

# NEWSLETTER

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## Editorial Note

Bilthoven, 3 July 2017

Dear colleague,

By the end of May we organised our annual EUR-*Salmonella* **workshop**. This year it was organised in Zaandam, the Netherlands, which was easier to reach than the location last year (Saint Malo, France). A total of 45 participants were present at the workshop. I have had a quick look at the completed evaluation forms and I am happy to notice that in general the participants were satisfied. The presentations given at the workshop are available at our website since 2 June 2017, see: [http://www.eurlsalmonella.eu/Workshops/Workshop\\_2017](http://www.eurlsalmonella.eu/Workshops/Workshop_2017)

During the workshop, the participants signed a card with greetings for Henny Maas who stopped working by the end of 2016 (see Newsletter 22-4). Henny was pleasantly surprised with this card and she would like to thank all NRLs for the many kind words and for the very nice cooperation for so many years.

At the workshop, the participants were informed about the publication of **the new Official Control Regulation (EU) 2017/625 (OCR)**. This new Regulation was published in the Official Journal on 7 April 2017. The Regulation replaces Regulation (EC) No 882/2004 (and Reg. 854/2004) and is the Framework legislation on all controls by competent authorities including official sampling and analyses and requirements for official laboratories, NRLs and EURL. The task and duties of EURLs and NRLs originally described in Reg. 882/2004 is now described in this new Reg. 2017/625. Information on the new Regulation can be found in the presentation of Pamina Suzuki given at the EURL-*Salmonella* workshop 2017: <http://www.eurlsalmonella.eu/dsresource?type=pdf&disposition=inline&objectid=rivmp:331084&versionid=&subobjectname=>. The Regulation itself can be found at the following link: <http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32017R0625>

In May 2017, we organised a follow-up study for serotyping of *Salmonella* isolates of the **21<sup>st</sup> interlaboratory comparison study on typing of *Salmonella***. Unfortunately the participating laboratory was still facing problems with serotyping *Salmonella* and the EURL is in contact with the NRL in trying to find the cause of the problems.

The analysis of the PFGE results of this study has been delayed and are expected to be reported to the participants in the coming weeks.

Also in May 2017, the participants of the 20<sup>th</sup> interlaboratory comparison study on **detection of *Salmonella* in samples from the primary production stage** received their own results, followed by the interim summary with the results of all laboratories in June. The majority of the participating laboratories found good results in this study. The results of one laboratory were indicated as moderate (due to an error in reporting the results of the control samples) and only one laboratory scored a poor performance. The interim summary report of the study can also be found at the EURL-*Salmonella* website: [http://www.eurlsalmonella.eu/Publications/Interlaboratory\\_comparison\\_study\\_Reports/Faeces\\_study\\_reports](http://www.eurlsalmonella.eu/Publications/Interlaboratory_comparison_study_Reports/Faeces_study_reports)

From 19 to 23 June 2016, the annual plenary meetings of **ISO/TC34/SC9 and CEN/TC275/WG6** were organised in Tokyo, Japan. A summary on the '*Salmonella*-related items' as discussed at these meetings is given in this Newsletter.

In this Newsletter also the timetables of the two interlaboratory comparison studies to be organised in fall 2017 are given. This concerns the **combined interlaboratory comparison study on detection of *Salmonella* in Food and samples from the Primary Production Stage (PPS)** in October 2017 and the **22<sup>nd</sup> interlaboratory comparison study on typing of *Salmonella*** in November 2017.

The reason for a **combined Food and PPS study** is the fact that we want to change the order of the PPS and the Food studies. Up till now, the PPS studies have been organised in February/March of each year. However, regularly we have been facing problems with the choice of the matrix due to Avian Influenza outbreaks in poultry, related to migration of wild birds in fall and winter. For that reason we want to reverse the order of the PPS and the Food studies. However, doing this may cause the following problems:

- Two studies with similar matrix would be organised closely after each other;
- In one year, a study for either PPS or food/feed would not be organised.

The proposed solution is to organise a study with a matrix that can be tested as 'food sample' and as 'PPS sample', namely swabs or hygienic sponges. This type of matrix is used in different fields:

- Sampling after cleaning and disinfection in the primary production stage (ISO 13307);
- Carcass sampling (ISO 17604);
- Sampling of surfaces in the food industry environment and food processing plants (ISO 18593).

Early 2017, EURL-*Salmonella* already performed some experiments by contaminating sponges with a mixture of *Salmonella* and 'background flora' and the results were promising.

For this combined study, NRLs-*Salmonella* which analyse food samples as well as NRLs-*Salmonella* which analyse samples from the primary production stage are invited to participate in this study. This may result in participation of more than one NRL per country. Each NRL will need to indicate in advance if they will participate as NRL for food analysis or as NRL for PPS analysis, including the use of the relevant method.

After the organisation of the combined Food and PPS study in fall 2017, the following studies are foreseen in 2018-2019 to come to the reverse order of the studies:

- February/March 2018: Detection of *Salmonella* in **animal feed**;
- September/October 2018: Detection of *Salmonella* in samples from the **primary production stage (PPS)**;
- February/March 2019: Detection of *Salmonella* in a **food matrix**.

For sure we will keep you informed about the planning and details of each study.

In the first half of 2017 the following **reports and article** were published in which the EURL-*Salmonella* had the lead or participated:

Pol-Hofstad, I.E. and Mooijman, K.A. The 19<sup>th</sup> EU Interlaboratory comparison study in Primary Production (2016) - Detection of *Salmonella* in chicken faeces adhering to boot socks. National Institute for Public Health and the Environment, Bilthoven, the Netherlands. RIVM Report no.: 2016-0044. (February 2017; errata March 2017) <http://www.rivm.nl/dsresource?objectid=9dae5131-3a49-41f7-9d67-e3617bf4f295&type=pdf&disposition=inline>

Kuijpers A.F.A. and Mooijman, K.A. EU Interlaboratory comparison study Food VII (2015) - Detection of *Salmonella* whole liquid chicken egg. National Institute for Public Health and the Environment, Bilthoven, the Netherlands. RIVM Report no.: 2014-042. (May 2017) <http://www.rivm.nl/bibliotheek/rapporten/2016-0042.pdf>

Rizzi, V., Da Silva Felicio, T., Felix, B., Gossner, C.M., Jacobs, W., Johansson, K., Kotila, S., Michelon, D., Monguidi M., Mooijman, K., Morabito, S., Pasinato, L., Torgny Björkman, J., Torpdahl, M., Tozzoli, R and Van Walle, I. The ECDC-EFSA molecular typing database for European Union public health protection.

Euroreference 2, March 2017.

[http://euroreference.mg.anses.fr/sites/default/files/17%2003%20ED%20ER%2002%201\\_RIZZI.pdf](http://euroreference.mg.anses.fr/sites/default/files/17%2003%20ED%20ER%2002%201_RIZZI.pdf)

In the last month(s) we received information from several NRLs on **misuse of the names of staff members of the EURL-*Salmonella* in emails** we never have sent. Please delete emails which you do not trust. These emails come from incorrect email addresses (not ours) and include links which you better do not open. We have asked our IT department if there is a way to prevent these emails from being sent, but it is difficult to find out how the misuse started. It may be the case that the email addresses of the NRLs published on our EURL-*Salmonella* website were (mis)used. Although it is not sure this is the case, we may consider deleting the email addresses of the NRLs from our website. If you prefer us to delete your email address(es) please let us know.

I would like to wish you all a very sunny and relaxing summer period!

Best wishes,  
Kirsten Mooijman  
Coordinator EURL-*Salmonella*

## Contribution of the EURL-*Salmonella*

**Time table of the combined interlaboratory comparison study for Food and Primary Production Stage (2017)  
Detection of *Salmonella* in hygienic sponges**

Week (2017)	Dates	Subject
39	25 – 29 September	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.
40	2 October	Mailing of parcels to the NRLs as Biological Substance Cat. B (UN3373) by door-to-door courier service  Preparation of media by the NRLs
41	9 October	Performance of the study
44	<u>2</u> November	Deadline for completing the electronic submission of results: <b>2 November 2017</b> (23:59 h CET) After this deadline the electronic submission form will be closed.

**Timetable of the 22<sup>nd</sup> interlaboratory comparison study (2017) on serotyping and optional PFGE typing of *Salmonella* for NRLs-*Salmonella***

Week (2017)	Date	Topic
38	18-22 September	Request for participation PFGE typing and check on contact details (serotyping is obligatory for EU NRLs).
42	16 - 20 October	Emailing of the protocol 2017 to the participants.
44	30 October – 3 November	Shipment of the parcels to the participants as Biological Substance Category B (UN 3373) by door-to-door courier service. Sending the link and the password for the web based test reports to the participants.
44	30 October – 3 November	<i>Upon receipt:</i> Starting the identification of the strains.
50	<b>15 December 2017</b>	Deadline for reporting <b>serotyping</b> results.
51	<b>22 December 2017</b>	Deadline for reporting <b>PFGE typing</b> results.
	January 2018	Serotyping: Reporting of individual laboratory results and Interim Summary Report.
	April 2018	PFGE typing: Reporting of individual laboratory results and Summary Report.
	Summer 2018	Final report.

## For Information

### **Summary of '*Salmonella*-related items' as discussed at the annual meetings of ISO/TC34/SC9 and CEN/TC275/WG6 Tokyo, Japan, 19-23 June 2017**

#### **General**

The abbreviations ISO/TC34/SC9 and CEN/TC275/WG6 stand for:

ISO: International Standardisation Organisation

TC34: Technical Committee 34 on Food products

SC9: Subcommittee 9: Microbiology

CEN: European Committee for Standardisation

TC275: Technical Committee 275 for Food analysis – Horizontal methods

WG6: Working Group 6 for Microbial contaminants

TAG: Task Group

Both meetings were organised by AFNOR (French standardization organization), and were hosted by the Japanese Industrial Standards Committee (JISC), Tokyo, Japan. Approximately 50 delegates attended the 36<sup>th</sup> SC9 meeting and 24<sup>th</sup> WG6 meeting, representing 15 member countries, other ISO committees and international organizations in liaison (like IDF, NMKL and 6 EURLs).

A summary on the *Salmonella*-related items is given below.

#### **EN ISO 6579-1 'Detection of *Salmonella*' (CEN lead)**

The group leader, Kirsten Mooijman of the EURL-*Salmonella*, informed the members of WG6 on the final activities of this Task Group (TAG).

After a second FDIS (Final Draft International Standard) ballot by the end of 2016, the final EN ISO 6579-1 was published in March 2017.

During the (second) FDIS ballot, a remark was made concerning the temperature at which pH measurement should be performed. Currently it is prescribed to perform pH measurement at 25 °C, but it was questioned if this could be a temperature range (e.g. 20-25 °C). It was decided to retain 25 °C in EN ISO 6579-1 and to ask ISO-WG5 to consider this item when EN ISO 11133 is under revision.

As the activities of TAG8 have been finished, this TAG will become dormant.

#### **PCR for identification of monophasic *Salmonella* Typhimurium**

In May 2016, this activity was registered in ISO as Preliminary Work Item (PWI), ISO/PWI 6579-4, to become a Technical Specification (TS). As soon as the technical work is finished, the work will be moved from CEN-TAG3 to ISO-WG10 after which ISO-WG10 will launch the New Work Item Proposal (NWIP). In the past year, several draft versions of the standard has been prepared and discussed with the EURL-*Salmonella* and the experts of TAG3. Earlier it has been agreed that the performance characteristics of the standard will be determined in an interlaboratory study with a 'standard set of strains', to be organised by the EURL-*Salmonella*. In November 2016, the EURL-*Salmonella* has made a call for test strains to create this 'standard set of strains'. By March 2017, the EURL received approximately 400 strains. The identity of all strains is verified by the EURL. Next, a selection of the 400 strains will be used to verify the 3 PCR procedures described in draft ISO/TS 6579-4 by the NRL-*Salmonella* in Germany (Burkhard Malorny; project leader in TAG3) and by the EURL-*Salmonella*. After this, the draft document may need further amendments and the technical work is likely to be finished so that the work will be moved to ISO-WG10. After the NWIP has been launched and a final draft version of ISO/TS 6579-4 is available, the interlaboratory study will be planned to determine the performance characteristics. The timing of this ILS is not yet sure.

### **Harmonisation of incubation temperatures; comparative trials on selective media for ISO 6579-1 at 35 °C and at 37 °C**

In 2016, a protocol was drafted for comparing incubation of MKTTn broth (for detection of *Salmonella*) at 35 °C and at 37 °C by Daniele Sohier and Kirsten Mooijman. In September 2016, the final protocol was sent to the members of ISO-SC9 and CEN-WG6 and invited to perform experiments, following the protocol. By June 2017, results were received from 9 laboratories, representing 6 countries, resulting in a total of 855 tests! Kirsten, together with Daniele Sohier (Germany) and Maryse Rannou (France) have done a first analysis of the data, which was presented at the meeting of SC9. It was made clear that this is only a first analysis and that it is necessary to look at the data more carefully, especially as there was one laboratory with a relatively high amount of positive deviating results (negative with MKTTn-37 and positive with MKTTn-35). The relevant laboratory will be asked if the given results were confirmed results or whether there is a possible technical explanation for the deviating results. After this is clarified, Kirsten will do a further analysis (together with Daniele and Maryse). If necessary, ISO-WG3 (on validation of microbiological methods) will also help with the further data analysis and interpretation of the results. The final outcome of the data analysis will be reported to the SC9 members for a decision at the next meeting (in 2018) on whether to enlarge the temperature of incubation of selective enrichments to 34-38 °C to include both 35 °C and 37 °C.

## From the Literature

### Salmonella-related Literature from Scopus: April – June 2017

**Wang, L., Wang, R., Chen, F., Jiang, T., Wang, H., Slavik, M., Wei, H., Li, Y.**

*QCM-based aptamer selection and detection of Salmonella typhimurium*  
(2017) *Food Chemistry*, 221, pp. 776-782.

**ABSTRACT:** In this study, quartz crystal microbalance (QCM) was used to select aptamers against *Salmonella typhimurium*. To increase the success rate of Systematic Evolution of Ligands Exponential Enrichment (SELEX), the affinity of DNA pool in each round was simultaneously tracked using QCM in order to avoid the loss of high-quality aptamers. When the frequency change reached a maximum value after several rounds of selection and counter-selection, the candidate pool was cloned and sequenced. Out of three aptamer candidates, aptamer B5 showed high specificity and binding affinity with dissociation constant (Kd value) of 58.5 nM, and was chosen for further studies. Subsequently, a QCM-based aptasensor was developed to detect *S. typhimurium*. This aptasensor was able to detect 103 CFU/mL of *S. typhimurium* with less than 1 h. This study demonstrated QCM-based selection could be more effective selection of aptamers and QCM-based aptasensor could be more sensitive in detecting *S. typhimurium*. ISSN: 03088146

**Husmaini, Sabrina, Arlina, F., Purwati, E., Aritonang, S.N., Abbas, H.**

*Impact of administration age of probiotic lactococcus plantarum on the intestinal microflora and performance of broilers*  
(2017) *Pakistan Journal of Nutrition*, 16 (5), pp. 359-363.

**ABSTRACT:** Objective: A trial was conducted to evaluate the effects of age at administration of *Lactococcus plantarum* isolates from virgin coconut oil processing waste on the number of Lactic Acid Bacteria (LAB) in the intestine and the growth performance of broilers. Methodology: The research used 160 day old cobb broilers divided into 4 treatment groups: T0 (without LP), T1 (*Lactococcus plantarum* administered at 1 week of age), T2 (2 weeks of age) and T3 (3 weeks of age). The basal diet consisted of corn, rice bran, fish meal, soy bean meal, bone meal, vegetable fat and premix (21.1% crude protein and 3038 kcal/kg energy metabolism). Chickens were given *Lactococcus plantarum* only one time and were slaughtered every week until 5 weeks old. Variables included the number of LAB, *E. coli* and *Salmonella* in the intestine, thickness and length of the intestine, carcass weight, fat and cholesterol content of carcass, body weight, feed intake and feed efficiency. The data were evaluated using a one-way ANOVA. Results: The results showed that *Lactococcus plantarum* administration affected the balance of microflora in the gut and the length of the intestine. *Lactococcus plantarum* treatment significantly increased the number of LAB in the intestine ( $p < 0.01$ ) up to 2 weeks after administration, conversely, the number of *E. coli* and *Salmonella* decreased. When given at 2 and 3 weeks, the effect of *Lactococcus plantarum* increased intestinal length and broiler growth performance was highly significant ( $p < 0.01$ ). Probiotic treatment did not affect carcass percentage but affected both the abdominal fat and cholesterol of broiler meat. Conclusion: Optimal body weight, feed conversion ratio (1.78) and cholesterol content were observed when *Lactococcus plantarum* was given at 2 weeks of age. ISSN: 16805194

**Lalsiamthara, J., Lee, J.H.**

*Pathogenic traits of Salmonella Montevideo in experimental infections in vivo and in vitro*  
(2017) *Scientific Reports*, 7, art. no. 46232, .

**ABSTRACT:** *Salmonella* serovar Montevideo (SM) is frequently associated with human *Salmonella* infections and causes gastrointestinal disease, cases are common particularly among individuals who come in close contact with live poultry or poultry meat products. To characterize SM disease in chickens, the pathogenic traits and tissue predilections of the disease were investigated. Dissemination of fluorescent-tagged SM (JOL1575GFP) was monitored after oral and intramuscular mock infections of specific-pathogen-free chickens. The spleen was predominantly affected by intramuscular infection while the cecum, spleen, and minimally liver were affected by oral infection. No conspicuous illness was observed in infected birds, and histopathological examination showed minimal damage of the intestinal epithelium and splenic parenchyma though SM was readily isolated from these tissues. Levels of SM internalization by primary chicken peritoneal macrophages were similar to that of *Salmonella Typhimurium*. SM was more sensitive to chicken than rabbit serum complement killing. Internal egg contamination of SM mock infected layers also occurred at trace levels and lasted for a week after inoculation. This study also confirmed that SM

infection in chickens is sub-clinical and asymptomatic, which suggests that latent asymptomatic carriers may excrete a large number of bacteria and transmit the pathogen by contaminating water or food sources. ISSN: 20452322

**Walia, K., Argüello, H., Lynch, H., Grant, J., Leonard, F.C., Lawlor, P.G., Gardiner, G.E., Duffy, G.**

*The efficacy of different cleaning and disinfection procedures to reduce Salmonella and Enterobacteriaceae in the lairage environment of a pig abattoir*  
(2017) *International Journal of Food Microbiology*, 246, pp. 64-71.

**ABSTRACT:** This study investigated several cleaning and disinfection protocols for their ability to eliminate *Salmonella* and to reduce levels of Enterobacteriaceae, within the lairage pens of a commercial pig abattoir. Eight protocols were evaluated in each of 12 lairage pens at the end of the slaughtering day on 3 occasions (36 pens/protocol): (P1) high-pressure cold water wash (herein referred to as high-pressure wash); (P2) high-pressure wash followed by a quaternary ammonium compound (QAC)-based disinfectant without rinsing; (P3) high-pressure wash followed by a chlorocresol-based disinfectant without rinsing; (P4) high-pressure wash followed by a sodium hydroxide/sodium hypochlorite detergent with rinsing; (P5) P4 followed by P2; (P6) P4 followed by P3; (P7) P5 with drying for 24–48 h; and (P8) P6 with drying for 24–48 h. Two floor swabs and one wall swab were taken from each lairage pen before and after each protocol was applied, and examined for the presence of *Salmonella* and enumeration of Enterobacteriaceae. High-pressure washing alone (P1) did not reduce the prevalence of *Salmonella* in the lairage pens. When high-pressure washing, the probability of detecting *Salmonella* following application of the chlorocresol-based disinfectant (P3) was lower than with the QAC-based disinfectant, P2 (14.2% versus 34.0%, respectively;  $p < 0.05$ ). The probability of detecting *Salmonella* after the combined use of detergent and the chlorocresol-based disinfectant (P6) was also lower than application of detergent followed by the QAC-based disinfectant, P5 (2.2% versus 17.1%, respectively;  $p < 0.05$ ). Drying of pens (P7 and P8) greatly reduced the probability of detecting *Salmonella*. Only 3.8% of swabs were *Salmonella*-positive 48 h after cleaning with detergent and the QAC-based disinfectant (P7); while an eradication of *Salmonella* was achieved 24 h after cleaning with detergent and the chlorocresol-based disinfectant, P8. A reduction in Enterobacteriaceae counts to below the limit of detection (LOD; 10 CFU/cm<sup>2</sup>) was achieved following cleaning with detergent and disinfection with the chlorocresol-based disinfectant, regardless of drying ( $p < 0.05$ ), whereas, applying detergent and the QAC-based disinfectant (P7) did not reduce Enterobacteriaceae counts to below the LOD. Therefore ensuring that lairage pens are allowed to dry after intensive cleaning with detergent and a chlorocresol-based disinfectant is recommended as the most effective hygiene routine to eliminate *Salmonella* and reduce Enterobacteriaceae counts. ISSN: 01681605

**Kogut, M.H., Arsenault, R.J.**

*Immunometabolic phenotype alterations associated with the induction of disease tolerance and persistent asymptomatic infection of Salmonella in the chicken intestine*  
(2017) *Frontiers in Immunology*, 8 (APR), art. no. 372, .

**ABSTRACT:** The adaptation of *Salmonella enterica* to the eukaryotic host is a key process that enables the bacterium to survive in a hostile environment. *Salmonella* have evolved an intimate relationship with its host that extends to their cellular and molecular levels. Colonization, invasion, and replication of the bacteria in an appropriate host suggest that modification of host functions is central to pathogenesis. Intuitively, this subversion of the cell must be a complex process, since hosts are not inherently programmed to provide an environment conducive to pathogens. Hosts have evolved countermeasures to pathogen invasion, establishment, and replication through two types of defenses: resistance and tolerance. Resistance functions to control pathogen invasion and reduce or eliminate the invading pathogen. Research has primarily concentrated on resistance mechanisms that are mediated by the immune system. On the other hand, tolerance is mediated by different mechanisms that limit the damage caused by a pathogen's growth without affecting or reducing pathogen numbers or loads. The mechanisms of tolerance appear to be separated into those that protect host tissues from the virulence factors of a pathogen and those that limit or reduce the damage caused by the host immune and inflammatory responses to the pathogen. Some pathogens, such as *Salmonella*, have evolved the capacity to survive the initial robust immune response and persist. The persistent phase of a *Salmonella* infection in the avian host usually involves a complex balance of protective immunity and immunopathology. *Salmonella* is able to stay in the avian ceca for months without triggering clinical signs. Chronic colonization of the intestinal tract is an important aspect of persistent *Salmonella* infection because it results in a silent propagation of bacteria in poultry stocks due to the impossibility to isolate contaminated animals. Data

from our lab promote the hypothesis that *Salmonella* have evolved a unique survival strategy in poultry that minimizes host defenses (disease resistance) during the initial infection and then exploits and/or induces a dramatic immunometabolic reprogramming in the cecum that alters the host defense to disease tolerance. Unfortunately, this disease tolerance results in the ongoing human food safety dilemma. ISSN: 16643224

**Chen, J., Park, B., Eady, M.**

*Simultaneous Detection and Serotyping of Salmonellae by Immunomagnetic Separation and Label-Free Surface-Enhanced Raman Spectroscopy*  
(2017) *Food Analytical Methods*, pp. 1-13. Article in Press.

ABSTRACT: Current detection and characterization techniques for *Salmonellae* are time consuming, and rapid methods could benefit investigation and control of foodborne outbreaks. In this study, the potential of surface-enhanced Raman spectroscopy (SERS) in label-free detection and serotyping of *Salmonella* was evaluated. After immunomagnetic separation (IMS) and overnight culture, SERS spectra were collected from multiple replicates and experiments and analyzed by chemometrics. The detection/characterization accuracies were evaluated in real unknown mixture samples, which were confirmed by plating on selective agar plates and anti-sera agglutination tests. Prediction accuracies were found between 93 and 100%, 87 and 100%, and 67 and 100% for detecting *Salmonella* from other species, characterization of *Salmonella* serotypes, and simultaneous detection and characterization, respectively. When validated in mixture samples consisting of six bacteria, accuracies were 65–100% with increased misclassification. Overall, the approach may provide an inexpensive alternative within similar or slightly longer periods of time, but further improvement in spectral reproducibility and accuracy is needed. ISSN: 19369751

**Ghoneim, N.H., Abdel-Moein, K.A., Zaher, H.**

*Camel as a transboundary vector for emerging exotic Salmonella serovars*  
(2017) *Pathogens and Global Health*, 111 (3), pp. 143-147.

ABSTRACT: The current study was conducted to shed light on the role of imported camels as a transboundary vector for emerging exotic *Salmonella* serovars. Fecal samples were collected from 206 camels directly after slaughtering including 25 local camels and 181 imported ones as well as stool specimens were obtained from 50 slaughterhouse workers at the same abattoir. The obtained samples were cultured while *Salmonella* serovars were identified through Gram's stain films, biochemical tests and serotyping with antisera kit. Moreover, the obtained *Salmonella* serovars were examined by PCR for the presence of *invA* and *stn* genes. The overall prevalence of *Salmonella* serovars among the examined camels was 8.3%. *Stn* gene was detected in the vast majority of exotic strains (11/14) 78.6% including emerging serovars such as *Salmonella* Saintpaul, *S. Chester*, *S. Typhimurium* whereas only one isolate from local camels carried *stn* gene (1/3) 33.3%. On the other hand, none of the examined humans yielded positive result. Our findings highlight the potential role of imported camels as a transboundary vector for exotic emerging *Salomonella* serovars. ISSN: 20477724

**Pethlerdprao, P., Supa-amornkul, S., Panvisavas, N., Chaturongakul, S.**

*Salmonella enterica multilocus sequence typing and its correlation with serotypes*  
(2017) *Food Biotechnology*, 31 (2), pp. 73-79.

ABSTRACT: *Salmonella enterica* is a foodborne pathogen of significant public health concern worldwide. In Thailand, *S. enterica* has also been ranked among the top five most significant bacterial agents of foodborne illnesses by the Ministry of Public Health. Conventionally, biochemical tests and antigen-antibody agglutination have been used to identify and subtype *S. enterica*, respectively. The objective of this study was to identify the serotypes of 180 *S. enterica* isolates. Multilocus sequence typing (MLST) was used to deduce the *S. enterica* serotypes based on sequence type (ST) correlation as shown in the MLST database (<http://mlst.warwick.ac.uk/mlst/>). Initially, MLST was used to confirm serotypes of 53 previously identified isolates of *S. Enteritidis*, *S. Typhimurium*, *S. Hadar*, *S. Virchow*, and *S. Infantis* isolated in Thailand. MLST and serotype correlation confirmed 52 (of 53) known isolates. MLST was performed in 127 *S. enterica* isolates of unknown serotypes from various sources. Serotypes of all 127 *S. enterica* isolates were successfully deduced based on STs. With MLST and PCR-based identification, we have shown that the majority of isolates are of monophasic *S. Typhimurium* (ST34; 43 isolates) and serotype Rissen (ST469; 37 isolates), in agreement with the top serotypes commonly found in Thailand based on the WHO National *Salmonella* and *Shigella* Center. We have also confirmed that MLST is a powerful *Salmonella* subtyping method which could be used not only as a tracking tool for an outbreak investigation at nucleotide level but also as a serotype predictor for making correlations with food safety regulations. ISSN: 08905436

**Sharma, M., Dashiell, G., Handy, E.T., East, C., Reynnells, R., White, C., Nyarko, E., Micallef, S., Hashem, F., Millner, P.D.**

*Survival of salmonella newport on whole and fresh-cut cucumbers treated with lytic bacteriophages*

(2017) *Journal of Food Protection*, 80 (4), pp. 668-673.

**ABSTRACT:** *Salmonella enterica* associated with consumption of cucumbers (*Cucumis sativus*) has led to foodborne outbreaks in the United States. Whole and fresh-cut cucumbers are susceptible to *S. enterica* contamination during growing, harvesting, and postharvest handling. The application of lytic bacteriophages specific for *S. enterica* was evaluated to reduce *Salmonella* populations on cucumbers. Unwaxed cucumbers ('Lisboa' variety, or mini-cucumbers purchased at retail) were inoculated with *Salmonella* Newport (5 log CFU per cucumber) and were sprayed with 3.2 mL of phosphate-buffered saline (control) or 10 log PFU/ml of SalmoFresh, a *Salmonella*-specific bacteriophage preparation (phage), to deliver  $4.76 \times 10^7$  PFU/cm<sup>2</sup>. Cucumbers were stored at 10 or 228C for 7 days. Inoculated mini-cucumbers were sliced with a sterile knife to investigate *Salmonella* transfer to mesocarp, and cut pieces were stored at 48C for 2 days. Populations (log CFU per cucumber) of *Salmonella* Newport on phage-treated whole cucumbers were significantly ( $P < 0.05$ ) smaller ( $2.44 \pm 0.94$ ) than on control-treated cucumbers ( $4.27 \pm 0.37$ ) on day 0. Populations on phage-treated cucumbers stored at 108C were  $1.72 \pm 0.77$  and  $1.56 \pm 0.46$ , which were significantly lower than those on control-treated cucumbers ( $3.20 \pm 0.48$  and  $2.33 \pm 0.25$ ) on days 1 and 4, respectively. Between days 0 and 1, populations on control-treated cucumbers stored at 10 and 228C declined by 1.07 and 2.47 log CFU per cucumber, respectively. At 228C, *Salmonella* Newport populations declined by 2.37 log CFU per cucumber between days 0 and 1. Phage application to whole cucumbers before slicing did not reduce the transfer of *Salmonella* Newport to fresh-cut slices. Lytic phage application may be a potential intervention to reduce *Salmonella* populations on whole cucumbers. ISSN: 0362028X

**Gamble, G.R., Berrang, M.E., Buhr, R.J., Hinton, A., Bourassa, D.V., Ingram, K.D., Adams, E.S., Feldner, P.W., Johnston, J.J.**

*Neutralization of bactericidal activity related to antimicrobial carryover in broiler carcass rinse samples*

(2017) *Journal of Food Protection*, 80 (4), pp. 685-691.

**ABSTRACT:** Studies were conducted to examine the ability of three chemicals to neutralize residual antibacterial activity of commercial antimicrobial chemicals used in poultry processing. Chemical antimicrobial interventions used in poultry processing may have potential for carryover into whole poultry carcass buffered peptone water (BPW) rinses collected for monitoring *Salmonella* contamination. Such carryover may lead to false-negative results due to continuing bactericidal action of the antimicrobial chemicals in the rinse. To simulate testing procedures used to detect *Salmonella* contamination, studies were conducted by separately adding test neutralizers (highly refined soy lecithin, sodium thiosulfate, or sodium bicarbonate) to BPW and using these solutions as carcass rinses. Control samples consisted of BPW containing no additional neutralizing agents. One of four antimicrobial solutions (cetylpyridinium chloride, peroxyacetic acid, acidified sodium chlorite, and a pH 1 hydrochloric: citric acid mix) was then added to the rinses. The four antimicrobial solutions were prepared at maximum allowable concentrations and diluted with modified BPW rinses to volumes simulating maximum carryover. These solutions were then inoculated with a mixed culture of five nalidixic acid-resistant *Salmonella* serovars at 106 CFU/mL. The inoculated rinse was stored at 48C for 24 h, and *Salmonella* was enumerated by direct plating on brilliant green sulfa agar supplemented with nalidixic acid. Results indicate that incorporation of optimal concentrations of three neutralizing agents into BPW neutralized the demonstrated carryover effects of each of the four antimicrobial solutions tested, allowing recovery of viable *Salmonella* at 106 CFU/mL ( $P < 0.05$ ), equivalent to recovery from carcass rinses with no antimicrobial carryover. Incorporation of these neutralizers in BPW for *Salmonella* monitoring may reduce false-negative results and aid regulatory agencies in accurate reporting of *Salmonella* contamination of poultry. ISSN: 0362028X

**Li, L., Dai, X., Wang, Y., Yang, Y., Zhao, X., Wang, L., Zeng, M.**

*RNA-seq-based analysis of drug-resistant Salmonella enterica serovar Typhimurium selected in vivo and in vitro*

(2017) *PLoS ONE*, 12 (4), art. no. e0175234, .

**ABSTRACT:** The aim of this study was to characterize the mechanism of fluoroquinolone (FQ) resistance in *Salmonella* Typhimurium. We established the *Caenorhabditis elegans*-*Salmonella* Typhimurium model to select for ciprofloxacin resistance in *Salmonella* Typhimurium colonizing *C. elegans*, generating the resistant strains TN4. Gradient doses of

ciprofloxacin were used to generate the resistant strain TW4 in vitro. RNA sequencing was used to establish the whole-transcriptome profile of three strains of *Salmonella* Typhimurium. The gene expression patterns of resistant strains TN4 and TW4 differed from those of the parental strain. In TN4, 2,277 genes were differentially expressed (1,833 upregulated and 444 downregulated) relative to the parental strain, and in TW4, 3,464 genes were differentially expressed (3,433 upregulated and 31 downregulated). Among these differentially expressed genes, 28 were associated with drug resistance and 26 were associated with the two-component systems in the two resistant strains. Seven different pathways were significantly affected in two strains. Efflux pump overexpression was identified as one of the main mechanisms underlying FQ resistance in the two resistant strains. TW4 differentially expressed more efflux pump genes than TN4 and most of these genes were more strongly expressed than in TN4. However, expression of the efflux pump repressor gene and the *mar* operon was downregulated in TN4 but not in TW4. Two-component systems are also important in drug resistance. Our findings provide an important basis for further studies of the complex network that regulate FQ resistance in *Salmonella*. ISSN: 19326203

**Henao-Herreño, L.X., López-Tamayo, A.M., Ramos-Bonilla, J.P., Haas, C.N., Husserl, J.**

*Risk of Illness with Salmonella due to Consumption of Raw Unwashed Vegetables Irrigated with Water from the Bogotá River*  
(2017) *Risk Analysis*, 37 (4), pp. 733-743.

ABSTRACT: The Bogotá River receives untreated wastewater from the city of Bogotá and many other towns. Downstream from Bogotá, water from the river is used for irrigation of crops. Concentrations of indicator organisms in the river are high, which is consistent with fecal contamination. To investigate the probability of illness due to exposure to enteric pathogens from the river, specifically *Salmonella*, we took water samples from the Bogotá River at six sampling locations in an area where untreated water from the river is used for irrigation of lettuce, broccoli, and cabbage. *Salmonella* concentrations were quantified by direct isolation and qPCR. Concentrations differed, depending on the quantification technique used, ranging between 107.7 and 109.9 number of copies of gene *invA* per L and 105.3 and 108.4 CFU/L, for qPCR and direct isolation, respectively. A quantitative microbial risk assessment model that estimates the daily risk of illness with *Salmonella* resulting from consuming raw unwashed vegetables irrigated with water from the Bogotá River was constructed using the *Salmonella* concentration data. The daily probability of illness from eating raw unwashed vegetables ranged between 0.62 and 0.85, 0.64 and 0.86, and 0.64 and 0.85 based on concentrations estimated by qPCR (0.47–0.85, 0.47–0.86, and 0.41–0.85 based on concentrations estimated by direct isolation) for lettuce, cabbage, and broccoli, respectively, which are all above the commonly propounded benchmark of 10–4 per year. Results obtained in this study highlight the necessity for appropriate wastewater treatment in the region, and emphasize the importance of postharvest practices, such as washing, disinfecting, and cooking. ISSN: 02724332

**Hobbs, J.L., Warshawsky, B., Maki, A., Zittermann, S., Murphy, A., Majury, A., Middleton, D.**

*Nuggets of wisdom: Salmonella enteritidis outbreaks and the case for new rules on uncooked frozen processed chicken*  
(2017) *Journal of Food Protection*, 80 (4), pp. 703-709.

ABSTRACT: In 2014 and 2015, three Canadian *Salmonella* serotype Enteritidis outbreak investigations implicated uncooked, frozen, processed chicken products produced at the same establishment, namely establishment A. In November 2014, a sustained increase in the number of reported domestically acquired *Salmonella* Enteritidis cases in Ontario led to the first outbreak investigation, which implicated uncooked, frozen, processed chicken products produced at establishment A. In June 2015, the identification of pulsed-field gel electrophoresis patterns that had not been previously reported in Canada led to a national *Salmonella* Enteritidis investigation. Of 51 cases reported nationally, 35 were from Ontario. Uncooked, frozen, processed chicken products produced at establishment A were identified as the source of the outbreak, and public health action was taken as a result of this second investigation. In September 2015, a sustained increase in the number of domestically acquired *Salmonella* Enteritidis PT13a cases in Ontario led to a third outbreak investigation, which identified a total of 36 PT13a cases. Uncooked, frozen, processed chicken products produced at establishment A were again identified as the source of the outbreak. Outbreaks have been linked to uncooked, frozen, processed chicken products since the late 1990s. Information collected during the three outbreak investigations, and from other jurisdictions, suggests that the breaded and prebrowned appearance of the product, as well as factors related to product packaging and marketing, result in consumer

misperception that this raw product is cooked. This misperception may result in mishandling and improper cooking. The three outbreaks described in this article highlight the potential ongoing risks to consumers from these products and support interventions to prevent contamination at the source level and infection at the consumer level.  
ISSN: 0362028X

**Glawischnig, W., Lazar, J., Wallner, A., Kornschöber, C.**

*Cattle-derived salmonella enterica serovar dublin infections in red foxes (*Vulpes vulpes*) in Tyrol, Austria*

(2017) *Journal of Wildlife Diseases*, 53 (2), pp. 361-363.

ABSTRACT: *Salmonella enterica* serovar Dublin is endemic in the cattle population in some areas of the Austrian province Tyrol, and each year single dairy farms have experienced clinical infections. To ascertain if Tyrolean red foxes (*Vulpes vulpes*) act as a reservoir for *Salmonella* spp., we tested hepatic tissue and intestinal content from foxes hunted in the years 2015–16 by using microbiological methods. In addition, we included several fox fecal samples collected on a mountain pasture near chamois carcasses in the investigation. Of 434 foxes tested, nine animals (2.1%) were positive for *Salmonella* spp. Serotyping revealed five foxes positive with *S. Dublin*, demonstrating that this serovar exists in the Tyrolean fox population. The fecal samples collected in the area surrounding skeletonized chamois (*Rupicapra rupicapra*) also tested positive for *S. Dublin*. These chamois were probably victims of a waterborne outbreak caused by *S. Dublin*-shedding cattle. Our results indicate that the *S. Dublin* infections in red foxes were primarily acquired through ingestion of infected cattle material such as abortion tissues, but also by feeding on dead chamois. The findings underline the importance of interspecies transmission in this domestic/ wildlife interface. ISSN: 00903558

**Crowe, S.J., Green, A., Hernandez, K., Peralta, V., Bottichio, L., Defibaugh-Chavez, S., Douris, A., Gieraltowski, L., Hise, K., La-Pham, K., Neil, K.P., Simmons, M., Tillman, G., Tolar, B., Wagner, D., Wasilenko, J., Holt, K., Trees, E., Wise, M.E.**

*Utility of combining whole genome sequencing with traditional investigational methods to solve foodborne outbreaks of salmonella infections associated with chicken: A new tool for tackling this challenging food vehicle*

(2017) *Journal of Food Protection*, 80 (4), pp. 654-660.

ABSTRACT: High consumption rates and a multitude of brands make multistate foodborne outbreaks of *Salmonella* infections associated with chicken challenging to investigate, but whole genome sequencing is a powerful tool that can be used to assist investigators. Whole genome sequencing of pathogens isolated from clinical, environmental, and food samples is increasingly being used in multistate foodborne outbreak investigations to determine with unprecedented resolution how closely related these isolates are to one another genetically. In 2014, federal and state health officials investigated an outbreak of 146 *Salmonella* Heidelberg infections in 24 states. A follow-up analysis was conducted after the conclusion of the investigation in which 27 clinical and 24 food isolates from the outbreak underwent whole genome sequencing. These isolates formed seven clades, the largest of which contained clinical isolates from a subcluster of case patients who attended a catered party. One isolate from a chicken processed by a large producer was closely related genetically (zero to three single-nucleotide polymorphism differences) to the clinical isolates from these subcluster case patients. Chicken from this large producer was also present in the kitchen of the caterer on the day before the event, thus providing additional evidence that the chicken from this producer was the outbreak source. This investigation highlights how whole genome sequencing can be used with epidemiologic and traceback evidence to identify chicken sources of foodborne outbreaks. ISSN: 0362028X

**Lynch, H., Leonard, F.C., Walia, K., Lawlor, P.G., Duffy, G., Fanning, S., Markey, B.K., Brady, C., Gardiner, G.E., Argüello, H.**

*Investigation of in-feed organic acids as a low cost strategy to combat Salmonella in grower pigs*

(2017) *Preventive Veterinary Medicine*, 139, pp. 50-57.

ABSTRACT: *Salmonella* carriage in pigs is a significant food safety issue. Dietary supplementation with organic acids has previously been shown to reduce shedding and transmission of *Salmonella*. Therefore, this study aimed to examine the effect of three commercially available organic acid-based products on *Salmonella* levels in grower pigs, using a model of experimental infection that closely mimics natural exposure to the organism. Seven week old trial pigs (n = 40) with a mean weight of 14.7 kg were placed in one of four pens with 10 pigs/pen. Pens had previously been contaminated with *Salmonella* Typhimurium 4,[5],12;i;- via seeder pigs. Trial pigs received one of four diets for 28 days: 1, control diet; 2, sodium butyrate supplemented diet; 3, benzoic acid supplemented diet

and 4, formic-citric acid supplemented diet. A further 10 pigs were placed in a *Salmonella*-free pen receiving the control diet. Pigs were weighed and blood sampled on days 0 and 28. Faeces was collected on day 0, 2, 3, 5, 7, 14, 21 and 28 and examined for *Salmonella*. On day 28, 5 pigs/group were euthanised and ileocaecal lymph nodes (ILN) and caecal contents sampled for culture. The remaining 5 pigs/pen were then fed the control diet and faeces were collected on days 35 and 42. On day 42 pigs were euthanised and ILN and caecal contents tested for *Salmonella* levels. The trial was repeated once. Within the first two days of exposure to the contaminated environment, 96% (77/80) of pigs became infected. Most pigs shed *Salmonella* at levels of between 100–103 CFU/g faeces for at least 7 days post-exposure. A significant reduction in *Salmonella* faecal concentration was observed after supplementation with sodium butyrate ( $p = 0.001$ ) and a formic citric acid blend ( $p < 0.0001$ ). Average daily weight gain (ADWG) was significantly increased in all groups fed the supplemented feed when compared to the positive control group. The use of sodium butyrate or a blend of formic and citric acid in feed could be considered a cost-effective control measure to reduce *Salmonella* faecal shedding and improve ADWG in *Salmonella* infected herds. ISSN: 01675877

**Andreoli, G., Merla, C., Valle, C.D., Corpus, F., Morganti, M., D'Incau, M., Colmegna, S., Marone, P., Fabbi, M., Barco, L., Carra, E.**

*Foodborne salmonellosis in Italy: Characterization of salmonella enterica serovar typhimurium and monophasic variant 4,[5],12:i isolated from salami and human patients (2017) Journal of Food Protection, 80 (4), pp. 632-639.*

**ABSTRACT:** *Salmonella enterica* serovar Typhimurium (STm) and its monophasic variant 4,[5],12:i (VMSTm) have been responsible for an increased number of foodborne infections in humans in Europe in recent years. The aim of this study was to investigate the origin of three foodborne salmonellosis outbreaks that occurred in Pavia Province (Lombardy region, northern Italy) in 2010. Phenotypic and genetic characteristics of the STm and VMSTm isolates from patients and from food that were recovered in the framework of the three outbreaks were evaluated through serotyping, phage typing, antimicrobial susceptibility testing, pulsed-field gel electrophoresis (PFGE), and multiple-locus variable-number tandem repeat analysis (MLVA). Salami from three artisan producers, which had all purchased meat from the same slaughterhouse, was the food source of infection in outbreak I. STm isolates were recovered from salami and patients with symptoms of gastroenteritis. These isolates had the same PFGE type and the same rare MLVA profile (3-18-9-NA-211). The same molecular profiles were found in an STm isolate from a salami, which likely was the source of another family outbreak (II). A VMSTm strain with common phenotypic and molecular profiles was isolated from three hospitalized patients and identified as the cause of another putative outbreak (III). During the following 3 years (2011 through 2013), 360 salami produced in Pavia Province were monitored for the presence of *S. enterica*. In 2011, no STm and VMSTm isolates were recovered from 159 salami tested. During 2012 and 2013, 13.9% of 201 tested salami harbored *S. enterica*, and half of the isolates were VMSTm, mainly in salami from those artisan producers involved in the previous outbreaks. These isolates were genetically variable, especially in terms of MLVA profiles. The data collected suggest that from 2012, VMSTm has replaced STm in the environments of the salami producers monitored in this study, and these data confirm the dominance of this emergent serovar along the pork supply chain. ISSN: 0362028X

**Borrisso, P.A., Quinlan, J.J.**

*Prevalence of pathogens and indicator organisms in home kitchens and correlation with unsafe food handling practices and conditions (2017) Journal of Food Protection, 80 (4), pp. 590-597.*

**ABSTRACT:** Despite education efforts, consumers often practice unsafe food handling and storage behaviors. Little is known about how these unsafe practices contribute to contamination of the home kitchen with foodborne pathogens. In addition, only a limited number of studies have examined the role of the kitchen as a reservoir for pathogens. The purpose of this study was to characterize microbial contamination and foodborne pathogens found in home kitchens and determine whether contamination was significantly associated with unsafe or unsanitary conditions observed in the kitchen. Swab samples were collected from food contact and preparation surfaces in homes (n100) in Philadelphia, PA. The samples were tested for coliforms, fecal coliforms, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella*, *Campylobacter*, and *Listeria*. Fecal coliforms were found in 44% of homes (most often in samples from kitchen sinks, sponges, and dishcloths), and *E. coli* was found in 15% of homes (mostly in samples from kitchen sinks). Nearly half (45%) of the homes tested positive for a foodborne pathogen, and 12% had multiple pathogens present in the kitchen. *S. aureus* was isolated from 39% of homes, most often from

countertops and refrigerator door handles. *Listeria* spp., including *L. monocytogenes* and *L. innocua*, were present in 15% of homes, most often in samples from refrigerator meat drawers. *C. jejuni* was isolated from 3% of homes. Contamination with *Listeria* was significantly associated with higher refrigerator temperatures. The contamination of surfaces with fecal coliforms and *S. aureus* was significantly associated with a lack of cleaning materials: dish soap and paper or cloth towels in the kitchen, and any type of towel in the nearest bathroom. The contamination of a sponge or dishcloth with either fecal coliforms or *S. aureus* was predictive of other surfaces in the kitchen having the same contamination, indicating that sponges and dishcloths are both reservoirs and vectors for bacteria in the kitchen. ISSN: 0362028X

**Chin, W.H., Sun, Y., Høgberg, J., Quyen, T.L., Engelsmann, P., Wolff, A., Bang, D.D.**

*Direct PCR – A rapid method for multiplexed detection of different serotypes of Salmonella in enriched pork meat samples*

(2017) *Molecular and Cellular Probes*, 32, pp. 24-32.

ABSTRACT: Salmonellosis, an infectious disease caused by *Salmonella* spp., is one of the most common foodborne diseases. Isolation and identification of *Salmonella* by conventional bacterial culture method is time consuming. In response to the demand for rapid on line or at site detection of pathogens, in this study, we developed a multiplex Direct PCR method for rapid detection of different *Salmonella* serotypes directly from pork meat samples without any DNA purification steps. An inhibitor-resistant Phusion Pfu DNA polymerase was used to overcome PCR inhibition. Four pairs of primers including a pair of newly designed primers targeting *Salmonella* spp. at subtype level were incorporated in the multiplex Direct PCR. To maximize the efficiency of the Direct PCR, the ratio between sample and dilution buffer was optimized. The sensitivity and specificity of the multiplex Direct PCR were tested using naturally contaminated pork meat samples for detecting and subtyping of *Salmonella* spp. Conventional bacterial culture methods were used as reference to evaluate the performance of the multiplex Direct PCR. Relative accuracy, sensitivity and specificity of 98.8%; 97.6% and 100%, respectively, were achieved by the method. Application of the multiplex Direct PCR to detect *Salmonella* in pork meat at slaughter reduces the time of detection from 5 to 6 days by conventional bacterial culture and serotyping methods to 14 h (including 12 h enrichment time). Furthermore, the method poses a possibility of miniaturization and integration into a point-of-need Lab-on-a-chip system for rapid online pathogen detection. ISSN: 08908508

**Amrutha, B., Sundar, K., Shetty, P.H.**

*Study on E. coli and Salmonella biofilms from fresh fruits and vegetables*

(2017) *Journal of Food Science and Technology*, 54 (5), pp. 1091-1097.

ABSTRACT: Foodborne outbreaks associated with fresh fruits and vegetables are on the rise worldwide. Biofilm formation is one of the important traits of pathogens making them strongly attached to substrates as well as express virulence phenotypes. Present study investigates the biofilm forming ability of *E. coli* and *Salmonella* sp. isolated from fresh fruits and vegetables. A total of 53 strains, including 35 *E. coli* and 18 *Salmonella* sp. isolated from different fruit and vegetable samples were taken into account for the study. Initial screening for biofilm formation was done using Congo Red agar plate test. Results revealed that 22.8% *E. coli* and 22.2% *Salmonella* sp. were potential biofilm formers. However, the MTP (Micro-Titre Plate) assay suggested more isolates of both *E. coli* and *Salmonella* sp. were moderate to strong biofilm producers. Agar plate diffusion assay with *Agrobacterium tumefaciens* NTL-4 showed the production of quorum signaling molecules (AHLs) by three isolates of *E. coli* and one *Salmonella* sp. Two *E. coli* isolates showed a significant amount of EPS production indicating higher biofilm forming potential. The Presence of LUX R homologue gene (*sdiA*) in two of the *Salmonella* isolates were confirmed by PCR which demonstrated their potential pathogenicity. Results of the work underline the biofilm forming and potentially virulent capacities of isolates from the surface of fruits and vegetables. ISSN: 00221155

**Cui, Y., Walcott, R., Chen, J.**

*Differential attachment of Salmonella enterica and enterohemorrhagic Escherichia coli to alfalfa, fenugreek, lettuce, and tomato seeds*

(2017) *Applied and Environmental Microbiology*, 83 (7), art. no. e03170-16, .

ABSTRACT: Vegetable seeds have the potential to disseminate and transmit foodborne bacterial pathogens. This study was undertaken to assess the abilities of selected *Salmonella* and enterohemorrhagic *Escherichia coli* (EHEC) strains to attach to fungicide-treated versus untreated, and intact versus mechanically damaged, seeds of alfalfa, fenugreek, lettuce, and tomato. Surface-sanitized seeds (2 g) were exposed to four

individual strains of *Salmonella* or EHEC at 20°C for 5 h. Contaminated seeds were rinsed twice, each with 10 ml of sterilized water, before being soaked overnight in 5 ml of phosphate-buffered saline at 4°C. The seeds were then vortexed vigorously for 1 min, and pathogen populations in seed rinse water and soaking buffer were determined using a standard plate count assay. In general, the *Salmonella* cells had higher attachment ratios than the EHEC cells. Lettuce seeds by unit weight had the highest numbers of attached *Salmonella* or EHEC cells, followed by tomato, alfalfa, and fenugreek seeds. In contrast, individual fenugreek seeds had more attached pathogen cells, followed by lettuce, alfalfa, and tomato seeds. Significantly more *Salmonella* and EHEC cells attached to mechanically damaged seeds than to intact seeds ( $P < 0.05$ ). Although, on average, significantly more *Salmonella* and EHEC cells were recovered from untreated than fungicide-treated seeds ( $P < 0.05$ ), fungicide treatment did not significantly affect the attachment of individual bacterial strains to vegetable seeds ( $P > 0.05$ ), with a few exceptions. This study fills gaps in the current body of literature and helps explain bacterial interactions with vegetable seeds with differing surface characteristics. ISSN: 00992240

**Suijkerbuijk, A.W.M., Bouwknegt, M., Mangen, M.-J.J., De Wit, G.A., Van Pelt, W., Bijkerk, P., Friesema, I.H.M.**

*The economic burden of a Salmonella Thompson outbreak caused by smoked salmon in the Netherlands, 2012-2013*

(2017) *European Journal of Public Health*, 27 (2), pp. 325-330.

**ABSTRACT:** Background: In 2012, the Netherlands experienced the most extensive food-related outbreak of *Salmonella* ever recorded. It was caused by smoked salmon contaminated with *Salmonella* Thompson during processing. In total, 1149 cases of salmonellosis were laboratory confirmed and reported to RIVM. Twenty percent of cases was hospitalised and four cases were reported to be fatal. The purpose of this study was to estimate total costs of the *Salmonella* Thompson outbreak. Methods: Data from a case-control study were used to estimate the cost-of-illness of reported cases (i.e. healthcare costs, patient costs and production losses). Outbreak control costs were estimated based on interviews with staff from health authorities. Using the Dutch foodborne disease burden and cost-of-illness model, we estimated the number of underestimated cases and the associated cost-of-illness. Results: The estimated number of cases, including reported and underestimated cases was 21 123. Adjusted for underestimation, the total cost-of-illness would be €6.8 million (95% CI €2.5-€16.7 million) with productivity losses being the main cost driver. Adding outbreak control costs, the total outbreak costs are estimated at €7.5 million. Conclusion: In the Netherlands, measures are taken to reduce salmonella concentrations in food, but detection of contamination during food processing remains difficult. As shown, *Salmonella* outbreaks have the potential for a relatively high disease and economic burden for society. Early warning and close cooperation between the industry, health authorities and laboratories is essential for rapid detection, control of outbreaks, and to reduce disease and economic burden. ISSN: 11011262

**Ferrato, C., Chui, L., King, R., Louie, M.**

*Utilization of a molecular serotyping method for Salmonella enterica in a routine laboratory in Alberta Canada*

(2017) *Journal of Microbiological Methods*, 135, pp. 14-19.

**ABSTRACT:** *Salmonella* is one of the most common enteric pathogens related to foodborne illness. Alberta's Provincial Laboratory for Public Health (ProvLab) provides Outbreak and Surveillance support by performing serotyping. The Check&Trace *Salmonella*<sup>™</sup> (CTS) assay (Check-Points, Netherlands), a commercial DNA microarray system, can determine the serotype designation of a *Salmonella* isolate with automated interpretation. Here we evaluate 1028 *Salmonella* isolates of human clinical or environmental sources in Alberta, Canada with the CTS assay. CTS was able to assign a serovar to 98.7% of the most frequently occurring human clinical strains in Alberta (82.5% overall), and 71.7% of isolates which were inconclusive by conventional methods. There was 99.7% concordance in environmental isolates. The CTS database has potential to expand to identify rare serovars. With the anticipated shift to molecular methods for identification, CTS provides an easy transition and demonstrates ease-of-use and reduces the turn-around-time of a reported result significantly compared to classical serotyping. ISSN: 01677012

**Berrios-Rodriguez, A., Olanya, O.M., Annous, B.A., Cassidy, J.M., Orellana, L., Niemira, B.A.**

*Survival of Salmonella Typhimurium on soybean sprouts following treatments with gaseous chlorine dioxide and biocontrol Pseudomonas bacteria*

(2017) *Food Science and Biotechnology*, 26 (2), pp. 513-520.

**ABSTRACT:** Control of Salmonella Typhimurium on sprouts is crucial for food and consumer safety. In this study, natural microflora on soybean seed was assessed and effects of gaseous chlorine dioxide (ClO<sub>2</sub>) and biocontrol Pseudomonas on the survival of S. Typhimurium on soybean sprouts were evaluated. Sprouts were dip-inoculated with S. Typhimurium prior to the application of the biocontrol (*P. chlororaphis* and *P. fluorescens*). After inoculation with S. Typhimurium, the sprouts were treated with ClO<sub>2</sub> at 0.4 mg/L for 1 h (90% R.H., 13°C). Pseudomonas strains and Salmonella were recovered on Pseudomonas Agar F (PAF) and xylose lysine tergitol-4 (XLT-4) media, respectively. Pseudomonas strains reduced Salmonella by <math>\approx 1</math> log colony forming units (CFU)/g of sprouts, whereas S. Typhimurium on soybean sprouts was reduced from 2.55 to 5.35 logs CFU/g by ClO<sub>2</sub>. Gaseous ClO<sub>2</sub> treatment reduced S. Typhimurium by 3.90 (0 h), 4.47 (24 h), and 3.61 log CFU/g (168 h). It was concluded that ClO<sub>2</sub> and biocontrol treatment can enhance sprout safety. ISSN: 12267708

**Edirmanasinghe, R., Finley, R., Parmley, E.J., Avery, B.P., Carson, C., Bekal, S., Golding, G., Mulvey, M.R.**

*A whole-genome sequencing approach to study cefoxitin-resistant Salmonella enterica serovar Heidelberg isolates from various sources*

(2017) *Antimicrobial Agents and Chemotherapy*, 61 (4), art. no. e01919, .

**ABSTRACT:** This study characterized cefoxitin-resistant and -susceptible Salmonella enterica serovar Heidelberg strains from humans, abattoir poultry, and retail poultry to assess the molecular relationships of isolates from these sources in Québec in 2012. Isolates were collected as part of the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS). All isolates were subjected to antimicrobial susceptibility testing, PCR for CMY-2, pulsed-field gel electrophoresis (PFGE), and whole-genome sequencing (WGS). A total of 113 S. Heidelberg isolates from humans (n = 51), abattoir poultry (n = 18), and retail poultry (n = 44) were studied. All cefoxitin-resistant isolates (n = 65) were also resistant to amoxicillin-clavulanic acid, ampicillin, ceftiofur, and ceftriaxone, and all contained the CMY-2 gene. PFGE analysis showed that 111/113 (98.2%) isolates clustered together with  $\geq 90\%$  similarity. Core genome analysis using WGS identified 13 small clusters of isolates with 0 to 4 single nucleotide variations (SNVs), consisting of cefoxitin-resistant and -susceptible human, abattoir poultry, and retail poultry isolates. CMY-2 plasmids from cefoxitin-resistant isolates all belonged to incompatibility group I1. Analysis of IncI1 plasmid sequences revealed high identity (95 to 99%) to a previously described plasmid (pCVM29188-101) found in Salmonella Kentucky. When compared to pCVM29188-101, all sequenced cefoxitin-resistant isolates were found to carry 1 of 10 possible variant plasmids. Transmission of S. Heidelberg may be occurring between human, abattoir poultry, and retail poultry sources, and transmission of a common CMY-2 plasmid may be occurring among S. Heidelberg strains with variable genetic backgrounds. ISSN: 00664804

**Sun, C., Wang, Z.-W., Li, J.-X., Fan, W.-L., Qiao, X.-Y., Liu, Z.-M., Li, S.-L., Tang, L.-J., Li, Y.-J., Xu, Y.-G.**

*A Rapid and Sensitive Method for Simultaneous Screening of Nine Foodborne Pathogens Using High-Performance Liquid Chromatography Assay*

(2017) *Food Analytical Methods*, 10 (4), pp. 1117-1127.

**ABSTRACT:** We developed a multiplex PCR-based procedure followed by high-performance liquid chromatography (mPCR-HPLC) assay for high-throughput screening foodborne pathogens, including Salmonella spp., Listeria monocytogenes, Enterobacter sakazakii, Staphylococcus aureus, Shigella spp., Escherichia coli O157:H7, Vibrio parahaemolyticus, Vibrio cholerae, and Vibrio vulnificus. Vibrio species-specific primers were designed targeting dnaJ gene, and pathogen-specific primers for L. monocytogenes, Salmonella spp., E. sakazakii, E. coli O157:H7, Shigella spp., and S. aureus were designed targeting iap, fimY, 16S ribosomal RNA (rRNA), rfbE, ipaH, and 442 genes, respectively. PCR products were analyzed using WAVE-4500 DNA system equipped with PS-DVB-C18 particles DNASep column and each specific amplicon generated a characteristic chromatographic profile. Detection limit of mPCR-HPLC assay was ca. 101 CFU/mL in pure cultures and less than 102 CFU/g in contaminated matrixes. A total of 395 bacterial strains were used for specificity testing of the mPCR-HPLC assay, and specific HPLC profiles were only produced in strains belonging to the target, showing a high specificity. Applying the assay to 2677 samples collected from clinical, food, and environmental sources revealed that 917 samples were positive, in accordance with bacterial isolation. The high sensitivity and specificity of mPCR-HPLC assay indicate its great potential to be a powerful tool for high-throughput screening foodborne pathogens. ISSN: 19369751

**Xu, Y.-G., Sun, B., Zhao, H.-Y., Liu, Z.-M., Jiang, Y.-P., Wang, L., Qiao, X.-Y., Li, Y.-J., Tang, L.-J.**

*Development and evaluation of a dual priming oligonucleotide system-based multiplex PCR assay for simultaneous detection of six foodborne pathogens*

(2017) *European Food Research and Technology*, 243 (4), pp. 555-563.

**ABSTRACT:** In this study, a multiplex PCR assay with dual priming oligonucleotide system (DPO system-based mPCR) was developed for the simultaneous detection of *Salmonella* spp., *Listeria* (L.) *monocytogenes*, *Shigella* spp., *Staphylococcus* (S.) *aureus*, *Campylobacter* (C.) *jejuni* and *Yersinia* (Y.) *enterocolitica* from food. Pathogen-specific DPO systems were designed targeting *Salmonella* spp. *fimY* gene, *L. monocytogenes* *iap* gene, *Shigella* spp. *ipaH* gene, *S. aureus* 442 gene, *C. jejuni* *gyrA* gene and *Y. enterocolitica* 16 s–23 s rRNA gene, respectively. Our optimized DPO system-based mPCR assay allowed a wide range of annealing temperature from 48 to 68 °C to efficiently amplify multi-genes followed by a nearly identical pattern with an analytical detection limit of 10<sup>2</sup>–10<sup>3</sup> CFU/mL (g) for the simultaneous detection of the six target bacteria in pure cultures or artificially contaminated food matrixes. Applying the DPO system-based mPCR assay to 238 target and 83 nontarget bacterial strains revealed that only target bacterial strains were positive in this assay, indicating a high specificity. Moreover, the DPO system-based mPCR assay showed a potential diagnostic capability evaluated by testing 2419 samples collected from food, clinical and environmental sources. The DPO system-based mPCR assay may provide a useful tool for the detection of these six foodborne pathogens in laboratory diagnosis. ISSN: 14382377

**Papadopoulos, T., Petridou, E., Zdragas, A., Mandilara, G., Vafeas, G., Passiotou, M., Vatopoulos, A.**

*Multiple clones and low antimicrobial resistance rates for Salmonella enterica serovar Infantis populations in Greece*

(2017) *Comparative Immunology, Microbiology and Infectious Diseases*, 51, pp. 54-58.

**ABSTRACT:** All the *Salmonella enterica* ser. *Infantis* strains isolated under official control programs in Greece during a four year period were studied, 23 of human origin, 16 from food animals and one from food. Molecular analyses (PFGE) in combination with antimicrobial susceptibility testing were used to study whether the occurrence *S. Infantis* in Greece resulted from different biotypes or a successful spread of one clone. Low rates of antimicrobial resistance were observed, except for streptomycin among human isolates (48%), indicating that selective pressure due to consumption of antimicrobials has not resulted the spread of dominant clones. Pulsed Field Gel Electrophoresis revealed 31 XbaI distinct pulsotypes among the 40 strains with 60% overall similarity reflecting diversity. Four main clusters were constructed, using an 85% cut off value, clusters A, B, C and D consisting of 14, 6, 8 and 8 isolates respectively. Point source of transmission was not hypothesized as multiple reservoirs of the serovar seem to be present in Greece during the study period. ISSN: 01479571

**Rohde, A., Hammerl, J.A., Boone, I., Jansen, W., Fohler, S., Klein, G., Dieckmann, R., Al Dahouk, S.**

*Overview of validated alternative methods for the detection of foodborne bacterial pathogens*

(2017) *Trends in Food Science and Technology*, 62, pp. 113-118.

**ABSTRACT:** Background Despite huge efforts to combat foodborne bacterial pathogens, the number of foodborne infections remains high throughout the world. Culture-dependent gold standard detection methods with their tedious and time-consuming procedures have approached their limits whereas the use of rapid and user-friendly alternative methods is on the rise. Validation by independent institutions, e.g. AOAC, AFNOR, MicroVal and NordVal, is a key element to demonstrate the applicability of a new method and its equivalence with standard procedures (e.g. DIN, ISO). Scope and approach In this review, the suitability of currently available validated methods for the qualitative and quantitative bacteriological analysis of food is presented and discussed with special emphasis on the method-inherent strengths and weaknesses. Furthermore, an overview on general validation characteristics as well as further promising tools for the detection of foodborne pathogens is given. Key findings and conclusions Improved cultivation methods as well as nucleic acid based and immunological methods dominate the market of alternative methods while emerging techniques like mass spectrometry, microarrays and phage-based techniques have yet to be thoroughly validated. Harmonized validation procedures are highly desirable as well as enhanced efforts to develop validated tests for a greater variety of pathogens, since current validated tests are mainly confined to the detection of *Salmonella enterica* and *Listeria* spp. ISSN: 09242244

**Park, S., Beuchat, L.R., Kim, H., Ryu, J.-H.**

*Inactivation of Salmonella enterica in chicken feces on the surface of eggshells by simultaneous treatments with gaseous chlorine dioxide and mild wet heat (2017) Food Microbiology, 62, pp. 202-206.*

ABSTRACT: The aim of this study was to investigate the lethal effects of simultaneous treatments with gaseous chlorine dioxide (ClO<sub>2</sub>) and mild wet heat (55 °C at 100% relative humidity [RH]) on *Salmonella enterica* in chicken feces on the surface of eggshells. Gaseous ClO<sub>2</sub> production decreased significantly ( $P \leq 0.05$ ) as the RH (23, 43, 68, 85, and 100%) at 25 °C was increased. The lethality of gaseous ClO<sub>2</sub> against *S. enterica* in feces on eggshells increased significantly ( $P \leq 0.05$ ) as RH increased. For example, when treated with gaseous ClO<sub>2</sub> at 85 and 100% RH at 25 °C, *S. enterica* (5.9 log CFU/egg) was inactivated within 4 h. In contrast, at 23, 43, and 68% RH, the pathogen remained at 5.1, 5.0, and 2.8 log CFU/egg, respectively, after 6 h. Finally, when eggshells surface-contaminated with *S. enterica* (5.8 log CFU/egg) were treated with gaseous ClO<sub>2</sub> (peak concentration of ClO<sub>2</sub>: 185.6 ppm) at 100% RH and 55 °C, inactivation occurred within 1 h. These results indicate that treatment of surface-contaminated shell eggs with gaseous ClO<sub>2</sub> at elevated RH and temperature is effective in inactivating *S. enterica*. These observations will be useful when developing an effective sanitation program to enhance the microbiological safety of shell eggs. ISSN: 07400020

**Li, M., Huang, L., Yuan, Q.**

*Growth and survival of Salmonella Paratyphi A in roasted marinated chicken during refrigerated storage: Effect of temperature abuse and computer simulation for cold chain management (2017) Food Control, 74, pp. 17-24.*

ABSTRACT: This research was conducted to evaluate the feasibility of using a one-step dynamic numerical analysis and optimization method to directly construct a tertiary model to describe the growth and survival of *Salmonella Paratyphi A* (SPA) in a marinated roasted chicken product. Multiple dynamic growth and survival curves obtained under different fluctuating temperature conditions between 4 and 35 °C were used to determine the growth kinetics of SPA. In combination with appropriate secondary models, the study examined both growth and survival of SPA simultaneously by an integrated one-step approach using a set of differential equations. The estimated minimum growth temperature ( $T_{min}$ ) of SPA was 8.91 °C, matching well with the growth characteristics of this microorganism. The growth at temperatures above  $T_{min}$  and the survival below  $T_{min}$  was accurately simulated by the predictive models. For model development, the root mean square error (RMSE) was 0.26 log CFU/g. The predictive models and kinetic parameters were validated using two dynamic growth and survival curves along with one isothermal thermal growth curve. The validation also showed that the models were accurate in predicting the growth and survival of the bacterium, with the RMSE of predictions only 0.52 log CFU/g. The errors of predictions were within normal experimental errors. The results of this work may be used to predict the change in the population of SPA in the marinated roasted chickens in the cold chain and during temperature abuse and to conduct risk assessment of this pathogen. ISSN: 09567135

**Lianou, A., Nychas, G.-J.E., Koutsoumanis, K.P.**

*Variability in the adaptive acid tolerance response phenotype of Salmonella enterica strains (2017) Food Microbiology, 62, pp. 99-105.*

ABSTRACT: The objective of this study was the assessment of the stationary-phase, low-pH-inducible acid tolerance response (ATR) of different *Salmonella enterica* strains. For this purpose, 30 strains of the pathogen were grown in tryptone soy broth in the absence (non-adapted cultures) and presence (1% w/v; acid-adapted cultures) of glucose, and then subjected to 4-h acid challenge trials at pH 3.0. Surviving populations of each strain were determined at 1-h intervals, and the Weibull model was fitted to the derived microbiological data. Extensive variability in the acid stress responses of the tested *S. enterica* strains was observed, with the total population reductions (log CFU/ml) attained in 4 h of acid challenge ranging from 0.9 to 5.5 and from 0.6 to 7.0 for the non-adapted and acid-adapted cultures, respectively. As demonstrated by the model scale parameter  $\delta$  and shape parameter  $p$ , the effect of acid adaptation on the inactivation curves was strain-specific. Although acid adaptation resulted in enhanced acid survival for the majority of the tested strains, there were strains exhibiting similar or decreased acid resistance compared to their non-adapted counterparts. Moreover, acid adaptation appeared to decrease the strain variability of  $\delta$  whereas increasing the strain variability of  $p$ : the coefficient of variation of  $\delta$  among the tested strains was 97.2 and 54.9% for the non-adapted and acid-adapted cultures, respectively, while the corresponding values for  $p$  were 12.7 and 48.1%. The data of the present study, which is the first one to

systematically evaluate the adaptive ATR of multiple *S. enterica* strains, clearly demonstrate that this phenotype (attempted to be induced by growing the pathogen in the presence of glucose) is strain-dependent. ISSN: 07400020

**Græsbøll, K., Andresen, L.O., Halasa, T., Toft, N.**

*Opportunities and challenges when pooling milk samples using ELISA*  
(2017) *Preventive Veterinary Medicine*, 139, pp. 93-98.

**ABSTRACT:** Testing large quantities of samples in order to detect one or more test-positive sample(s) is expensive and time-consuming. It is possible to optimize this process by pooling samples. Two frameworks to produce different hierarchical and non-hierarchical pooling schemes were tested and compared to standard pooling. Their efficiency and the potential savings were determined as a function of prevalence and the number of pooled samples. The potential benefit of pooling samples is dependent upon the changes in the analytical sensitivity and specificity of the test used when diluting test-positive samples by pooling. To illustrate this, the sensitivity of antibody ELISA on pooled samples of bovine milk for *Salmonella* Dublin, *Mycobacterium avium* spp. paratuberculosis, and bovine virus diarrhoea was tested. For these milk assays, the analytical sensitivity decreased rapidly with increasing pool sizes. The efficiency of pooling is usually only measured by the number of tests performed, yet real savings depend on all the costs involved in the pooling process. These may differ between laboratories depending on the available equipment and the salaries of the technicians, among other factors. Therefore, several cost parameters were introduced to describe the total cost and thereby calculate the total savings. In terms of overall savings, both tested schemes were potentially optimal depending on the prevalence, possible pool size, and the cost of retesting. For the pool sizes of interest in this study, the three-stage hierarchical pooling scheme was often marginally more efficient in terms of the total number of tests. However, if the price of re-pooling was high, the two-stage scheme performed better in terms of total savings. In addition, for low prevalences and the possibility of pooling a large number of samples, the two-stage non-hierarchical test may be more efficient, both in terms of number of tests and overall cost. In order to apply these results in different laboratory settings, a free Shiny WebApp was developed, to compare several pooling schemes with different cost parameters. ISSN: 01675877

**Jahne, M.A., Schoen, M.E., Garland, J.L., Ashbolt, N.J.**

*Simulation of enteric pathogen concentrations in locally-collected greywater and wastewater for microbial risk assessments*  
(2017) *Microbial Risk Analysis*, 5, pp. 44-52.

**ABSTRACT:** As decentralized water reuse continues to gain popularity, risk-based treatment guidance is increasingly sought for the protection of public health. However, efforts to evaluate pathogen risks and log-reduction requirements have been hindered by an incomplete understanding of pathogen occurrence and densities in locally-collected wastewaters (i.e., from decentralized collection systems). Of particular interest is the potentially high enteric pathogen concentration in small systems with an active infected excreter, but generally lower frequency of pathogen occurrences in smaller systems compared to those with several hundred contributors. Such variability, coupled with low concentrations in many source streams (e.g., sink, shower/bath, and laundry waters), has limited direct measurement of pathogens. This study presents an approach to modeling pathogen concentrations in variously sized greywater and combined wastewater collection systems based on epidemiological pathogen incidence rates, user population size, and fecal loadings to various residential wastewater sources. Pathogen infections were modeled within various population sizes (5-, 100-, and 1,000-person) for seven reference pathogens (viruses: adenoviruses, Norovirus, and Rotavirus; bacteria: *Campylobacter* and *Salmonella* spp.; and protozoa: *Cryptosporidium* and *Giardia* spp.) on each day of 10,000 possible years, accounting for intermittent infection and overlap of infection periods within the population. Fecal contamination of fresh greywaters from bathroom sinks, showers/baths, and laundry, as well as combined greywater and local combined wastewater (i.e., including toilets), was modeled based on reported fecal indicators in the various sources. Simulated daily infections and models of fecal contamination were coupled with pathogen shedding characteristics to generate distributions of pathogen densities in the various waters. The predicted frequency of pathogen occurrences in local wastewaters was generally low due to low infection incidence within small cohort groups, but increased with collection scale (population size) and infection incidence rate (e.g., Norovirus). When pathogens did occur, a decrease in concentrations from 5- to 100- and from 100- to 1,000-person systems was observed; nonetheless, overall mean concentrations (i.e., including non-occurrences) remained the same due to the increased number of occurrences. This highlights value of the model for characterizing scaling effects over averaging methods, which overestimate the frequency of pathogen occurrence in small

systems while underestimating concentration peaks that likely drive risk periods. Results of this work will inform development of risk-based pathogen reduction requirements for decentralized water reuse. ISSN: 23523522

**Kosa, K.M., Cates, S.C., Godwin, S., Chambers, E., IV**

*Barriers to using a food thermometer when cooking poultry at home: Results from a national survey*

(2017) *Food Protection Trends*, 37 (2), pp. 116-125.

ABSTRACT: Raw poultry may be contaminated with *Salmonella* and *Campylobacter*, so it is important that consumers properly handle and prepare poultry. Using a food thermometer is the only reliable way to ensure that poultry has reached a safe internal temperature. A nationally representative Web-enabled panel survey of U.S. adult grocery shoppers (n = 1,504) was conducted to describe consumers' handling and preparation practices for raw poultry. About 62% of consumers reported owning a thermometer. Among thermometer owners, the majority reported using one to determine doneness of whole Turkeys (73.2%) and chickens (56.7%), but fewer used one to determine doneness of Turkey breasts (42.6%), chicken breasts/other parts (26.3%), or patties (11.7%) made with raw ground poultry. Among owners who were nonusers, the majority reported using another method to determine doneness or reported they "never thought to use one." Few respondents expressed concerns on how to use a thermometer, or on ease or practicality of using one. Educators should address the unreliability of visual cues to determine doneness and emphasize that use of a thermometer is the only reliable way to ensure that bacteria are destroyed. It is also important to convey the risk of contracting *Salmonella* and *Campylobacter* infection from eating raw/undercooked poultry. ISSN: 15419576

**Park, S.H., Kim, S.A., Rubinelli, P.M., Roto, S.M., Ricke, S.C.**

*Microbial compositional changes in broiler chicken cecal contents from birds challenged with different *Salmonella* vaccine candidate strains*

(2017) *Vaccine*, 35 (24), pp. 3204-3208.

ABSTRACT: Previously, we constructed and characterized the vaccine efficacy of *Salmonella* Typhimurium mutant strains in poultry with either inducible *mviN* expression (PBAD-*mviN*) or methionine auxotrophy ( $\Delta\Delta$ metRmetD). The aim of the present study was to assess potential impact of these *Salmonella* vaccine strains on the cecal microbiota using a next generation sequencing (NGS). The cecal microbial community obtained from unvaccinated (group 1) and vaccinated chickens (group 2, vaccinated with PBAD-*mviN*; group 3, vaccinated with wild type; group 4, vaccinated with  $\Delta\Delta$ metRmetD) were subjected to microbiome sequencing analysis with an Illumina MiSeq platform. The NGS microbiome analysis of chicken ceca revealed considerable changes in microbial composition in the presence of the different vaccine strains and exhibited detectable patterns of distinctive clustering among the respective groups (the R value of unweighted PCoA plot was 0.68). The present study indicates that different *S. Typhimurium* vaccine strains can differentially influence the microbiota of the ceca in terms of presence but not in the relative abundance of microbiota. ISSN: 0264410X

**Anukampa, Shagufta, B., Sivakumar, M., Kumar, S., Agarwal, R.K., Bhilegaonkar, K.N., Kumar, A., Dubal, Z.B.**

*Antimicrobial resistance and typing of *Salmonella* isolated from street vended foods and associated environment*

(2017) *Journal of Food Science and Technology*, pp. 1-8. Article in Press.

ABSTRACT: The present study was carried out to find out the occurrence and types of *Salmonella* present in street vended foods and associated environment, and their resistance pattern against various antibiotics. About 1075 street vended food and associated environment samples were processed for isolation and confirmation of different *Salmonella* spp. by targeting gene specific *invA* gene and serotype specific Sdf I, Via B and Spy genes by PCR. Selected *Salmonella* isolates were screened for antibiotic resistance by using Baur-Kirby disk diffusion test. Out of 1075 samples, only 31 (2.88%) isolates could be amplified the *invA* gene of which 19 could be recovered from meat vendors; 8 from egg vendors while remaining 4 from milk vendors. Though, majority of *Salmonella* recovered from raw foods the ready-to-eat food like chicken gravy and rasmalai also showed its presence which pose a serious public health threat. Overall, 19, 6 and 1 isolates of *S. Typhimurium*, *S. Enteritidis* and *S. Typhi* could be detected by PCR while remaining 5 isolates could not be amplified suggesting other type of *Salmonella*. Selected *Salmonella* isolates were completely resistance to Oxacillin (100%) followed by Cefoxitin (30.43%) and Ampicillin (26.10%). Thus, it is observed that the street vended foods of animal origin and associated environment play an important role in transmission of food borne pathogens including *Salmonella*. ISSN: 00221155

**Kiflu, B., Alemayehu, H., Abdurahaman, M., Negash, Y., Eguale, T.**

*Salmonella* serotypes and their antimicrobial susceptibility in apparently healthy dogs in Addis Ababa, Ethiopia

(2017) *BMC Veterinary Research*, 13 (1), art. no. 134, .

y&partnerID=40&md5=d8a8b0823098d70c50480496660cd09f

**ABSTRACT:** Background: The close bond between pet animals and family members poses risk of infection with zoonotic bacterial pathogens such as *Salmonella*. No data is available on occurrence of *Salmonella* in dogs in Ethiopia. The aim of this study was therefore to determine the prevalence, serotype distribution and antimicrobial resistance of *Salmonella* from feces of apparently healthy dogs in Addis Ababa, Ethiopia. Results: Of the total 360 dogs examined, 42 (11.7%; 95% Confidence limit of 8.5%-15.4%) were positive for *Salmonella*. Fourteen serotypes were detected and the predominant ones were *S. Bronx* (n = 7; 16.7%), *S. Newport* (n = 6; 14.3%), followed by *S. Typhimurium*, *S. Indiana*, *S. Kentucky*, *S. Saintpaul* and *S. Virchow* (n = 4; 9.5%) each. *Salmonella* infection status was significantly associated with history of symptom of diarrhea during the past 60 days (OR = 3.78; CI = 1.76-8.13; p = 0). Highest resistance rates were found for oxytetracycline (59.5%), neomycin (50%), streptomycin (38.1%), cephalothin (33.3%), doxycycline (30.9%), ampicillin (30.9%) and amoxicillin + clavulanic acid (26.2%). Thirty eight (90.5%) of the isolates were resistant or intermediately resistant to at least one of the 16 antimicrobials tested. Resistance to two or more antimicrobials was detected in 30 (71.4%) of the isolates. Resistance to three or more antimicrobials was detected in 19 (45.2%) of the isolates. Conclusion: This study demonstrated high carriage rate of *Salmonella* serotypes known for causing human salmonellosis and large proportion of them were resistant to antimicrobials used in public and veterinary medicine for management of various bacterial infections, suggesting the possible risk of infection of human population in close contact with these dogs by drug resistant pathogens. Therefore, it is vital to work on raising public awareness on zoonotic canine diseases prevention measures and good hygienic practices. ISSN: 17466148

**Smith, R.P., Andres, V., Dormer, L., Gosling, R., Oastler, C., Davies, R.H.**

*Study of the impact on Salmonella of moving outdoor pigs to fresh land*

(2017) *Epidemiology and Infection*, 145 (10), pp. 1983-1992.

**ABSTRACT:** Anecdotal evidence has suggested that outdoor-kept pigs show an improvement to health and productivity after being moved to a new site. This study explores whether *Salmonella* occurrence reduced and was sustained after moving to a new site. Nine farms were followed for a year in which four sampling visits were completed. The highest detection of *Salmonella* was from pooled faecal dropping from pigs, run-off/ pooled water, rodents and wild birds. Descriptive summaries showed that the prevalence of both all *Salmonella* and serovars of public health importance were lower at all visits after the move. Some variability was shown in results from individual farms, but a year after the move, six farms still maintained a lower prevalence. A risk factor model showed that the prevalence at visits 2 and 3 after the move was significantly lower than baseline, after accounting for a number of significant factors that were included in the model. These were sample type and seasonality (included as a priori), presence of coughing in the sampled group and Glasser's disease on the farm, and the use of tent or kennel accommodation. This finding provides important evidence that more frequent site moves may help reduce *Salmonella* prevalence in outdoor herds. ISSN: 09502688

**Hernández, J.R.R., Gómez-Lucas, I., Navarro-Pedreño, J., Jordán, M.M., Bech, J., Nieto Asencio, V.M., Iñiguez, N.P.**

*Environmental consequences from the use of sewage sludge in soil restoration related to microbiological pollution*

(2017) *Journal of Soils and Sediments*, pp. 1-7. Article in Press.

**ABSTRACT:** Purpose: This article analyzed the survival of *Escherichia coli*, total coliforms, and *Salmonella* spp. in a soil amended with urban sewage sludge due to its potential use in soil rehabilitation and to the risk of microbial pollution. Materials and methods: The survival of *E. coli*, total coliforms, and *Salmonella* spp. was determined in a soil amended with different doses of four different urban sewage sludge based on equivalent nitrogen fertilization of 0, 85, 170, and 340 kg N/ha. After the topsoil/sludge mixtures were made, they were wet to 18% moisture and analyzed for 2 months to determine the presence of bacteria, and then again after 1 year. Results and discussion: The results indicate that the presence of microorganisms was strongly conditioned by the type of biosolid and the dose applied. Soil moisture diminished as the experiment progressed and seemed to play a role in controlling the presence of the bacteria. Conclusions: The initial concentrations of bacteria depend on the sewage sludge treatment. The evolution of *E. coli* had a similar trend as total coliforms, and *Salmonella* spp. was absent after 8 weeks although a positive

presence was detected in some soils after a year. As a conclusion, long periods of time reduce the risk from the presence of pathogens in soils, and the persistence may be closely related to the treatment of sewage sludge and the initial amount of microorganisms in the sewage sludge. ISSN: 14390108

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

*Global gene-expression analysis of the response of Salmonella enteritidis to egg white exposure reveals multiple egg white-imposed stress responses*  
(2017) *Frontiers in Microbiology*, 8 (MAY), art. no. 829, .

ABSTRACT: Chicken egg white protects the embryo from bacterial invaders by presenting an assortment of antagonistic activities that combine together to both kill and inhibit growth. The key features of the egg white anti-bacterial system are iron restriction, high pH, antibacterial peptides and proteins, and viscosity. *Salmonella enterica* serovar Enteritidis is the major pathogen responsible for egg-borne infection in humans, which is partly explained by its exceptional capacity for survival under the harsh conditions encountered within egg white. However, at temperatures up to 42°C, egg white exerts a much stronger bactericidal effect on *S. Enteritidis* than at lower temperatures, although the mechanism of egg white-induced killing is only partly understood. Here, for the first time, the impact of exposure of *S. Enteritidis* to egg white under bactericidal conditions (45°C) is explored by global-expression analysis. A large-scale (18.7% of genome) shift in transcription is revealed suggesting major changes in specific aspects of *S. Enteritidis* physiology: induction of egg white related stress-responses (envelope damage, exposure to heat and alkalinity, and translation shutdown); shift in energy metabolism from respiration to fermentation; and enhanced micronutrient provision (due to iron and biotin restriction). Little evidence of DNA damage or redox stress was obtained. Instead, data are consistent with envelope damage resulting in cell death by lysis. A surprise was the high degree of induction of hexonate/hexuronate utilization genes, despite no evidence indicating the presence of these substrates in egg white. ISSN: 1664302X

**Jacxsens, L., Uyttendaele, M., Luning, P., Allende, A.**

*Food safety management and risk assessment in the fresh produce supply chain*  
(2017) *IOP Conference Series: Materials Science and Engineering*, 193 (1), art. no. 012020, .

ABSTRACT: This paper is the output of several years of scientific research coordinated by Laboratory of Food Preservation and Food Microbiology at UGent, within the EU FP7 Research project Veg-i-trade ([www.vegitrade.org](http://www.vegitrade.org)), in collaboration with among other partners, Wageningen University and Cebas-CSIC. Fresh produce and derived products are globally traded and subjected to an inherent sensitive towards enteric pathogens as *Salmonella* and pathogenic *E. coli* due to their cultivation practices. As fruits and vegetables are increasingly being consumed raw, a potential health risk towards consumers is present. In the Veg-i-Trade project the extend of presence of pathogens in leafy greens and strawberry fruit and their cultivation environment (as water, soil, manured soil, etc.) was analysed. Insight in the food safety management system enlightened the need for further fostering and guidance towards farmers in good practices in order to reduce the potential pressure of the presence of the pathogens both in EU and non EU countries. Exposure assessment calculations demonstrated the usefulness of mathematic modelling to gain more insight in fragmented microbiological analysis and information of cultivation practices, as such the impact of contamination of irrigation water and the impact of a flooding event. Veg-i-Trade was a challenging project both in scientific and management perspective as 23 partners collaborated. ISSN: 17578981

**Rajan, K., Shi, Z., Ricke, S.C.**

*Current aspects of Salmonella contamination in the US poultry production chain and the potential application of risk strategies in understanding emerging hazards*  
(2017) *Critical Reviews in Microbiology*, 43 (3), pp. 370-392.

ABSTRACT: One of the leading causes of foodborne illness in poultry products is *Salmonella enterica*. *Salmonella* hazards in poultry may be estimated and possible control methods modeled and evaluated through the use of quantitative microbiological risk assessment (QMRA) models and tools. From farm to table, there are many possible routes of *Salmonella* dissemination and contamination in poultry. From the time chicks are hatched through growth, transportation, processing, storage, preparation, and finally consumption, the product could be contaminated through exposure to different materials and sources. Examination of each step of the process is necessary as well as an examination of the overall picture to create effective countermeasures against contamination and prevent disease. QMRA simulation models can use either point

estimates or probability distributions to examine variables such as *Salmonella* concentrations at retail or at any given point of processing to gain insight on the chance of illness due to *Salmonella* ingestion. For modeling *Salmonella* risk in poultry, it is important to look at variables such as *Salmonella* transfer and cross contamination during processing. QMRA results may be useful for the identification and control of critical sources of *Salmonella* contamination. ISSN: 1040841X

**Uejio, C.K.**

*Temperature Influences on Salmonella Infections across the Continental United States (2017) Annals of the American Association of Geographers, 107 (3), pp. 751-764.*

ABSTRACT: *Salmonella* spp. are one of the most common causes of gastrointestinal illness in humans. Elevated temperatures increase *Salmonella* spp.'s growth rate and likelihood that the food consumer will develop a severe illness. Climate and *Salmonella* associations have only been reported for a few U.S. states. This study investigated associations between temperature and reported human *Salmonella* infections from 2006 to 2014. The study analyzed state-level relationships across the contiguous United States. States voluntarily report weekly human *Salmonella* cases to the Nationally Notifiable Diseases Surveillance System. Representative weather conditions were created by population weighting temperature from the North American Land Data Assimilation System. Time series analysis using generalized additive models associated temperature against *Salmonella* infections while controlling for temporal patterns and the size of the population at risk. The study also investigated temperature and *Salmonella* infection transmission thresholds. In twenty-five states, higher weekly temperatures increased reported *Salmonella* infections. Each degree (°C) rise in temperature increased the risk of reporting a case by 1.3 to 5.9 percent. Many of these states were located in the Southwest, east central states, Midwest and Great Plains, and Northeast regions. Only temperatures above a state-specific threshold increased cases in four states. Above each threshold, a 1°C temperature increase translated into 5.6 to 22.8 percent more cases. Weekly temperatures increased reported human *Salmonella* infections across a much larger portion of the United States than published research suggests. Knowledge of places and periods of time where climate increases *Salmonella* risk can help target surveillance and health interventions. ISSN: 24694452

**Saeidabadi, M.S., Nili, H., Dadras, H., Sharifiyazdi, H., Connolly, J., Valcanis, M., Raidal, S., Ghorashi, S.A.**

*Evaluation of PCR and high-resolution melt curve analysis for differentiation of Salmonella isolates*

*(2017) Avian Pathology, 46 (3), pp. 319-331.*

ABSTRACT: Consumption of poultry products contaminated with *Salmonella* is one of the major causes of foodborne diseases worldwide and therefore detection and differentiation of *Salmonella* spp. in poultry is important. In this study, oligonucleotide primers were designed from hemD gene and a PCR followed by high-resolution melt (HRM) curve analysis was developed for rapid differentiation of *Salmonella* isolates. Amplicons of 228 bp were generated from 16 different *Salmonella* reference strains and from 65 clinical field isolates mainly from poultry farms. HRM curve analysis of the amplicons differentiated *Salmonella* isolates and analysis of the nucleotide sequence of the amplicons from selected isolates revealed that each melting curve profile was related to a unique DNA sequence. The relationship between reference strains and tested specimens was also evaluated using a mathematical model without visual interpretation of HRM curves. In addition, the potential of the PCR–HRM curve analysis was evaluated for genotyping of additional *Salmonella* isolates from different avian species. The findings indicate that PCR followed by HRM curve analysis provides a rapid and robust technique for genotyping of *Salmonella* isolates to determine the serovar/serotype. ISSN: 03079457

**Schielke, A., Rabsch, W., Prager, R., Simon, S., Fruth, A., Helling, R., Schnabel, M., Siffczyk, C., Wiczorek, S., Schroeder, S., Ahrens, B., Oppermann, H., Pfeiffer, S., Merbecks, S.S., Rosner, B., Frank, C., Weiser, A.A., Lubber, P., Gilsdorf, A., Stark, K., Werber, D.**

*Two consecutive large outbreaks of salmonella muenchen linked to pig farming in germany, 2013 to 2014: Is something missing in our regulatory framework?*

*(2017) Eurosurveillance, 22 (18), 10 p.*

ABSTRACT: In 2013, raw pork was the suspected vehicle of a large outbreak (n = 203 cases) of *Salmonella* Muenchen in the German federal state of Saxony. In 2014, we investigated an outbreak (n = 247 cases) caused by the same serovar affecting Saxony and three further federal states in the eastern part of Germany. Evidence from epidemiological, microbiological and trace-back investigations strongly implicated different

raw pork products as outbreak vehicles. Trace-back analysis of *S. Muenchen*-contaminated raw pork sausages narrowed the possible source down to 54 pig farms, and *S. Muenchen* was detected in three of them, which traded animals with each other. One of these farms had already been the suspected source of the 2013 outbreak. *S. Muenchen* isolates from stool of patients in 2013 and 2014 as well as from food and environmental surface swabs of the three pig farms shared indistinguishable pulsed-field gel electrophoresis patterns. Our results indicate a common source of both outbreaks in the primary production of pigs. Current European regulations do not make provisions for *Salmonella* control measures on pig farms that have been involved in human disease outbreaks. In order to prevent future outbreaks, legislators should consider tightening regulations for *Salmonella* control in causative primary production settings. ISSN: 1025496X

**Zhao, Y., Jiang, X., Qu, Y., Pan, R., Pang, X., Jiang, Y., Man, C.**

*Salmonella* detection in powdered dairy products using a novel molecular tool (2017) *Journal of Dairy Science*, 100 (5), pp. 3480-3496.

ABSTRACT: In this study, we developed a rapid, specific, and sensitive loop-mediated isothermal amplification technique combined with a lateral flow dipstick (LAMP-LFD) method to detect *Salmonella* targeting the *siIA* gene in powdered infant formula (PIF). The specificity of the detection method (LAMP-LFD) approached 100% using 21 *Salmonella* and 31 non-*Salmonella* bacterial strains. This detection method exhibited high sensitivity limits for pure cultures at 3.7 cfu/mL and in PIF at 2.2 cfu/g without enrichment. To evaluate the applicability of the LAMP-LFD method, we detected 60 positive PIF samples and 20 negative PIF samples. The results showed that the method of LAMP-LFD had a high diagnostic specificity of 100% for detection of *Salmonella* in PIF. To reduce incidence of LAMP contamination, we applied propidium monoazide (PMA) to eliminate carryover contamination of LAMP. At the same time, we found that PMA does not affect observation of LFD for measurement of LAMP signal. The results verified that the method of LAMP-LFD targeting the *siIA* gene is rapid, accurate, and sensitive for *Salmonella* detection in PIF, and that PMA shows great potential to be widely used to eliminate the amplicon contamination risk generated by the highly sensitive LAMP reaction in the detection process. ISSN: 00220302

**Catford, A., Ganz, K., Tamber, S.**

*Enumerative analysis of Salmonella in outbreak-associated breaded and frozen comminuted raw chicken products* (2017) *Journal of Food Protection*, 80 (5), pp. 814-818.

ABSTRACT: A significant data gap exists with respect to the levels of pathogens in foods implicated in foodborne outbreaks. These data are essential for the quantification of pathogen exposure via the ingestion of contaminated food. Here we report the levels of the foodborne pathogen *Salmonella* in comminuted raw chicken products that had been breaded and then frozen. The products investigated were collected during four food safety investigations of foodborne outbreaks that occurred in Canada from 2014 to 2016. Most-probable-number (MPN) distribution analysis of the food samples revealed *Salmonella* levels of 0.0018 to 3 MPN/g, which is equivalent to 1 MPN per 0.33 to 556 g of product. These data suggest low levels of *Salmonella* may be associated with foodborne outbreaks. ISSN: 0362028X

**Tasmin, R., Hasan, N.A., Grim, C.J., Grant, A., Choi, S.Y., Alam, M.S., Bell, R., Cavanaugh, C., Balan, K.V., Babu, U.S., Parveen, S.**

*Genotypic and phenotypic characterization of multidrug resistant Salmonella Typhimurium and Salmonella Kentucky strains recovered from chicken carcasses* (2017) *PLoS ONE*, 12 (5), art. no. e0176938, .

ABSTRACT: *Salmonella* Typhimurium is the leading cause of human non-typhoidal gastroenteritis in the US. *S. Kentucky* is one the most commonly recovered serovars from commercially processed poultry carcasses. This study compared the genotypic and phenotypic properties of two *Salmonella enterica* strains Typhimurium (ST221-31B) and Kentucky (SK222-32B) recovered from commercially processed chicken carcasses using whole genome sequencing, phenotype characterizations and an intracellular killing assay. Illumina MiSeq platform was used for sequencing of two *Salmonella* genomes. Phylogenetic analysis employing homologous alignment of a 1,185 non-duplicated protein-coding gene in the *Salmonella* core genome demonstrated fully resolved bifurcating patterns with varying levels of diversity that separated ST221-31B and SK222-32B genomes into distinct monophyletic serovar clades. Single nucleotide polymorphism (SNP) analysis identified 2,432 (ST19) SNPs within 13 Typhimurium genomes including ST221-31B representing Sequence Type ST19 and 650 (ST152) SNPs were detected within 13 Kentucky genomes including SK222-32B representing Sequence Type ST152. In addition to serovar-specific

conserved coding sequences, the genomes of ST221-31B and SK222-32B harbor several genomic regions with significant genetic differences. These included phage and phage-like elements, carbon utilization or transport operons, fimbriae operons, putative membrane associated proteinencoding genes, antibiotic resistance genes, siderophore operons, and numerous hypothetical protein-encoding genes. Phenotype microarray results demonstrated that ST221-31B is capable of utilizing certain carbon compounds more efficiently as compared to SK222-3B; namely, 1,2-propanediol, M-inositol, L-threonine,  $\alpha$ -D-lactose, D-tagatose, adonitol, formic acid, acetoacetic acid, and L-tartaric acid. ST221-31B survived for 48 h in macrophages, while SK222-32B was mostly eliminated. Further, a 3-fold growth of ST221-31B was observed at 24 hours post-infection in chicken granulosa cells while SK222-32B was unable to replicate in these cells. These results suggest that *Salmonella* Typhimurium can survive host defenses better and could be more invasive than *Salmonella* Kentucky and provide some insights into the genomic determinants responsible for these differences.

ISSN: 19326203

**Sarjit, A., Dykes, G.A.**

*Transfer of Campylobacter and Salmonella from poultry meat onto poultry preparation surfaces*

(2017) *Journal of Food Protection*, 80 (5), pp. 750-757.

ABSTRACT: Thermophilic *Campylobacter* and *Salmonella enterica* are major causes of gastrointestinal foodborne infection. Survival of these pathogens on food-associated surfaces is a risk contributing to their spread through the food system. This study examined the transfer of two strains each of *C. jejuni*, *C. coli*, *Salmonella* Enteritidis, and *Salmonella* Typhimurium from chicken meat to a knife or scissors used on either a plastic or wooden cutting board. Each strain of *Campylobacter* and *Salmonella* at  $\sim 10^8$  CFU mL<sup>-1</sup> was inoculated (5 mL) onto 25 g of chicken meat with skin and allowed to attach (for 10 min). The meat was then cut (20 times per implement) into 1-cm<sup>2</sup> pieces with either a knife or scissors on either a plastic or wooden cutting board. The numbers of pathogens transferred from meat onto cutting implements and cutting board surfaces were enumerated. The surfaces were subsequently either rinsed with water or rinsed with water and wiped with a kitchen towel to mimic commonly used superficial cleaning practices for these implements, and the numbers of pathogens were enumerated again. The bacterial numbers for both pathogens were determined on thin-layer agar. The attachment of the *Salmonella* strains to chicken meat ( $\sim 7.0$  to  $7.8$  log CFU cm<sup>-2</sup>) was higher than the attachment of the *Campylobacter* strains ( $\sim 4.6$  to  $6.6$  log CFU cm<sup>-2</sup>). All four *Salmonella* strains transferred in higher numbers ( $\sim 1.9$  to  $6.3$  log CFU cm<sup>-2</sup>) to all surfaces than did the *Campylobacter* strains ( $\sim 1.1$  to  $3.9$  log CFU cm<sup>-2</sup>). The transfer rates of both pathogens from the chicken meat to all the surfaces examined varied substantially between  $\sim 0$  and 21.1%. The highest rate of transfer ( $\sim 21.1\%$ ) observed was for *C. coli* 2875 when transferred from the chicken meat to the scissors. Most cleaning treatments reduced the numbers of both pathogens ( $\sim 0.3$  to  $4.1$  log CFU cm<sup>-2</sup>) transferred to all the surfaces. Our study gives insights into the risks associated with the transfer of *Campylobacter* and *Salmonella* from poultry to the surfaces used in poultry preparation.

ISSN: 0362028X

**Berrang, M.E., Cox, N.A., Cosby, D.E., Frye, J.G., Jackson, C.R.**

*Detection of Salmonella Serotypes by Overnight Incubation of Entire Broiler Carcass*

(2017) *Journal of Food Safety*, 37 (2), art. no. e12298, .

ABSTRACT: There are multiple ways to sample broiler chicken carcasses for the prevalence of *Salmonella*. A common method in the USA is a whole carcass rinse and culture of an aliquot of the rinse. The objective of this study was to compare the sensitivity of the rinse aliquot method to overnight enrichment of the entire carcass in the rinse liquid. Fourteen replicate samplings of eight carcasses each were done at two commercial broiler processing plants. Carcasses were subjected to a whole carcass rinse in buffered peptone from which 30 mL was removed and added to 30 mL fresh buffered peptone (rinse aliquot sample). The aliquot sample and the carcass, in the remaining buffered peptone, were incubated overnight prior to standard selective enrichment and plating for *Salmonella* detection. *Salmonella* was detected in 15% of rinse aliquot samples and 59% of whole carcass enrichment samples. When detected by both methods, for the most part, the same serotypes were found on individual carcasses. Whole carcass enrichment was shown to be more sensitive than rinse aliquot method, and likely detects *Salmonellae* even when tightly bound to the carcass or present in very low numbers. As such, whole carcass enrichment is a useful research tool to determine *Salmonella* prevalence. Practical Applications: Whole broiler carcass enrichment is a more sensitive method for detection of *Salmonella* than

whole carcass rinse and aliquot incubation. Logistical requirements, however, make it impractical for routine or large scale investigations. Whole carcass enrichment has utility for research purposes to determine relative sensitivity of other methods or for testing of broiler processing microbial interventions. ISSN: 01496085

**He, P., Zhu, G., Luo, J., Wang, H., Yan, Y., Chen, L., Gao, W., Chen, Z.**

*Development and Application of a One-Tube Multiplex Real-Time PCR with Melting Curve Analysis for Simultaneous Detection of Five Foodborne Pathogens in Food Samples* (2017) *Journal of Food Safety*, 37 (2), art. no. e12297, .

ABSTRACT: This study was aimed at developing a one-tube multiplex real-time PCR system for simultaneous detection of five major foodborne pathogens (*Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella enterica*, *Vibrio parahaemolyticus* and *Shigella* spp.) in food samples. The newly developed multiplex real-time PCR was performed using fluorescent dye EvaGreen and melting curve analysis for pathogen discrimination. The specificity of the PCR method was evaluated by testing with DNA extracted from different strains. The detection limits of genomic DNA per reaction for the five pathogens ranged from 0.39 pg (*Shigella* spp.) to 40 pg (*Listeria monocytogenes*). Sensitivity and specificity of this multiplex real-time PCR were further confirmed using artificially and naturally contaminated samples. The newly developed multiplex real-time PCR was useful for rapid, specific and cost-effective detection of the five target pathogens in contaminated food samples. Practical Applications: The newly developed multiplex real-time PCR assay could detect five major foodborne pathogens (*Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella enterica*, *Vibrio parahaemolyticus* and *Shigella* spp.) in food samples in a single reaction tube. This method is simple, cost-effective, specific, sensitive and is capable of high-throughput detection for five foodborne pathogens simultaneously. Therefore, it has a potential application as a useful tool in food testing laboratories for quick identification of foodborne pathogens. ISSN: 01496085

**Bollerslev, A.M., Nauta, M., Hald, T., Hansen, T.B., Aabo, S.**

*A risk-based approach for evaluation of hygiene performance at pig slaughter* (2017) *Food Control*, 75, pp. 116-125.

ABSTRACT: In Denmark, the pig slaughterhouses have a daily input of pigs infected and/or contaminated with *Salmonella*, and the slaughter hygiene has major influence on the level of *Salmonella* contamination on the meat leaving the slaughterhouse. However, the relationship between the effect of improved hygiene performance and the consequential reduction of human health risk has not been estimated so far. In this study, swab samples from 2702 pig carcasses were collected, originally for other purposes, from five large Danish slaughterhouses in a period from 2005 to 2007, covering all seasons of the year. The samples were analysed quantitatively for *E. coli* and semi-quantitatively for *Salmonella*. A positive association between the number of *E. coli* on carcasses and the prevalence of *Salmonella* positive carcasses was shown. For carcasses positive for *Salmonella*, a positive association was also shown between the number of *E. coli* and the number of *Salmonella* on the carcass. As no biological association has been reported between faecal shedding of *E. coli* and presence of *Salmonella*, the relationship was considered to be associated with the level of faecal contamination. The positive association between *E. coli* and *Salmonella* was used as basis for developing a quantitative risk assessment model for *Salmonella*, using the level *E. coli* as model input. The model output associated the hygiene performance with a relative risk estimate of human salmonellosis. The overall objective was to develop a decision support tool that can be used to support risk-based hygiene interventions in pig slaughterhouses. ISSN: 09567135

**Kim, J.H., Min, S.C.**

*Microwave-powered cold plasma treatment for improving microbiological safety of cherry tomato against Salmonella*

(2017) *Postharvest Biology and Technology*, 127, pp. 21-26.

ABSTRACT: Microwave-powered cold plasma treatment (CPT) has been investigated as a nonthermal intervention technology for improving the microbiological safety of cherry tomatoes (*Solanum lycopersicum* var. *cerasiforme*) against *Salmonella* (approximately 7 log CFU/tomato). Cherry tomatoes were subjected to CPT using helium (He) or a He-oxygen (O<sub>2</sub>) gas mixture for 2–10 min at 400–900 W of plasma generation power. A central composite method was applied to investigate the interactions between treatment conditions and the *Salmonella* reduction rate, weight loss, or temperature of the tomatoes. CPTs using He and a He-O<sub>2</sub> gas mixture at 827 W for 9 min resulted in the greatest reduction in *Salmonella* numbers (3.5 ± 0.1 and 3.5 ± 0.5 log CFU/tomato, respectively) and temperature increases of 3.0 ± 0.3 and 3.5 ± 0.4 °C, respectively. He-CPT at 900 W for 10 min, determined as the optimal conditions for *Salmonella* inactivation in this study,

did not appreciably influence the surface morphology of cherry tomatoes. While the optimal He-CPT did not effectively inhibit the growth of *Salmonella* on the tomatoes at 25 °C, the treatment prevented the *Salmonella* growth during storage at 5 °C, without affecting the tomato respiration rate ( $P < 0.05$ ). These results demonstrate the potential of CPT as a postharvest technology to improve the microbiological safety of cherry tomatoes against *Salmonella*, without affecting their biological properties. ISSN: 09255214

**Park, S., Harrison, M.A., Berrang, M.E.**

*Postchill antimicrobial treatments to control Salmonella, Listeria, and Campylobacter contamination on chicken skin used in ground chicken*  
(2017) *Journal of Food Protection*, 80 (5), pp. 857-862.

ABSTRACT: Ground poultry products are frequently contaminated with foodborne pathogens. With the potential for increased regulatory scrutiny, it is important to use sufficient intervention strategies to control pathogen levels effectively. A large proportion of the bacteria introduced to ground chicken are likely to come from broiler skin, which is added to achieve target fat content and maintain product texture and taste. In this research, antimicrobials, including 50 ppm of chlorine and 1,200 ppm of peracetic acid (PAA), were applied in a postchill system to reduce the number of *Salmonella* Typhimurium, *Listeria monocytogenes*, and *Campylobacter coli* inoculated on chicken skin used to formulate ground chicken. Results showed that chlorine provided no significant effect in reducing the number of pathogens in ground chicken made with treated skin compared with water treatment but that it did help decrease pathogens in postchill water. PAA was found to be an effective ( $P \leq 0.05$ ) antimicrobial agent, not only in reducing the number of pathogens on ground chicken, but also in postchill water. Treating chicken skin with PAA prior to inclusion in ground chicken can be an effective intervention strategy to lessen contamination in a ground chicken meat product. ISSN: 0362028X

**Hu, L., Ma, L.M., Zheng, S., He, X., Wang, H., Brown, E.W., Hammack, T.S., Zhang, G.**

*Evaluation of 3M Molecular Detection System and ANSR Pathogen Detection System for rapid detection of Salmonella from egg products*  
(2017) *Poultry Science*, 96 (5), pp. 1410-1418.

ABSTRACT: Isothermal amplification assay is a novel simple detection technology that amplifies DNA with high speed, efficiency, and specificity under isothermal conditions. The objective of this study was to evaluate the effectiveness of the 3M Molecular Detection System (MDS) and ANSR Pathogen Detection System (PDS) for the detection of *Salmonella* in egg products as compared to the Food and Drug Administration's Bacteriological Analytical Manual (BAM) culture method and a modified culture method (3M MDS and ANSR PDS preferred method). Two *Salmonella* ser. Enteritidis (18579, PT4; CDC 2010K 1441, PT8), one *Salmonella* ser. Heidelberg (607310-1), and one *Salmonella* ser. Typhimurium (0723) isolates were used in this study. Seven wet egg products and 13 dry egg products were inoculated with these strains individually at 1 to 5 CFU/25 g. One set of test portions was prepared following FDA BAM procedures [with lactose broth (LB) as pre-enrichment broth]. Another set of test portions was prepared using buffered peptone water (BPW) as pre-enrichment broth, as instructed by the 2 detection systems. Results from 3M MDS and ANSR PDS were 100% in agreement with their BPW-based culture method results. When LB was used as pre-enrichment broth, the number of *Salmonella* positive test portions (80 tested), identified with the BAM, 3M MDS, and ANSR PDS, were 63, 61, and 60, respectively. In conclusion, both 3M MDS and ANSR PDS *Salmonella* assays were as effective as their BPW based culture methods and were equivalent to the BAM culture method for the detection of *Salmonella* in egg products. These sensitive isothermal assays can be used as rapid detection tools for *Salmonella* in egg products provided that BPW is used as pre-enrichment broth. ISSN: 00325791

**Jacobson, A.P., Wang, H., Gill, V.S., Duvall, R., Arce, G., Chirtel, S., Hammack, T.S.**

*Relative effectiveness of selected preenrichment media for the detection of Salmonella from leafy green produce and herbs*  
(2017) *Food Microbiology*, 63, pp. 123-128.

ABSTRACT: Four buffered preenrichment media (BAX® System MP Media (BAX)), Universal Preenrichment Broth (UPB), modified Buffered Peptone Water (mBPW), and Buffered Peptone Water (BPW) were compared with lactose broth (LB) in the Bacteriological Analytical Manual's (BAM) *Salmonella* culture method for the analysis of 9 leafy green produce and herb types. Artificially contaminated test portions were pre-enriched in each medium and the results were analyzed statistically using Fisher's Exact 2-tailed F test ( $p < 0.05$ ) with pairwise comparisons. There was no difference in recovery of *Salmonella* from curly parsley and basil among the five media ( $p > 0.05$ ). UPB was

consistently among the most effective media for recovery of Salmonella from the nine produce types; however, *S. Typhimurium* and *S. Newport* were isolated from cabbage more frequently with mBPW than with UPB ( $p < 0.05$ ). Comparisons of the results among the preenrichment media from all experimental trials, with leafy green produce and herbs, demonstrate that Salmonella is more effectively detected and isolated using buffered enrichments than with the currently recommended LB ( $p < 0.05$ ). There were no significant differences among the buffered preenrichments for the detection of Salmonella-positive test portions of the produce tested (BAX (160 Salmonella-positive test portions/480 test portions), UPB (176/480), mBPW (184/480), BPW (169/480), LB (128/480)) ( $p > 0.05$ ). ISSN: 07400020

**Walia, K., Lynch, H., Grant, J., Duffy, G., Leonard, F.C., Lawlor, P.G., Gardiner, G.E.**  
*The efficacy of disinfectant misting in the lairage of a pig abattoir to reduce Salmonella and Enterobacteriaceae on pigs prior to slaughter*  
(2017) *Food Control*, 75, pp. 55-61.

**ABSTRACT:** Water misting/showers are used in abattoir lairages to improve meat quality, and to cool and calm pigs after transport and during hot weather. One novel approach, which has not been investigated to date, is to add a disinfectant to the misting water as a means of topically reducing Salmonella on pigs prior to slaughter, thereby potentially controlling this organism in the abattoir. The objective of this study was therefore to evaluate misting with water or with Virkon® S (an approved disinfectant for use in the presence of animals), for their ability to topically reduce Salmonella on high seroprevalence pig herds before stunning and to reduce Enterobacteriaceae. Three experimental groups were investigated: control group (i.e., no misting); water group (misting with cold, 15–17 °C, water, herein referred to as water); and a disinfectant group (misting with 0.5% Virkon® S). As pigs entered the abattoir, each animal was swabbed along its back before being allocated to its experimental group. Each group was randomly assigned to one of 3 lairage pens that were separated by non-trial pens. After 30 min of misting with water or disinfectant, pigs were moved to the stunning area, where each pig was again swabbed, as above. Swabs were analyzed for the presence of Salmonella and enumeration of Enterobacteriaceae. Before misting, Salmonella prevalence on the pigs was 79.0%, 72.1% and 83.6% for the control, water and disinfectant groups, respectively. After misting, Salmonella prevalence increased to 94.3% in the water group; whereas for the disinfectant group, the prevalence increased marginally to 85.9%. No change in Salmonella prevalence was detected for the control group. In line with the Salmonella results, no significant differences were observed in Enterobacteriaceae counts in the control group at either time point (4.37 and 5.01 log<sub>10</sub> CFU/cm<sup>2</sup>, respectively) or in the disinfectant group before and after misting (4.02 and 4.26 log<sub>10</sub> CFU/cm<sup>2</sup>, respectively). However, a 2.3 log<sub>10</sub> CFU/cm<sup>2</sup> increase in Enterobacteriaceae was recorded for the water group after misting as compared to before misting ( $p < 0.05$ ). Since misting with water alone increased topical Salmonella contamination on pigs before slaughter, a risk assessment based on known Salmonella data, meat quality and welfare is recommended to determine whether its use is justifiable. On the other hand, the findings from this study suggest that misting with Virkon® S at 0.5% could have a role in topical antiseptics of pigs contaminated with Salmonella prior to slaughter and as such this warrants further investigation. ISSN: 09567135

**Gast, R.K., Guraya, R., Jones, D.R., Guard, J., Anderson, K.E., Karcher, D.M.**  
*Colonization of internal organs by Salmonella serovars Heidelberg and Typhimurium in experimentally infected laying hens housed in enriched colony cages at different stocking densities*  
(2017) *Poultry Science*, 96 (5), pp. 1402-1409.

**ABSTRACT:** Contaminated eggs produced by infected commercial laying flocks are often implicated as sources of human infections with Salmonella Enteritidis, but Salmonella serovars Heidelberg and Typhimurium have also been associated with egg-transmitted illness. Contamination of the edible contents of eggs is a consequence of the colonization of reproductive tissues in systemically infected hens. In recent years, the animal welfare implications of diverse poultry housing and management systems have been vigorously debated, but the food safety significance of laying hen housing remains uncertain. The present study evaluated the effects of 2 different bird stocking densities on the invasion of internal organs by Salmonella serovars Heidelberg and Typhimurium in groups of experimentally infected laying hens housed in colony cages enriched with perching and nesting areas. Laying hens were distributed at 2 different stocking densities (648 and 973 cm<sup>2</sup>/bird) into colony cages and (along with a group housed in conventional cages at 648 cm<sup>2</sup>/bird) orally inoculated with doses of 10<sup>7</sup> cfu of 2-strain cocktails of either Salmonella Heidelberg or Salmonella Typhimurium. At 5 to 6 d postinoculation, hens were euthanized

and samples of internal organs (cecum, liver, spleen, ovary, and oviduct) were removed for bacteriologic culturing. The overall frequency of *Salmonella* isolation from ceca after inoculation with strains of serovar Heidelberg (83.3%) was significantly ( $P < 0.001$ ) greater than the corresponding value for strains of serovar Typhimurium (53.8%), whereas *Salmonella* was recovered significantly more often from both livers (85.2% vs. 53.7%;  $P < 0.0001$ ) and spleens (78.7% vs. 56.5%;  $P = 0.0008$ ) after inoculation with strains of serovar Typhimurium than strains of serovar Heidelberg. However, there were no significant differences ( $P > 0.05$ ) between stocking densities or cage systems in the frequencies of isolation of either *Salmonella* serovar from any of the five sampled tissues. These results contrast with prior studies, which reported increased susceptibility to internal organ invasion by *Salmonella* Enteritidis among hens in conventional cages at higher stocking densities. ISSN: 00325791

**Fabiani, L., Pucci, E., Delibato, E., Volpe, G., Piermarini, S., De Medici, D., Capuano, F., Palleschi, G.**

*ELIME assay vs Real-Time PCR and conventional culture method for an effective detection of Salmonella in fresh leafy green vegetables*  
(2017) *Talanta*, 166, pp. 321-327.

**ABSTRACT:** The detection of *Salmonella* according to EC regulation is still primarily based on traditional microbiological culture methods that may take several days to be completed. The purpose of this work is to demonstrate the applicability of an Enzyme-Linked-Immuno-Magnetic-Electrochemical (ELIME) assay, recently developed by our research group for the detection of salmonella in irrigation water, in fresh (raw and ready-to-eat) leafy green vegetables by comparison with Real-Time PCR (RTi-PCR) and ISO culture methods. Since vegetables represent a more complex matrix than irrigation water, preliminary experiments were carried out on two leafy green vegetables that resulted negative for salmonella by the ISO method. 25 g of these samples were experimentally inoculated with 1–10 CFU of *S. Napoli* or *S. Thompson* and pre-enriched for 20 h in two different broths. At this time aliquots were taken, concentrated at different levels by centrifugation, and analyzed by ELIME and RTi-PCR. Once selected the best culture medium for salmonella growth, and the optimal concentration factor suitable to reduce the sample matrix effect, enhancing the out-put signal, several raw and ready-to-eat leafy green vegetables were artificially inoculated and pre-enriched. Aliquots were then taken at different incubation times and analyzed with both techniques. Results obtained showed that 20 and 8 h of pre-enrichment were required to allow the target salmonella (1–10 CFU/25 g) to multiply until reaching a detectable concentration by ELIME and RTi-PCR assays, respectively. A confirmation with the ISO culture method was carried out. Based on the available literature, this is the first report of the application of an ELISA based method for the detection of *Salmonella* in vegetables. ISSN: 00399140

**Gosling, R.J., Mawhinney, I., Vaughan, K., Davies, R.H., Smith, R.P.**

*Efficacy of disinfectants and detergents intended for a pig farm environment where Salmonella is present*

(2017) *Veterinary Microbiology*, 204, pp. 46-53.

**ABSTRACT:** Disinfection is a useful component of disease control, although products and chemical groups vary in their activity against different pathogens. This study investigated the ability of fifteen disinfectants to eliminate pig-associated *Salmonella*. Active compounds of products included chlorocresol, glutaraldehyde/formaldehyde, glutaraldehyde/quaternary ammonium compounds (QAC), iodine, peracetic acid and potassium peroxomonosulphate. Six detergents were also tested for their ability to dislodge faecal material, and interactions with specific disinfectants. Eight serovars were screened against all products using dilution tests and a monophasic *Salmonella* Typhimurium strain was selected for further testing. The disinfectants were tested using models to replicate boot dip (faecal suspension) and animal housing (surface contamination) disinfection respectively at the Department for Environment, Food and Rural Affairs Approved Disinfectant General Orders (GO) concentration, half GO and twice GO. Stability over time and ability to eliminate *Salmonella* in biofilm was also assessed. The most effective products were then field tested. Most products at GO concentration eliminated *Salmonella* in the faecal suspension model. One glutaraldehyde/QAC and one glutaraldehyde/formaldehyde-based product at GO concentration eliminated *Salmonella* in the surface contamination model. Chlorocresol-based products were more stable in the faecal suspension model. One chlorocresol and the glutaraldehyde/formaldehyde-based product were most successful in eliminating *Salmonella* from biofilms. All products tested on farm reduced bacterial log counts; the glutaraldehyde/QAC based product produced the greatest reduction. The type of product and the application concentration can impact on

efficacy of farm disinfection; therefore, clearer guidance is needed to ensure the appropriate programmes are used for specific environments. ISSN: 03781135

**Hyeon, J.-Y., Deng, X.**

*Rapid detection of Salmonella in raw chicken breast using real-time PCR combined with immunomagnetic separation and whole genome amplification*  
(2017) *Food Microbiology*, 63, pp. 111-116.

ABSTRACT: We presented the first attempt to combine immunomagnetic separation (IMS), whole genome amplification by multiple displacement amplification (MDA) and real-time PCR for detecting a bacterial pathogen in a food sample. This method was effective in enabling real-time PCR detection of low levels of *Salmonella enterica* Serotype Enteritidis (SE) (~10 CFU/g) in raw chicken breast without culture enrichment. In addition, it was able to detect refrigeration-stressed SE cells at lower concentrations (~0.1 CFU/g) in raw chicken breast after a 4-h culture enrichment, shortening the detection process from days to hours and displaying no statistical difference in detection rate in comparison with a culture-based detection method. By substantially improving performance in SE detection over conventional real-time PCR, we demonstrated the potential of IMS-MDA real-time PCR as a rapid, sensitive and affordable method for detecting *Salmonella* in food.  
ISSN: 07400020

**Ren, X., Fu, Y., Xu, C., Feng, Z., Li, M., Zhang, L., Zhang, J., Liao, M.**

*High resolution melting (HRM) analysis as a new tool for rapid identification of Salmonella enterica serovar Gallinarum biovars Pullorum and Gallinarum*  
(2017) *Poultry Science*, 96 (5), pp. 1088-1093.

ABSTRACT: *Salmonella enterica* serovar *Gallinarum* biovars *Pullorum* and *Gallinarum* represent the most common causative agents of chicken salmonellosis, which result in high mortality and morbidity throughout the world. It is difficult and laborious to discriminate these diseases based on biochemical or phenotypic methods. Herein, we report the development of a single nucleotide polymorphism (SNP) PCR-high resolution melt (PCR-HRM) assay for the detection and discrimination of both *S. Pullorum* and *S. Gallinarum*. The gene *rfbS*, which encodes a factor involved in the biosynthesis of ADP paratose in serogroup D of *Salmonella*, has been identified as a robust genetic marker for the identification of *S. Pullorum* and *S. Gallinarum* based on polymorphisms at positions 237 and 598. Therefore, PCR-HRM analyses were used to characterize this gene. A total of 15 reference and 33 clinical isolates of *Salmonella* and related Gram-negative bacteria were detected using 2 sets of primers. Our PCR-HRM assay could distinguish *S. Pullorum* from *S. Gallinarum* and other strains using the primer pair SP-237F/237R. Similarly, *S. Gallinarum* could be distinguished from *S. Pullorum* and other strains using primer set SG- 598F/598R. These 2 assays showed high specificity (100%) for both *S. Pullorum* and *S. Gallinarum*; the sensitivity of these 2 assays was at least 100-fold greater than that of the allele-specific PCR assay. This present study demonstrated that HRM analysis represents a potent, simple, and economic tool for the rapid, specific, and sensitive detection of *S. Pullorum* and *S. Gallinarum*. Our approach also may aid efforts for purification of Avian *Salmonella* disease. ISSN: 00325791

**Vojkovská, H., Myšková, P., Gelbíčová, T., Skočková, A., Kolářková, I., Karpíšková, R.**

*Occurrence and characterization of food-borne pathogens isolated from fruit, vegetables and sprouts retailed in the Czech Republic*  
(2017) *Food Microbiology*, 63, pp. 147-152.

ABSTRACT: Food of non-animal origin is a major component of the human diet and has been considered to pose a low risk from the point of view of bacteriological safety. However, an increase in the number of outbreaks of illness caused by such pathogens and linked to the consumption of fresh fruit and vegetables have been reported from around the world recently. *Salmonella* spp., STEC (Shiga toxin producing *Escherichia coli*) and *Listeria monocytogenes* are among the most frequently identified agents. Additionally, the transmission of antibiotic resistant strains including also the methicillin resistant *S. aureus* (MRSA) to humans via the food chain is one of the greatest public health problems being confronted today. Therefore, we focused on the bacterial safety of fruit, vegetables and sprouts on sale in the Czech Republic. One strain (0.3%) of *Salmonella* Enteritidis phage type PT8, one strain (0.3%) of MRSA and 17 strains (5.0%) of *L. monocytogenes* were isolated from a total of 339 collected samples. The most problematic commodities were frozen fruit and vegetables (packed and unpacked) and fresh-cut vegetables. Our findings indicate deficiencies in hygiene practices during harvesting, processing and distribution of these commodities. Although sprouts and berries are the most likely to be contaminated

by human pathogens, only two samples were positive for the presence of *L. monocytogenes*. ISSN: 07400020

**Jorgensen, F., Sadler-Reeves, L., Shore, J., Aird, H., Elviss, N., Fox, A., Kaye, M., Willis, C., Amar, C., De Pinna, E., McLaughlin, J.**

*An assessment of the microbiological quality of lightly cooked food (including sous-vide) at the point of consumption in England*

(2017) *Epidemiology and Infection*, 145 (7), pp. 1500-1509.

**ABSTRACT:** This observational study aims to investigate the microbiological quality of commercially prepared lightly cooked foods with a major component of food of animal origin and collected as would be served to a consumer. A total of 356 samples were collected from catering (92%), retail (7%) or producers (1%) and all were independent of known incidents of foodborne illness. Using standard methods, all samples were tested for: the presence of *Campylobacter* spp. and *Salmonella* spp. and enumerated for levels of, *Bacillus* spp. including *B. cereus*, *Clostridium perfringens*, *Listeria* spp. including *L. monocytogenes*, *Staphylococcus aureus*, *Escherichia coli*, Enterobacteriaceae and aerobic colony count (ACC). Results were interpreted as unsatisfactory, borderline or satisfactory according to the Health Protection Agency guidelines for assessing the microbiological safety of ready-to-eat foods placed on the market. Amongst all samples, 70% were classified as satisfactory, 18% were borderline and 12% were of unsatisfactory microbiological quality. Amongst the unsatisfactory samples, six (2%) were potentially injurious to health due to the presence of: *Salmonella* spp. (one duck breast); *Campylobacter* spp. (two duck breast and one chicken liver pâté); *L. monocytogenes* at  $4.3 \times 10^3$  cfu (colony-forming units)/g (one duck confit with foie gras ballotin) and *C. perfringens* at  $2.5 \times 10^5$  cfu/g (one chicken liver pâté). The remaining unsatisfactory samples were due to high levels of indicator *E. coli*, Enterobacteriaceae or ACC. ISSN: 09502688

**Mritunjay, S.K., Kumar, V.**

*A study on prevalence of microbial contamination on the surface of raw salad vegetables* (2017) *3 Biotech*, 7 (1), art. no. 13, .

**ABSTRACT:** The present work evaluates the microbiological quality of raw salad vegetables (RSV) consumed in Dhanbad city, India. A total of 480 samples of 8 different raw salad vegetables from local market were examined for overall microbial quality in terms of aerobic mesophilic, psychrotrophic counts, yeast, mould and total coliform levels. *E. coli* O157:H7, *Listeria monocytogenes* and *Salmonella* sp. were detected by real-time polymerase chain reaction (qPCR) subsequent to isolation. Results showed that all the samples were found positive for total coliform; however, *E. coli* was detected in 16.7% of the total samples. Pathogenic microorganisms such as *E. coli* O157:H7, *L. monocytogenes* and *Salmonella* spp. were detected in 1.3, 3.5 and 4.0%, respectively, of the total samples. However, pathogens were not detected in any of the cabbage samples. The *Exiguobacterium* sp. (Strain ISM SP 2014) was detected in the spinach sample while studying the bacterial contamination, reported for the first time on the surface of RSV. The 16S rRNA gene sequencing showed less than 92% similarity with sequences available in the public domain. ISSN: 2190572X

**Harich, M., Maherani, B., Salmieri, S., Lacroix, M.**

*Antibacterial activity of cranberry juice concentrate on freshness and sensory quality of ready to eat (RTE) foods*

(2017) *Food Control*, 75, pp. 134-144.

**ABSTRACT:** Ready-to-eat (RTE) foods are not further treated before consumption in such a way that may significantly reduce the microbial load, therefore the risk of foodborne disease must be considered. In this regard, the use of natural antimicrobial compounds is an interesting method to be considered. On this topic, the antibacterial activity of cranberry juice concentrate (CJC) have been evaluated in vitro and in situ against 3 foodborne pathogenic bacteria. Results showed a high antimicrobial effect with a noticeable inhibition capacity against *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella typhimurium*. Acid sensitivity studies of bacteria indicated that at the same pH level (pH = 2.4) in presence of organic acid solution (citric and quinic acids), cranberry juice concentrate showed greater antibacterial effects than the acids due to their phenolic compounds. In situ studies showed 2.5, 1.8 and 5 log reduction of *E. coli*, *L. monocytogenes* and *S. typhimurium*, respectively in presence of cranberry juice concentrate, on pre-cut red peppers after 7 days of storage at 4 °C. A total inhibition of *L. monocytogenes* on fresh cranberry fruits in primary day of storage, was observed. Cranberries treated with CJC also showed a 3 log reduction of *S. typhimurium* after 4 days of storage at 4 °C. The results suggest that CJC can be an effective preservation, source of

natural antibacterial, to protect the RTE foods from foodborne pathogens contamination without effecting on sensorial properties of treated samples and allow to maintain the freshness, sensory and the nutritional quality of RTE foods. ISSN: 09567135

**Szabo, I., Grafe, M., Kemper, N., Junker, E., Malorny, B.**

*Genetic basis for loss of immuno-reactive O-chain in Salmonella enterica serovar Enteritidis veterinary isolates*

(2017) *Veterinary Microbiology*, 204, pp. 165-173.

ABSTRACT: Fifty-two rough *Salmonella enterica* serovar Enteritidis (*S. Enteritidis*) isolates from broilers and the environment were characterized for their serological and genotypic properties. Under routine diagnostic serotyping methods such isolates lack the immuno-reactivity of the O-chain of the lipopolysaccharide (LPS), and are referred to as non-typeable. Using a modified slide agglutination method, the isolates could be differentiated into three different serological variants. Twenty-six isolates (50%) were defined as semi-rough, nineteen isolates (37%) as deep-rough, four isolates (8%) as rough and three isolates could not be assigned. Genetically, all semi-rough isolates lacked the *wzyB* gene encoding the O-antigen polymerase. Two isolates carried a frameshift mutation in *wzyB*. In 15 of 23 cases deep-rough or rough isolates had a single point mutation, a single – or double-nucleotide insert or deletion in the *wbaP* gene. The mutational changes lead to expression of truncated (premature) protein, resulting in the loss of the immuno-reactive O-chain. Both rough and smooth *S. Enteritidis* isolates showed identical or highly similar XbaI-PFGE profiles. Our results indicate that the loss of a functional LPS in *S. Enteritidis* isolates is caused by a variety of different mutation events within the *wzyB* (semi-rough) or the *wbaP* (deep-rough) gene and is not a result of a vertical spread of a specific *S. Enteritidis* subtype. The defect of the LPS may be a common evolutionary mechanism through which host defence can be escaped. ISSN: 03781135

**Dong, H.-J., Cho, S., Boxrud, D., Rankin, S., Downe, F., Lovchik, J., Gibson, J., Erdman, M., Saeed, A.M.**

*Single-nucleotide polymorphism typing analysis for molecular subtyping of Salmonella Tennessee isolates associated with the 2007 nationwide peanut butter outbreak in the United States*

(2017) *Gut Pathogens*, 9 (1), art. no. 25, .

ABSTRACT: Background: In 2007, a nationwide *Salmonella Tennessee* outbreak occurred via contaminated peanut butter. Here, we developed a single-nucleotide polymorphism (SNP)-typing method for *S. Tennessee* to determine the clonal subtypes of *S. Tennessee* that were associated with the peanut butter outbreak. Methods and results: One seventy-six *S. Tennessee* isolates from various sources, including humans, animals, food, and the environment, were analyzed by using the SNP technique. Eighty-four representative SNP markers were selected by comparing the sequences of three representative *S. Tennessee* strains with different multi-locus sequence typing and variable number tandem repeats from our collection. The set of eighty-four SNP markers showed 100% typeability for the 176 strains, with the nucleotide diversity ranging from 0.011 to 0.107 (mean =  $0.049 \pm 0.018$ , median = 0.044) for each marker. Among the four clades and nine subtypes generated by the SNP typing, subtype 1, which comprised 142 *S. Tennessee* strains, was the most predominant. The dominance of single-strain clones in subtype 1 revealed that *S. Tennessee* is highly clonal regardless of outbreak-association, source, or period of isolation, suggesting the presence of an *S. Tennessee* strain prototype. Notably, a minimum 18 SNP set was able to determine clonal *S. Tennessee* strains with similar discrimination power, potentially allowing more rapid and economic strain genotyping for both outbreaks and sporadic cases. Conclusions: The SNP-typing method described here might aid the investigation of the epidemiology and microevolution of pathogenic bacteria by discriminating between outbreak-related and sporadic clinical cases. In addition, this approach enables us to understand the population structure of the bacterial subtypes involved in the outbreak. ISSN: 17574749

**Xie, X., Hu, Y., Xu, Y., Yin, K., Li, Y., Chen, Y., Xia, J., Xu, L., Liu, Z., Geng, S., Li, Q., Jiao, X., Chen, X., Pan, Z.**

*Genetic analysis of Salmonella enterica serovar Gallinarum biovar Pullorum based on characterization and evolution of CRISPR sequence*

(2017) *Veterinary Microbiology*, 203, pp. 81-87.

ABSTRACT: *Salmonella enterica* serovar Gallinarum biovar Pullorum (*S. Pullorum*) is the cause of pullorum disease, characterized by white diarrhea, which leads to high mortality in poultry. In this study, we aimed to assess the genetic diversity of 655 *S. Pullorum* strains from 1962 to 2015 in China, Europe, and South America. A sequence typing scheme based on clustered regularly interspaced short palindromic repeats (CRISPR) was

used to reveal the genetic relationships among these strains in this study. Overall, a total of 20 Pullorum sequence types (PSTs) of CRISPR were identified in the 655 isolates with PST7 (74%, 486/655) and PST3 (13%, 86/655) to be the most two frequent PSTs belonging to two different lineages, which confirmed the genetic conservation of *S. Pullorum* strains isolated from six provinces and two direct-controlled municipalities (Beijing and Shanghai) in China. However, the identification of seven new PSTs distributed in strains isolated since 2001 implied that genetic variation continues to develop in *S. Pullorum*. Interestingly, the whole-genome single-nucleotide polymorphism typing (WGST) of 96 strains out of the 655 isolates divided them into four lineages based on SNP analysis of core genomic sequence and exhibit good correspondence with the CRISPR subtyping method. Notably, 22 out of 26 isolates from Europe and South America were distributed in five distinctive PSTs (with no Chinese strains). Additionally, CRISPR data of spacers and their arrangement exhibit subtle but distinct specificity between different strains, and the dynamic adaptive nature of CRISPR loci provides critical insights into the evolution of *S. Pullorum* as the bacteria are influenced by their environment. ISSN: 03781135

**Arafat, N., Eladi, A.H., Mahgoub, H., El-shafei, R.A.**

*Effect of infectious bursal disease (IBD) vaccine on Salmonella Enteritidis infected chickens (2017) Vaccine, 35 (29), pp. 3682-3689.*

**ABSTRACT:** Background Chickens infected with both infectious bursal disease virus (IBDV) and *Salmonella* had higher mortality. In this work, we investigated the effect of IBDV vaccine (modified live-virus bursal disease vaccine, Nobilis strain 228E®) on experimentally infected chickens with *Salmonella Enteritidis* (SE). Methods Four experimental groups were included in this study, negative control group, 228E® group, 228E® + SE infected group, and SE infected group. Chickens were ocularly administrated 228E® at 12 days of age and orally infected with *S. Enteritidis* at 13 days of age. Sera, intestinal fluid, blood, cloacal swabs and tissue samples were collected at 1, 2 and 3 weeks post vaccination (PV). Results The recorded mortalities were higher in the 228E® + SE infected group, compared to the SE infected group. The anti-*S. Enteritidis* serum antibody titer and the intestinal mucosal IgA level were higher in the SE infected group at 2 and 3 weeks PV, compared to 228E® + SE infected group. *S. Enteritidis* fecal shedding and organ colonization were significantly higher in the 228E® + SE infected group than the SE infected group at 2 and 3 weeks PV. The 228E® + SE group had significantly lower bursa to body weight ratios at 2 and 3 weeks PV, as well as had higher bursal lesion scores than the SE infected group. IBDV vaccine depressed the specific-SE systemic and mucosal antibody responses, but did not affect the specific-SE cellular immune responses. Conclusion Chickens administrated IBDV vaccine, followed by *S. Enteritidis* infection, could cause a significant effect on the bursa of Fabricius, resulting in failure of systemic and mucosal antibody responses to the *S. Enteritidis* and reduce the elimination and the clearance of *S. Enteritidis*. ISSN: 0264410X

**Melo, A.N.F.D., Souza, G.T.D., Schaffner, D., Oliveira, T.C.M.D., Maciel, J.F., Souza, E.L.D., Magnani, M.**

*Changes in thermo-tolerance and survival under simulated gastrointestinal conditions of Salmonella Enteritidis PT4 and Salmonella Typhimurium PT4 in chicken breast meat after exposure to sequential stresses*

*(2017) International Journal of Food Microbiology, 251, pp. 15-23.*

**ABSTRACT:** This study assessed changes in thermo-tolerance and capability to survive to simulated gastrointestinal conditions of *Salmonella Enteritidis* PT4 and *Salmonella Typhimurium* PT4 inoculated in chicken breast meat following exposure to stresses (cold, acid and osmotic) commonly imposed during food processing. The effects of the stress imposed by exposure to oregano (*Origanum vulgare* L.) essential oil (OVEO) on thermo-tolerance were also assessed. After exposure to cold stress (5 °C for 5 h) in chicken breast meat the test strains were sequentially exposed to the different stressing substances (lactic acid, NaCl or OVEO) at sub-lethal amounts, which were defined considering previously determined minimum inhibitory concentrations, and finally to thermal treatment (55 °C for 30 min). Resistant cells from distinct sequential treatments were exposed to simulated gastrointestinal conditions. The exposure to cold stress did not result in increased tolerance to acid stress (lactic acid: 5 and 2.5 µL/g) for both strains. Cells of *S. Typhimurium* PT4 and *S. Enteritidis* PT4 previously exposed to acid stress showed higher ( $p < 0.05$ ) tolerance to osmotic stress (NaCl: 75 or 37.5 mg/g) compared to non-acid-exposed cells. Exposure to osmotic stress without previous exposure to acid stress caused a salt-concentration dependent decrease in counts for both strains. Exposure to OVEO (1.25 and 0.62 µL/g) decreased the acid and osmotic tolerance of both *S. Enteritidis* PT4 and *S. Typhimurium* PT4. Sequential exposure to acid and osmotic stress conditions after cold exposure increased ( $p < 0.05$ ) the thermo-tolerance in both strains. The cells that

survived the sequential stress exposure (resistant) showed higher tolerance ( $p < 0.05$ ) to acidic conditions during continuous exposure (182 min) to simulated gastrointestinal conditions. Resistant cells of *S. Enteritidis* PT4 and *S. Typhimurium* PT4 showed higher survival rates ( $p < 0.05$ ) than control cells at the end of the in vitro digestion. These results show that sequential exposure to multiple sub-lethal stresses may increase the thermo-tolerance and enhance the survival under gastrointestinal conditions of *S. Enteritidis* PT4 and *S. Typhimurium* PT4. ISSN: 01681605

**Yachison, C.A., Yoshida, C., Robertson, J., Nash, J.H.E., Kruczkiewicz, P., Taboada, E.N., Walker, M., Reimer, A., Christianson, S., Nichani, A., Nadon, C.**

*The validation and implications of using whole genome sequencing as a replacement for traditional serotyping for a national Salmonella reference laboratory*  
(2017) *Frontiers in Microbiology*, 8 (JUN), art. no. 1044, .

ABSTRACT: *Salmonella* serotyping remains the gold-standard tool for the classification of *Salmonella* isolates and forms the basis of Canada's national surveillance program for this priority foodborne pathogen. Public health officials have been increasingly looking toward whole genome sequencing (WGS) to provide a large set of data from which all the relevant information about an isolate can be mined. However, rigorous validation and careful consideration of potential implications in the replacement of traditional surveillance methodologies with WGS data analysis tools is needed. Two in silico tools for *Salmonella* serotyping have been developed, the *Salmonella* in silico Typing Resource (SISTR) and SeqSero, while seven gene MLST for serovar prediction can be adapted for in silico analysis. All three analysis methods were assessed and compared to traditional serotyping techniques using a set of 813 verified clinical and laboratory isolates, including 492 Canadian clinical isolates and 321 isolates of human and non-human sources. Successful results were obtained for 94.8, 88.2, and 88.3% of the isolates tested using SISTR, SeqSero, and MLST, respectively, indicating all would be suitable for maintaining historical records, surveillance systems, and communication structures currently in place and the choice of the platform used will ultimately depend on the users need. Results also pointed to the need to reframe serotyping in the genomic era as a test to understand the genes that are carried by an isolate, one which is not necessarily congruent with what is antigenically expressed. The adoption of WGS for serotyping will provide the simultaneous collection of information that can be used by multiple programs within the current surveillance paradigm; however, this does not negate the importance of the various programs or the role of serotyping going forward. ISSN: 1664302X

**Channaiah, L.H., Michael, M., Acuff, J.C., Phebus, R.K., Thippareddi, H., Olewnik, M., Milliken, G.**

*Validation of the baking process as a kill-step for controlling Salmonella in muffins*  
(2017) *International Journal of Food Microbiology*, 250, pp. 1-6.

ABSTRACT: This research investigates the potential risk of *Salmonella* in muffins when contamination is introduced via flour, the main ingredient. Flour was inoculated with a 3-strain cocktail of *Salmonella* serovars (Newport, Typhimurium, and Senftenberg) and re-dried to achieve a target concentration of  $\sim 8 \log$  CFU/g. The inoculated flour was then used to prepare muffin batter following a standard commercial recipe. The survival of *Salmonella* during and after baking at 190.6 °C for 21 min was analyzed by plating samples on selective and injury-recovery media at regular intervals. The thermal inactivation parameters (D and z values) of the 3-strain *Salmonella* cocktail were determined. A  $\geq 5 \log$  CFU/g reduction in *Salmonella* population was demonstrated by 17 min of baking, and a 6.1 log CFU/g reduction in *Salmonella* population by 21 min of baking. The D-values of *Salmonella* serovar cocktail in muffin batter were  $62.2 \pm 3.0$ ,  $40.1 \pm 0.9$  and  $16.5 \pm 1.7$  min at 55, 58 and 61 °C, respectively; and the z-value was  $10.4 \pm 0.6$  °C. The water activity (aw) of the muffin crumb (0.928) after baking and 30 min of cooling was similar to that of pre-baked muffin batter, whereas the aw of the muffin crust decreased to (0.700). This study validates a typical commercial muffin baking process utilizing an oven temperature of 190.6 °C for at least 17 min as an effective kill-step in reducing a *Salmonella* serovar population by  $\geq 5 \log$  CFU/g. ISSN: 01681605

**Martelli, F., Lambert, M., Butt, P., Cheney, T., Tatone, F.A., Callaby, R., Rabie, A., Gosling, R.J., Fordon, S., Crocker, G., Davies, R.H., Smith, R.P.**

*Evaluation of an enhanced cleaning and disinfection protocol in Salmonella contaminated pig holdings in the United Kingdom*  
(2017) *PLoS ONE*, 12 (6), art. no. e0178897, .

ABSTRACT: *Salmonella* is the second most commonly reported zoonotic gastrointestinal pathogen in the European Union, and a significant proportion of the cases are linked to the consumption of contaminated pork. Reduction of *Salmonella* at the farm level helps to

minimise the contamination pressure at the slaughterhouse, and therefore the number of *Salmonella* bacteria entering the food chain. Cleaning and disinfection (C&D) between batches of pigs is an intervention measure that has potential to reduce the transmission of *Salmonella* contamination within farms. In this study, two pig finisher buildings in each of 10 *Salmonella* positive farms were sampled pre-C&D, post-C&D, post-restocking with the following batch of pigs, and shortly before these pigs were sent to slaughter. The incoming batch of pigs was also sampled before it reached the study building (pre-restocking). At each visit, pooled and individual faecal samples were collected and *Salmonella* isolation was carried out according to an ISO 6579: 2002 Annex D-based method. One building on each farm (intervention) was cleaned and disinfected according to a rigorous protocol consisting of several steps and a Defra-approved disinfectant used at the General Orders concentration, whilst the other building (control) was cleaned and disinfected as per normal farm routine. At the post-C&D visit, Enterobacteriaceae and total bacterial counts were determined to evaluate residual faecal contamination and general hygiene levels. Rodent specialists visited the farms before and after C&D and rodent carcasses were collected for *Salmonella* testing. The intervention buildings were significantly less likely ( $p = 0.004$ ) to be positive for *Salmonella* after C&D. The pre-restocking pigs had the highest likelihood ( $p < 0.001$ ) of being *Salmonella* positive (often with multiple serovars) and there was no significant difference between intervention and control buildings in *Salmonella* prevalence at the post-restocking visit ( $p = 0.199$ ). However, the pigs housed in the intervention buildings were significantly less likely ( $p = 0.004$ ) to be positive for *Salmonella* at slaughter age. Multivariable analysis suggested that cleaning all fixtures of buildings, leaving the pens empty for 2-3 days and using an effective disinfectant are factors significantly improving the likelihood of removing *Salmonella* contamination during C&D. Signs of rodents were recorded in all farms, but rodent activity and harbourage availability decreased between visits. All the rats tested were *Salmonella* negative. *S. Typhimurium* or its monophasic variants were isolated from 6 mouse carcasses in 3 farms where the same serovars were isolated from pigs. This study demonstrates that an appropriate C&D programme significantly reduces the likelihood of residual contamination in *Salmonella* positive pig buildings, and suggests a significant reduction in the prevalence of *Salmonella* in the pigs in appropriately cleaned and disinfected buildings when sampled before slaughter. Due to a high prevalence of infection in replacement pigs, control of *Salmonella* in pig farms is challenging. Rodents may also contribute to the carry-over of infection between batches. C&D is a useful measure to help reduce the number of infected pigs going to the slaughterhouse, but should be supplemented by other control measures along the pig breeding and production chain. ISSN: 19326203

**Jung, J., Friedrich, L.M., Danyluk, M.D., Schaffner, D.W.**

*Quantification of transfer of salmonella from citrus fruits to peel, edible portion, and gloved hands during hand peeling*

(2017) *Journal of Food Protection*, 80 (6), pp. 933-939.

**ABSTRACT:** Although studies have quantified bacterial transfer between hands and various materials, cross-contamination between the surface of fresh citrus fruit and the edible portions during hand peeling has not been reported. This study quantifies transfer of *Salmonella* to the edible portion of citrus fruit from a contaminated peel during hand peeling. Citrus fruits used for this study were *Citrus sinensis* (sweet orange) cultivars 'Valencia' and 'Navel', *Citrus unshiu* (Satsuma mandarins), *Citrus reticulata* 3 *Citrus paradisi* ('Minneola' tangelo or 'Honeybell'), and *C. paradisi* (grapefruit) cultivar 'Marsh'. An avirulent *Salmonella Typhimurium* LT2 (ATCC 700720) resistant to rifampin was used for all experiments. The inoculum containing approximately  $9 \log$  CFU/mL (50 IL) was spot inoculated onto the equator, stem, or styler of each fruit and allowed to dry for 24 h. Six volunteers put on single-use latex gloves and peeled inoculated fruit. Peel, edible fruit portion, and gloves were collected and enumerated separately. Three replicates of the study were performed in which each volunteer peeled two inoculated fruit of each variety (n 36 fruit per variety). Cross-contamination from contaminated surface of citrus fruits to edible portion or gloved hands during peeling was affected by inoculation sites. Average *Salmonella* transfer to the edible portion ranged from 0.16% (Valencia inoculated at the equator) to 5.41% (navel inoculated at the stem). Average *Salmonella* transfer to gloved hands ranged from 0.41% (grapefruit inoculated at the stem) to 8.97% (navel inoculated at the stem). Most *Salmonella* remained on the peel of citrus fruits. The average level of *Salmonella* remaining on the peel ranged from 5.37% (Minneola inoculated at the equator) to 66.3% (Satsuma inoculated at the styler). When grapefruit was inoculated, the *Salmonella* that remained on the peel showed a bimodal pattern in which some individuals left almost all *Salmonella* on the peel, while others left substantially less.

ISSN: 0362028X

**Youn, S.Y., Jeong, O.M., Choi, B.K., Jung, S.C., Kang, M.S.**

*Development of a Real-Time Multiplex PCR Assay with Propidium Monoazide Treatment for Simultaneous Detection of Live Salmonella, and Salmonella Enteritidis, S. Typhimurium, S. Pullorum, and S. Gallinarum, in Rinse Water of Chicken Carcasses*  
(2017) *Food Analytical Methods*, 10 (6), pp. 1681-1689.

**ABSTRACT:** Salmonella is a major food-borne pathogen in humans and a cause of local or systemic disease in animals. Therefore, rapid and reliable methods to detect these poultry-associated Salmonella serotypes are necessary for efficient control of Salmonella in poultry. The present study aimed to develop a real-time multiplex PCR (MqPCR) method to simultaneously detect and/or differentiate Salmonella sp. and poultry-associated serotypes, including Salmonella Enteritidis, Salmonella Typhimurium, Salmonella Pullorum, and Salmonella Gallinarum. A MqPCR method was designed using four specific primer pairs and probes for the detection of Salmonella sp., including S. Enteritidis, S. Typhimurium, S. Pullorum, and S. Gallinarum. Additionally, a novel TaqMan-based MqPCR method combined with propidium monoazide (PMA) treatment was developed for the simultaneous quantification of viable cells of Salmonella sp. and these four Salmonella serotypes in rinse water of chicken carcasses. The MqPCR assay specifically detected Salmonella sp., S. Enteritidis, S. Typhimurium, S. Pullorum, and S. Gallinarum, showing 100% sensitivity and 100% specificity. This optimized PMA-MqPCR assay could detect live Salmonella (100–106 CFU/reaction) without enrichment in live/dead cell mixtures from spiked rinse water of chicken carcasses. The procedure for detecting live Salmonella required less than 2 h to complete. This PMA TaqMan-based MqPCR technique facilitates accurate and rapid monitoring of contamination with viable Salmonella. Also, the assay enables simultaneous identification of S. Enteritidis, S. Typhimurium, S. Pullorum, and S. Gallinarum in rinse water of chicken carcasses. The assay developed in this study will be useful in diagnostic laboratories for improving Salmonella control in poultry and poultry products.

ISSN: 19369751

**Anderson, T.C., Marsden-Haug, N., Morris, J.F., Culpepper, W., Bessette, N., Adams, J.K., Bidol, S., Meyer, S., Schmitz, J., Erdman, M.M., Gomez, T.M., Barton Behravesh, C.**

*Multistate Outbreak of Human Salmonella Typhimurium Infections Linked to Pet Hedgehogs – United States, 2011–2013*  
(2017) *Zoonoses and Public Health*, 64 (4), pp. 290-298.

**ABSTRACT:** Zoonotic Salmonella infections cause approximately 130 000 illnesses annually in the United States. Of 72.9 million US households owning at least one pet, five million own small mammals; 3000 hedgehogs were documented by USDA in USDA-licensed breeding facilities and pet stores in 2012. State health department collaborators and PulseNet, the national bacterial subtyping network, identified human infections of a Salmonella Typhimurium outbreak strain, which were investigated by CDC, USDA-APHIS and state public and animal health officials. A case was defined as an illness in a person infected with the outbreak strain identified between 1 December 2011 and 3 June 2013. Investigators collected information on patient exposures, cultured animal and environmental specimens for Salmonella, and conducted traceback investigations of USDA-licensed hedgehog facilities. There were 26 cases in 12 states. Illness onset dates ranged from 26 December 2011 to 8 April 2013. The median patient age was 15 years (range = <1–91 years); 58% were female. Among 23 persons with available information, 8 (35%) were hospitalized and one outbreak strain-associated death was reported. Of 25 patients with available information, 20 (80%) reported pet hedgehog contact in the week before illness onset. The outbreak strain was isolated from animal and environmental samples collected from three ill persons' homes in three states. Hedgehogs were purchased in geographically distant states from USDA-licensed breeders (10/17, 59%); a USDA-licensed pet store (1/17, 6%); unlicensed or unknown status breeders (3/17, 18%); and private individuals (3/17, 18%). Traceback investigations of USDA-licensed facilities did not reveal a single source of infection. Public and animal health collaboration linked pet hedgehog contact to human infections of Salmonella Typhimurium, highlighting the importance of a One Health investigative approach to zoonotic salmonellosis outbreaks. More efforts are needed to increase awareness among multiple stakeholders on the risk of illness associated with pet hedgehogs. ISSN: 18631959

**Costard, S., Espejo, L., Groenendaal, H., Zagmutt, F.J.**

*Outbreak-related disease burden associated with consumption of unpasteurized cow's milk and cheese, United States, 2009–2014*  
(2017) *Emerging Infectious Diseases*, 23 (6), pp. 957-964.

**ABSTRACT:** The growing popularity of unpasteurized milk in the United States raises public health concerns. We estimated outbreak-related illnesses and hospitalizations caused by

the consumption of cow's milk and cheese contaminated with Shiga toxin-producing *Escherichia coli*, *Salmonella* spp., *Listeria monocytogenes*, and *Campylobacter* spp. using a model relying on publicly available outbreak data. In the United States, outbreaks associated with dairy consumption cause, on average, 760 illnesses/year and 22 hospitalizations/year, mostly from *Salmonella* spp. and *Campylobacter* spp. Unpasteurized milk, consumed by only 3.2% of the population, and cheese, consumed by only 1.6% of the population, caused 96% of illnesses caused by contaminated dairy products. Unpasteurized dairy products thus cause 840 (95% CrI 611–1,158) times more illnesses and 45 (95% CrI 34–59) times more hospitalizations than pasteurized products. As consumption of unpasteurized dairy products grows, illnesses will increase steadily; a doubling in the consumption of unpasteurized milk or cheese could increase outbreak-related illnesses by 96%. ISSN: 10806040

**Chen, Z., Jiang, X.**

*Selection of indigenous indicator micro-organisms for validating desiccation-adapted Salmonella reduction in physically heat-treated poultry litter*  
(2017) *Journal of Applied Microbiology*, 122 (6), pp. 1558-1569.

**ABSTRACT:** Aims: The thermal resistance of desiccation-adapted *Salmonella* Senftenberg 775/W was compared with those of indigenous enterococci and total aerobic bacteria in poultry litter. Methods and Results: Aged broiler litter and composted turkey litter with 20, 30, 40 and 50% moisture contents were inoculated with desiccation-adapted *Salm.* Senftenberg 775/W, and then heat-treated at 75 and 85°C. Compared to total aerobic bacteria, there were better correlations between mean log reductions of desiccation-adapted *Salm.* Senftenberg 775/W and indigenous enterococci in broiler litter samples with 20, 30, 40 and 50% moisture contents at 75°C ( $R^2 > 0.91$ ), and 20, 30 and 40% moisture contents at 85°C ( $R^2 > 0.87$ ). The mean log reductions of *Salm.* Senftenberg 775/W were better correlated with those of indigenous enterococci in turkey litter samples with 20, 30, 40 and 50% moisture contents at 75°C ( $R^2 > 0.88$ ), and 20 and 30% moisture contents at 85°C ( $R^2 = 0.83$ ) than those of total aerobic bacteria, which had a better correlation in turkey litter sample with 40% ( $R^2 = 0.98$ ) moisture content at 85°C. Conclusion: Indigenous enterococci may be used to validate the thermal processing of poultry litter, as it predicts the survival behaviour of *Salmonella* under some treatment conditions. Significance and the Impact of the Study: This study provides some scientific data for poultry litter processors when validating the effectiveness of thermal processing. ISSN: 13645072

**Acosta, O., Usaga, J., Churey, J.J., Worobo, R.W., Padilla-Zakour, O.I.**

*Effect of water activity on the thermal tolerance and survival of salmonella enterica serovars Tennessee and senftenberg in goat's milk caramel*  
(2017) *Journal of Food Protection*, 80 (6), pp. 922-927.

**ABSTRACT:** The low thermal tolerance of *Salmonella enterica* in foods with intermediate moisture levels, such as caramel sauces, ensures that mild heat treatment is sufficient to achieve 5-log reductions of this pathogen. This treatment mitigates the risk posed by salmonellae in raw materials; however, recontamination might occur because of survival of the pathogen in products that are not heated before consumption. This study was conducted to evaluate the effect of water activity ( $a_w$ ) on the thermal tolerance and survival of *S. enterica* serovars Tennessee and Senftenberg. The D-values at 76, 78, and 80°C, z-values, and survival at 20.0  $\pm$  0.58°C for 32 weeks of these two serovars were determined in goat's milk caramel at three  $a_w$  values (0.85, 0.90, and 0.93). The highest thermal tolerance was observed at  $a_w$  0.85 for *Salmonella* Senftenberg (D76°C 2.9  $\pm$  0.3 min), and the lowest was at  $a_w$  0.93 for *Salmonella* Tennessee (D80°C 0.131  $\pm$  0.007 min). After a logarithmic transformation of the z-values, a significant interaction between serovar and  $a_w$  was found ( $P < 0.0001$ ), but no consistent trends were observed at the three evaluated  $a_w$  levels for either serovar. Survival response was modeled using two sigmoidal three-parameter models. A significant interaction was found between nominal variables  $a_w$  and serovar when comparing inflection points of the resulting curves:  $P < 0.0016$  for the logistic model ( $R^2$  0.91) and  $P < 0.0014$  for the Gompertz model ( $R^2$  0.92). Although a .8-log reduction was observed at week 20 of storage, regardless of the product's  $a_w$  and the serovar, low levels of salmonellae were found in the product up to week 32 of storage. Our findings may assist the food industry with the establishment of critical limits for the safe thermal treatment of milk- and sugar-based foods with intermediate moisture levels. The survival data presented here highlight the relevance of implementing and effectively maintaining good sanitation and hygiene practices during the production of goat's milk caramel and similar food products. ISSN: 0362028X

**Lee, K.H., Ab Samad, L.S., Lwin, P.M., Riedel, S.F., Magin, A., Bashir, M., Vaishampayan, P.A., Lin, W.-J.**

*On the rocks: Microbiological quality and microbial diversity of packaged ice in southern California*

(2017) *Journal of Food Protection*, 80 (6), pp. 1041-1049.

**ABSTRACT:** Ice is defined as a food and is frequently used in direct contact with food and beverages. Packaged ice is commercially produced and can be easily found in grocery and convenience stores. However, the quality and safety of packaged ice products is not consistent. The Packaged Ice Quality Control Standards manual (PIQCS) published by the International Packaged Ice Association provides the quality and processing standards for packaged ice produced by its members. Packaged ice produced on the premise of stores (on-site packaged ice) is not required to be in compliance with these standards. In this study, packaged ice produced by manufacturing plants or by in-store bagger (ISB) machines and on-site packaged ice were compared for their microbiological quality and microbial diversity. Our results revealed that 19% of the 120 on-site packaged ice samples did not meet the PIQCS microbial limit of 500 CFU/mL (or g) and also the absence of coliforms and *Escherichia coli*. Staphylococci were found in 34% of the on-site packaged ice samples, most likely through contamination from the packaging workers. None of the ISB and manufactured packaged ice samples had unacceptable microbial levels, and all were devoid of staphylococci. *Salmonella* was absent in all samples analyzed in this study. Microbial community analysis of ice based on 16S/18S rRNA targeted sequencing revealed a much higher microbial diversity and abundance in the on-site packaged ice than in the ISB ice. Proteobacteria, especially Alphaproteobacteria and Betaproteobacteria, were the dominant bacterial groups in all samples tested. Most of these bacteria were oligotrophic; however, a few opportunistic or potential pathogens were found at low levels in the on-site packaged ice but not in the ISB packaged ice. The types of microbes identified may provide information needed to investigate potential sources of contamination. Our data also suggest a need for enforcement of processing standards during the on-site packaging of ice. ISSN: 0362028X

**Bonardi, S.**

*Salmonella in the pork production chain and its impact on human health in the European Union*

(2017) *Epidemiology and Infection*, 145 (8), pp. 1513-1526.

**ABSTRACT:** *Salmonella* spp. comprise the second most common food-borne pathogens in the European Union (EU). The role of pigs as carriers of *Salmonella* has been intensively studied both on farm and at slaughter. *Salmonella* infection in pigs may cause fever, diarrhoea, prostration and mortality. However, most infected pigs remain healthy carriers, and those infected at the end of the fattening period could pose a threat to human health. Contamination of pig carcasses can occur on the slaughter line, and it is linked to cross-contamination from other carcasses and the presence of *Salmonella* in the environment. Therefore, *Salmonella* serovars present on pig carcasses can be different from those detected in the same fates on the farm. In recent years, *S. Typhimurium*, *S. Derby* and *S. serotype 4,[5],12:i:-* (a monophasic variant of *S. Typhimurium*) have been the most common serovars to be detected in pigs in EU countries, but *S. Rissen*, *S. Infantis*, *S. Enteritidis* and *S. Brandenburg* have also been reported. In humans, several cases of salmonellosis have been linked to the consumption of raw or undercooked pork and pork products. Among the main serovars of porcine origin detected in confirmed human cases, *S. Typhimurium*, the monophasic variant *S. 4,[5],12:i:-* and *S. Derby* are certainly the most important. ISSN: 09502688

**Li, L., Cepeda, J., Subbiah, J., Froning, G., Juneja, V.K., Thippareddi, H.**

*Dynamic predictive model for growth of Salmonella spp. in scrambled egg mix*  
(2017) *Food Microbiology*, 64, pp. 39-46.

**ABSTRACT:** Liquid egg products can be contaminated with *Salmonella* spp. during processing. A dynamic model for the growth of *Salmonella* spp. in scrambled egg mix - high solids (SEM) was developed and validated. SEM was prepared and inoculated with ca. 2 log CFU/mL of a five serovar *Salmonella* spp. cocktail. *Salmonella* spp. growth data at isothermal temperatures (10, 15, 20, 25, 30, 35, 37, 39, 41, 43, 45, and 47 °C) in SEM were collected. Baranyi model was used (primary model) to fit growth data and the maximum growth rate and lag phase duration for each temperature were determined. A secondary model was developed with maximum growth rate as a function of temperature. The model performance measures, root mean squared error (RMSE, 0.09) and pseudo-R<sup>2</sup> (1.00) indicated good fit for both primary and secondary models. A dynamic model was developed by integrating the primary and secondary models and validated using two sinusoidal temperature profiles, 5–15 °C (low temperature) for 480 h and 10–40 °C (high

temperature) for 48 h. The RMSE values for the sinusoidal low and high temperature profiles were 0.47 and 0.42 log CFU/mL, respectively. The model can be used to predict *Salmonella* spp. growth in case of temperature abuse during liquid egg processing.  
ISSN: 07400020

**Harvey, R.R., Heiman Marshall, K.E., Burnworth, L., Hamel, M., Tataryn, J., Cutler, J., Meghnath, K., Wellman, A., Irvin, K., Isaac, L., Chau, K., Locas, A., Kohl, J., Huth, P.A., Nicholas, D., Traphagen, E., Soto, K., Mank, L., Holmes-Talbot, K., Needham, M., Barnes, A., Adcock, B., Honish, L., Chui, L., Taylor, M., Gaulin, C., Bekal, S., Warshawsky, B., Hobbs, L., Tschetter, L.R., Surin, A., Lance, S., Wise, M.E., Williams, I., Gieraltowski, L.**

*International outbreak of multiple Salmonella serotype infections linked to sprouted chia seed powder - USA and Canada, 2013-2014*

(2017) *Epidemiology and Infection*, 145 (8), pp. 1535-1544.

ABSTRACT: *Salmonella* is a leading cause of bacterial foodborne illness. We report the collaborative investigative efforts of US and Canadian public health officials during the 2013-2014 international outbreak of multiple *Salmonella* serotype infections linked to sprouted chia seed powder. The investigation included open-ended interviews of ill persons, traceback, product testing, facility inspections, and trace forward. Ninety-four persons infected with outbreak strains from 16 states and four provinces were identified; 21% were hospitalized and none died. Fifty-four (96%) of 56 persons who consumed chia seed powder, reported 13 different brands that traced back to a single Canadian firm, distributed by four US and eight Canadian companies. Laboratory testing yielded outbreak strains from leftover and intact product. Contaminated product was recalled. Although chia seed powder is a novel outbreak vehicle, sprouted seeds are recognized as an important cause of foodborne illness; firms should follow available guidance to reduce the risk of bacterial contamination during sprouting. ISSN: 09502688

**Carroll, L.M., Wiedmann, M., den Bakker, H., Siler, J., Warchocki, S., Kent, D., Lyalina, S., Davis, M., Sischo, W., Besser, T., Warnick, L.D., Pereira, R.V.**

*Whole-genome sequencing of drug-resistant Salmonella enterica isolates from dairy cattle and humans in New York and Washington States reveals source and geographic associations*

(2017) *Applied and Environmental Microbiology*, 83 (12), art. no. e00140-17, .

ABSTRACT: Multidrug-resistant (MDR) *Salmonella enterica* can be spread from cattle to humans through direct contact with animals shedding *Salmonella* as well as through the food chain, making MDR *Salmonella* a serious threat to human health. The objective of this study was to use whole-genome sequencing to compare antimicrobial-resistant (AMR) *Salmonella enterica* serovars Typhimurium, Newport, and Dublin isolated from dairy cattle and humans in Washington State and New York State at the genotypic and phenotypic levels. A total of 90 isolates were selected for the study (37 S. Typhimurium, 32 S. Newport, and 21 S. Dublin isolates). All isolates were tested for phenotypic antibiotic resistance to 12 drugs using Kirby-Bauer disk diffusion. AMR genes were detected in the assembled genome of each isolate using nucleotide BLAST and ARG-ANNOT. Genotypic prediction of phenotypic resistance resulted in a mean sensitivity of 97.2 and specificity of 85.2. Sulfamethoxazole-trimethoprim resistance was observed only in human isolates ( $P < 0.05$ ), while resistance to quinolones and fluoroquinolones was observed only in 6 S. Typhimurium isolates from humans in Washington State. S. Newport isolates showed a high degree of AMR profile similarity, regardless of source. S. Dublin isolates from New York State differed from those from Washington State based on the presence/absence of plasmid replicons, as well as phenotypic AMR susceptibility/nonsusceptibility ( $P < 0.05$ ). The results of this study suggest that distinct factors may contribute to the emergence and dispersal of AMR S. enterica in humans and farm animals in different regions.  
ISSN: 00992240

**Sommers, C., Gunther, N.W., IV, Sheen, S.**

*Inactivation of Salmonella spp., pathogenic Escherichia coli, Staphylococcus spp., or Listeria monocytogenes in chicken purge or skin using a 405-nm LED array*

(2017) *Food Microbiology*, 64, pp. 135-138.

ABSTRACT: Raw poultry are sometimes contaminated with foodborne pathogens, which can lead to illness in humans. In recent years research has focused on a variety of light technologies to decontaminate food and food contact surfaces during meat and poultry processing. In this study we evaluated the ability of 405-nm light generated from an LED array to inactivate multi-isolate cocktails of either *Salmonella* spp., pathogenic *Escherichia coli*, *Staphylococcus* spp., or *Listeria monocytogenes* suspended in chicken purge or skin. When exposed to 180 J/cm<sup>2</sup> 405-nm light at two separate light intensities (300 mW/cm<sup>2</sup>/s

or 150 mW/cm<sup>2</sup>/s) the maximum pathogen reduction on chicken skin was ca. 0.4 log. When the pathogens were suspended in chicken purge the maximum log reductions ranged from 0.23 to 0.68 log (180 J/cm<sup>2</sup>; 150 mW/cm<sup>2</sup>/s) versus 0.69 to 1.01 log (180 J/cm<sup>2</sup>; 300 mW/cm<sup>2</sup>/s). Log reductions of each pathogen, when they were subjected to heat shock prior to 405-nm light treatment, were reduced, indicating that thermal effects accounted for much of the bacterial inactivation. ISSN: 07400020

**Jeong, S.-G., Kang, D.-H.**

*Inactivation of Escherichia coli O157:H7, Salmonella Typhimurium, and Listeria monocytogenes in ready-to-bake cookie dough by gamma and electron beam irradiation (2017) Food Microbiology, 64, pp. 172-178.*

ABSTRACT: This study was conducted to investigate the efficacy of gamma and electron beam irradiation to inactivate foodborne pathogens in ready-to-bake cookie dough and to determine the effect on quality by measuring color and texture changes. Cookie dough inoculated with *Escherichia coli* O157:H7, *Salmonella Typhimurium*, or *Listeria monocytogenes* was subjected to gamma and electron beam irradiation, with doses ranging from 0 to 3 kGy. As the radiation dose increased, the inactivation effect increased among all tested pathogens. After 3.0 kGy of gamma and electron beam irradiation, numbers of inoculated pathogens were reduced to below the detection limit (1 log CFU/g). The D10-values of *E. coli* O157:H7, *S. Typhimurium*, and *L. monocytogenes* in cookie dough treated with gamma rays were 0.53, 0.51, and 0.71 kGy, respectively, which were similar to those treated by electron beam with the same dose. Based on the D10-value of pathogens in cookie dough, *L. monocytogenes* showed more resistance to both treatments than did *E. coli* O157:H7 and *S. Typhimurium*. Color values and textural characteristics of irradiated cookie dough were not significantly ( $P > 0.05$ ) different from the control. These results suggest that irradiation can be applied to control pathogens in ready-to-bake cookie dough products without affecting quality. ISSN: 07400020

**Adhikari, P., Cosby, D.E., Cox, N.A., Kim, W.K.**

*Colonization of mature laying hens with Salmonella Enteritidis by oral or intracloacal inoculation (2017) Journal of Applied Poultry Research, 26 (2), pp. 286-294.*

ABSTRACT: Evidence of *Salmonella Enteritidis* (SE) in internal organs of White Leghorns once they are inoculated via the oral (OR) or intracloacal (IC) route has not been consistently demonstrated. The aim of the current study was to evaluate OR or IC inoculation route of a nalidixic acid (Nal) resistant SE (SENAR) on the SE colonization of ceca and the invasion of internal organs in mature White Leghorns. Five experiments were conducted, and hens were inoculated with 10<sup>8</sup> colony-forming units (cfu) of SENAR. Hens were euthanized at 7 and 14 d post inoculation (dpi), and the ceca, spleen, liver with gall bladder (L/GB), and ovaries were collected for bacteriological analyses. The recovery of SENAR in ceca was 100% at 7 dpi. Recovery from the ovaries was lower than the other organs for both routes of inoculation. The SE recovery of L/GB, spleen, and ovaries at 7 dpi was not different between the two routes. By 14 dpi, all organs approached negative, and the recovery rate was similar between OR and IC. Fecal shedding was 100% positive at 3 dpi and reduced to almost 0% by 14 dpi. Mature hens were colonized by SENAR with either OR or IC inoculation when using a larger volume and a higher cfu/mL (0.1 mL OR in experiment 1 vs. 1.0 mL OR and IC in the rest). SENAR showed some translocation into other organs, to a greater extent with IC. The colonization did not persist either in ceca or the internal organs at 14 dpi. ISSN: 10566171

**Chen, Z., Jiang, X.**

*Thermal resistance and gene expression of both desiccation-adapted and rehydrated Salmonella enterica serovar Typhimurium cells in aged broiler litter (2017) Applied and Environmental Microbiology, 83 (12), art. no. e00367-17, .*

ABSTRACT: The objective of this study was to investigate the thermal resistance and gene expression of both desiccation-adapted and rehydrated *Salmonella enterica* serovar *Typhimurium* cells in aged broiler litter. *S. Typhimurium* was desiccation adapted in aged broiler litter with a 20% moisture content (water activity [aw], 0.81) for 1, 2, 3, 12, or 24 h at room temperature and then rehydrated for 3 h. As analyzed by quantitative real-time reverse transcriptase PCR (qRT-PCR), the *rpoS*, *proV*, *dnaK*, and *grpE* genes were upregulated ( $P < 0.05$ ) under desiccation stress and could be induced after 1 h but in less than 2 h. Following rehydration, fold changes in the levels of these four genes became significantly lower ( $P < 0.05$ ). The desiccation-adapted  $\Delta$ *rpoS* mutant was less heat resistant at 75°C than was the desiccation-adapted wild type ( $P < 0.05$ ), whereas there were no differences in heat resistance between desiccation-adapted mutants in two nonregulated genes (*otsA* and *PagfD*) and the desiccation-adapted wild type ( $P > 0.05$ ).

Survival characteristics of the desiccation-adapted  $\Delta$ PagfD (rdar [red, dry, and rough] morphotype) and  $\Delta$ agfD (saw [smooth and white] morphotype) mutants were similar ( $P > 0.05$ ). Trehalose synthesis in the desiccation-adapted wild type was not induced compared to a nonadapted control ( $P > 0.05$ ). Our results demonstrated the importance of the *rpoS*, *proV*, *dnaK*, and *grpE* genes in the desiccation survival of *S. Typhimurium*. By using an  $\Delta$ rpoS mutant, we found that the *rpoS* gene was involved in the crossprotection of desiccation-adapted *S. Typhimurium* against high temperatures, while trehalose synthesis or rdar morphology did not play a significant role in this phenomenon. In summary, *S. Typhimurium* could respond rapidly to low-aw conditions in aged broiler litter while developing cross-protection against high temperatures, but this process could be reversed upon rehydration. ISSN: 00992240