. 50.48% isolates showed resistance to at least one antibiotic. Resistance against the aminoglycoside streptomycin was most common while 9 isolates were found to be multi-drug resistant having resistance against more than three antibiotics. Determination of virulence gene profile revealed that the genes belonging to *csg* operons, the *fim* genes that encode for type 1 fimbriae and the genes belonging to type III secretion system were predominant among the isolates. The universal presence of fimbrial genes and the genes encoded by pathogenicity islands 1-2 among the isolates we report here indicates that these isolates could potentially cause disease in humans. Therefore, the genomes we report here could be a valuable reference point for future traceback investigations when wildlife is considered to be the potential source of human *Salmonellosis*. ISSN: 17574749

Andersen, S.C., Fachmann, M.S.R., Kiil, K., Nielsen, E.M., Hoorfar, J.

Gene-based pathogen detection: Can we use qPCR to predict the outcome of diagnostic metagenomics?

(2017) *Genes*, 8 (11), art. no. 332, .

ABSTRACT: In microbial food safety, molecular methods such as quantitative PCR (qPCR) and next-generation sequencing (NGS) of bacterial isolates can potentially be replaced by diagnostic shotgun metagenomics. However, the methods for pre-analytical sample preparation are often optimized for qPCR, and do not necessarily perform equally well for qPCR and sequencing. The present study investigates, through screening of methods, whether qPCR can be used as an indicator for the optimization of sample preparation for NGS-based shotgun metagenomics with a diagnostic focus. This was used on human fecal samples spiked with 103 or 106 colony-forming units (CFU)/g *Campylobacter jejuni*, as well as porcine fecal samples spiked with 103 or 106 CFU/g *Salmonella typhimurium*. DNA was extracted from the samples using variations of two widely used kits. The following quality parameters were measured: DNA concentration, qPCR, DNA fragmentation during library preparation, amount of DNA available for sequencing, amount of sequencing data, distribution of data between samples in a batch, and data insert size; none showed any correlation with the target ratio of the spiking organism detected in sequencing data. Surprisingly, diagnostic metagenomics can have better detection sensitivity than qPCR for samples spiked with 103 CFU/g *C. jejuni*. The study also showed that qPCR and sequencing results may be different due to inhibition in one of the methods. In conclusion, qPCR cannot uncritically be used as an indicator for the optimization of sample preparation for diagnostic metagenomics. ISSN: 20734425

Palmer, A.D., Slauch, J.M.

Mechanisms of Salmonella pathogenesis in animal models

(2017) *Human and Ecological Risk Assessment*, 23 (8), pp. 1877-1892.

ABSTRACT: Animal models play an important role in understanding the mechanisms of bacterial pathogenesis. Here we review the recent studies of *Salmonella* infection in various animal models. Although mice are a classic animal model for *Salmonella*, mice do not normally get diarrhea, raising the question of how well the model represents normal human infection. However, pre-treatment of mice with oral streptomycin, which apparently reduces the normal microbiota, leads to an inflammatory diarrheal response upon oral infection with *Salmonella*. This has led to a re-evaluation of the role of various *Salmonella* virulence factors in colonization of the intestine and induction of diarrhea. Indeed, it is now clear that *Salmonella* purposefully induces inflammation, which leads to the production of both carbon sources and terminal electron acceptors by the host that allow *Salmonella* to outgrow the normal intestinal microbiota. Overall use of this modified mouse model provides a more nuanced understanding of *Salmonella* intestinal infection in the context of the microbiota with implications for the ability to predict human risk. ISSN: 10807039

Theuß, T., Ueberham, E., Lehmann, J., Lindner, T., Springer, S.

Immunogenic potential of a Salmonella Typhimurium live vaccine for pigs against monophasic Salmonella Typhimurium DT 193

(2017) *BMC Veterinary Research*, 13 (1), art. no. 343, .

ABSTRACT: Background: Monophasic *Salmonella Typhimurium* (mSTM) strains account for up to 8.6% of all human salmonellosis cases. They have an increasing prevalence during recent years and several human cases with hospitalisation were reported. These strains are often isolated from pigs and pork - one primary source of human infection. A *Salmonella Typhimurium* (STM) live vaccine has been proven successful in controlling of STM infections in pigs for many years. The aim of this study was to test the immunogenicity of the vaccine in weaners during oral challenge with a virulent mSTM strain and to examine the kinetics of STM-specific IgA, IgM and IgG antibodies induced by vaccination and infection. Results: Despite clinical signs being present in both groups, the vaccination led to a significant reduction of diarrhoea, overall clinical symptoms and a milder elevation of the body temperature. Necropsy revealed fewer pathological lesions in the gastrointestinal tract of vaccinated compared to control animals. Moreover, in the ileal and caecal mucosa and in the ileocaecal lymph nodes the challenge strain burden was significantly reduced by vaccination. Significant differences in the antibody responses of both groups were present during the vaccination period and after infection. In vaccinated animals *Salmonella*-specific IgA and IgG antibody levels increased significantly after vaccination and were even more pronounced in response to challenge. In contrast, similarly low levels of IgM antibodies were detected during the vaccination period in both vaccinated and non-vaccinated animals. However, after challenge IgM antibody levels increased significantly in control pigs while neither IgA nor IgG antibodies were detectable. Conclusion: The data demonstrate that mSTM can evoke clinical signs in weaners. Due to the vaccination their incidence and magnitude were significantly milder. Vaccination also led to a significantly reduced challenge strain burden in the intestine and the lymph nodes which is comparable to previous studies using the same vaccine in a challenge with

biphasic STM. Therefore, it is concluded that this vaccine induces immunity against monophasic and biphasic STM strains. Furthermore, the results of antibody profiles in response to vaccination and infection provide additional evidence for humoral immune mechanisms triggered during *Salmonella* infection or vaccination. ISSN: 17466148

Mantha, S., Anderson, A., Acharya, S.P., Harwood, V.J., Weidhaas, J.

Transport and attenuation of Salmonella enterica, fecal indicator bacteria and a poultry litter marker gene are correlated in soil columns
(2017) *Science of the Total Environment*, 598, pp. 204-212.

ABSTRACT: Millions of tons of fecal-contaminated poultry litter are applied to U.S. agricultural fields annually. Precipitation and irrigation facilitate transport of fecal-derived pathogens and fecal indicator bacteria (FIB) to groundwater. The goal of this study was to compare transport of pathogens, FIB, and a microbial source tracking marker gene for poultry litter (LA35) in a simulated soil-to-groundwater system. Nine laboratory soil columns containing four different soil types were used to evaluate microbial transport to groundwater via infiltration. Quantitative polymerase chain reaction was used to monitor *Salmonella enterica* Typhimurium, *Escherichia coli*, *Enterococcus* spp., *Brevibacterium* sp. LA35 and Bacteroidales leached from soil columns inoculated with poultry litter. *S. enterica* was correlated with LA35 poultry litter marker gene and FIB concentrations in column soils containing organic matter, but not in acid washed sands. In contrast, *S. enterica* was found to correlate with LA35 and FIB in the leachate from columns containing sand, but not with leachate from organic soil columns. The majority of recovered DNA was found in leachate of predominately sandy soil columns, and in the soil of loamy columns. At least 90% of the DNA retained in soils for each microbial target was found in the top 3 cm of the column. These studies suggest that poultry litter associated pathogens and FIB are rapidly released from litter, but are influenced by complex attenuation mechanisms during infiltration, including soil type. This study advances our understanding of the potential for subsurface transport of poultry litter associated pathogens and FIB, and support the use of the LA35 marker gene for evaluating poultry litter impacts on groundwater.
ISSN: 00489697

Hughes, R.-A., Ali, R.A., Mendoza, M.A., Hassan, H.M., Koci, M.D.

Impact of dietary galacto-oligosaccharide (GOS) on chicken's gut microbiota, mucosal gene expression, and Salmonella colonization
(2017) *Frontiers in Veterinary Science*, 4 (NOV), art. no. 192, .

ABSTRACT: Preventing *Salmonella* colonization in young birds is key to reducing contamination of poultry products for human consumption (eggs and meat). While several *Salmonella* vaccines have been developed that are capable of yielding high systemic antibodies, it is not clear how effective these approaches are at controlling or preventing *Salmonella* colonization of the intestinal tract. Effective alternative control strategies are needed to help supplement the bird's ability to prevent *Salmonella* colonization, specifically by making the cecum less hospitable to *Salmonella*. In this study, we investigated the effect of the prebiotic galacto-oligosaccharide (GOS) on the cecal microbiome and ultimately the carriage of *Salmonella*. Day-old pullet chicks were fed control diets or diets supplemented with GOS (1% w/w) and then challenged with a cocktail of *Salmonella* Typhimurium and *Salmonella* Enteritidis. Changes in cecal tonsil gene expression, cecal microbiome, and levels of cecal and extraintestinal *Salmonella* were assessed at 1, 4, 7, 12, and 27 days post infection. While the *Salmonella* counts were generally lower in the GOS-treated birds, the differences were not significantly different at the end of the experiment. However, these data demonstrated that treatment with the prebiotic GOS can modify both cecal tonsil gene expression and the cecal microbiome, suggesting that this type of treatment may be useful as a tool for altering the carriage of *Salmonella* in poultry.
ISSN: 22971769

Larivière-Gauthier, G., Thibodeau, A., Letellier, A., Yergeau, É., Fravallo, P.

Reduction of Salmonella Shedding by sows during gestation in relation to its fecal microbiome
(2017) *Frontiers in Microbiology*, 8 (NOV), art. no. 2219, .

ABSTRACT: Pork meat is estimated to be responsible for 10-20% of human salmonellosis cases in Europe. Control strategies at the farm could reduce contamination at the slaughterhouse. One of the targeted sectors of production is maternity, where sows could be *Salmonella* reservoirs. The aim of this study was to assess the dynamics of shedding of *Salmonella* in terms of variation in both shedding prevalence and strains excreted during gestation in Quebec's maternity sector. The evolution of the fecal microbiota of these sows during gestation was also assessed to detect bacterial populations associated with these variations. A total of 73 sows both at the beginning and the end of the gestation were

randomly selected and their fecal matter was analyzed. *Salmonella* detection was conducted using a method that includes two selective enrichment media (MSRV and TBG). Nine isolates per positive samples were collected. Among the 73 sows tested, 27 were shedding *Salmonella*. Sows in the first third of their gestation shed *Salmonella* significantly more frequently (21/27) than those in the last third (6/46) ($X^2 P < 0.05$). The shedding status of 19 of the sows that were previously sampled in the first third of their gestation was followed, this time in the last third of their gestation, which confirmed reduction of shedding. Using 16S rRNA gene sequencing and qPCR, significant differences between the fecal flora of sows at the beginning and the end of the gestation, shedding *Salmonella* or not and with different parity number were detected. Using MaAsLin, multiple OTUs were found to be associated with the time of gestation, the status of *Salmonella* excretion and parity number. Some of the identified taxa could be linked to the reduction of the shedding of *Salmonella* at the end of gestation. In this study, we showed that the level of *Salmonella* shedding was variable during gestation with significantly higher shedding at the beginning rather than at the end of gestation. We also observed for the first time a significant change in the microbiota during sow gestation and identified interesting taxa which could be linked to a reduced *Salmonella* shedding. ISSN: 1664302X

Webb, H.E., Brichta-Harhay, D.M., Brashears, M.M., Nightingale, K.K., Arthur, T.M., Bosilevac, J.M., Kalchayanand, N., Schmidt, J.W., Wang, R., Granier, S.A., Brown, T.R., Edrington, T.S., Shackelford, S.D., Wheeler, T.L., Loneragan, G.H.
Salmonella in peripheral lymph nodes of healthy cattle at Slaughter
(2017) *Frontiers in Microbiology*, 8 (NOV), art. no. 2214, .

ABSTRACT: To more fully characterize the burden of *Salmonella enterica* in bovine peripheral lymph nodes (PLN), PLN (n = 5,450) were collected from healthy cattle at slaughter in 12 commercial abattoirs that slaughtered feedlot-fattened (FF) cattle exclusively (n = 7), cattle removed (or culled) from breeding herds (n = 3), or both FF and cull cattle (n = 2). Qualitative and quantitative methods were used to estimate prevalence and concentration of *Salmonella* in PLN. Isolates were subjected to a variety of phenotypic, serological, and molecular assays. Overall, *Salmonella* prevalence in PLN from FF and cull cattle was 7.1 and 1.8%. However, burden varied by season in that observed prevalence in PLN collected in cooler or warmer seasons was 2.4 and 8.2%, respectively. Prevalence in PLN from cull cattle in the southwest region of the US was 2.1 and 1.1% for cool and warm seasons, respectively; however, prevalence in FF PLN was far greater in that it was 6.5 and 31.1%, respectively. *Salmonella* was recovered from 289 (5.6%) PLN and 2.9% (n = 160) of all PLN tested had quantifiable concentrations that varied from 1.6 to 4.9 log₁₀ colony forming units/PLN. The most common serotypes isolated from PLN were Montevideo (26.9%), Lille (14.9%), Cerro (13.0%), Anatum (12.8%), and Dublin (6.9%). In all, 376 unique isolates were collected from the 289 *Salmonella*-positive PLN. Antimicrobial susceptibility testing revealed the majority (80.6%) of these isolates were pansusceptible; however, 10.7% of isolates were found to be resistant to two or more antimicrobial classes. We were able to document an observed increased in prevalence of *Salmonella* in PLN during the warmer season, particularly in FF cattle from the southwest region of the US. The mechanisms underlying the observed association between season, region, and production source have yet to be elucidated. Nevertheless, these findings increase our understanding of the sources of contamination of beef products and shed light on transmission dynamics that may be useful in targeting these sources.
ISSN: 1664302X

Mohammed, M.

Phage typing or CRISPR typing for epidemiological surveillance of Salmonella Typhimurium?
(2017) *BMC Research Notes*, 10 (1), art. no. 578, .

ABSTRACT: Objective: *Salmonella Typhimurium* is the most dominant *Salmonella* serovar around the world. It is associated with foodborne gastroenteritis outbreaks but has recently been associated with invasive illness and deaths. Characterization of *S. Typhimurium* is therefore very crucial for epidemiological surveillance. Phage typing has been used for decades for subtyping of *S. Typhimurium* to determine the epidemiological relation among isolates. Recent studies however have suggested that high throughput clustered regular interspaced short palindromic repeats (CRISPR) typing has the potential to replace phage typing. This study aimed to determine the efficacy of high-throughput CRISPR typing over conventional phage typing in epidemiological surveillance and outbreak investigation of *S. Typhimurium*. Results: In silico analysis of whole genome sequences (WGS) of well-documented phage types of *S. Typhimurium* reveals the presence of different CRISPR type among strains belong to the same phage type. Furthermore, different phage types of *S. Typhimurium* share identical CRISPR type. Interestingly,

identical spacers were detected among outbreak and non-outbreak associated DT8 strains of *S. Typhimurium*. Therefore, CRISPR typing is not useful for the epidemiological surveillance and outbreak investigation of *S. Typhimurium* and phage typing, until it is replaced by WGS, is still the gold standard method for epidemiological surveillance of *S. Typhimurium*. ISSN: 17560500

Garrido-Maestu, A., Azinheiro, S., Carvalho, J., Abalde-Cela, S., Carbó-Argibay, E., Diéguez, L., Piotrowski, M., Kolen'ko, Y.V., Prado, M.

Combination of microfluidic loop-mediated isothermal amplification with gold nanoparticles for rapid detection of Salmonella spp. in food samples
(2017) *Frontiers in Microbiology*, 8 (NOV), art. no. 2159, .

ABSTRACT: Foodborne diseases are an important cause of morbidity and mortality. According to the World Health Organization, there are 31 main global hazards, which caused in 2010 600 million foodborne illnesses and 420000 deaths. Among them, *Salmonella* spp. is one of the most important human pathogens, accounting for more than 90000 cases in Europe and even more in the United States per year. In the current study we report the development, and thorough evaluation in food samples, of a microfluidic system combining loop-mediated isothermal amplification with gold nanoparticles (AuNPs). This system is intended for low-cost, in situ, detection of different pathogens, as the proposed methodology can be extrapolated to different microorganisms. A very low limit of detection (10 cfu/25 g) was obtained. Furthermore, the evaluation of spiked food samples (chicken, turkey, egg products), completely matched the expected results, as denoted by the index kappa of concordance (value of 1.00). The results obtained for the relative sensitivity, specificity and accuracy were of 100% as well as the positive and negative predictive values. ISSN: 1664302X

Rubio, M.D.S., Penha Filho, R.A.C., Almeida, A.M.D., Berchieri, A., Jr.

Development of a multiplex qPCR in real time for quantification and differential diagnosis of Salmonella Gallinarum and Salmonella Pullorum
(2017) *Avian Pathology*, 46 (6), pp. 644-651.

ABSTRACT: Currently there are 2659 *Salmonella* serovars. The host-specific biovars *Salmonella Pullorum* and *Salmonella Gallinarum* cause systemic infections in food-producing and wild birds. Fast diagnosis is crucial to control the dissemination in avian environments. The present work describes the development of a multiplex qPCR in real time using a low-cost DNA dye (SYBr Green) to identify and quantify these biovars. Primers were chosen based on genomic regions of difference (RoD) and optimized to control dimers. Primers pSGP detect both host-specific biovars but not other serovars and pSG and pSP differentiate biovars. Three amplicons showed different melting temperatures (T_m), allowing differentiation. The pSGP amplicon (97 bp) showed T_m of 78°C for both biovars. The pSG amplicon (273 bp) showed a T_m of 86.2°C for *S. Gallinarum* and pSP amplicon (260 bp) dissociated at 84.8°C for *S. Pullorum* identification. The multiplex qPCR in real time showed high sensitivity and was capable of quantifying 108–101 CFU of these biovars. ISSN: 03079457

Axmann, S., Kolar, V., Adler, A., Strnad, I.

Efficiency of organic acid preparations for the elimination of naturally occurring Salmonella in feed material
(2017) *Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment*, 34 (11), pp. 1915-1924.

ABSTRACT: *Salmonella* can enter animal stocks via feedstuffs, thus posing not only an infection risk for animals, but also threatening to contaminate food of animal origin and finally humans. *Salmonella* contamination in feedstuffs is still a recurring and serious issue in animal production (especially for the poultry sector), and is regularly detected upon self-monitoring by feed companies (self-checks) and official inspections authorities. Operators within the feed chain in certain cases need to use hygienic condition enhancers, such as organic acids, to improve the quality of feed for animal nutrition, providing additional guarantees for the protection of animal and public health. The present study investigated the efficiencies of five organic acid preparations. The acid products were added to three different feed materials contaminated with *Salmonella* (contamination occurred by recontamination in the course of the production process) at seven different inclusion rates (1–7%) and analysed after 1, 2, and 7 days' exposure time using culture method (tenfold analysis). A reliable standard was established for defining a successful decontamination under the prevailing test conditions: 10 *Salmonella*-negative results out of 10 tested samples (0/10: i.e. 0 positive samples and 10 negative samples). The results demonstrated that the tested preparations showed significant differences with regard to the reduction in *Salmonella* contamination. At an inclusion rate of 7% of the feed

materials, two out of five acid preparations showed an insufficient, very small, decontamination effect, whereas two others had a relatively large partial effect. Reliable decontamination was demonstrated only for one acid preparation, however, subject to the use of the highest acid concentration. ISSN: 19440049

St. Amand, J.A., Cassis, R., King, R.K., Annett Christianson, C.B.

Prevalence of Salmonella spp. in environmental samples from table egg barns in Alberta (2017) Avian Pathology, 46 (6), pp. 594-601.

ABSTRACT: Some *Salmonella* spp. are zoonotic, a frequent cause of foodborne illness in Canada, and known to infect humans through contaminated poultry and poultry products. Certain serotypes of *Salmonella* spp. have been demonstrated to be vertically transmitted from hen to egg. The incidence of *Salmonella* spp. isolation in the flock has been correlated to its isolation from the environment. Twenty-one producers were enrolled in this study to examine the occurrence of *Salmonella* spp. in 48 table egg layer flocks housed in 35 barns in Alberta. The purpose of this study was to: (i) identify *Salmonella* serotypes isolated from the environment of table egg layer facilities in Alberta and (ii) record the prevalence of *Salmonella* spp. across eight defined environmental sampling points. *Salmonella* spp. were isolated from the environment of 20/35 barns representing 29/48 flocks. The most common serotypes isolated were *S. Heidelberg*, *S. Kentucky* and *S. Mbandaka*. The order of most to least contaminated sample location was manure belts (54.1%), feeders (47.9%), feed motors (45.8%), egg belts and walls (41.7%), fans (35.0%), cage bottoms (31.3%) and lobbies (27.1%). *Salmonella* spp. were isolated from 7/7 barns post cleaning and disinfection, demonstrating the persistence of this organism in the environment and the need for effective eradication protocols. ISSN: 03079457

Beneduce, L., Gatta, G., Bevilacqua, A., Libutti, A., Tarantino, E., Bellucci, M., Troiano, E., Spano, G.

Impact of the reusing of food manufacturing wastewater for irrigation in a closed system on the microbiological quality of the food crops (2017) International Journal of Food Microbiology, 260, pp. 51-58.

ABSTRACT: In order to evaluate if the reuse of food industry treated wastewater is compatible for irrigation of food crops, without increased health risk, in the present study a cropping system, in which ground water and treated wastewater were used for irrigation of tomato and broccoli, during consecutive crop seasons was monitored. Water, crop environment and final products were monitored for microbial indicators and pathogenic bacteria, by conventional and molecular methods. The microbial quality of the irrigation waters influenced sporadically the presence of microbial indicators in soil. No water sample was found positive for pathogenic bacteria, independently from the source. *Salmonella* spp. and *Listeria monocytogenes* were detected in soil samples, independently from the irrigation water source. No pathogen was found to contaminate tomato plants, while *Listeria monocytogenes* and *E. coli* O157:H7 were detected on broccoli plant, but when final produce were harvested, no pathogen was detected on edible part. The level of microbial indicators and detection of pathogenic bacteria in field and plant was not dependent upon wastewater used. Our results, suggest that reuse of food industry wastewater for irrigation of agricultural crop can be applied without significant increase of potential health risk related to microbial quality. ISSN: 01681605

Muniz, E.C., Verdi, R., Leão, J.A., Back, A., Nascimento, V.P.D.

Evaluation of the effectiveness and safety of a genetically modified live vaccine in broilers challenged with Salmonella Heidelberg (2017) Avian Pathology, 46 (6), pp. 676-682.

ABSTRACT: Salmonellosis ranks among the major diseases of commercial poultry, and its presence in poultry flocks is responsible for economic losses and risks related to public health. Vaccines are an important tool within integrated programmes to control salmonellosis. The purpose of this study was to assess cross-protection provided by the Poulvac® ST vaccine in the control of *Salmonella Heidelberg* in experimentally challenged 3- and 21-day-old birds. Eighty birds were identified and separated into four treatments (T1: vaccinated and challenged at 3 days of age, T2: unvaccinated and challenged at 3 days of age, T3: vaccinated and challenged at 21 days of age, and T4: unvaccinated and challenged at 21 days of age). The inoculum was produced from a Brazilian field strain of SH. At the end of the experiment, caecum and liver/spleen samples were collected for quantitative and qualitative analysis of SH, respectively. Analysis of the liver/spleen showed that Poulvac® ST significantly ($P \leq 0.05$) reduced the percentage of SH positivity in the group challenged at 3 days of age, while in the group challenged at 21 days this difference was almost considered significant ($P = 0.1818$). On the other hand, there was no statistically significant difference in SH count in the caecum (CFU/g) in the group

challenged at 3 days, but for the group challenged at 21 days the SH counts were significantly ($P \leq 0.05$) lower in the vaccinated group when compared to the positive control. ISSN: 03079457

Afroj, S., Aldahami, K., Reddy, G., Guard, J., Adesiyun, A., Samuel, T., Abdela, W.
Simultaneous detection of multiple salmonella serovars from milk and chicken meat by real-Time PCR using unique genomic target regions
(2017) *Journal of Food Protection*, 80 (11), pp. 1944-1957.

ABSTRACT: A novel genomic and plasmid target-based PCR platform was developed for the detection of *Salmonella* serovars Heidelberg, Dublin, Hadar, Kentucky, and Enteritidis. Unique genome loci were obtained through extensive genome mining of protein databases and comparative genomic analysis of these serovars. Assays targeting *Salmonella* serovars Hadar, Heidelberg, Kentucky, and Dublin had 100% specificity and sensitivity, whereas those for *Salmonella* Enteritidis had 97% specificity and 88% sensitivity. The limits of detection for *Salmonella* serovars Heidelberg, Kentucky, Hadar, Enteritidis, and Dublin were 12, 9, 40, 13, and 5,280 CFU, respectively. A sensitivity assay was also performed by using milk artificially inoculated with pooled *Salmonella* serovars, yielding a detection limit of 1 to 10 CFU/25 mL of milk samples after enrichment. The minimum DNA detected using the multiplexed TaqMan assay was 75.8 fg (1.53×10^1 genomic equivalents [GE]) for *Salmonella* Heidelberg, 140.8 fg (2.83101 GE) for *Salmonella* Enteritidis, and 3.48 pg (6.96×10^2 GE) for *Salmonella* Dublin. PCR efficiencies were 89.8% for *Salmonella* Heidelberg, 94.5% for *Salmonella* Enteritidis, and 75.5% for *Salmonella* Dublin. Four types of 30 pasteurized milk samples were tested negative by culture techniques and with a genus-specific *Salmonella* *invA* gene PCR assay. Among 30 chicken samples similarly tested, 12 (40%) were positive by both culture and the *invA* PCR. Testing of these 12 samples with the serovar-specific PCR assay detected single and mixed contamination with *Salmonella* Kentucky, *Salmonella* Enteritidis, and *Salmonella* Heidelberg. Five unique primers were designed and tested by multiplex conventional PCR in conjunction with the use of the multiplex TaqMan assay with three of the primers. The diagnostic assays developed in this study could be used as tools for routine detection of these five *Salmonella* serovars and for epidemiological investigations of foodborne disease outbreaks. ISSN: 0362028X

Zhang, G., Hu, L., Pouillot, R., Tatavarthy, A., Van Doren, J.M., Kleinmeier, D., Ziobro, G.C., Melka, D., Wang, H., Brown, E.W., Strain, E., Bunning, V.K., Musser, S.M., Hammack, T.S.

Prevalence of salmonella in 11 spices offered for sale from retail establishments and in imported shipments offered for entry to the United States
(2017) *Journal of Food Protection*, 80 (11), pp. 1791-1805.

ABSTRACT: The U.S. Food and Drug Administration conducted a survey to evaluate *Salmonella* prevalence and aerobic plate counts in packaged (dried) spices offered for sale at retail establishments in the United States. The study included 7,250 retail samples of 11 spice types that were collected during November 2013 to September 2014 and October 2014 to March 2015. No *Salmonella*-positive samples (based on analysis of 125 g) were found among retail samples of cumin seed (whole or ground), sesame seed (whole, not roasted or toasted, and not black), and white pepper (ground or cracked), for prevalence estimates of 0.00% with 95% Clopper and Pearson's confidence intervals of 0.00 to 0.67%, 0.00 to 0.70%, and 0.00 to 0.63%, respectively. *Salmonella* prevalence estimates (confidence intervals) for the other eight spice types were 0.19% (0.0048 to 1.1%) for basil leaf (whole, ground, crushed, or flakes), 0.24% (0.049 to 0.69%) for black pepper (whole, ground, or cracked), 0.56% (0.11 to 1.6%) for coriander seed (ground), 0.19% (0.0049 to 1.1%) for curry powder (ground mixture of spices), 0.49% (0.10 to 1.4%) for dehydrated garlic (powder, granules, or flakes), 0.15% (0.0038 to 0.83%) for oregano leaf (whole, ground, crushed, or flakes), 0.25% (0.03 to 0.88%) for paprika (ground or cracked), and 0.64% (0.17 to 1.6%) for red pepper (hot red pepper, e.g., chili, cayenne; ground, cracked, crushed, or flakes). *Salmonella* isolates were serotyped, and genomes were sequenced. Samples of these same 11 spice types were also examined from shipments of imported spices offered for entry to the United States from 1 October 2011 to 30 September 2015. *Salmonella* prevalence estimates (based on analysis of two 375-g composite samples) for shipments of imported spices were 1.7 to 18%. The *Salmonella* prevalence estimates for spices offered for sale at retail establishments for all of the spice types except dehydrated garlic and basil were significantly lower than estimates for shipments of imported spice offered for entry. ISSN: 0362028X

Salazar, F., Garcia, S., Lagunas-Solar, M., Pan, Z., Cullor, J.

Efficacy of a heat-spray and heat-double spray process on inoculated nuts with Salmonella enteritidis ATCC 1045

(2017) *Food Control*, 81, pp. 74-79.

ABSTRACT: Due to *Salmonella* outbreaks in almonds, regulatory standards have been established, requiring that almonds for human consumption in North America must achieve a minimum of 4 log₁₀ CFU/g reduction of *Salmonella*. This study investigated a system using a combination of heating and transient application of ethanol to reduce bacterial load. This approach used a small scale heat-spray and heat-double spray process that included a two factor block design with heat (25±2 °C and 125±2 °C) and spray levels (0,1,2); One factor design with nut levels of almonds, pistachios, pecans, and walnuts for each heat-spray and heat-double spray process; a two factor experiment included a dip contact time (5s, 1800s) and ethanol evaporation time (5 s, 1800 s). Also, to evaluate the interaction of a heat-spray process on moisture content of almonds, a two factor design with levels of heat (25±2 °C and 125 ± 2 °C) and spray (0,1) was used. Additionally, the spray evaporation rate was evaluated. The heat-spray process shows additivity, while the heat-double spray process shows synergism. The heat-double spray process on almonds achieved a 6.1 mean log₁₀ CFU/g reduction of *Salmonella* that was 35% higher than that of the heat-spray. For other nuts, the heat-double spray process led to a 4.8, 3.0, and 4.0 log₁₀ reduction for pecan, pistachio, and walnut, respectively. The dip time (p < 0.05) had a greater effect than ethanol evaporation (p > 0.05) on log₁₀ reduction of *Salmonella* in almonds. By applying ethanol 70%, the moisture increases by ~0.5% w.b., whereas applying temperature decreases moisture by ~2% w.b. The implication of these findings is that both the heat-spray and heat-double-spray process may be alternatives to the current almond disinfection processes in achieving a high log₁₀ reduction.

ISSN: 09567135

Maserati, A., Fink, R.C., Lourenco, A., Julius, M.L., Diez-Gonzalez, F.

General response of Salmonella enterica serovar Typhimurium to desiccation: A new role for the virulence factors sopD and sseD in survival

(2017) *PLoS ONE*, 12 (11), art. no. e0187692, .

ABSTRACT: *Salmonella* can survive for long periods under extreme desiccation conditions. This stress tolerance poses a risk for food safety, but relatively little is known about the molecular and cellular regulation of this adaptation mechanism. To determine the genetic components involved in *Salmonella*'s cellular response to desiccation, we performed a global transcriptomic analysis comparing *S. enterica* serovar Typhimurium cells equilibrated to low water activity (aw 0.11) and cells equilibrated to high water activity (aw 1.0). The analysis revealed that 719 genes were differentially regulated between the two conditions, of which 290 genes were up-regulated at aw 0.11. Most of these genes were involved in metabolic pathways, transporter regulation, DNA replication/repair, transcription and translation, and, more importantly, virulence genes. Among these, we decided to focus on the role of *sopD* and *sseD*. Deletion mutants were created and their ability to survive desiccation and exposure to aw 0.11 was compared to the wild-type strain and to an *E. coli* O157:H7 strain. The *sopD* and *sseD* mutants exhibited significant cell viability reductions of 2.5 and 1.3 Log (CFU/g), respectively, compared to the wild-type after desiccation for 4 days on glass beads. Additional viability differences of the mutants were observed after exposure to aw 0.11 for 7 days. *E. coli* O157:H7 lost viability similarly to the mutants. Scanning electron microscopy showed that both mutants displayed a different morphology compared to the wild-type and differences in production of the extracellular matrix under the same conditions. These findings suggested that *sopD* and *sseD* are required for *Salmonella*'s survival during desiccation. ISSN: 19326203

Costa, A., Gusmara, C., Gardoni, D., Zaninelli, M., Tambone, F., Sala, V., Guarino, M.

The effect of anaerobic digestion and storage on indicator microorganisms in swine and dairy manure

(2017) *Environmental Science and Pollution Research*, 24 (31), pp. 24135-24146.

ABSTRACT: The aim of this experimental study was to evaluate the influence of anaerobic digestion and storage on indicator microorganisms in swine and dairy excreta. Samples were collected every 90 days for 15 months at eight farms, four pig, and four dairy farms, four of them having a biogas plant. Moreover, to evaluate storage effects on samples, 20 l of manure and slurry taken at each farm (digested manure only in farms with a biogas plant) were stored in a controlled climatic chamber at 18 °C, for 6 months. The bacterial load and the chemical-physical characteristics of excreta were evaluated at each sampling time, stored slurry, and manure were sampled and analyzed every 2 months. A high variability of the concentration of bacteria in the different excreta types was observed

during the experiment, mainly depending on the type and time of treatment. No sample revealed either the presence of *Escherichia coli* O157:H7 or of *Salmonella*, usually linked to the temporary rearing of infected animals in facilities. Anaerobic digestion and storage affected in a significant way the reduction of indicator bacteria like lactobacilli, coliforms, and streptococci. Anaerobic digestion lowered coliforms in pig slurry (-2.80 log, $P < 0.05$), streptococci in dairy manure (-2.44 log, $P < 0.001$) and in pig slurry (-1.43 log, $P < 0.05$), and lactobacilli in pig slurry (-3.03 log, $P < 0.05$). Storage lowered coliforms and the other indicators counts, in particular in fresh wastes, while clostridia did not show a reduction in concentration. ISSN: 09441344

Gavrilovici, C., Pânzaru, C.-V., Cozma, S., Mârțu, C., Lupu, V.V., Ignat, A., Miron, I., Stârcea, M.

"Message from a turtle": otitis with Salmonella arizonae in children: Case report (2017) Medicine, 96 (44), p. e8455.

ABSTRACT: RATIONALE: *Salmonella enterica* subsp *arizonae* is a common gut inhabitant of reptiles (snakes are the most common reservoir, but it also occurs in turtles). Although human cases owing to this organism are exceedingly rare, it may occasionally infect young infants and immunocompromised individuals with a history of intimate associations with reptiles. Our case is the 20th one among the infections with *S arizonae* in children, but the 2nd one of otitis and the first of mastoiditis. The other cases had different anatomical locations, such as gastroenteritis, osteomyelitis, meningitis, ankle infection, wound infection, and sinusitis.

PATIENT CONCERNS AND DIAGNOSIS: We report a rare case of otitis with *Salmonella* in a previously healthy adolescent, which was most likely acquired after bathing in a lake. The ear infection was complicated with mastoiditis. Audiometric testing showed a moderately conductive hearing loss (60dB on pure-tone average).

INTERVENTION: Standard therapy for *S arizonae* was initiated. The surgery revealed a "hidden" cholesteatoma. Surgical management comprised of canal wall up mastoidectomy with attico-antrotomy and posterior tympanotomy followed by tympanoplasty.

OUTCOMES: Daily postoperative dressing care of the incision, along with antibiotic lavage of the external auditory canal packing, ensured a favorable evolution. The functional gain was important; the 1-month postsurgical pure tone audiogram indicated nearly normal hearing (a mean of 25dB for air conduction thresholds).

LESSON: *Salmonella enterica* serotype *arizonae* is a rare cause of human infection, being a common organism in reptiles, like snakes and turtles. Young children are at a particular risk for acquiring such infections. Our study might encourage further epidemiologic investigations into these infections to generate a more effective strategy among public health agencies. ISSN: 15365964

Jambalang, A.R., Buys, E.M., Botha, F.S.

Bacterial species from retailed poultry eggs in Tshwane, South Africa: Implication for consumers

(2017) South African Journal of Science, 113 (11-12), art. no. 2016-0232, .

ABSTRACT: Food safety is an important public health issue and governments across the world are intensifying their efforts to improve the quantity, quality and the safety of national food supplies. Bacteria, especially *Salmonella* species, present in or on chicken meat and hens' eggs in particular are the most common causes of food poisoning and the major sources of human salmonellosis. Literature reveals little information on the risk factors for salmonellae infection in Africa. The aim of this study was to determine which, if any, bacteria, especially *Salmonella* species, are present in and on hens' eggs.

Representative bacterial colonies were confirmed with Gram staining and then identified using the MALDI-TOF Biotyper assay. The genera identified were *Escherichia coli* (34%), *Enterococcus faecalis* (14%), *Proteus mirabilis* (9%), *Klebsiella pneumoniae* (7%), *Salmonella Typhimurium* (6%), *Enterobacter cloacae* (1%), *Stenotrophomonas maltophilia* (0.6%), *Salmonella Dublin* (0.6%) and *Salmonella Braenderup* (0.2%). Raw hens' eggs and products containing raw hens' eggs may contain pathogenic bacteria, thereby exposing a large number of consumers to the risk of contracting food poisoning when undercooked or uncooked hens' eggs are consumed. ISSN: 19967489

Osaili, T.M., Al-Nabulsi, A.A., Nazzal, D.S., Shaker, R.R.

Effect of storage temperatures and stresses on the survival of Salmonella spp. in halva (2017) Letters in Applied Microbiology, 65 (5), pp. 403-409.

ABSTRACT: The presence of *Salmonella* spp. in halva has been associated with foodborne illnesses and product recalls from the markets. This study investigated the effect of environmental stresses on the survival of *Salmonella* spp. in halva during storage for 12 months at 10 and 25°C (log (N0/N) g⁻¹). Halva samples were inoculated with a

cocktail of four strains of unstressed, desiccation stressed or heat stressed *Salmonella* (106–107 CFU per gram). In general, survival of *Salmonella* spp. in halva decreased significantly ($P < 0.05$) as storage time and temperature increased. At the end of halva shelf life at 10°C, the initial populations of unstressed, desiccation stressed or heat stressed *Salmonella* spp. decreased by 2.7, 2.6 or 2.8 log CFU per gram (reduction rate c. 0.2 log CFU per month), respectively. While at 25°C, the populations decreased 5.2, 6.7 or 6.3 log CFU per gram, respectively (reduction rate c. 0.4–0.5 log CFU per month). The populations of stressed *Salmonella* spp. in halva samples were not significantly different ($P \geq 0.05$) from populations of unstressed cells during storage at 10 and 25°C, except during the last 3 months of storage at 25°C when populations of unstressed cells were higher ($P < 0.05$). Exposing *Salmonella* spp. to desiccation or heat stress prior product contamination may play a role in *Salmonella* spp. survival in halva during storage. Significance and Impact of the Study: Contamination of halva (tahini halva) with *Salmonella* from raw materials or during production was documented. Halva and tahini have been involved in salmonellosis outbreaks in different countries. The study demonstrated enhanced survivability of stressed and unstressed *Salmonella* spp. in halva over a 12-month storage period at 10 and 25°C with lower log reductions than expected. Exposing *Salmonella* spp. to desiccation or heat stress prior product contamination may play a role in microbial survival in halva during storage. These findings serve as a model to halva producers to implement control measures to prevent *Salmonella* spp. contamination in halva. ISSN: 02668254

Goodman, L.B., McDonough, P.L., Anderson, R.R., Franklin-Guild, R.J., Ryan, J.R., Perkins, G.A., Thachil, A.J., Glaser, A.L., Thompson, B.S.

Detection of Salmonella spp. in veterinary samples by combining selective enrichment and real-time PCR

(2017) *Journal of Veterinary Diagnostic Investigation*, 29 (6), pp. 844-851.

ABSTRACT: Rapid screening for enteric bacterial pathogens in clinical environments is essential for biosecurity. *Salmonella* found in veterinary hospitals, particularly *Salmonella enterica* serovar Dublin, can pose unique challenges for culture and testing because of its poor growth. Multiple *Salmonella* serovars including Dublin are emerging threats to public health given increasing prevalence and antimicrobial resistance. We adapted an automated food testing method to veterinary samples and evaluated the performance of the method in a variety of matrices including environmental samples ($n = 81$), tissues ($n = 52$), feces ($n = 148$), and feed ($n = 29$). A commercial kit was chosen as the basis for this approach in view of extensive performance characterizations published by multiple independent organizations. A workflow was established for efficiently and accurately testing veterinary matrices and environmental samples by use of real-time PCR after selective enrichment in Rappaport–Vassiliadis soya (RVS) medium. Using this method, the detection limit for *S. Dublin* improved by 100-fold over subculture on selective agars (eosin–methylene blue, brilliant green, and xylose–lysine–deoxycholate). Overall, the procedure was effective in detecting *Salmonella* spp. and provided next-day results. ISSN: 10406387

Boonyarittichaijij, R., Verbrugge, E., Dekeukeleire, D., De Beelde, R., Rouffaer, L.O., Haesendonck, R., Strubbe, D., Mattheus, W., Bertrand, S., Pasmans, F., Bonte, D., Verheyen, K., Lens, L., Martel, A.

Salmonella Typhimurium DT193 and DT99 are present in great and blue tits in Flanders, Belgium

(2017) *PLoS ONE*, 12 (11), art. no. e0187640, .

ABSTRACT: Endemic infections with the common avian pathogen *Salmonella enterica* subspecies *enterica* serovar Typhimurium (*Salmonella Typhimurium*) may incur a significant cost on the host population. In this study, we determined the potential of endemic *Salmonella* infections to reduce the reproductive success of blue (*Cyanistes caeruleus*) and great (Parus major) tits by correlating eggshell infection with reproductive parameters. The fifth egg of each clutch was collected from nest boxes in 19 deciduous forest fragments. Out of the 101 sampled eggs, 7 *Salmonella Typhimurium* isolates were recovered. The low bacterial prevalence was reflected by a similarly low serological prevalence in the fledglings. In this study with a relatively small sample size, presence of *Salmonella* did not affect reproductive parameters (egg volume, clutch size, number of nestlings and number of fledglings), nor the health status of the fledglings. However, in order to clarify the impact on health and reproduction a larger number of samples have to be analyzed. Phage typing showed that the isolates belonged to the definitive phage types (DT) 193 and 99, and multi-locus variable number tandem repeat analysis (MLVA) demonstrated a high similarity among the tit isolates, but distinction to human isolates. These findings suggest the presence of passerine-adapted *Salmonella* strains in free-ranging tit populations with host pathogen co-existence. ISSN: 19326203

Benahmed, F., Wang, H., Beaubrun, J.J.-G., Gopinath, G.R., Cheng, C.-M., Hanes, D.E., Hammack, T.S., Rasmussen, M., Davidson, M.K.

Detection of salmonella enterica subsp. enterica serovar cubana from naturally contaminated chick feed

(2017) *Journal of Food Protection*, 80 (11), pp. 1815-1820.

ABSTRACT: Because some significant outbreaks of human salmonellosis have been traced to contaminated animal feed, the rapid and efficient detection of *Salmonella* in feed is essential. However, the current U.S. Food and Drug Administration Bacteriological Analytical Manual (BAM) culture method that uses lactose broth as a preenrichment medium has not reliably supported the results of real-Time PCR assays for certain foods. We evaluated the BAM culture method and a quantitative real-Time PCR (qPCR) assay using two preenrichment media, modified buffered peptone water and lactose broth, to detect *Salmonella enterica* subsp. *enterica* serovar *Cubana* in naturally contaminated chick feed. After 24 h of incubation, the qPCR method was as sensitive as the culture method when modified buffered peptone water was used as the preenrichment medium but less sensitive than culture when lactose broth was used. After 48 h of incubation, detection of *Salmonella Cubana* by qPCR and by culture in either preenrichment medium was equivalent. We also compared the performance of the traditional serotyping method, which uses pure cultures of *Salmonella* grown on blood agar, to two molecular serotyping methods. The serotyping method based on whole genome sequencing also requires pure cultures, but the PCR-based molecular serotyping method can be done directly with the enriched culture medium. The PCR-based molecular serotyping method provided simple and rapid detection and identification of *Salmonella Cubana*. However, whole genome sequencing allows accurate identification of many *Salmonella* serotypes and highlights variations in the genomes, even in tight genomic clusters. We also compared the genome of the chick feed isolate with 58 *Salmonella Cubana* strains in GenBank and found that the chick feed isolate was very closely related to an isolate from a foodborne outbreak involving alfalfa sprouts. ISSN: 0362028X

Liu, Y., Zhang, D.-F., Zhou, X., Xu, L., Zhang, L., Shi, X.

Comprehensive analysis reveals two distinct evolution patterns of Salmonella flagellin gene clusters

(2017) *Frontiers in Microbiology*, 8 (DEC), art. no. 2604, .

ABSTRACT: *Salmonella* is one of the primary causes of foodborne disease, especially *Salmonella enterica* subsp. *enterica* (I) which has caused ~99% of clinical salmonellosis cases for humans and domestic mammals. The flagella genes, *fliC* and *fljB*, which encode the *Salmonella* phase 1 and phase 2 antigens respectively, are considered as the *Salmonella* serotype determinant genes, and contribute to the virulence of *Salmonella*. However, the evolution of the two flagellin genes is still not well-understood. In this study, the *fliC* and *fljB* gene clusters were analyzed among 205 *S. enterica* subspecies I genomes. The dataset covered 87 different serovars of *S. enterica* subsp. *enterica* and included 9 genomes (six serovars) of four other *Salmonella* subspecies. Based on a pan-genome definition and flanked gene linkages, the *fliC* and *fljB* gene clusters were identified in 207 (91 serovars) and 138 (61 serovars) genomes, respectively. A phylogenetic tree constructed based on SNPs (Single Nucleotide Polymorphisms) of core genes were used to reflect the essential evolutionary relationships among various serovars. Congruence analysis was performed among the core genome and each gene of *fliC* and *fljB* gene clusters, with only *fliA* and *fliS* showing congruence to *Salmonella* core genome. Congruence was also observed among *fliB*, *fliC/fljB*, and *fliD* genes, and their phylogeny revealed a division into two major groups, which strongly corresponded to monophasic and biphasic serovars. Besides, homologous recombination events referring *fliB*, *fliC*, and *fliD* were found to have mainly occurred within each group. These results suggested two distinct evolutionary patterns of *Salmonella* flagellin gene clusters. Further insight on the evolutionary implication of the two patterns and a framework for phase variation mechanism are needed to be further processed. ISSN: 1664302X

Ferrari, R.G., Panzenhagen, P.H.N., Conte-Junior, C.A.

Phenotypic and genotypic eligible methods for Salmonella Typhimurium source tracking
(2017) *Frontiers in Microbiology*, 8 (DEC), art. no. 2587, .

ABSTRACT: Salmonellosis is one of the most common causes of foodborne infection and a leading cause of human gastroenteritis. Throughout the last decade, *Salmonella enterica* serotype Typhimurium (ST) has shown an increase report with the simultaneous emergence of multidrug-resistant isolates, as phage type DT104. Therefore, to successfully control this microorganism, it is important to attribute salmonellosis to the exact source. Studies of *Salmonella* source attribution have been performed to determine the main

food/food-production animals involved, toward which, control efforts should be correctly directed. Hence, the election of a ST subtyping method depends on the particular problem that efforts must be directed, the resources and the data available. Generally, before choosing a molecular subtyping, phenotyping approaches such as serotyping, phage typing, and antimicrobial resistance profiling are implemented as a screening of an investigation, and the results are computed using frequency-matching models (i.e., Dutch, Hald and Asymmetric Island models). Actually, due to the advancement of molecular tools as PFGE, MLVA, MLST, CRISPR, and WGS more precise results have been obtained, but even with these technologies, there are still gaps to be elucidated. To address this issue, an important question needs to be answered: what are the currently suitable subtyping methods to source attribute ST. This review presents the most frequently applied subtyping methods used to characterize ST, analyses the major available microbial subtyping attribution models and ponders the use of conventional phenotyping methods, as well as, the most applied genotypic tools in the context of their potential applicability to investigate ST source tracking. ISSN: 1664302X

Zhang, H., Qi, Y., Wang, L., Zhang, S., Deng, X.

Salmonella survival during thermal dehydration of fresh garlic and storage of dehydrated garlic products

(2017) *International Journal of Food Microbiology*, 263, pp. 26-31.

ABSTRACT: *Salmonella* survival was characterized and modeled during thermal dehydration of fresh garlic and storage of dehydrated garlic products. In our experiments that simulated commercial dehydration processing at 80 ± 5 °C, moderate level of *Salmonella* contamination (4–5 log CFU/g) on fresh garlic was reduced below the enumeration limit (1.7 log CFU/g) after 4.5 h of dehydration and not detectable by culture enrichment after 7 h. With high level of contamination (7–8 log CFU/g), the *Salmonella* population persisted at 3.6 log CFU/g after 8 h of processing. By increasing the dehydration temperature to 90 ± 5 °C, the moderate and high levels of initial *Salmonella* load on fresh garlic dropped below the enumeration limit after 1.5 and 3.75 h of processing and became undetectable by culture enrichment after 2.5 and 6 h, respectively. During the storage of dried garlic products, *Salmonella* was not able to grow under all tested combinations of temperature (25 and 35 °C) and water activity (0.56–0.98) levels, suggesting active inhibition. Storage temperature played a primary role in determining *Salmonella* survival on dehydrated garlic flakes. Under a typical storage condition at 25 °C and ambient relative humidity, *Salmonella* could persist over months with the population gradually declining (4.3 log reduction over 88 days). Granular size of dehydrated garlic had an impact on *Salmonella* survival, with better survival of the pathogen observed in smaller granules. At the early stage of dehydrated garlic storage (until 7 days), rising water activity appeared to initially promote but then inhibited *Salmonella* survival, resulting in a water activity threshold at 0.73 where *Salmonella* displayed strongest persistence. However, this phenomenon was less apparent during extended storage (after 14 days). ISSN: 01681605

Hörmansdorfer, S., Messelhäuser, U., Rampp, A., Schönberger, K., Dallman, T., Allerberger, F., Kornschöber, C., Sing, A., Wallner, P., Zapf, A.

Re-evaluation of a 2014 multi-country European outbreak of salmonella enteritidis phage type 14b using recent epidemiological and molecular data

(2017) *Eurosurveillance*, 22 (50), art. no. 17-00196, 7 p.

ABSTRACT: A European multi-country outbreak of *Salmonella* Enteritidis phage type (PT) 14b occurred from March to November 2014 associated with the consumption of eggs. The outbreak involved more than 400 human cases from France, Luxembourg, Austria and the United Kingdom. In 2016–2017, it has been re-evaluated combining recent epidemiological results with latest molecular data. The outbreak was traced back to one large Bavarian egg producer with four distinct premises, three located in Bavaria, one in the Czech Republic. The outbreak isolates of *S. Enteritidis* PT 14b were grouped into three closely related clades by whole genome sequencing. Two of these clades could be referred to two Bavarian premises of the egg producer on the basis of epidemiological and molecular data, while epidemiological data presumably linked the third clade to another premises of the egg producer. Interestingly and in contrast to the situation in other European countries where several outbreaks were documented, all notified 91 laboratory-confirmed cases of *S. Enteritidis* PT 14b from Bavaria were sporadic, singular cases not belonging to any epidemiological outbreaks. In conclusion, as demonstrated here, the resolution of food-related outbreaks with such a high discriminatory power is rare in outbreak investigation. ISSN: 1025496X

Swaggerty, C.L., Kogut, M.H., He, H., Genovese, K.J., Johnson, C., Arsenault, R.J.
Differential levels of cecal colonization by Salmonella Enteritidis in chickens triggers distinct immune kinome profiles

(2017) *Frontiers in Veterinary Science*, 4 (DEC), art. no. 214, .

ABSTRACT: *Salmonella enterica* serovar Enteritidis are facultative intracellular bacteria that cause disease in numerous species. *Salmonella*-related infections originating from poultry and/or poultry products are a major cause of human foodborne illness with *S. Enteritidis* the leading cause worldwide. Despite the importance of *Salmonella* to human health and chickens being a reservoir, little is known of the response to infection within the chicken gastrointestinal tract. Using chicken-specific kinome immune peptide arrays we compared a detailed kinomic analysis of the chicken jejunal immune response in a single line of birds with high and low *Salmonella* loads. Four-day-old chicks were challenged with *S. Enteritidis* (10⁵ cfu) and cecal content and a section of jejunum collected at three times: early [4-7 days post-infection (dpi)], middle (10-17 dpi), and late (24-37 dpi). *Salmonella* colonization was enumerated and birds with the highest (n = 4) and lowest (n = 4) loads at each time were selected for kinomic analyses. Key biological processes associated with lower loads of *Salmonella* clustered around immune responses, including cell surface receptor signaling pathway, positive regulation of cellular processes, defense response, innate immune response, regulation of immune response, immune system process, and regulation of signaling. Further evaluation showed specific pathways including chemokine, Jak-Stat, mitogen activated protein kinase, and T cell receptor signaling pathways were also associated with increased resistance. Collectively, these findings demonstrate that it is possible to identify key mechanisms and pathways that are associated with increased resistance against *S. Enteritidis* cecal colonization in chickens. Therefore, providing a foundation for future studies to identify specific proteins within these pathways that are associated with resistance, which could provide breeders additional biomarkers to identify birds naturally more resistant to this important foodborne pathogen. ISSN: 22971769

Siala, M., Barbana, A., Smaoui, S., Hachicha, S., Marouane, C., Kammoun, S., Gdoura, R., Messadi-Akrout, F.

Screening and detecting Salmonella in different food matrices in Southern Tunisia using a combined enrichment/real-time PCR method: Correlation with conventional culture method (2017) *Frontiers in Microbiology*, 8 (DEC), art. no. 2416, .

ABSTRACT: A combined enrichment/ newly developed *invA* TaqMan® real-time PCR (qPCR) method as a screening assay to detect *Salmonella* spp. in 500 naturally food matrices is evaluated. DNA template for qPCR was extracted from an overnight pre-enriched sample in buffered peptone water using lysis-guanidine isothiocyanate method. Heterologous internal amplification control (IAC) was incorporated during qPCR assays and co-amplified with the *invA* gene of the target pathogen. *InvA* qPCR exhibited 100% specificity when testing 94 *Salmonella* strains (inclusivity) and 32 non-*Salmonella* strains (exclusivity). The qPCR showed a consistent detection of two copies of the *invA* gene/PCR reaction, a good intra- and inter-run reproducibility with a good PCR efficiency (89.6%). QPCR was sensitive and showed *Salmonella* detection at 8.5 × 10⁰ CFU mL⁻¹ of artificially spiked poultry meat -BWP solution in less than 40 cycles. When analyzing 500 different food matrices and comparing the results with the ISO 6579:2002 conventional culture method, the sensitivity and specificity were 100 and 76.6%, respectively. QPCR showed *Salmonella* spp. DNA in raw poultry meat 27/45 (60%), milk 31/93 (33.3%), raw red meat 5/13 (38.5%), and fish 11/46 (23.9%) samples. The prevalence of *Salmonella* spp. in cakes, dairy, cooked meals, charcuterie products using qPCR was 11/14 (26.8%), 5/22 (22.7%), 32/150 (21.3%), and 5/20 (25%), respectively, compared to 0% as demonstrated by culture. *S. Anatum* was the most common serovar found associated with red meat compared to *S. kentucky* isolated from fish and poultry meat. In conclusion, our study is the first to use a combined enrichment/*invA* qPCR method as a screening assay to detect *Salmonella* DNA in different types of commercialized food in Southern Tunisia. QPCR results indicate that *Salmonella* contamination is common in milk and in other types of food samples. ISSN: 1664302X

Mair-Jenkins, J., Borges-Stewart, R., Harbour, C., Cox-Rogers, J., Dallman, T., Ashton, P., Johnston, R., Modha, D., Monk, P., Puleston, R.

Investigation using whole genome sequencing of a prolonged restaurant outbreak of Salmonella Typhimurium linked to the building drainage system, England, february 2015 to march 2016

(2017) *Eurosurveillance*, 22 (49), art. no. 17-00037, 9 p.

ABSTRACT: Following notification of a *Salmonella enterica* serovar Typhimurium gastroenteritis outbreak, we identified 82 cases linked to a restaurant with symptom onset

from 12 February 2015 to 8 March 2016. Seventy-two cases had an isolate matching the nationally unique whole genome sequencing profile (single nucleotide polymorphism (SNP) address: 1.1.1.124.395.395). Interviews established exposure to the restaurant and subsequent case-control analysis identified an association with eating carvery buffet food (adjusted odds ratios (AOR): 20.9; 95% confidence interval (CI): 2.2 – ∞). Environmental inspections, food/ water testing, and a food trace-back investigation were inconclusive. Repeated cycles of cleaning were undertaken, including hydrogen peroxide fogging, however, transmission continued. After 7 months of investigation, environmental swabbing identified 106 isolates from kitchen surfaces and restaurant drains matching the outbreak profile. We found structural faults with the drainage system and hypothesised that a reservoir of bacteria in drain biofilm and underfloor flooded areas may have sustained this outbreak. Ineffective drain water-traps (U-bends) may have also contributed by allowing transmission of contaminated aerosols into the kitchen environment. These findings suggest that routine swabbing of sink drain points and inspection of drainage systems should be considered in future outbreak scenarios. ISSN: 1025496X

Wei, W., Wang, X., Xie, Z., Wang, W., Xu, J., Liu, Y., Gao, H., Zhou, Y.

Evaluation of sanitizing methods for reducing microbial contamination on fresh strawberry, cherry tomato, and red bayberry

(2017) *Frontiers in Microbiology*, 8 (DEC), art. no. 2397, .

ABSTRACT: Strawberries, cherry tomatoes, and red bayberries, which are the most popular types of fresh produce in China, are vulnerable to microbial contamination. In this study, different sanitizing methods [treatment with 2% organic acids, 0.02% sodium hypochlorite (SH), 0.1% sodium chlorite (SC), and 0.1% acidified sodium chlorite (ASC)] were applied to fresh strawberry, cherry tomato, and red bayberry, and their abilities to reduce aerobic bacteria, *Escherichia coli* O157:H7, mold, yeast, and *Salmonella* Typhimurium were evaluated. The commercially used SH method reduced the background microbiota on strawberry, cherry tomato, and red bayberry by 0.20-2.07 log cfu/g. The ASC method reduced background microbiota (except for mold) on strawberry and cherry tomato by more than 3.0 log cfu/g. ASC was the only sanitizer that significantly reduced mold on red bayberry, and lactic acid was the only organic acid sanitizer that effectively reduced yeast on red bayberry. The ASC method had the best sterilizing effect on the three fresh fruits and also required the shortest sanitizing time and low chlorite content. The application of ASC method significantly reduced the microbiota on retail grocery samples, and the effect was similar to that achieved by sanitizing methods comparison. ISSN: 1664302X

Lake, I.R.

Food-borne disease and climate change in the United Kingdom

(2017) *Environmental Health: A Global Access Science Source*, 16, art. no. 117, .

ABSTRACT: This review examined the likely impact of climate change upon food-borne disease in the UK using *Campylobacter* and *Salmonella* as example organisms. *Campylobacter* is an important food-borne disease and an increasing public health threat. There is a reasonable evidence base that the environment and weather play a role in its transmission to humans. However, uncertainty as to the precise mechanisms through which weather affects disease, make it difficult to assess the likely impact of climate change. There are strong positive associations between *Salmonella* cases and ambient temperature, and a clear understanding of the mechanisms behind this. However, because the incidence of *Salmonella* disease is declining in the UK, any climate change increases are likely to be small. For both *Salmonella* and *Campylobacter* the disease incidence is greatest in older adults and young children. There are many pathways through which climate change may affect food but only a few of these have been rigorously examined. This provides a high degree of uncertainty as to what the impacts of climate change will be. Food is highly controlled at the National and EU level. This provides the UK with resilience to climate change as well as potential to adapt to its consequences but it is unknown whether these are sufficient in the context of a changing climate. ISSN: 1476069X

Bonardi, S., Bruini, I., Bolzoni, L., Cozzolino, P., Pierantoni, M., Brindani, F., Bellotti, P., Renzi, M., Pongolini, S.

Assessment of Salmonella survival in dry-cured Italian salami

(2017) *International Journal of Food Microbiology*, 262, pp. 99-106.

ABSTRACT: The inactivation of *Salmonella* during curing of Italian traditional pork salami was investigated. A total of 150 batches of ground raw meat (GRM) used for salami manufacturing by four producers were tested for *Salmonella* by real-time PCR followed by ISO 6579 cultural confirmation and MPN enumeration. Salami produced with *Salmonella*

positive GRMs were re-tested at the end of their curing period. Aw, pH and NaCl content were also measured. Detection of *Salmonella* was performed testing both 25 and 50 g of the samples. By Real-Time PCR 37% of the GRMs resulted positive, but cultural detection of *Salmonella* was obtained in 14% of the samples only. *Salmonella* enumeration ranged from 31 MPN/g to < 1.3 MPN/g. The difference between testing 50 g and 25 g of the samples was statistically significant (p value ≤ 0.01). In particular, ISO-50 g detected *Salmonella* in 100% of all positive samples, vs. 62% of ISO-25 g. Salami made of the contaminated GRMs were 29% *Salmonella*-positive, as most batches of salami produced with *Salmonella*-positive GRMs resulted negative after regular curing (20–48 days). Overall, 13% of salami produced with *Salmonella*-contaminated GRMs were positive. They belonged to six batches, which turned out negative after prolonged curing ranging between 49 and 86 days. *Salmonella* enumeration in salami ranged from 8.7 MPN/g to < 1.3 MPN/g. Unlike GRMs, no significant difference was observed between the ISO-50 g and the ISO-25 g in detecting *Salmonella* in cured salami (p value: > 0.05). The most common *Salmonella* serovars in GRMs were Derby (52%), Typhimurium monophasic variant 4, (Barbuti et al., 1993), 12:i:- (19%) and Stanley (10%). *Salmonella* Derby (56%), London, Branderup, Panama (13%, respectively) and Goldcoast (6%) were most frequent in cured salami. The study showed negative correlation between real-time CT values and cultural confirmation of *Salmonella*, as well as the importance of sample size for *Salmonella* detection. Among considered factors with possible effect on the occurrence of *Salmonella* in salami, statistical analysis revealed a role for aw in salami and for *Salmonella* load in GRMs, while pH and NaCl content did not significantly affect the probability of finding *Salmonella* in dry-cured salami in the context of this study. In particular the lower aw values due to longer curing were associated with lower *Salmonella* presence in traditional dry-cured salami. ISSN: 01681605

Cook, K.L., Givan, E.C., Mayton, H.M., Parekh, R.R., Taylor, R., Walker, S.L.

Using the agricultural environment to select better surrogates for foodborne pathogens associated with fresh produce

(2017) *International Journal of Food Microbiology*, 262, pp. 80-88.

ABSTRACT: Despite continuing efforts to reduce foodborne pathogen contamination of fresh produce, significant outbreaks continue to occur. Identification of appropriate surrogates for foodborne pathogens facilitates relevant research to identify reservoirs and amplifiers of these contaminants in production and processing environments. Therefore, the objective of this study was to identify environmental *Escherichia coli* isolates from manures (poultry, swine and dairy) and surface water sources with properties similar to those of the produce associated foodborne pathogens *E. coli* O157:H7 and *Salmonella enterica* serotype Typhimurium. The most similar environmental *E. coli* isolates were from poultry ($n = 3$) and surface water ($n = 1$) sources. The best environmental *E. coli* surrogates had cell surface characteristics (zeta potential, hydrophobicity and exopolysaccharide composition) that were similar (i.e., within 15%) to those of *S. Typhimurium* and/or formed biofilms more often when grown in low nutrient media prepared from lettuce lysates (24%) than when grown on high nutrient broth (7%). The rate of attachment of environmental isolates to lettuce leaves was also similar to that of *S. Typhimurium*. In contrast, *E. coli* O157:H7, a commonly used *E. coli* quality control strain and swine isolates behaved similarly; all were in the lowest 10% of isolates for biofilm formation and leaf attachment. These data suggest that the environment may provide a valuable resource for selection of surrogates for foodborne pathogens. ISSN: 01681605

Neira, C., Laca, A., Laca, A., Díaz, M.

Microbial diversity on commercial eggs as affected by the production system. A first approach using PGM

(2017) *International Journal of Food Microbiology*, 262, pp. 3-7.

ABSTRACT: A novel DNA-based technique (PGM) has been employed for first time to analyse commercial eggs with the advantage of allowing an exhaustive identification of the microbiota present. Eggs from two different production systems, i.e. a free range system and a cage system, were analysed. Twenty-one and twenty-two phyla were identified on the surface of cage system and free range system eggs, respectively. In both cases, Firmicutes was the dominant phylum (representing around 50% of total phyla), being found families frequently reported to be present in the intestinal microbiota of chickens or hens, such as Clostridiaceae, Ruminococcaceae and Lachnospiraceae. Additionally, other phyla and families not previously described in association with eggshells could also be identified in this work. Most of the potential pathogenic genera associated with eggs (*Salmonella*, *Clostridium*, *Helicobacter*, *Pseudomonas* and *Staphylococcus*) showed higher incidence in eggs coming from cage systems than in eggs coming from free range systems,

although the abundance of these genera were very low in both cases (< 5% of total bacteria). ISSN: 01681605

Hesse, M., Stamm, A., Berndt, A., Glünder, G., Weber, R.

Immune response to Salmonella infections in vaccinated and non-vaccinated turkeys (2017) Research in Veterinary Science, 115, pp. 165-173.

ABSTRACT: Vaccination has been widely used to reduce the *Salmonella* burden in poultry and subsequently the transmission to humans. Concerning turkey, there is little knowledge on the immune response to colonization and invasion by *Salmonella* species or about efficacy of vaccination and involved immune mechanisms. In the present study, turkeys were vaccinated at the day of hatch and infected with *Salmonella* Typhimurium (ST) or Enteritidis (SE) field strains three weeks later. A control group was kept uninfected. After challenge infection, bacterial counts in the cecal content, liver and spleen were determined 7 and 14 days post infection. They were often statistically significantly lower in vaccinated poult than in non-vaccinated ones. Production of iNOS, and the cytokines IL-8, IL-10 and IFN- γ were reduced in vaccinated birds. However, neither the influx of CD4 +, CD8 α + and CD28 + cells into cecal mucosa after infection nor the antibody response were statistically significantly altered in vaccinated birds. ISSN: 00345288

Ricke, S.C.

Insights and challenges of Salmonella infection of laying hens (2017) Current Opinion in Food Science, 18, pp. 43-49.

ABSTRACT: *Salmonella* infection of laying hens and subsequent contamination of eggs continues to be a public health concern. The focus of this review is to discuss some of the current and future issues that impact *Salmonella* association with laying hens and egg contamination. Among these issues are the impact of shifting to alternative cage free layer hen housing and away from cage batteries. Most of the focus will be on *Salmonella* Enteritidis as the serotype primarily associated with laying hens and the mechanisms that ensure its successful colonization, infection and subsequent contamination of table eggs. The variability in virulence and survival characteristics among *S. Enteritidis* strains will also be discussed including studies on detailed characterization at the molecular level. Finally, the comprehensive assessment of the laying hen gastrointestinal tract microbiota to better understand *S. Enteritidis* colonization and the subsequent host response will be examined. ISSN: 22147993

Goto, R., Miki, T., Nakamura, N., Fujimoto, M., Okada, N.

Salmonella Typhimurium PagP- and UgtL-dependent resistance to antimicrobial peptides contributes to the gut colonization (2017) PLoS ONE, 12 (12), art. no. e0190095, .

ABSTRACT: Mucosal barrier formed by cationic antimicrobial peptides (CAMPs) is believed to be crucial for host protection from pathogenic gut infection. However, some pathogens can develop resistance to the CAMPs to survive in hosts. *Salmonella enterica* is a common cause of acute diarrhea. During the course of this disease, the pathogen must continuously colonize the gut lumen, which contains CAMPs. However, it is incompletely understood whether the resistance of *Salmonella* strains to CAMPs contributes to the development of gut infections. PhoPQ two-component system-dependent lipid A modifications confer resistance to CAMPs in *S. enterica* serovar Typhimurium. Therefore, we introduced mutations into the PhoPQ-regulated genes in an *S. Typhimurium* strain, obtaining pagP ugtL and pmrA mutant strains. Each mutant strain demonstrated a distinct spectrum of the resistance to CAMPs. Using streptomycin mouse model for *Salmonella* diarrhea, we show that the pagP ugtL, but not pmrA, mutant strain had a gut colonization defect. Furthermore, the pagP ugtL, but not pmrA, mutant strain had decreased outer membrane integrity and susceptibility to magainin 2, an alpha-helical CAMP. Taken together, the PagP- and UgtL-dependent resistance to CAMPs was demonstrated to contribute to sustained colonization in the gut. This may be due to the robust outer membrane of *S. Typhimurium*, inducing the resistance to alpha-helical CAMPs such as α -defensins. Our findings indicate that the development of resistance to CAMPs is required for the *S. Typhimurium* gut infection. ISSN: 19326203

Gerlach, R.G., Walter, S., McClelland, M., Schmidt, C., Steglich, M., Prager, R., Bender, J.K., Fuchs, S., Schoerner, C., Rabsch, W., Lang, W., Jantsch, J.

Comparative whole genome analysis of three consecutive Salmonella diarizonae isolates (2017) International Journal of Medical Microbiology, 307 (8), pp. 542-551.

ABSTRACT: Infections of very young children or immunocompromised people with *Salmonella* of higher subspecies are a well-known phenomenon often associated with contact to cold-blooded animals. We describe the molecular characterization of three *S.*

enterica subsp. diarizonae strains, isolated consecutively over a period of several months from a hospital patient suffering from diarrhea and sepsis with fatal outcome. With the initial isolate the first complete genome sequence of a member of subsp. diarizonae is provided and based on this reference we revealed the genomic differences between the three isolates by use of next-generation sequencing and confirmed by phenotypical tests. Genome comparisons revealed mutations within *gpt*, *hfq* and *purK* in the first isolate as a sign of clonal variation rather than host-directed evolution. Furthermore, our work demonstrates that *S. enterica* subsp. *diarizonae* possess, besides a conserved set of known *Salmonella* Pathogenicity Islands, a variable portfolio of additional genomic islands of unknown function. ISSN: 14384221

Fu, S., Hiley, L., Octavia, S., Tanaka, M.M., Sintchenko, V., Lan, R.

Comparative genomics of Australian and international isolates of Salmonella Typhimurium: Correlation of core genome evolution with CRISPR and prophage profiles
(2017) *Scientific Reports*, 7 (1), art. no. 9733, .

ABSTRACT: *Salmonella enterica* subsp. *enterica* serovar *Typhimurium* (*S. Typhimurium*) is a serovar with broad host range. To determine the genomic diversity of *S. Typhimurium*, we sequenced 39 isolates (37 Australian and 2 UK isolates) representing 14 Repeats Groups (RGs) determined primarily by clustered regularly interspaced short palindromic repeats (CRISPR). Analysis of single nucleotide polymorphisms (SNPs) among the 39 isolates yielded an average of 1,232 SNPs per isolate, ranging from 128 SNPs to 11,339 SNPs relative to the reference strain LT2. Phylogenetic analysis of the 39 isolates together with 66 publicly available genomes divided the 105 isolates into five clades and 19 lineages, with the majority of the isolates belonging to clades I and II. The composition of CRISPR profiles correlated well with the lineages, showing progressive deletion and occasional duplication of spacers. Prophage genes contributed nearly a quarter of the *S. Typhimurium* accessory genome. Prophage profiles were found to be correlated with lineages and CRISPR profiles. Three new variants of HP2-like P2 prophage, several new variants of P22 prophage and a plasmid-like genomic island *StmGI-0323* were found. This study presents evidence of horizontal transfer from other serovars or species and provides a broader understanding of the global genomic diversity of *S. Typhimurium*.
ISSN: 20452322

Fatunla, K., Inam, E., Essien, J., Dan, E., Odon, A., Kang, S., Semple, K.T.

Influence of composting and thermal processing on the survival of microbial pathogens and nutritional status of Nigeria sewage sludge
(2017) *International Journal of Recycling of Organic Waste in Agriculture*, 6 (4), pp. 301-310.

ABSTRACT: Purpose: Sewage sludge samples from a water treatment plant in Nigeria were subjected to an in-vessel composting (using sawdust as a bulking agent) and thermal sludge processing to improve its quality for agricultural applications. Methods: Treated samples were analyzed for physicochemical and microbiological properties using standard analytical and aerobic culture protocols. Results: Microbiological analysis of the initial fresh mixture (sewage sludge/sawdust) showed that the total heterotrophic bacteria was 1.17×10^6 CFU/g of fresh compost, coliforms 4.7×10^4 CFU/g, *Salmonella* sp., and *Shigella* sp. 7.3×10^4 CFU/g, yeasts and moulds 9.0×10^4 CFU/g. These values were significantly ($p = 0.05$) reduced after 40 days of in-vessel composting to 4.3×10^4 CFU/g for total heterotrophic bacteria, 7.4×10^2 CFU/g for coliforms, while yeasts and moulds, *Salmonella* and *Shigella* sp. were not detected in the final compost. The results of the physicochemistry revealed variation in pH, temperature, and nutrients status of treated sludge. Conclusion: *Salmonella* sp., *Staphylococcus aureus*, and *Shigella* sp. were eliminated, while a 2-log reduction in coliform counts occurred after 40 days of composting. Composting had a better processing impact by increasing the ash as well as reducing the carbon/nitrogen ratio of treated sludge, while thermal processing improved the sulfate and phosphate components of treated sludge. The treated sludge (biosolids) met the permissible limits of microbiological and nutritional standards recommended by US EPA for land application of sludge and could, therefore, be used as a biofertilizer, soil conditioner and also for land reclamation. ISSN: 21953228

Kase, J.A., Zhang, G., Chen, Y.

Recent foodborne outbreaks in the United States linked to atypical vehicles — lessons learned
(2017) *Current Opinion in Food Science*, 18, pp. 56-63.

ABSTRACT: The past decade has seen atypical vehicles linked to foodborne outbreaks in the United States, including low moisture foods, frozen foods, and certain produce commodities which were not or rarely associated with outbreaks before. We reviewed

selected recent outbreaks involving *Salmonella*, Shiga toxin-producing *Escherichia coli*, and *Listeria monocytogenes*. Recognition of these outbreaks was partially due to improvements in outbreak response and surveillance, partially attributable to whole genome sequencing and related tools. Depending on the pathogen and vehicle, the contamination events leading to these outbreaks could occur before, during, and after food processing. Important data for root cause analyses are not always available because of inadequate traceability records or sampling throughout production chains, and challenges in detecting low levels of potentially injured pathogens heterogeneously distributed in foods. Further understanding of pathogen behaviors in these foods will enable better risk assessments and improve pathogen control strategies. ISSN: 22147993

Thanh, M.D., Agustí, G., Mader, A., Appel, B., Codony, F.

Improved sample treatment protocol for accurate detection of live Salmonella spp. in food samples by viability PCR

(2017) *PLoS ONE*, 12 (12), art. no. e0189302, .

ABSTRACT: Culture-based detection is still considered as the standard way for detection of *Salmonella* in foods, although molecular methods, such as viability PCR (vPCR), have been introduced to overcome some disadvantages of traditional culture methods. Despite the success of the vPCR methodology, the problem of false-positive results is a major drawback, especially when applied to environmental samples, hindering the interpretation of the results. To improve the efficiency of vPCR, many approaches have been introduced by several authors during the last years. In the present work, the combination of PEMAX dye, double tube change, and double photo-activation step was established as a strategy to improve vPCR protocol. By combining these approaches, we developed an improved sample treatment protocol able to neutralize DNA signals of up to 5.0×10^7 dead cells/sample from both pure culture and artificially contaminated food samples. Our results indicate that vPCR can work reliable and has a potential for high throughput detection of live *Salmonella* cells in food samples, minimizing false-positive signals. ISSN: 19326203

Singh, A., Barnard, T.G.

Adaptations in the physiological heterogeneity and viability of Shigella dysenteriae, Shigella flexneri and Salmonella typhimurium, after exposure to simulated gastric acid fluid (2017) *Microbial Pathogenesis*, 113, pp. 378-384.

ABSTRACT: Stomach acidity is an important barrier of the human body to protect itself from microbial pathogens entering the small intestine and causing infection. This study examined the survival adaptations of non-acid adapted diarrheal *Shigella* and *Salmonella* strains in an environment mimicking the human stomach. The bacterial responses to the challenge of acidic simulated gastric fluid were studied using flow cytometry physiological heterogeneity, membrane integrity and survival (culturability) respectively. Flow cytometry showed that bacterial cells, when exposed to gastric fluid, transformed distinctly, into physiologically heterogeneous sub-populations: intact, stressed and damaged cells, when stained with propidium iodide and thiazole orange. *Shigella* and *Salmonella* cells became membrane compromised during initial acid shock (0–30 min), and 80% of these cells shifted to the stressed state throughout gastric fluid exposure. Approximately 10–30% of bacterial strains remained culturable after 60 min of gastric fluid exposure at pH 2.5–4.5, with the percentage increasing with an inoculum size of 102 CFU/ml. This ability of non-acid adapted *Shigella* and *Salmonella* sp. to adapt and survive low pH gastric fluid, even though the bacterial numbers decreased or changed to a stressed state, further supports the possible risk of infection when consumed. ISSN: 08824010

Ohta, N., Norman, K.N., Norby, B., Lawhon, S.D., Vinasco, J., Den Bakker, H., Loneragan, G.H., Scott, H.M.

Population dynamics of enteric Salmonella in response to antimicrobial use in beef feedlot cattle

(2017) *Scientific Reports*, 7 (1), art. no. 14310, .

ABSTRACT: A randomized controlled longitudinal field trial was undertaken to assess the effects of injectable ceftiofur crystalline-free acid (CCFA) versus in-feed chlortetracycline on the temporal dynamics of *Salmonella enterica* spp. *enterica* in feedlot cattle. Two replicates of 8 pens (total 176 steers) received one of 4 different regimens. All, or one, out of 11 steers were treated with CCFA on day 0 in 8 pens, with half of the pens later receiving three 5-day regimens of chlortetracycline from day 4 to day 20. *Salmonella* was isolated from faecal samples and antimicrobial susceptibility was analysed via microbroth dilution. Serotype was determined by whole-genome sequencing. On day 0, mean *Salmonella* prevalence was 75.0% and the vast majority of isolates were pansusceptible. Both antimicrobials reduced overall prevalence of *Salmonella*; however, these treatments increased the proportion of multi-drug resistant (MDR) *Salmonella* from day 4 through day

26, which was the last day of faecal collection. Only six *Salmonella* serotypes were detected. *Salmonella* serotype Reading isolates were extensively MDR, suggesting a strong association between serotype and resistance. Our study demonstrates that the selection pressures of a 3rd generation cephalosporin and chlortetracycline during the feeding period contribute to dynamic population shifts between antimicrobial susceptible and resistant *Salmonella*. ISSN: 20452322

Borges, K.A., Furian, T.Q., De Souza, S.N., Menezes, R., Salle, C.T.P., De Souza Moraes, H.L., Tondo, E.C., Do Nascimento, V.P.

Phenotypic and Molecular Characterization of Salmonella Enteritidis SE86 Isolated from Poultry and Salmonellosis Outbreaks (2017) Foodborne Pathogens and Disease, 14 (12), pp. 742-754.

ABSTRACT: *Salmonella* Enteritidis remains a standout among the leading causes of foodborne diseases worldwide. Previous studies have demonstrated that a unique clonal group of *Salmonella* Enteritidis, named SE86, is involved in foodborne outbreaks in southern Brazil and is frequently identified among strains isolated from poultry. The aim of this study was to determine the influence of the isolation source (food products involved in salmonellosis outbreaks and poultry sources) on the phenotypic and molecular characteristics of *Salmonella* Enteritidis SE86. A biofilm formation assay, antimicrobial susceptibility test, polymerase chain reaction identification of virulence-associated genes, and phage type 4 (PT4) assessment were performed to characterize *Salmonella* Enteritidis SE86. The human strains presented less antimicrobial resistance than the poultry strains. Resistance to some substances was related to the isolation source of the strain. Strains of the same clonal group presented different biofilm production abilities. Biofilm formation was independent of the isolation source at all temperatures. Temperature influenced biofilm formation only by the poultry strains. Most of the investigated genes presented a high frequency and a regular distribution, regardless of the isolation source. The *spvB*, *spiA*, *pagC*, *sipB*, *prgH*, *spaN*, *sitC*, and *lpfC* genes were associated with the avian strains, whereas *iroN* was associated with the strains isolated from food products involved in salmonellosis outbreaks. Most strains belonged to PT4. No relationship was found between biofilm production and antimicrobial resistance or between the virulence profile and biofilm production or antimicrobial resistance. ISSN: 15353141

Ommi, D., Hemmatinezhad, B., Hafshejani, T.T., Khamesipour, F.

Incidence and Antimicrobial Resistance of Campylobacter and Salmonella from Houseflies (Musca Domestica) in Kitchens, Farms, Hospitals and Slaughter Houses (2017) Proceedings of the National Academy of Sciences India Section B - Biological Sciences, 87 (4), pp. 1285-1291.

ABSTRACT: Carriage status of *Campylobacter* and *Salmonella* was investigated in houseflies in Shahrekord and Isfahan provinces of Iran. This was a longitudinal study conducted from June 2013 to May 2014. Flies were collected from household kitchens, animal farms, slaughter houses and hospitals and put in sample bottles filled with peptone water. Bacteria were isolated and DNA was extracted from bacterial isolates using a commercial kit. Confirmation of the organisms was carried out by polymerase chain reaction using primer sets for detection of these pathogens. Out of 600 houseflies 19.5 % (117/600) were positive for *Campylobacter* and 15.8 % (95/600) were positive for *Salmonella* organisms. The recovery frequencies of the two organisms in different locations were similar. Higher proportions of infected flies were obtained during summer whereas low proportions were obtained during winter of all the organisms ($P < 0.05$). The organisms had low to moderate resistance to different antimicrobial agents. It is concluded that houseflies do harbor antimicrobial resistant diarrheagenic pathogens including *Campylobacter* and *Salmonella*, more so during summer. The data support the importance of taking into account the houseflies in future plans aimed at stemming infections caused by these organisms. ISSN: 03698211

Alhenaky, A., Abdelqader, A., Abuajamieh, M., Al-Fataftah, A.-R.

The effect of heat stress on intestinal integrity and Salmonella invasion in broiler birds (2017) Journal of Thermal Biology, 70, pp. 9-14.

ABSTRACT: The intestinal mucosa works as a barrier to protect the internal environment of the animal from bacteria and bacterial toxins found in the gut lumen. Heat stress may harm this function. Therefore, we designed the current experiment to investigate the effect of heat stress on intestinal integrity, physiological and immunological responses and *Salmonella* invasion in broiler chickens. At 26 days of age, 72 birds were randomly distributed into 3 treatments, with 8 replicates per treatment and 3 birds per replicate. The three treatments were control treatment; kept at thermoneutral environmental conditions (20 ± 2 °C), chronic heat stress treatment (exposed to 30 ± 2 °C; 24 h/day) and acute

heat stress treatment (exposed to 35 ± 2 °C from 09:00 to 13:00 and kept at 20 ± 1 °C from 13:00 to 09:00). The heat stress exposure was conducted for 10 successive days. Compared with the control treatment, birds subject to chronic and acute heat stress had reduced ($P < 0.05$) body weight and body gain and increased ($P < 0.05$) feed conversion ratio. However, feed intake and mortality rate were only increased ($P < 0.05$) in the acute heat stress treatment. Rectal temperature and Δ rectal temperature (°C/h) increased ($P < 0.05$) sharply during the first 2 days of exposure followed by gradual decreases until a plateau was achieved. Heat-stressed birds had increased ($P < 0.05$) serum concentrations of corticosterone, endotoxin lipopolysaccharide and the systemic inflammatory cytokine: TNF- α and IL-2, as well as a higher ($P < 0.05$) prevalence of *Salmonella* spp. in meat and livers, as compared with control treatment. It can be concluded that heat stress impaired intestinal integrity which resulted in increased intestinal permeability to endotoxin, translocation of intestinal pathogens (*Salmonella* spp.) and serum inflammatory cytokines. Therefore, avoiding thermal dysfunction of intestinal barrier is a significant factor in maintaining welfare, immune status and meat safety of broiler birds. ISSN: 03064565

Silva, B.N., Cadavez, V., Teixeira, J.A., Gonzales-Barron, U.

Meta-analysis of the incidence of foodborne pathogens in vegetables and fruits from retail establishments in Europe

(2017) *Current Opinion in Food Science*, 18, pp. 21-28.

ABSTRACT: In this study, a systematic review and meta-analysis were conducted to summarise available information on the occurrence of *Salmonella* spp. *Listeria monocytogenes* and shigatoxin-producing *Escherichia coli* (STEC) in fruits and vegetables sold at retail establishments in several European countries. Overall, *L. monocytogenes* was the main pathogen detected in all kinds of vegetables, packaged or not (3.4%; 95% CI: 2.1–5.4%) with *Salmonella* spp. being the pathogen of lowest incidence (0.9%; 95% CI: 0.5–1.2%). The pooled occurrence rate of pathogens in either packed or unpacked vegetables was estimated at 1.9% (95% CI: 1.2–3.1%), with 2.1% of prevalence (95% CI: 1.3–3.4%) for unpacked vegetables and 1.7% (95% CI: 0.9–2.9%) for packed ones. For the three pathogens, the category of spices and herbs was the most frequently contaminated with pathogens, whereas salads presented the lowest occurrence. The vegetable category with highest incidence of *Salmonella* spp. (1.7%; 95% CI: 0.7–4.1%) and *L. monocytogenes* (2.2%; 95% CI: 1.0–4.7%) is leafy greens whilst STEC is more frequently recovered from sprouts (1.9%; 95% CI: 0.5–5.9%). In the case of fruits, the pooled prevalence estimates for *Salmonella* spp., *L. monocytogenes* and STEC were 1.60% (0.54%; 95% CI: 0.55–4.60%), 1.91% (0.50%; 95% CI: 0.93–3.88%) and 4.71% (1.52%; 95% CI: 1.73–12.2%), correspondingly. ISSN: 22147993

de Curraize, C., Amoureux, L., Bador, J., Chapuis, A., Siebor, E., Clément, C., Sauge, J., Aho-Glélé, L.-S., Neuwirth, C.

"Does the Salmonella Genomic Island 1 (SGI1) confer invasiveness properties to human isolates?"

(2017) *BMC Infectious Diseases*, 17 (1), art. no. 741, .

ABSTRACT: Background: In the eighties, a multidrug resistant clone of *Salmonella* Typhimurium DT104 emerged in UK and disseminated worldwide. This clone harbored a *Salmonella* genomic island 1 (SGI1) that consists of a backbone and a multidrug resistant region encoding for penta-resistance (ampicillin, chloramphenicol/florfenicol, streptomycin/spectinomycin, sulphonamides and tetracycline (ACSSuT)). Several authors suggested that SGI1 might have a potential role in enhancement of virulence properties of *Salmonella enterica*. The aim of this study was to investigate whether nontyphoidal *S. enterica* isolates carrying SGI1 cause more severe illness than SGI1 free ones in humans. Methods: From 2011 to 2016, all patients infected with nontyphoidal *S. enterica* in our hospital were retrospectively included. All nontyphoidal *S. enterica* isolates preserved in our University Hospital (Dijon, France) were screened for the presence of SGI1. Clinical and biological data of patients were retrospectively collected to evaluate illness severity. Statistical analysis of data was performed by Kruskal-Wallis test or Fisher's exact test for univariate analysis, and by logistic regression for multivariate analysis. Results: A total of 100 isolates of *S. enterica* (22 serovars) were collected. Twelve isolates (12%) belonging to 4 serovars harbored SGI1: *S. Typhimurium*, *S. Infantis*, *S. Kentucky*, *S. St Paul*. The severity of the disease was age-related (for invasive infection, sepsis and inflammatory response) and was associated with immunosuppression (for invasive infection, sepsis and bacteremia) but not with the presence of SGI1 or with antimicrobial resistance. Conclusion: A rather high proportion (12%) of human clinical isolates belonging to various serovars (for the first time serovar *St Paul*) and harboring various antimicrobial resistance profile carried SGI1. Diseases due to SGI1-positive *S. enterica* or to antimicrobial resistant

isolates were not more severe than the others. This first clinical observation should be confirmed by a multicenter and prospective study. ISSN: 14712334

De Carli, S., Gräf, T., Kipper, D., Lehmann, F.K.M., Zanetti, N., Siqueira, F.M., Cibulski, S., Fonseca, A.S.K., Ikuta, N., Lunge, V.R.

Molecular and phylogenetic analyses of Salmonella Gallinarum trace the origin and diversification of recent outbreaks of fowl typhoid in poultry farms (2017) Veterinary Microbiology, 212, pp. 80-86.

ABSTRACT: Fowl typhoid (FT) and pullorum disease (PD) are two important poultry infections caused by *Salmonella enterica* subsp. *enterica* serotype *Gallinarum* (*S. Gallinarum*). *S. Gallinarum* strains are adapted to birds and classified into biovars *Gallinarum* (bvGA) and *Pullorum* (bvPU) as they are the causative agent of FT and PD, respectively. In Brazil, FT/PD outbreaks have been reported along the last 50 years, but there was a recent increase of FT field reports with the suspicion it could be due to virulence reversion of the attenuated live vaccine SG9R. In this study, we applied molecular biology assays and phylogenetic methods to detect and investigate *S. Gallinarum* isolates from commercial poultry flocks in order to understand the evolutionary history and origin of the recent FT outbreaks in Brazil. *S. Gallinarum* isolates were obtained from thirteen different poultry flocks with clinical signs of FT/PD from 2013 to 2015. These isolates were serotyped, tested with three specific PCR (for the detection of bvGA, bvPU and live vaccine strain SG9R) and submitted to sequencing of a variable genome region (ISR analysis). The complete genome of one bvGA strain (BR_RS12) was also compared to other *S. Gallinarum* complete genomes (including other two Brazilian ones: bvGA 287/91 and bvPU FCVA198). PCR detected all thirteen isolates as *S. Gallinarum* (eight bvGA and five bvPU), none positive for SG9R strain. ISR analysis revealed that all eight bvGA isolates showed exactly the same nucleotide sequences with 100% similarity to reference strains, while two patterns were observed for bvPU. Genome phylogeny demonstrated distinct clades for bvGA and bvPU, with the bvGA clade showing a clear subdivision including three genomes: SG9R vaccine, the respective SG9 parent strain and one SG9R revertant field isolate (MB4523). The evolutionary rate of the total *S. Gallinarum* genome was calculated at 6.15×10^{-7} substitutions/site/year, with 2.8 observed substitutions per year per genome (1 SNP per 4292 bases). Phylodynamics analysis estimated that at least two introductions of *S. Gallinarum* bvGA happened in Brazil, the first in 1885 and the second in 1950. The Brazilian bvGA genomes 287/91 and BR_RS12 analyzed here were related to the early and the late introductions, respectively. In conclusion, these results indicate the occurrence of *S. Gallinarum* strains associated with FT outbreaks that have been circulating for more than 50 years in Brazil and are not originated from virulence reversion of the SG9R vaccine. ISSN: 03781135

Li, Q., Yin, K., Xie, X., Zhao, F., Xia, J., Chen, Y., Hu, Y., Xu, L., Chen, X., Jiao, X.

Detection and CRISPR subtyping of Salmonella spp. isolated from whole raw chickens in Yangzhou from China (2017) Food Control, 82, pp. 291-297.

ABSTRACT: This study was undertaken to acquire data on *Salmonella* contamination of whole raw chickens, eggs, and vegetables available to consumers in Yangzhou city, China, between April 2011 and March 2012. In total, 240 chicken carcasses were tested, and the overall contamination rate for *Salmonella* was 33.8%. While the prevalence of *Salmonella* in 100 eggs and 155 vegetable samples was 7.0% and 3.2%, respectively. The 84 isolated strains were identified in 19 different serotypes with *Salmonella enterica* serovar *Indiana* (*S. Indiana*) (25.0%), *S. Typhimurium* (21.4%) and *S. Enteritidis* (17.9%) as the predominant serovars. Moreover, the median load of the contaminated chicken samples reached 6.4 MPN/100 g with 3.6 MPN/100 g as the 25th percentile and 15.0 MPN/100 g as the 75th percentile. Chicken carcasses collected in October had not only the highest prevalence of *Salmonella* (70%), but also the highest median load (33 MPN/100 g) and 75th percentile load (460 MPN/100 g), while the lowest prevalence (10%) was in April. The clustered regularly interspaced short palindromic repeats (CRISPR) subtyping method was then used to identify serotypes of *Salmonella* and distinguish strains from the same *Salmonella* serotypes. We found that 85.7% of strains were distributed in 11 serotypes speculated by CRISPR typing, which corresponded to the identified serotypes by O and H antiserum. The speculated serotypes of 7.1% of the strains by CRISPR typing are very close to the identified ones, as they belong to the same O group with a small difference in the O or H antigen. All of the above findings implied that CRISPR typing could be applied to serotyping of *Salmonella*. In addition, CRISPR typing method could be used to subtype different strains from the same serotype, specifically *S. Hadar*. ISSN: 09567135

Li, J., Hao, H., Cheng, G., Wang, X., Ahmed, S., Shabbir, M.A.B., Liu, Z., Dai, M., Yuan, Z.

The effects of different enrofloxacin dosages on clinical efficacy and resistance development in chickens experimentally infected with Salmonella Typhimurium (2017) Scientific Reports, 7 (1), art. no. 11676, .

ABSTRACT: To investigate the optimal dosage which can improve clinical efficacy and minimize resistance, pharmacokinetics/pharmacodynamics model of enrofloxacin was established. Effect of enrofloxacin treatments on clearance of *Salmonella* in experimentally infected chickens and simultaneously resistance selection in *Salmonella* and coliforms were evaluated in three treatment groups (100, PK/PD designed dosage of 4, 0.1 mg/kg b.w.) and a control group. Treatment duration was three rounds of 7-day treatment alternated with 7-day withdrawal. Results showed that 100 mg/kg b.w. of enrofloxacin completely eradicated *Salmonella*, but resistant coliforms (4.0-60.8%) were selected from the end of the second round's withdrawal period till the end of the experiment (days 28-42). PK/PD based dosage (4 mg/kg b.w.) effectively reduced *Salmonella* for the first treatment duration. However upon cessation of medication, *Salmonella* repopulated chickens and persisted till the end with reduced susceptibility (MICCIP = 0.03-0.25 mg/L). Low frequency (5-9.5%) of resistant coliforms was selected (days 39-42). Enrofloxacin at dosage of 0.1 mg/kg b.w. was not able to eliminate *Salmonella* and selected coliforms with slight decreased susceptibility (MICENR = 0.25 mg/L). In conclusion, short time treatment (7 days) of enrofloxacin at high dosage (100 mg/kg b.w.) could be effective in treating *Salmonella* infection while minimizing resistance selection in both *Salmonella* and coliforms. ISSN: 20452322

Hashemi, A., Baghbani-arani, F., Ahmadiyan, S., Ghavami-Nejad, S.

Multiple-locus variable-number tandem-repeat analysis in Salmonella isolates as an effective molecular subtyping method (2017) Microbial Pathogenesis, 113, pp. 11-16.

ABSTRACT: Due to the limitations of serotyping, to differentiate closely related microbial isolates and to investigate disease outbreaks, molecular genotyping methods including multiple loci variable number of tandem repeats (VNTR) analysis (MLVA) has been developed. The usefulness of MLVA was recently demonstrated for *Salmonella* Infantis and *Salmonella* Enteritidis isolated from human sources in Iran. In the present study. The discriminatory ability of this method was investigated in 78 Iranian *Salmonella* enterica isolates. *Salmonella* strains isolated from human urine, stool, bone marrow, blood, ascites and synovial fluid sources in Iran during the years 2012 and 2015 were analyzed. Among these 78 *Salmonella* isolates, 70 isolates belonging to eight serotypes/serogroups, while eight were nontypeable. Six VNTR loci were amplified from all isolates. The isolates were distributed into 67 genotypes. Two out of the 6 markers (Sal20 and Sal16) were highly discriminatory for all strains (DI > 0.80) while composition of all VNTR loci produced 67 different types with 0.995 D value. The high discrimination power of MLVA in *Salmonella* molecular typing via combination of VNTR loci studied here, suggesting that this method is highly valuable for molecular epidemiology of *Salmonella* strains. ISSN: 08824010

Fijalkowski, K., Rorat, A., Grobelak, A., Kacprzak, M.J.

The presence of contaminations in sewage sludge – The current situation (2017) Journal of Environmental Management, 203, pp. 1126-1136.

ABSTRACT: Sewage sludge/biosolids are by-wastes of municipal and industrial wastewater treatment. As sources of nutrients (C, N, P) they are widely used in intensive farming where large supplementation of organic matter to maintain fertility and enhance crop yields is needed. However, according to the report of European Commission published in 2010, only 39% of produced sewage sludge is recycled into agriculture in the European Union. This situation occurs mainly due to the fact, that the sewage sludge may contain a dangerous volume of different contaminants. For over decades, a great deal of attention has been focused on total concentration of few heavy metals and pathogenic bacteria *Salmonella* and *Escherichia coli*. The Sewage Sludge Directive (86/278/EEC) regulates the allowable limits of Zn, Cu, Ni, Pb, Cd, Cr and Hg and pathogens and allows for recovery of sludge on land under defined sanitary and environmentally sound conditions. In this paper, a review on quality of sewage sludge based on the publications after 2010 has been presented. Nowadays there are several papers focusing on new serious threats to human health and ecosystem occurring in sewage sludge – both chemicals (such as toxic trace elements – Se, Ag, Ti; nanoparticles; polyaromatic hydrocarbons; polychlorinated biphenyl; perfluorinated surfactants, polycyclic musks, siloxanes, pesticides, phenols, sweeteners, personal care products, pharmaceuticals, benzotriazoles) and biological traits (*Legionella*, *Yersinia*, *Escherichia coli* O157:H7). ISSN: 03014797

Zhu, X., Chen, L., Wu, J., Tang, H., Wang, Y.

Salmonella typhimurium Infection Reduces *Schistosoma japonicum* Worm Burden in Mice (2017) *Scientific Reports*, 7 (1), art. no. 1349, .

ABSTRACT: Coinfection of microorganisms is a common phenomenon in humans and animals. In order to further our understanding of the progress of coinfection and the possible interaction between different pathogens, we have built a coinfection mouse model with *Schistosoma japonicum* and *Salmonella typhimurium*, and used this model to investigate the systemic metabolic and immune responses using NMR-based metabolomics and immunological techniques. Our results show that *Salmonella typhimurium* (ATCC14028) infection reduces the number of adult schistosomal worms and eggs, relieves symptoms of schistosomiasis and also abates the mortality of mice infected by *Schistosoma japonicum*. In addition, *Salmonella typhimurium* infection counteracts the metabolic disturbances associated with schistosomiasis, which was reflected by the reverted levels of metabolites in coinfecting mice, compared with the *Schistosoma japonicum* infected mice. Furthermore, immune analyses also indicate that shift of the immune response to different pathogens is a result of indirect interactions between *Schistosoma japonicum* and *Salmonella typhimurium* within the host. *Salmonella typhimurium* infection can ameliorate *Schistosoma japonicum*-caused schistosomiasis in BALB/c mice, which is most likely due to inverse immune polarization. Our work provides an insight into coinfection between *Schistosoma japonicum* and *Salmonella typhimurium*, and may further contribute to the development of new tools for controlling *Schistosoma japonicum*-associated diseases. ISSN: 20452322

Dai, F., Zhang, M., Xu, D., Yang, Y., Wang, J., Li, M., Du, M.

The development of methods for the detection of Salmonella in chickens by a combination of immunomagnetic separation and PCRs (2017) *Biotechnology and Applied Biochemistry*, 64 (6), pp. 888-894.

ABSTRACT: Micro- and nanoimmunomagnetic beads (MIMBs and NIMBs) used for immunomagnetic separation (IMS) with PCR were studied for the rapid detection of *Salmonella*. The capture efficiency of the two different IMBs was evaluated by a conventional plate counting method, and the binding pattern was studied using scanning electron microscopy. The specificity of the IMBs was tested with *Salmonella*, *Shigella flexneri*, enterohemorrhagic *Escherichia coli* O157:H7, and *Listeria monocytogenes*. By comparing the pre-enrichment IMS and the IMS enrichment steps with a 5.5-H enrichment time, this study developed a rapid and sensitive method for the detection of *Salmonella* in chicken. The method was implemented by IMS enrichment and PCR with MIMBs and NIMBs, with a total analysis time of 8 H. We showed that the method was sensitive based on NIMBs with a detection limit of 10³ CFU for *Salmonella* in 25 g of chicken. ISSN: 08854513