

NEWSLETTER

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Editorial Note

Bilthoven, 1 October 2018

Dear colleague,

I hope you all have had a wonderful and relaxing summer! Here in the Netherlands the temperatures regularly touched 30 °C or more during this summer, which is quite extreme.

Shortly before the summer break, our **colleague** Angelina Kuijpers informed us that she had found a new job and would leave the RIVM per 1 October 2018. We are happy for her that she has found a new challenge, but we are a bit sad that 12 years of good cooperation came to an end. We would like to thank her very much for all her great work for the EURL-*Salmonella* and would like to wish her all the best in her new job!

To make sure that the activities for the EURL-*Salmonella* would continue, we started a search for a new colleague immediately after the summer break. We are very happy that we can already introduce to you our new colleague for the EURL-*Salmonella*: Robin Diddens. Since 2015, Robin works at the RIVM and he was very interested in a new challenge at the EURL-*Salmonella*. In the coming months Robin will need to get settled in the job and he may approach you later this year to inform you about a next interlaboratory comparison study for detection of *Salmonella* in food or animal feed. We would like to wish Robin much success in his new job!

In our previous Newsletter I already informed you that we had to change to new software for management of our **website**. In the meantime this process has been finalised and you may already have noticed that the lay-out of the website has changed. If you did not yet notice, please have a look at www.eurlsalmonella.eu. With the migration to the new version of the website, we were no longer able to include all 'old documents' on the website. However, all documents published until July 2018 can still be found in the archive of the 'old' website. You can find a link to this archive at the bottom of each page of the website.

Also in the previous Newsletter, I promised you to give a summary on relevant '*Salmonella*-related items' as discussed at **the annual meeting of ISO/TC34/SC9 and CEN/TC275/WG6** organised in Lausanne, Switzerland, from 18 to 22 June 2018. To fulfil this promise, please find this summary enclosed in this Newsletter.

At the EURL-*Salmonella* workshop in May this year, Henry Kuronen from the NRL-*Salmonella* in Finland informed us about a document he drafted to give hints in case of problems with **MSRV-agar**. He kindly shared with us this document on **troubleshooting**, which is enclosed in this Newsletter as well.

Also included (again) in this Newsletter, is the (updated) timetable of the **23rd interlaboratory comparison study on typing of *Salmonella***, which will be organised in fall 2018. If we will receive a sufficient number of applications for it, this typing study will also include the first pilot study on MLVA typing.

Last, but not least, I need to inform you that still emails are sent around in name of one or more of the staff members of the EURL-*Salmonella*, which we did not send. Very annoying and, according to our IT department, very difficult to avoid. Therefore, I need to warn you again to be careful with dodgy emails which seem to be sent by one of us, but are not. Especially, take care not to open

'strange' links and/or attachments to emails. In case you have doubts with an email sent in our name, please ask us to confirm if the email is genuine. Better safe than sorry. I am very sorry for this inconvenience.

Best wishes,
Kirsten Mooijman
Coordinator EURL-*Salmonella*

Contribution of EURL-*Salmonella*

Timetable of the 23rd interlaboratory comparison study (2018) on serotyping and optional PFGE typing and/or MLVA typing of *Salmonella* for NRLs-*Salmonella*

Week	Date	Topic
39	25 September	Emailing of the link to the registration form for the typing study. Please register by 17 October at the latest.
43	22-26 October	Emailing of the protocol 2018.
45	5-9 November	Shipment of the parcels to the participants as Biological Substance Category B (UN 3373). If you did not receive the parcel by 9 November , please contact the EURL- <i>Salmonella</i> .
45	5-9 November	Sending the link and the password for the web based test report on serotyping to the participants.
45	5-9 November	Sending the link for the dedicated web based test report on PFGE typing to the participants in a separate email. The pre-configured database and instructions for use in case of (optional) analysis of a provided gel in Bionumerics will be included in this email as well.
45	5-9 November	Sending the link for the dedicated web based test report on MLVA typing to the participants in a separate email.
45	5-9 November	<i>Upon receipt:</i> Starting the identification of the strains, according to the usual practice of the laboratory.
50	14 December 2018 at the latest	Deadline for completing the electronic submission of serotyping results: 14 December 2018 (23:59 h CET) After this deadline, the electronic submission form for serotyping results will be closed.
51	21 December 2018 at the latest	Deadline for completing the electronic submission of PFGE typing results: 21 December 2018 Deadline for completing the electronic submission of MLVA typing results: 21 December 2018
	February 2019	Serotyping: Reporting of individual laboratory results and Interim Summary Report.
	April 2019	Pilot MLVA typing: Reporting of individual laboratory results and Summary Report.
	May 2019	PFGE typing: Reporting of individual laboratory results and Summary Report.
	Summer 2019	Final report.

Summary of '*Salmonella*-related items' as discussed at the annual meetings of ISO/TC34/SC9 and CEN/TC275/WG6 Lausanne, Switzerland, 18-22 June 2018

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 standardisation organisation), and were hosted by the Swiss Association for Standardization (SNV), held at Nestlé Research, Lausanne, Switzerland. Approximately 70 delegates attended the 37th SC9 meeting and 25th WG6 meeting, representing 24 organisations (including 8 microbiological EURLs).

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

CEN Mandate M/381 (CEN lead)

All 15 EN ISO standards under the CEN Mandate were published in 2017, including performance characteristics. The final report was sent to EC DG-SANTE in September 2017. It was agreed to share this final report with the members of WG6.

For the validation study of each individual standard under the CEN Mandate, manuscripts have been drafted to become published in a special issue of the International Journal of Food Microbiology (including the validation of EN ISO 6579-1 for detection of *Salmonella*).

ISO Ad hoc group '*Harmonisation of selective incubation temperatures (35 °C vs 37 °C)*'

The project leader of the Ad hoc group, Kirsten Mooijman (EURL-*Salmonella*), presented the report of the experiments performed by the members of ISO/TC34/SC9 to compare incubation of selective media for detection of *Salmonella* at 35°C and at 37 °C.

By June 2017, results were received from 9 laboratories, representing 6 countries, resulting in a total of 855 tests! Kirsten, together with Daniele Sohler (Germany) and Maryse Rannou (France) performed the analysis of the (confirmed) data, which was presented at the meeting of SC9. The members of SC9 agreed with the conclusions of the Ad hoc group:

- The overall results showed similar sensitivity results: 97,5% for incubation of the selective media at 37 °C and 98,3% for incubation at 35 °C.
- The data interpretation in relation to the deviating results fulfilled both proposed 'amended' acceptability limits.
- The reported amount of background flora after incubation of the selective media at 35 °C or at 37 °C was comparable.

These results indicate that comparable results are obtained when incubating selective media for detection of *Salmonella* at 35 °C and at 37 °C. It can therefore be concluded that for incubation of these selective media also a temperature range of 34-38 °C can be used (like earlier agreed for the incubation of non-selective media).

It was agreed that the information on extension of the temperature range for incubation of selective media will be added to the amendment to be drafted for EN ISO 6579-1.

Additionally it was discussed whether there is a need to perform similar studies for other pathogens. It was agreed that in case harmonisation of incubation temperatures of selective media is needed, this needs to be tested per pathogen. However, there is no immediate need to start similar studies for other pathogens. As soon as a standard will be revised it will also be considered to perform a comparison study for incubation of selective media at 35 °C and at 37 °C.

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

Earlier in 2018, a written consultation of Recommendation N.472 took place to ask for agreement to publish a correction or amendment of two EN ISO standards because of mistakes detected in these documents after publication. This concerned EN ISO 6579-1 (Detection of *Salmonella*) and EN ISO 21528-2 (Enumeration of *Enterobacteriaceae*). During the consultation it was also possible to indicate other mistakes.

A few additional comments were given to Annex D of EN ISO 6579-1 (detection of *Salmonella* Typhi and *Salmonella* Paratyphi) concerning possible mistakes, of which one concerns the French version of EN ISO 6579-1. Another comment concerns the harmonisation with the full text of the EN ISO document and it was agreed to include this in the amendment together with the correction of the composition of Selenite Cystine broth (original correction of Recommendation N.472). This latter correction concerns the fact that in EN ISO 6579-1:2017 it is indicated that 100 ml L-cystine solution (D.3.1.2) should be added to 1000 ml base medium, but this has to be only 10 ml. In addition to the current comments it was indicated that many questions were posed whether Annex D should always be followed when analysing 'routine' samples as Annex D is normative. Kirsten clarified that this is not the case and that Annex D should only be used in case *S. Typhi* and *S. Paratyphi* are specifically sought. However, as it does not seem to be clear to the users of EN ISO 6579-1 it was agreed to publish an amendment, including the corrections indicated above and changing Annex D from normative to informative. This proposal was agreed by at least 5 countries (FR, DE, NL, CH, BE) and it was agreed that Kirsten Mooijman (EURL-*Salmonella*) will become the project leader.

In addition to the corrections mentioned above, also the broadening of the temperature range for incubation of selective media from 37 °C ± 1 °C to 34-38 °C will be introduced in the amendment.

PCR identification of monophasic *S. Typhimurium* (ISO/TS 6579-4)

In May 2016, SC9 agreed to register the Preliminary Work Item (PWI) of ISO 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).

The EURL-*Salmonella* has made a selection of 172 out of 400 test strains (target and non-target strains) to test the three PCR protocols of 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. When the technical work is finished 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 (ILS) will be planned to determine the performance characteristics. The timing of this ILS is not yet sure.

Contribution of NRL-*Salmonella*

Troubleshooting of Modified semi-solid Rappaport Vassiliadis (MSRV) agar

Henry Kuronen, NRL-*Salmonella* Finland, Finnish Food Safety Authority Evira

Possible problems with MSRV agar

Modified semi-solid Rappaport Vassiliadis (MSRV) agar is a selective enrichment medium and is used for the detection of *Salmonella* in food, animal feed and samples from the primary production stage (EN ISO 6579-1:2017).

Occasionally problems may occur when using MSRV agar which can be caused by:

1. Preparation of MSRV-agar plates.
2. Storage of MSRV-agar plates.
3. Use of MSRV agar.

Below the different causes of possible problems are discussed in more detail.

1. Possible problems caused during preparation of MSRV agar plates

Below possible causes for deviating results related to the preparation of MSRV agar plates are listed:

- Too long heating time:
 - It should be avoided to keep the medium at high temperatures longer than necessary, as too long heating can result in liquefaction of the semi-solid agar (during storage or incubation). MSRV agar which is liquified during storage shall not be used.
 - Too long heating can result in 'loose' composition of the medium, resulting in handling problems and higher risks for cross-contamination.
- Deviating pH:
 - The prescribed pH is 5,1-5,4. Some media producers sell MSRV agar with a pH range of $5,4 \pm 0,2$. In these cases the pH of the MSRV agar can be higher than prescribed and can result in unspecific swarming of other bacteria than *Salmonella*.
- Deviating concentration of novobiocin:
 - The prescribed concentration novobiocin in MSRV agar is 10 mg/l. Some producers make MSRV agar with the 'old' concentration of 20 mg/l. Higher concentrations novobiocin than prescribed can negatively affect the swarming of *Salmonella* and thus may have an effect on the detection of *Salmonella*.
- Plates are moved during solidification:
 - The concentration of agar is very low so that the construction can break easily.
 - It is advisable not to move the plates after preparation and to keep the plates at room temperature before moving them to cold storage the day after preparation.
- Plates are too dry:
 - There is large risk of over-drying when the plates are dried in a laminar air flow cabinet or in an incubator. It may be better to store the plates at room temperature during solidification before moving them to cold storage the day after preparation.

2. Possible problems caused during storage of MSRV agar plates

Below possible causes for deviating results related to the storage of MSRV agar plates are listed:

- Too many movements of the plates:
 - Limit the actions which can influence the construction of the semi-solid agar, like moving, shaking, over-drying.
 - The plates shall not be inverted, the agar is too sloppy to do so.
 - If plates can be inverted without affecting the construction of the semi-solid agar, something went wrong with the preparation of the medium (e.g. too high concentration of agar).
- Too long storage time:
 - The prescribed storage time of the plates is 2 weeks. Longer storage time may affect the quality of the plates, like drying, fragmentation, crystallisation of the medium which negatively affects the swarming of *Salmonella* on the plate.

3. Possible problems when using MSRV agar

Below possible causes for deviating results related to the use of MSRV agar plates are listed:

- Problems with BPW culture:
 - If the amount of BPW was too low in relation to the amount of sample (no 1 to 10 dilution or e.g. swab or boot sock not fully submerged in BPW), this will lower the resuscitation effect.
 - If the amount of BPW was too high in relation to the amount of sample, it will be more difficult to detect low numbers of *Salmonella*.
 - Do not mix, shake or swirl the BPW culture before taking the subculture, as floating particles can disturb the growth on MSRV agar. Take an inoculum from the largest volume of free fluid.
- Problems with subculturing from suspect MSRV plates:
 - For subculturing, material should be taken from the furthest point of spread of opaque growth from the inoculation point. If the inoculum is taken near the inoculation point this may give a higher chance on a mixed culture of *Salmonella* and other *Enterobacteriaceae*.
 - The presence of high amounts of *Enterobacteriaceae* (like *Enterobacter cloacae*) can influence the swarming of *Salmonella*.
 - If no swarming occurs on the MSRV agar, but the presence of non motile *Salmonella* (very rare) is suspected, it is advisable to take a subculture from the inoculation point and/or inoculate a selective enrichment broth (RVS, MKTTn) in parallel to MSRV agar.
- Evaporation of water from MSRV agar during 48 h incubation at 41,5 °C:
 - From an experiment in one Finnish laboratory it was noticed that 8-11% of water may evaporate from MSRV during incubation (measured by loss of weight). Too much evaporation may dry the plates and can influence the swarming on the plate.
- Possible risk factors for cross-contamination:
 - Too 'loose' MSRV agar.
 - Contaminated pipettes.
 - Dosing devices, e.g. for dosing BPW to the sample.
 - Aerosols or dust in the environment.

Reference

EN ISO 6579-1:2017. Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of *Salmonella* - Part 1: Detection of *Salmonella* spp. International Organization of Standardization, Switzerland.

From the Literature

Salmonella-related Literature from Scopus: July – September 2018

Sharma, P., Caraguel, C., Sexton, M., McWhorter, A., Underwood, G., Holden, K., Chousalkar, K.

Shedding of Salmonella Typhimurium in vaccinated and unvaccinated hens during early lay in field conditions: A randomised controlled trial

(2018) *BMC Microbiology*, 18 (1), art. no. 78, .

ABSTRACT: Background: Salmonella vaccination is one of the control measure that farmers can use to reduce bacterial shedding in their flocks. This study aimed to examine the efficacy of the Vaxsafe® ST (Strain STM-1) attenuated live vaccine administered as ocular and oral doses followed by an intramuscular (IM) dose in rearing, in reducing contamination by Salmonellae of both eggs and the environment in the commercial multi-age cage layer sheds. A randomised controlled trial was conducted up to 26 weeks post last vaccine on two different multi-age caged egg farms. Results: No clinical symptoms were observed following IM administration of STM-1 during rearing. Following the first two STM-1 doses, both vaccinated and unvaccinated birds exhibited antibody titres below the positive cut-off value, however after IM administration of STM-1, antibody titres in the vaccinated group were above the cut-off value. Wild type Salmonella Typhimurium was not detected during the rearing of pullets. During production, the antibody titres were significantly higher in the vaccinated group at all sampling points during this trial. There was no significant difference in the prevalence of Salmonella (detected by culture and PCR method) between the vaccinated and unvaccinated groups on the egg belt and faeces in early lay. Wild-type Salmonella spp. were consistently found in dust samples. Quantitative PCR (qPCR) assay was able to differentiate between the live vaccine strain and wild type Salmonella. The load of wild-type Salmonella in shed environment was relatively low (1.3 log₁₀ ± 0.48 CFU/m² of surface area). Conclusion: Given that Salmonella Typhimurium and other serovars are able to survive/persist in the shed environment (such as in dust), regular cleaning and or removal of dust from shed is important. Use of the Vaxsafe® ST vaccine in multi-age flocks is "not an ultimate intervention" for reduction of Salmonella Typhimurium because of the complexities involved in achieving control, such as the efficacy of cleaning of sheds, the lack of resting periods between batches and the possible carry over of contamination from existing flocks. Hence implementation of more than one or several interventions strategies is essential. ISSN: 14712180

Mancin, M., Barco, L., Losasso, C., Belluco, S., Cibin, V., Mazzucato, M., Bilei, S., Carullo, M.R., Decastelli, L., Di Giannatale, E., D'Incau, M., Goffredo, E., Lollai, S., Piraino, C., Scuota, S., Staffolani, M., Tagliabue, S., Ricci, A.

Salmonella serovar distribution from non-human sources in Italy; Results from the IT-Enter-Vet network

(2018) *Veterinary Record*, 183 (2), p. 69.

ABSTRACT: The study summarises the results obtained over the period 2002-2013 by the Italian IT-Enter-Vet network, aimed at collecting data on Salmonella isolates from non-human sources. A total of 42,491 Salmonella isolates were reported with a progressive decrease over the years. S. Typhimurium was the most frequent serovar up to 2011, but then, it was overtaken by S. 4,[5],12,:i:-, S. Derby, S. Livingstone and S. Enteritidis alternated as the third most commonly isolated serovars. With regard to the sources of isolation, S. Typhimurium was distributed ubiquitously among the animal species. On the contrary, S. 4,[5],12,:i:- and S. Derby were strictly associated with pigs, whereas S. Livingstone, S. Enteritidis and S. Infantis were clearly related to poultry. Intriguingly, when the frequency of serovar distribution along the food chain was considered, it was evident that S. Typhimurium and S. Derby tended to persist along the chain, as they were isolated even more frequently from foods than from animals. A similar distribution was found for S. Enteritidis and S. Hadar. Despite limitations related to non-mandatory participation of laboratories in the network, the data presented are valuable to obtain a picture of the evolution of Salmonella from non-human sources over time in Italy. ISSN: 00424900

Li, P., Fan, W., Everaert, N., Liu, R., Li, Q., Zheng, M., Cui, H., Zhao, G., Wen, J.

Messenger RNA sequencing and pathway analysis provide novel insights into the susceptibility to Salmonella enteritidis Infection in Chickens

(2018) *Frontiers in Genetics*, 9 (JUL), art. no. 256, .

ABSTRACT: *Salmonella enteritidis* (SE) is a foodborne pathogen that negatively affects both animal and human health. Controlling poultry SE infection will have great practical significance for human public health, as poultry are considered to be important sources and carriers of the disease. In this study, the splenic transcriptomes of challenged-susceptible (S), challenged-resistant (R) and non-challenged (C) chicks (3-days old, specific-pathogen-free White Leghorn) were characterized in order to identify the immune-related gene markers and pathways. A total of 934 significant differentially expressed genes (DEGs) were identified in comparisons among the C, R and S birds. First reported here, the DEGs involved in the Forkhead box O (FoxO) signaling pathway, especially FoxO3, were identified as potential markers for host resistance to SE infection. The challenged-susceptible birds exhibited strong activation of the FoxO signaling pathway, which may be a major defect causing immune cell apoptosis as part of SE-induced pathology; these S birds also showed weak activation of mitogen-activated protein kinase (MAPK)-related genes, contrasting with strong splenic activation in the R birds. Interestingly, suppression of several pathways in the immune response against *Salmonella*, including cytokine-cytokine receptor interaction and Jak-STAT, was only found in S birds and there was evidence of cross-talk among these pathways, perhaps contributing to susceptibility to *Salmonella* infection. These findings will help facilitate understanding resistance and susceptibility to SE infection in the earliest phases of the host immune response through *Salmonella*-induced pathways, provide new approaches to develop strategies for SE prevention and treatment, and may enhance innate resistance by genetic selection in animals. ISSN: 16648021

Mathew, E.N., Muyyarikkandy, M.S., Kuttappan, D., Amalaradjou, M.A.

Attachment of Salmonella enterica on mangoes and survival under conditions simulating commercial mango packing house and importer facility
(2018) *Frontiers in Microbiology*, 9 (JUL), art. no. 1519, .

ABSTRACT: Consumption of raw mangoes has led to multiple *Salmonella*-associated foodborne outbreaks in the United States. Although several studies have investigated the epiphytic fitness of *Salmonella* on fresh produce, there is sparse information available on the survival of *Salmonella* on mangoes under commercial handling and storage conditions. Hence, the objective of the study was to evaluate the survival of *Salmonella* on mangoes under ambient conditions simulating the mango packing house and importer facility. Further, the ability of the pathogen to adhere and attach on to the mango fructoplane was also investigated. For the attachment assays, mango skin sections were inoculated with fifty microliters of *S. Newport* suspension (6.5 log CFU/skin section) and minimum time required for adhesion and attachment were recorded. With the survival assays, unwaxed mangoes were spot inoculated with the *Salmonella* cocktail to establish approximately 4 and 6.5 log CFU/mango. The fruits were then subjected to different storage regimens simulating fruit unloading, waxing, and storage at the packing house and ripening and storage at the importer facility. Results of our study reveal that *Salmonella* was able to adhere on to the fructoplane immediately after contact. Further, formation of attachment structures was seen as early as 2 min following inoculation. With the survival assays, irrespective of the inoculum levels, no significant increase or decrease in pathogen population was observed when fruit were stored either at ambient (29-32°C and RH 85-95%, for 48 h), ripening (20-22°C and RH 90-95% for 9 days) or refrigerated storage (10-15°C and 85-95% for 24-48 h) conditions. Therefore, once contaminated, mangoes could serve as potential vehicles in the transmission of *Salmonella* along the post-harvest environment. Hence development and adoption of effective food safety measures are warranted to promote the microbiological safety of mangoes. ISSN: 1664302X

Xie, J., Wu, F., Xu, X., Yang, X., Zhao, R., Ma, Q., Li, P., Wang, L., Hao, R., Jia, L., Du, X., Qiu, S., Song, H.

Antibiotic resistance and molecular characterization of the hydrogen sulfide-negative phenotype among diverse Salmonella serovars in China
(2018) *BMC Infectious Diseases*, 18 (1), art. no. 292, .

ABSTRACT: Background: Among 2179 *Salmonella* isolates obtained during national surveillance for salmonellosis in China from 2005 to 2013, we identified 46 non-H₂S-producing strains originating from different sources. Methods: The isolates were characterized in terms of antibiotic resistance and genetic variability by pulsed-field gel electrophoresis and multilocus sequence typing. Mutation in the *phs* operon, which may account for the non-H₂S-producing phenotype of the isolated *Salmonella* strains, was performed in this study. Results: Among isolated non-H₂S-producing *Salmonella* strains, more than 50% were recovered from diarrhea patients, of which H₂S-negative *S. Gallinarum*, *S. Typhimurium*, *S. Choleraesuis* and *S. Paratyphi A* isolates constituted 76%. H₂S-negative isolates exhibited a high rate of resistance to ticarcillin, ampicillin, and

tetracycline, and eight of them had the multidrug resistance phenotype. Most H2S-negative *Salmonella* isolates had similar pulsed-field gel electrophoresis profiles and the same sequence type as H2S-positive strains, indicating a close origin, but carried mutations in the *phsA* gene, which may account for the non-H2S-producing phenotype. Conclusions: Our data indicate that multiple H2S-negative strains have emerged and persist in China, emphasizing the necessity to implement efficient surveillance measures for controlling dissemination of these atypical *Salmonella* strains. ISSN: 14712334

Sannö, A., Rosendal, T., Aspán, A., Backhans, A., Jacobson, M.

Distribution of enteropathogenic Yersinia spp. and Salmonella spp. in the Swedish wild boar population, and assessment of risk factors that may affect their prevalence (2018) Acta Veterinaria Scandinavica, 60 (1), art. no. 40, .

ABSTRACT: Background: Pure Eurasian wild boars and/or hybrids with domestic pigs are present in the wild on most continents. These wild pigs have been demonstrated to carry a large number of zoonotic and epizootic pathogens such as *Salmonella* spp., *Yersinia enterocolitica* and *Y. pseudotuberculosis*. Wild boar populations throughout Europe are growing and more and more wild boar meat is being consumed, the majority within the homes of hunters without having passed a veterinary inspection. The aim of this study was to investigate if factors such as population density, level of artificial feeding, time since establishment of a given population, and the handling of animal by-products from slaughtered animals could influence the presence of these pathogens in the wild boar. Results: In total, 90 wild boars from 30 different populations in Sweden were sampled and analysed using a protocol combining pre-cultivation and PCR-detection. The results showed that 27% of the sampled wild boars were positive for *Salmonella* spp., 31% were positive for *Y. enterocolitica* and 22% were positive for *Y. pseudotuberculosis*. In 80% of the sampled populations, at least one wild boar was positive for one of these enteropathogens and in total, 60% of the animals carried at least one of the investigated enteropathogens. The presumptive risk factors were analysed using a case-control approach, however, no significant associations were found. Conclusion: Human enteropathogens are commonly carried by wild boars, mainly in the tonsils, and can thus constitute a risk for contamination of the carcass and meat during slaughter. Based on the present results, the effect of reducing population densities and number of artificial feeding places might be limited. ISSN: 0044605X

Mąka, Ł., Maćkiw, E., Stasiak, M., Wołkowicz, T., Kowalska, J., Postupolski, J., Popowska, M.

Ciprofloxacin and nalidixic acid resistance of Salmonella spp. isolated from retail food in Poland

(2018) International Journal of Food Microbiology, 276, pp. 1-4.

ABSTRACT: Distribution of amino acid substitutions in the quinolone resistance-determining region (QRDR) of *gyrA*, *gyrB*, *parC*, *parE* and determinants of plasmid-mediated quinolone resistance (PMQR) were investigated among quinolone-resistant *Salmonella* spp. strains isolated from retail food in Poland in the years 2008–2013. Ten different amino acid substitutions were identified in QRDRs. Five different amino acid substitutions were identified in *gyrA*: Ser83Tyr, Ser83Phe, Asp87Tyr, Asp87Asn, Asp87Gly, two amino acid substitutions in *parC*: Thr57Ser, Ser80Ile and in *parE*: Leu445Phe, Arg511Ser. One substitution — Ser464Phe — was detected within *gyrB*. In *gyrA* a single substitution (Ser83Tyr) was identified the most frequently — 34.8% (63/181). Second most frequently identified variant (21.0%–38/181) was a co-existence of two single substitutions in *gyrA*: Ser83Tyr and *parC*: Thr57Ser. In four isolates co-existed three substitutions in three different genes: *gyrA*: Ser83Tyr + *parC*: Thr57Ser + *parE*: Leu445Phe (two isolates), *gyrA*: Ser83Phe + *parC*: Thr57Ser + *parE*: Leu445Phe, and *gyrA*: Ser83Tyr + *parC*: Thr57Ser + *parE*: Arg511Ser. In the two isolates four substitutions were identified — in *gyrA*: Ser83Phe + Asp87Tyr and in *parC*: Thr57Ser + Ser80Ile. Among resistant isolates, MIC values varied between 32 and 2048 mg/L (nalidixic acid) and between 0.125 and 16 mg/L (ciprofloxacin). MIC values of two isolates harboring *qnrS1* without any substitutions were 32 mg/L (NA) and 0.5–1.0 mg/L (CIP). The highest MIC values for NA and CIP were observed in two isolates of *Salmonella* spp. carrying double substitutions in *gyrA*: Ser83Phe + Asp87Tyr and *parC*: Thr57Ser + Ser80Ile. MIC value for NA was 2048 mg/L while for CIP — 16 mg/L. ISSN: 01681605

Verma, P., Saharan, V.V., Nimesh, S., Singh, A.P.

Phenotypic and virulence traits of Escherichia coli and Salmonella strains isolated from vegetables and fruits from India

(2018) Journal of Applied Microbiology, 125 (1), pp. 270-281.

ABSTRACT: Aims: The present study was designed to assess the phenotypic traits and virulence determinants of vegetable-/fruit-origin *Escherichia coli* and *Salmonella* strains. Methods and Results: A total of 520 fresh vegetables/fruits samples were analysed for the presence of *E. coli*, including Shiga toxin-producing *E. coli* (STEC), and *Salmonella*. The vegetable-/fruit-origin *E. coli* and *Salmonella* strains were further assessed for antimicrobial resistance, biofilm formation, extracellular matrix production and in vitro invasion/intracellular survivability assays. A total of 73 *E. coli*, including four STEC, and 26 *Salmonella* strains were recovered from vegetables/fruits in the present study. Most of the *E. coli* and *Salmonella* isolates were able to form biofilm with higher production of cellulose/curli-fimbriae. Furthermore, more resistance was observed in *E. coli* isolates (61.6%) than in *Salmonella* isolates (38.5%) against tested antimicrobials. Additionally, invasion/intracellular survival results showed that majority of the *E. coli* and *Salmonella* isolates were able to efficiently invade/replicate intracellularly in the human epithelial cells. Conclusions: Our results demonstrate that vegetable-/fruit-origin *E. coli* and *Salmonella* significantly exhibited distinct phenotypic/virulence traits which could be linked to their plant-associated lifestyle with food safety issues. Significance and Impact of the Study: The present study provides valuable baseline information that *E. coli* and *Salmonella* may use plants as an alternative host with significant clinical importance. ISSN: 13645072

Gurtler, J.B., Harlee, N.A., Smelser, A.M., Schneider, K.R.

Salmonella enterica contamination of market fresh tomatoes: A review (2018) *Journal of Food Protection*, 81 (7), pp. 1193-1213.

ABSTRACT: *Salmonella* contamination associated with market fresh tomatoes has been problematic for the industry and consumers. A number of outbreaks have occurred, and dollar losses for the industry, including indirect collateral impact to agriculturally connected communities, have run into the hundreds of millions of dollars. This review covers these issues and an array of problems and potential solutions surrounding *Salmonella* contamination in tomatoes. Some other areas discussed include (i) the use of case-control studies and DNA fingerprinting to identify sources of contamination, (ii) the predilection for contamination based on *Salmonella* serovar and tomato cultivar, (iii) internalization, survival, and growth of *Salmonella* in or on tomatoes and the tomato plant, in biofilms, and in niches ancillary to tomato production and processing, (iv) the prevalence of *Salmonella* in tomatoes, especially in endogenous regions, and potential sources of contamination, and (v) effective and experimental means of decontaminating *Salmonella* from the surface and stem scar regions of the tomato. Future research should be directed in many of the areas discussed in this review, including determining and eliminating sources of contamination and targeting regions of the country where *Salmonella* is endemic and contamination is most likely to occur. Agriculturalists, horticulturalists, microbiologists, and epidemiologists may make the largest impact by working together to solve other unanswered questions regarding tomatoes and *Salmonella* contamination. ISSN: 0362028X

Noviyanti, F., Hosotani, Y., Koseki, S., Inatsu, Y., Kawasaki, S.

Predictive Modeling for the Growth of Salmonella Enteritidis in Chicken Juice by Real-Time Polymerase Chain Reaction (2018) *Foodborne Pathogens and Disease*, 15 (7), pp. 406-412.

ABSTRACT: The goals of this study were to monitor the growth kinetics of *Salmonella* Enteritidis in chicken juice using real-time polymerase chain reaction (PCR) and to evaluate its efficacy by comparing the results with an experimental database. *Salmonella* Enteritidis was inoculated in chicken juice samples at an initial inoculum of 10⁴ CFU/mL with inoculated samples incubated at six different temperatures (10, 15, 20, 25, 30, and 35°C). Sampling was carried out for 36 h to observe the growth of *Salmonella* Enteritidis. The total DNA was extracted from the samples, and the copy number of the *Salmonella* invasion gene (*invA*) was quantified by real-time PCR and converted to *Salmonella* Enteritidis cell concentration. Growth kinetics data were analyzed by the Baranyi and Roberts model to obtain growth parameters, whereas the Ratkowsky's square-root model was used to describe the effect of the interactions between growth parameters and temperature on the growth of *Salmonella* Enteritidis. The growth parameters of *Salmonella* Enteritidis obtained from an experiment conducted at a constant temperature were validated with growth data from chicken juice samples that were incubated under fluctuating temperature conditions between 5°C and 30°C for 30-min periods. A high correlation was observed between maximum growth rate (μ_{max}) and storage temperature, indicating that the real-time PCR-monitoring method provides a precise estimation of *Salmonella* Enteritidis growth in food material with a microbial flora. Moreover, the μ_{max} data reflected data from microbial responses viewer database and ComBase. The results of

this study suggested that real-time PCR monitoring provides a precise estimation of *Salmonella* Enteritidis growth in food materials with a background microbial flora.
ISSN: 15353141

Parisi, A., Crump, J.A., Glass, K., Howden, B.P., Furuya-Kanamori, L., Vilkins, S., Gray, D.J., Kirk, M.D.

Health Outcomes from Multidrug-Resistant Salmonella Infections in High-Income Countries: A Systematic Review and Meta-Analysis
(2018) *Foodborne Pathogens and Disease*, 15 (7), pp. 428-436.

ABSTRACT: Background: *Salmonella* is a leading cause of foodborne enterocolitis worldwide. Antimicrobial use in food animals is the driving force for antimicrobial resistance among *Salmonella* particularly in high-income countries. Nontyphoidal *Salmonella* (NTS) infections that are multidrug resistant (MDR) (nonsusceptible to ≥ 1 agent in ≥ 3 antimicrobial categories) may result in more severe health outcomes, although these effects have not been systematically examined. We conducted a systematic review and meta-analysis to examine impacts of MDR NTS on disease outcomes in high-income settings. Methods: We systematically reviewed the literature from scientific databases, including PubMed, Scopus, and grey literature sources, using preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines. We included peer-reviewed publications of case-control and cohort studies, outbreak investigations, and published theses, imposing no language restriction. We included publications from January 1, 1990 through September 15, 2016 from high-income countries as classified by the World Bank, and extracted data on duration of illness, hospitalization, morbidity and mortality of MDR, and pan-susceptible NTS infections. Results: After removing duplicates, the initial search revealed 4258 articles. After further screening, 16 eligible studies were identified for the systematic review, but, only 9 of these were included in the meta-analysis. NTS serotypes differed among the reported studies, but serotypes Typhimurium, Enteritidis, Newport, and Heidelberg were the most often reported MDR pathogens. *Salmonella* infections that were MDR were associated with excess bloodstream infections (odds ratio [OR] 1.73; 95% confidence interval [CI] 1.32-2.27), more frequent hospitalizations (OR 2.51; 95% CI 1.38-4.58), and higher mortality (OR 3.54; 95% CI 1.10-11.40) when compared with pan-susceptible isolates. Conclusions: Our study suggests that MDR NTS infections have more serious health outcomes compared with pan-susceptible strains. With the emergence of MDR *Salmonella* strains in high-income countries, it is crucial to reduce the use of antimicrobials in animals and humans, and intervene to prevent foodborne infections.
ISSN: 15353141

Crabb, H.K., Lee Allen, J., Maree Devlin, J., Matthew Firestone, S., Reginald Wilks, C., Rudkin Gilkerson, J.

Salmonella spp. transmission in a vertically integrated poultry operation: Clustering and diversity analysis using phenotyping (serotyping, phage typing) and genotyping (MLVA)
(2018) *PLoS ONE*, 13 (7), art. no. e0201031, p. 1DUMMY.

ABSTRACT: The transmission of *Salmonella* enterica within a vertically integrated poultry operation was investigated longitudinally over an 18-month period (2013–2014). Thirty six percent of all samples collected (1503 of 4219) were positive for salmonellae with seven *Salmonella enterica* subsp. *enterica* serovars, and one *Salmonella enterica* subsp. *salamae* serovar detected. Both *Salmonella enterica* subsp. *enterica* serovars Infantis and Typhimurium were detected in all locations sampled. *Salmonella* Typhimurium was the most frequently detected serovar (63% of serotyped samples) with 8 phage types (PT) and 41 multiple-locus variable-number tandem-repeats analysis (MLVA) profiles identified. The most frequently identified phage types were PT135a and DT135. A total of 62 PT/MLVA combinations were observed. MLVA profiles 03-14-10-09-525 and 03-15-11-11-525 were the most frequently identified and 83% of the isolates shared at least one MLVA profile with an isolate from another phage type. The use of phage typing and MLVA profiling, on their own or in combination, were insufficient to understand the complexity of the epidemiological relationships between locations within this production system. Despite the high level of apparent diversity, cluster analysis was unable to differentiate the transmission pathways of all *S. Typhimurium* variants detected within the integrated enterprise. Using additional epidemiological information, the parent breeder rearing site was identified as the most likely point of introduction of two *S. Typhimurium* isolates into the production system with subsequent dissemination to the broiler flocks via the hatchery. This complexity is unable to be resolved in the absence of intensive sampling programs at all generations of the production system. ISSN: 19326203

Djordjević, J., Bošković, M., Starčević, M., Ivanović, J., Karabasil, N., Dimitrijević, M., Lazić, I.B., Baltić, M.Ž.

Survival of Salmonella spp. in minced meat packaged under vacuum and modified atmosphere

(2018) *Brazilian Journal of Microbiology*, 49 (3), pp. 607-613.

ABSTRACT: The effect of different modified atmosphere packaging regimes on the behavior of *Salmonella* spp. on minced meat was studied. Minced meat was experimentally contaminated with a *Salmonella* spp. cocktail (*S. Enteritidis*, *S. Typhimurium*, *S. Infantis* and *S. Arizonae*), packaged under vacuum or modified atmosphere with initial headspaces containing 20%O₂/50%CO₂/30%N₂ and 20%O₂/30%CO₂/50%N₂) and stored at 3 ± 1 °C for 12 days. Samples were analyzed for *Salmonella* spp., viable and lactic acid bacteria count every third day. *Salmonella* spp. counts decreased during storage in all packaging types, with reductions of about 1.5 log CFU/g. A significant difference (p < 0.01) was noted between *Salmonella* spp. counts in meat packaged in vacuum and modified atmospheres, although there was no significant difference in *Salmonella* spp. count between meat packaged in 50%CO₂, and meat packaged in 30%CO₂. At the end of the study, there were significant differences (p < 0.01; p < 0.05) in total viable and lactic acid bacterial counts between meat packaged in vacuum and modified atmosphere, and the lowest counts were noted in meat packaged in modified atmosphere with 50%CO₂.
ISSN: 15178382

Burgess, B.A., Morley, P.S.

Risk factors for veterinary hospital environmental contamination with Salmonella enterica
(2018) *Epidemiology and Infection*, 146 (10), pp. 1282-1292.

ABSTRACT: Healthcare-associated infections in veterinary hospitals are commonly attributed to *Salmonella enterica*, particularly in large animal facilities, and are characteristically associated with widespread environmental contamination. The objective of this study was to investigate factors influencing the likelihood of identifying environmental contamination of a veterinary hospital with *S. enterica*, while exploring different analytic methods to model complex factors that may influence this ecology. Environmental surveillance samples were collected in a large veterinary hospital as part of a long-term infection control programme. Data were collected retrospectively from the electronic medical records database. Many easily measured variables were complex in nature (i.e., they represented variance that is unmeasured or unidentified as a specific factor) necessitating the use of alternative analytic methods (variable cluster and principal components analyses) to provide perspective regarding the complex data structure and latent factors that may be contributing to this ecology. Subsequently, multivariable logistic regression was performed using generalised estimating equations. Results suggest the probability of detecting *Salmonella* in the environment increased as demand on personnel increased (e.g., in a busy hospital). Veterinary personnel need to remain vigilant in implementing practices that we believe empirically will mitigate risk for widespread environmental contamination and sustained transmission among patients (i.e., rigorous hygiene for personnel and the environment).
ISSN: 09502688

Sadekuzzaman, M., Mizan, M.F.R., Yang, S., Kim, H.-S., Ha, S.-D.

Application of bacteriophages for the inactivation of Salmonella spp. in biofilms
(2018) *Food Science and Technology International*, 24 (5), pp. 424-433.

ABSTRACT: Microbial biofilms pose a serious threat to food industry, as they are difficult to inactivate or remove owing to their inherent resistance to traditional physical and antimicrobial treatments. Bacteriophages have been suggested as promising biocontrol agents for eliminating biofilms within the food industry. The efficacy of phages (BP 1369 and BP 1370) was evaluated against *Salmonella* spp. in biofilms. Biofilms were grown on food (lettuce), food contact surfaces (stainless steel and rubber), and MBEC biofilm devices. The efficacy of these phages in reducing biofilms was examined following phage (108 PFU/mL) treatment for 2 h. Bacteriophage treatment reduced biofilm cells by 3.0, 2.0, and 3.0 log CFU/cm² on stainless steel, rubber, and an MBEC device, respectively. The adhered viable cells on lettuce were reduced by more than 1.0 log CFU/cm² with phage treatment. ISSN: 10820132

Cole, M.L., Singh, O.V.

Microbial occurrence and antibiotic resistance in ready-to-go food items
(2018) *Journal of Food Science and Technology*, 55 (7), pp. 2600-2609.

ABSTRACT: Foodborne pathogens, such as *Escherichia coli*, and *Salmonella*, are commonly prevalent in contaminated food products seen through annual food recalls. Excessive use of antibiotics through the past few decades has led to a multitude of antibiotic resistant bacteria, including foodborne pathogens. We investigated microbial occurrence and their antibiotic resistances in ready-to-go food items, i.e. canned food, bagged food, and baby food. A total of 112 isolates were isolated from varying food items, and 21 of these isolates

were identified through 16S rRNA sequencing revealing *Bacillus* sp., *Staphylococcus* sp. and *Micrococcus* sp. Bagged food items showed the most microbial diversity as well as the largest colony forming unit (log 20–25 CFU/g). Isolates showed antibiotic resistance to ampicillin, streptomycin, chloramphenicol, and kanamycin at concentrations of 100, 500, and 1000 µg/mL. 57% isolates were ampicillin resistance followed by kanamycin (26%). A variety of microorganisms present in ready-to-go food items may not be pathogenic, however their occurrence and multiple antibiotic resistance (MAR) poses risk of transferring their genes to foodborne pathogens. ISSN: 00221155

Shang, K., Wei, B., Kang, M.

Distribution and dissemination of antimicrobial-resistant Salmonella in broiler farms with or without enrofloxacin use

(2018) *BMC Veterinary Research*, 14 (1), art. no. 257, .

ABSTRACT: Background: *Salmonella* is a major zoonotic food-borne pathogen that persists on poultry farms, and animals undergo reinfection with endemic strains. The present study aimed to investigate the characteristics and dissemination of antimicrobial-resistant *Salmonella* within and between broiler farms that used enrofloxacin and those that did not. Results: Cloacal and environmental (litter, feed, and water) samples from two selected flocks in each of 12 farms owned by the same company were collected three times over a 30-day period of two production cycles during 2015-2016. The rate of *Salmonella* isolation was 7.8% (123/1584). Nine *Salmonella* serotypes (116 isolates) and seven untypable isolates were identified, and *Salmonella* Montevideo was the most prevalent serotype. Azithromycin-resistant (17.9%) and colistin-resistant (3.3%) isolates were detected, and multidrug-resistant isolates (43.1%) were also observed. No isolate was resistant to enrofloxacin or ciprofloxacin; however, intermediate resistance to enrofloxacin was significantly higher ($P < 0.05$) in farms that used enrofloxacin than in those that did not. The rate of multi-drug resistance among litter isolates (25/44, 56.8%) was significantly higher ($P < 0.05$) than that among cloacal swab (24/67, 35.8%) and feed (4/12, 33.3%) isolates. Pulsed-field gel electrophoresis (PFGE) analysis of strains of the same serotype was conducted to determine their epidemiological relationship. The PFGE types were classified into 31 groups with a 100% correlation cutoff in dendrograms for *Salmonella* Montevideo isolates, which showed 100% genomic identity based on age, sample type, flock, and production cycle within and between farms. Conclusion: The present study highlights the occurrence of horizontal transmission and cyclic contamination with antimicrobial-resistant *Salmonella* in broiler farms owned by the same company. Litter may be a good indicator of indoor environmental contamination with antimicrobial-resistant *Salmonella* on farms. Additionally, enrofloxacin use may be one of the factors promoting resistance towards it in *Salmonella*. ISSN: 17466148

Lamas, A., Regal, P., Vázquez, B., Miranda, J.M., Cepeda, A., Franco, C.M.

Salmonella and Campylobacter biofilm formation: a comparative assessment from farm to fork

(2018) *Journal of the Science of Food and Agriculture*, 98 (11), pp. 4014-4032.

ABSTRACT: It takes several steps to bring food from the farm to the fork (dining table), and contamination with food-borne pathogens can occur at any point in the process. *Campylobacter* spp. and *Salmonella* spp. are the main microorganisms responsible for foodborne disease in the EU. These two pathogens are able to persist throughout the food supply chain thanks to their ability to form biofilms. Owing to the high prevalence of *Salmonella* and especially of *Campylobacter* in the food supply chain and the huge efforts of food authorities to reduce these levels, it is of great importance to fully understand their mechanisms of persistence. Diverse studies have evaluated the biofilm-forming capacity of foodborne pathogens isolated at different steps of food production. Nonetheless, the principal obstacle of these studies is to reproduce the real conditions that microorganisms encounter in the food supply chain. While there are a wide number of *Salmonella* biofilm studies, information on *Campylobacter* biofilms is still limited. A comparison between the two microorganisms could help to develop new research in the field of *Campylobacter* biofilms. Therefore, this review evaluates relevant work in the field of *Salmonella* and *Campylobacter* biofilms and the applicability of the data obtained from these studies to real working conditions. ISSN: 00225142

Cherrie, M.P.C., Nichols, G., Iacono, G.L., Sarran, C., Hajat, S., Fleming, L.E.

Pathogen seasonality and links with weather in England and Wales: A big data time series analysis David Stieb, Cecile Boot, Michelle Turner, Osmar Zaiane

(2018) *BMC Public Health*, 18 (1), art. no. 1067, .

ABSTRACT: Background: Many infectious diseases of public health importance display annual seasonal patterns in their incidence. We aimed to systematically document the

seasonality of several human infectious disease pathogens in England and Wales, highlighting those organisms that appear weather-sensitive and therefore may be influenced by climate change in the future. Methods: Data on infections in England and Wales from 1989 to 2014 were extracted from the Public Health England (PHE) SGSS surveillance database. We conducted a weekly, monthly and quarterly time series analysis of 277 pathogen serotypes. Each organism's time series was forecasted using the TBATS package in R, with seasonality detected using model fit statistics. Meteorological data hosted on the MEDMI Platform were extracted at a monthly resolution for 2001-2011. The organisms were then clustered by K-means into two groups based on cross correlation coefficients with the weather variables. Results: Examination of 12.9 million infection episodes found seasonal components in 91/277 (33%) organism serotypes. *Salmonella* showed seasonal and non-seasonal serotypes. These results were visualised in an online Rshiny application. Seasonal organisms were then clustered into two groups based on their correlations with weather. Group 1 had positive correlations with temperature (max, mean and min), sunshine and vapour pressure and inverse correlations with mean wind speed, relative humidity, ground frost and air frost. Group 2 had the opposite but also slight positive correlations with rainfall (mm, > 1 mm, > 10 mm). Conclusions: The detection of seasonality in pathogen time series data and the identification of relevant weather predictors can improve forecasting and public health planning. Big data analytics and online visualisation allow the relationship between pathogen incidence and weather patterns to be clarified. ISSN: 14712458

Savas, S., Ersoy, A., Gulmez, Y., Kilic, S., Levent, B., Altintas, Z.

Nanoparticle enhanced antibody and DNA biosensors for sensitive detection of Salmonella (2018) *Materials*, 11 (9), art. no. 1541, .

ABSTRACT: Bacteria-related pathogenic diseases are one of the major health problems throughout the world. *Salmonella* is a genus of rod-shaped Gram-negative enterobacteria of which more than 2600 serotypes have been identified. Infection with *Salmonella* can cause salmonellosis, a serious bacterial toxin-infection syndrome associated with gastroenteritis, and paratyphoid and typhoid fevers. Its rapid and sensitive detection is a key to the prevention of problems related to health. This paper describes the development of antibody and DNA sensors for *Salmonella* detection using a microfluidic-based electrochemical system. Commercial *Salmonella typhimurium* and *Salmonella typhimurium* from human stool samples were investigated using standard and nanomaterial-amplified antibody sensors. *S. typhimurium* could be detected down to 1 cfu mL⁻¹. The specificity of immunoassay was tested by studying with non-specific bacteria including *E. coli* and *S. aureus* that revealed only 2.01% and 2.66% binding when compared to the target bacterium. On the other hand, the quantification of *Salmonella* DNA was investigated in a concentration range of 0.002-200 µM using the developed DNA biosensor that demonstrated very high specificity and sensitivity with a detection limit of 0.94 nM. Our custom-designed microfluidic sensor offers rapid, highly sensitive, and specific diagnostic assay approaches for pathogen detection. ISSN: 19961944

Micciche, A.C., Foley, S.L., Pavlidis, H.O., McIntyre, D.R., Ricke, S.C.

A review of prebiotics against Salmonella in poultry: Current and future potential for microbiome research applications

(2018) *Frontiers in Veterinary Science*, 5 (AUG), art. no. 191, .

ABSTRACT: Prebiotics are typically fermentable feed additives that can directly or indirectly support a healthy intestinal microbiota. Prebiotics have gained increasing attention in the poultry industry as wariness toward antibiotic use has grown in the face of foodborne pathogen drug resistance. Their potential as feed additives to improve growth, promote beneficial gastrointestinal microbiota, and reduce human-associated pathogens, has been well documented. However, their mechanisms remain relatively unknown. Prebiotics increasing short chain fatty acid (SCFA) production in the cecum have long since been considered a potential source for pathogen reduction. It has been previously concluded that prebiotics can improve the safety of poultry products by promoting the overall health and well-being of the bird as well as provide for an intestinal environment that is unfavorable for foodborne pathogens such as *Salmonella*. To better understand the precise benefit conferred by several prebiotics, "omic" technologies have been suggested and utilized. The data acquired from emerging technologies of microbiomics and metabolomics may be able to generate a more comprehensive detailed understanding of the microbiota and metabolome in the poultry gastrointestinal tract. This understanding, in turn, may allow for improved administration and optimization of prebiotics to prevent foodborne illness as well as elucidate unknown mechanisms of prebiotic actions. This review explores the use of prebiotics in poultry, their impact on gut *Salmonella* populations, and how

utilization of next-generation technologies can elucidate the underlying mechanisms of prebiotics as feed additives. ISSN: 22971769

Kiel, R.C., Martin, J.N., Woerner, D.R., Murphy, R., Geornaras, I., Levey, J.R., Yang, H., Delmore, R.J., Belk, K.E.

Influence of storage temperature, moisture content, and physical impurities on the distribution and survival of salmonella enterica in poultry fat intended for pet food use (2018) Journal of Food Protection, 81 (8), pp. 1364-1372.

ABSTRACT: Contamination of rendered products with *Salmonella* is a concern for the rendering industry, particularly when those products are intended for use in other foodstuffs, such as pet food. This study was conducted to understand the influence of compositional variation on the location and survivability of *Salmonella* in a poultry fat matrix. Specifically, this study aimed to (i) assess the influence of postinoculation time and moisture content on the distribution of *Salmonella* in rendered poultry fat and (ii) evaluate the impact of postinoculation time and physical parameters (i.e., impurity level and moisture content) on survival of three *Salmonella* strains in rendered poultry fat stored at two different temperatures. Three studies, designated as study I(a), I(b), and II, respectively, were conducted to address these objectives. In study I(a), a green fluorescent protein-expressing strain of *Salmonella* Typhimurium was used to map the organism within warmed (458C) poultry fat containing various levels of moisture. In study I(b), the influence of storage temperature on the survivability of green fluorescent protein-expressing *Salmonella* was evaluated. In study II, the impacts of physical impurities, moisture content, and storage temperature on the survivability of three *Salmonella* strains (*Enteritidis*, *Senftenberg*, and *Typhimurium*) were assessed. The results of this study demonstrated that composition (i.e., moisture and impurity contents) influences the survivability of *Salmonella* in poultry fat; specifically, *Salmonella* is more persistent in poultry fat with a greater moisture content and water activity. Nonetheless, although composition impacts the distribution and survivability of *Salmonella* in poultry fat, *Salmonella* generally does not survive in poultry fat maintained at high temperatures (458C and above). ISSN: 0362028X

San Román, B., Garrido, V., Sánchez, S., Martínez-Ballesteros, I., Garaizar, J., Mainar-Jaime, R.C., Migura-García, L., Grilló, M.J.

Relationship between Salmonella infection, shedding and serology in fattening pigs in low-moderate prevalence areas (2018) Zoonoses and Public Health, 65 (5), pp. 481-489.

ABSTRACT: *Salmonella* is a major foodborne pathogen causing important zoonosis worldwide. Pigs asymptotically infected in mesenteric lymph nodes (MLN) can be intermittent shedders of the pathogen through faeces, being considered a major source of human infections. European baseline studies of fattening pig salmonellosis are based on *Salmonella* detection in MLN. This work studies the relationship between *Salmonella* infection in MLN and intestinal content (IC) shedding at slaughter and the relationship between the presence of the pathogen and the serologic status at farm level. Mean *Salmonella* prevalence in the selected pigs (vertically integrated production system of Navarra, Spain) was 7.2% in MLN, 8.4% in IC and 9.6% in serum samples. In this low-moderate prevalence context, poor concordance was found between MLN infection and shedding at slaughter and between bacteriology and serology. In fact, most of shedders were found uninfected in MLN (83%) or carrying different *Salmonella* strains in MLN and in IC (90%). The most prevalent *Salmonellae* were Typhimurium resistant to ACSSuT ± Nx or ASSuT antibiotic families, more frequently found invading the MLN (70%) than in IC (33.9%). Multivariable analysis revealed that risk factors associated with the presence of *Salmonella* in MLN or in IC were different, mainly related either to good hygiene practices or to water and feed control, respectively. Overall, in this prevalence context, detection of *Salmonella* in MLN is an unreliable predictor of faecal shedding at abattoir, indicating that subclinical infections in fattening pigs MLN could have limited relevance in the IC shedding. ISSN: 18631959

Ricke, S.C., Kim, S.A., Shi, Z., Park, S.H.

Molecular-based identification and detection of Salmonella in food production systems: current perspectives

(2018) Journal of Applied Microbiology, 125 (2), pp. 313-327.

ABSTRACT: *Salmonella* remains a prominent cause of foodborne illnesses and can originate from a wide range of food products. Given the continued presence of pathogenic *Salmonella* in food production systems, there is a consistent need to improve identification and detection methods that can identify this pathogen at all stages in food systems. Methods for subtyping have evolved over the years, and the introduction of whole genome

sequencing and advancements in PCR technologies have greatly improved the resolution for differentiating strains within a particular serovar. This, in turn, has led to the continued improvement in *Salmonella* detection technologies for utilization in food production systems. In this review, the focus will be on recent advancements in these technologies, as well as potential issues associated with the application of these tools in food production. In addition, the recent and emerging research developments on *Salmonella* detection and identification methodologies and their potential application in food production systems will be discussed. ISSN: 13645072

Blázquez, E., Rodríguez, C., Ródenas, J., Saborido, N., Solà-Ginés, M., Pérez de Rozas, A., Campbell, J.M., Segalés, J., Pujols, J., Polo, J.

Combined effects of spray-drying conditions and postdrying storage time and temperature on Salmonella choleraesuis and Salmonella typhimurium survival when inoculated in liquid porcine plasma

(2018) *Letters in Applied Microbiology*, 67 (2), pp. 205-211.

ABSTRACT: The objective of this study was to determine the effectiveness of the spray-drying process on the inactivation of *Salmonella choleraesuis* and *Salmonella typhimurium* spiked in liquid porcine plasma and to test the additive effect of immediate postdrying storage. Commercial spray-dried porcine plasma was sterilized by irradiation and then reconstituted (1:9) with sterile water. Aliquots of reconstituted plasma were inoculated with either *S. choleraesuis* or *S. typhimurium*, subjected to spray-drying at an inlet temperature of 200°C and an outlet temperature of either 71 or 80°C, and each spray-drying temperature combinations were subjected to either 0, 30 or 60 s of residence time (RT) as a simulation of residence time typical of commercial dryers. Spray-dried samples were stored at either 4.0 ± 3.0°C or 23.0 ± 0.3°C for 15 days. Bacterial counts of each *Salmonella* spp., were completed for all samples. For both *Salmonella* spp., spray-drying at both outlet temperatures reduced bacterial counts about 3 logs at RT 0 s, while there was about a 5.5 log reduction at RT 60 s. Storage of all dried samples at either 4.0 ± 3.0°C or 23.0 ± 0.3°C for 15 days eliminate all detectable bacterial counts of both *Salmonella* spp. **Significance and Impact of the Study:** Safety of raw materials from animal origin like spray-dried porcine plasma (SDPP) may be a concern for the swine industry. Spray-drying process and postdrying storage are good inactivation steps to reduce the bacterial load of *Salmonella choleraesuis* and *Salmonella typhimurium*. For both *Salmonella* spp., spray-drying at 71°C or 80°C outlet temperatures reduced bacterial counts about 3 log at residence time (RT) 0 s, while there was about a 5.5 log reduction at RT 60 s. Storage of all dried samples at either 4.0 ± 3.0°C or 23.0 ± 0.3°C for 15 days was effective for eliminating detectable bacterial counts of both *Salmonella* spp. ISSN: 02668254

Gambino-Shirley, K., Stevenson, L., Concepción-Acevedo, J., Trees, E., Wagner, D., Whitlock, L., Roberts, J., Garrett, N., Van Duyne, S., McAllister, G., Schick, B., Schlater, L., Peralta, V., Reporter, R., Li, L., Waechter, H., Gomez, T., Fernández Ordenes, J., Ulloa, S., Ragimbeau, C., Mossong, J., Nichols, M.

Flea market finds and global exports: Four multistate outbreaks of human Salmonella infections linked to small turtles, United States—2015

(2018) *Zoonoses and Public Health*, 65 (5), pp. 560-568.

ABSTRACT: Zoonotic transmission of *Salmonella* infections causes an estimated 11% of salmonellosis annually in the United States. This report describes the epidemiologic, traceback and laboratory investigations conducted in the United States as part of four multistate outbreaks of *Salmonella* infections linked to small turtles. *Salmonella* isolates indistinguishable from the outbreak strains were isolated from a total of 143 ill people in the United States, pet turtles, and pond water samples collected from turtle farm A, as well as ill people from Chile and Luxembourg. Almost half (45%) of infections occurred in children aged <5 years, underscoring the importance of the Centers for Disease Control and Prevention recommendation to keep pet turtles and other reptiles out of homes and childcare settings with young children. Although only 43% of the ill people who reported turtle exposure provided purchase information, most small turtles were purchased from flea markets or street vendors, which made it difficult to locate the vendor, trace the turtles to a farm of origin, provide education and enforce the United States federal ban on the sale and distribution of small turtles. These outbreaks highlight the importance of improving public awareness and education about the risk of *Salmonella* from small turtles not only in the United States but also worldwide. ISSN: 18631959

Eggers, J., Feirtag, J.M., Olstein, A.D., Bosilevac, J.M.

A novel selective medium for simultaneous enrichment of Shiga toxin-Producing Escherichia coli and salmonella in ground beef

(2018) *Journal of Food Protection*, 81 (8), pp. 1252-1257.

ABSTRACT: Microbiological analysis of ground beef for contamination by both *Salmonella* and Shiga toxin-producing *Escherichia coli* (STEC) is performed by the U.S. Department of Agriculture, Food Safety Inspection Service (FSIS), as part of its Performance Standards Verification Testing program. FSIS has established a zero tolerance for STEC serotype O157:H7 and serogroups O26, O45, O103, O111, O121, and O145 because they are regarded as adulterants. The detection and isolation of these specific serogroups presents a technical challenge necessitating time-consuming and costly laboratory procedures that often exceed the technical capabilities of many small internal and reference laboratories. We describe here a method using a novel STEC and *Salmonella* selective (SSS) broth that allows for simultaneous selective enrichment of STEC and *Salmonella* sp., providing isolation and detection from the same broth. The method only involves direct plating from beef enrichments to detect suspect isolates that can be easily confirmed by using immunoassays or PCR, rendering the isolation simpler and less costly than the current described methods. In a side-by-side comparison with modified tryptic soy broth (mTSB), the use of SSS broth resulted in primarily isolating STEC and *Salmonella* sp., while substantially suppressing the growth of other gram-negative Enterobacteriaceae by 90%. Significantly more ($v_2 = 3.84$) samples containing *E. coli* O157:H7 and STEC O26, O111, O121, and O145 and a nondifferent ($v_2 = 3.84$) number of samples containing STEC O103 and O45 were identified when enriching in SSS broth. Coenrichment using six different *Salmonella* serovars showed numerically greater but not significant ($v_2 = 3.84$) positive samples by using SSS broth compared with mTSB for a majority of serotypes. ISSN: 0362028X

Ngoi, S.T., Yap, K.-P., Thong, K.L.

Genomic characterization of endemic Salmonella enterica serovar Typhimurium and Salmonella enterica serovar I 4,[5],12:i:- isolated in Malaysia (2018) Infection, Genetics and Evolution, 62, pp. 109-121.

ABSTRACT: *Salmonella enterica* serovar Typhimurium (*S. Typhimurium*) and the monophasic variant *Salmonella* I 4,[5],12:i:- are two clinically-important non-typhoidal *Salmonella* serovars worldwide. However, the genomic information of these two organisms, especially the monophasic variant, is still lacking in Malaysia. The objective of the study was to compare the genomic features of a monophasic variant and two endemic *S. Typhimurium* strains isolated from humans. All three strains were subjected to whole genome sequencing followed by comparative genomic and phylogenetic analyses. Extensive genomic deletion in the *fljAB* operon (from STM2757 to *iroB*) is responsible for the monophasic phenotype of STM032/04. The two *S. Typhimurium* genomes (STM001/70 and STM057/05) were essentially identical, despite being isolated 35 years apart. All three strains were of sequence type ST19. Both *S. Typhimurium* genomes shared unique prophage regions not identified in the monophasic STM032/04 genome. Core genome phylogenetic analyses showed that the monophasic STM032/04 was closely-related to the *S. Typhimurium* LT2, forming a distinctive clade separated from the two endemic *S. Typhimurium* strains in Malaysia. The presence of serovar Typhimurium-specific *mdh* gene, conserved Gifsy and Fels-1 prophages, and the close genomic resemblance with *S. Typhimurium* LT2 suggested that the monophasic STM032/04 was originated from an LT2-like *S. Typhimurium* ancestor in Malaysia, following an evolutionary path different from the *S. Typhimurium* strains. In conclusion, the monophasic *Salmonella* I 4,[5],12:i:- and the *S. Typhimurium* strains isolated in Malaysia descended from different phylogenetic lineages. The high genomic resemblance between the two *S. Typhimurium* strains isolated for at least 35 years apart indicated their successful evolutionary lineage. The identification of multiple virulence and antimicrobial resistance determinants in the *Salmonella* I 4,[5],12:i:- and *S. Typhimurium* genomes explained the pathogenic nature of the organisms. ISSN: 15671348

Lamas, A., Regal, P., Vázquez, B., Miranda, J.M., Cepeda, A., Franco, C.M.

Influence of milk, chicken residues and oxygen levels on biofilm formation on stainless steel, gene expression and small RNAs in Salmonella enterica (2018) Food Control, 90, pp. 1-9.

ABSTRACT: *Salmonella* spp. are highly versatile foodborne pathogens as they can adapt and shift from the aerobic environment outside the host to the anaerobic environment inside the host through changes in gene expression. These changes can be mediated by small RNAs (sRNAs), a class of post-transcriptional regulators that can modulate diverse functions from biofilm formation to motility and virulence. In this study, biofilm formation on stainless steel, motility, morphotype, and transcription of biofilm- and virulence-related genes and sRNAs were evaluated in fourteen *Salmonella enterica* strains under aerobiosis, microaerobiosis and anaerobiosis conditions. In order to mimic actual food industry conditions and to compare with laboratory media, chicken exudate and milk were used as

culture media. In all growth media tested, biofilm formation was significantly greater ($P < 0.05$) in aerobiosis than in microaerobiosis or anaerobiosis. The RDAR (red, dry, and rough) morphotype was only produced in aerobiosis, while motility was significantly higher in anaerobiosis ($P < 0.05$) than in microaerobiosis or aerobiosis. Whereas the gene *csgD*, which codifies the biofilm master regulator, was downregulated, the virulence genes *hilA* and *invA* were upregulated in microaerobiosis and anaerobiosis in all growth media. The transcription of sRNAs was highly influenced by both atmosphere and growth media. Positive regulators of biofilm formation *arcZ*, *sroC* and *csrB* were downregulated in microaerobiosis and anaerobiosis in comparison to aerobiosis. Curiously, the negative regulators of biofilm formation *oxyS* and *rprA* were also downregulated in microaerobiosis and anaerobiosis. However, the virulence promoter and negative regulator of biofilm formation *dsrA* was significantly upregulated in chicken juice. The results of this study indicate that oxygen levels have a considerable influence on biofilm formation and motility in *Salmonella*. Modification of the gene expression and transcription of sRNAs could be useful in understanding how *Salmonella* adapt to different conditions within the food chain. This information could then be taken into account in the development of new strategies with which to control the growth of this pathogen. ISSN: 09567135

Chen, Y., Pouillot, R., Santillana Farakos, S.M., Duret, S., Spungen, J., Fu, T.-J., Shakir, F., Homola, P.A., Dennis, S., Van Doren, J.M.

Risk Assessment of Salmonellosis from Consumption of Alfalfa Sprouts and Evaluation of the Public Health Impact of Sprout Seed Treatment and Spent Irrigation Water Testing (2018) Risk Analysis, 38 (8), pp. 1738-1757.

ABSTRACT: We developed a risk assessment of human salmonellosis associated with consumption of alfalfa sprouts in the United States to evaluate the public health impact of applying treatments to seeds (0–5-log₁₀ reduction in *Salmonella*) and testing spent irrigation water (SIW) during production. The risk model considered variability and uncertainty in *Salmonella* contamination in seeds, *Salmonella* growth and spread during sprout production, sprout consumption, and *Salmonella* dose response. Based on an estimated prevalence of 2.35% for 6.8 kg seed batches and without interventions, the model predicted 76,600 (95% confidence interval (CI) 15,400–248,000) cases/year. Risk reduction (by 5- to 7-fold) predicted from a 1-log₁₀ seed treatment alone was comparable to SIW testing alone, and each additional 1-log₁₀ seed treatment was predicted to provide a greater risk reduction than SIW testing. A 3-log₁₀ or a 5-log₁₀ seed treatment reduced the predicted cases/year to 139 (95% CI 33–448) or 1.4 (95% CI <1–4.5), respectively. Combined with SIW testing, a 3-log₁₀ or 5-log₁₀ seed treatment reduced the cases/year to 45 (95% CI 10–146) or <1 (95% CI <1–1.5), respectively. If the SIW coverage was less complete (i.e., less representative), a smaller risk reduction was predicted, e.g., a combined 3-log₁₀ seed treatment and SIW testing with 20% coverage resulted in an estimated 92 (95% CI 22–298) cases/year. Analysis of alternative scenarios using different assumptions for key model inputs showed that the predicted relative risk reductions are robust. This risk assessment provides a comprehensive approach for evaluating the public health impact of various interventions in a sprout production system. ISSN: 02724332

Hamilton, K.A., Chen, A., de-Graft Johnson, E., Gitter, A., Kozak, S., Niquice, C., Zimmer-Faust, A.G., Weir, M.H., Mitchell, J., Gurian, P.L.

Salmonella risks due to consumption of aquaculture-produced shrimp (2018) Microbial Risk Analysis, 9, pp. 22-32.

ABSTRACT: The use of aquaculture is increasing to meet the growing global demand for seafood. However, the use of aquaculture for seafood production incurs potential human health risks, especially from enteric bacteria such as *Salmonella* spp. *Salmonella* spp. was the most frequently reported cause of outbreaks associated with crustaceans from 1998 to 2004. Among crustacean species, shrimp are the most economically important, internationally traded seafood commodity, and the most commonly aquaculture-raised seafood imported to the United States. To inform safe aquaculture practices, a quantitative microbial risk assessment (QMRA) was performed, incorporating stochastic variability in pathogen growth, industrial shrimp processing, and consumer shrimp preparation. Several scenarios including gamma irradiation and cooking time were considered in order to examine the relative importance of these practices in terms of their impact on risk. Median annual infection risks for all scenarios considered were below 10⁻⁴ and median disability adjusted life year (DALY) metrics were below 10⁻⁶ DALY per person per year, however, 95th percentile risks were above 10⁻⁴ annual probability of infection and 10⁻⁶ DALY per person per year for scenarios with improper cooking and lack of gamma irradiation. The greatest difference between microbiological risks for the scenarios tested was observed when comparing proper vs. improper cooking (5–6 orders of magnitude) and gamma irradiation (4–5 orders of magnitude) compared to (up to less than 1 order of magnitude)

for peeling and “deveining” (removing the shrimp digestive tract) vs. peeling only. The findings from this research suggest that restriction of *Salmonella* spp. to low levels (median 5–30 per L aquaculture pond water) may be necessary for scenarios in which proper downstream food handling and processing cannot be guaranteed. ISSN: 23523522

Yang, P., Wong, C., Hash, S., Fung, F., Menon, S.

Rapid detection of Salmonella spp. using magnetic resonance (2018) Journal of Food Safety, 38 (4), art. no. e12473, .

ABSTRACT: The globalization of the world's food trade calls for rapid and accurate detection of foodborne pathogens to ensure safety of foods for human consumption, to prevent outbreaks and management of foodborne infectious diseases. Currently, commercial detection methods for pathogenic microbials require multiple days for sample-to-answer results. In this study, we demonstrated a highly sensitive and rapid detection of a microbial pathogen using Molecular Mirroring (M2) technology and Lab-in-the-Box system based on nuclear magnetic resonance that works rapidly and efficiently for the detection of *Salmonella*. This technology detected *Salmonella* at 1 cfu/reaction in water. In tuna, the M2 technology detected 1 cfu/g with 5 hr of enrichment and analysis with a T2 signal of 342 ms. In addition to sensitive detection and minimal enrichment, this methodology detected pathogens from inhibitory mediums. Therefore, this technology can be widely applied to other fields such as environmental monitoring, public health and safety, national security, and medical diagnosis. Practical applications: The combination of molecular biology and nuclear magnetic resonance technology represents a novel, rapid, sensitive, and highly specific methodology for the detection of *Salmonella* spp. in tuna compared to standard conventional methods. Practical applications of the M2 technology have been tested with human samples, animal samples, and food samples to detect microbial pathogens before and after food processing, thus is ideal to protect public health and to ensure food safety. Furthermore, this biosensor analytical technology can be applied to almost any medium or target of interest in the field of food safety, clinical diagnostics, and biosurveillance. ISSN: 01496085

Erickson, M.C., Liao, J.-Y., Habteselassie, M.Y., Cannon, J.L.

Inactivation of Escherichia coli O157:H7 and Salmonella during washing of contaminated gloves in levulinic acid and sodium dodecyl sulfate solutions (2018) Food Microbiology, 73, pp. 275-281.

ABSTRACT: Field workers often wear gloves harvesting ready-to-eat produce; however, fields are not sterile environments and gloves may become contaminated numerous times during a working shift. This study explored the potential for inactivation of *Escherichia coli* O157:H7 and *Salmonella* when contaminated gloves were washed in levulinic acid (LV) and sodium dodecyl sulfate (SDS) solutions. Washing nitrile gloves with increasing concentrations of LV above 1.0% led to a decreased prevalence of glove contamination by *Salmonella* ($P = 0.0000$). A higher level of prevalence occurred for solid agar-cultured pathogens than liquid broth-cultured pathogens after nitrile gloves were washed in LV/SDS ($P = 0.0000$). Pathogens residing on latex gloves were more likely to be completely inactivated by washing in 0.5% LV/0.1% SDS solutions than nitrile or Cannons gloves that exhibited inconsistent responses dependent on the pathogen strain. However, drying after washing nitrile gloves in 0.5% LV/0.1% SDS led to additional pathogen inactivation ($P = 0.0394$). Pathogen transfer from gloves to produce was implied as the pathogen prevalence on cantaloupe rind handled by LV/SDS-washed gloves was not statistically different from the prevalence on gloves ($P = 0.7141$). Hence, the risk of produce contamination may still exist but would be reduced by washing gloves in LV/SDS. ISSN: 07400020

Mook, P., Gardiner, D., Verlander, N.Q., McCormick, J., Usdin, M., Crook, P., Jenkins, C., Dallman, T.J.

Operational burden of implementing Salmonella Enteritidis and Typhimurium cluster detection using whole genome sequencing surveillance data in England: A retrospective assessment

(2018) Epidemiology and Infection, 146 (11), pp. 1452-1460.

ABSTRACT: Since April 2014 all presumptive *Salmonella* isolates received by Public Health England (PHE) have been characterised using whole genome sequencing (WGS) and the genomic data generated used to identify clusters of infection. To inform the implementation and development of a national gastrointestinal infection surveillance system based on WGS we have retrospectively identified genetically related clusters of *Salmonella* Enteritidis and *Salmonella* Typhimurium infection over a one year period and determined the distribution of these clusters by PHE operational levels. Using a constrained WGS cluster definition based on single nucleotide polymorphism distance, case frequency

and temporal spread we demonstrate that the majority of clusters spread to multiple PHE operational levels. The greatest investigative burden is on national level staff investigating small, geographically dispersed clusters. We also demonstrate that WGS identifies long-running, slowly developing clusters that may previously have remained undetected. This analysis also indicates likely increased workload for local health protection teams and will require an operational strategy to balance limited human resources with the public health importance of investigating small, geographically contained clusters of highly related cases. While there are operational challenges to its implementation, integrated cluster detection based on WGS from local to international level will provide further improvements in the identification of, response to and control of clusters of *Salmonella* spp. with public health significance. ISSN: 09502688

Hassan, R., Tecele, S., Adcock, B., Kellis, M., Weiss, J., Saupe, A., Sorenson, A., Klos, R., Blankenship, J., Blessington, T., Whitlock, L., Carleton, H.A., Concepción-Acevedo, J., Tolar, B., Wise, M., Neil, K.P.

Multistate outbreak of Salmonella Paratyphi B variant L(+) tartrate(+) and Salmonella Weltevreden infections linked to imported frozen raw tuna: USA, March-July 2015 (2018) Epidemiology and Infection, 146 (11), pp. 1461-1467.

ABSTRACT: Foodborne non-typhoidal salmonellosis causes approximately 1 million illnesses annually in the USA. In April 2015, we investigated a multistate outbreak of 65 *Salmonella* Paratyphi B variant L(+) tartrate(+) infections associated with frozen raw tuna imported from Indonesia, which was consumed raw in sushi. Forty-six (92%) of 50 case-patients interviewed ate sushi during the week before illness onset, and 44 (98%) of 45 who specified ate sushi containing raw tuna. Two outbreak strains were isolated from the samples of frozen raw tuna. Traceback identified a single importer as a common source of tuna consumed by case-patients; this importer issued three voluntary recalls of tuna sourced from one Indonesian processor. Four *Salmonella* Weltevreden infections were also linked to this outbreak. Whole-genome sequencing was useful in establishing a link between *Salmonella* isolated from ill people and tuna. This outbreak highlights the continuing foodborne illness risk associated with raw seafood consumption, the importance of processing seafood in a manner that minimises contamination with pathogenic microorganisms and the continuing need to ensure imported foods are safe to eat. People at higher risk for foodborne illness should not consume undercooked animal products, such as raw seafood. ISSN: 09502688

Petsong, K., Uddin, M.J., Vongkamjan, K., Ahn, J.

Combined effect of bacteriophage and antibiotic on the inhibition of the development of antibiotic resistance in Salmonella typhimurium (2018) Food Science and Biotechnology, 27 (4), pp. 1239-1244.

ABSTRACT: This study was designed to evaluate the combined effects of bacteriophage and antibiotic on the reduction of the development of antibiotic-resistance in *Salmonella* typhimurium LT2. The susceptibilities of *S. typhimurium* to ciprofloxacin and erythromycin were increased when treated with bacteriophages, showing more than 10% increase in clear zone sizes and greater than twofold decrease in minimum inhibitory concentration values. The growth of *S. typhimurium* was effectively inhibited by the combination of bacteriophage P22 and ciprofloxacin. The combination treatment effectively reduced the development of antibiotic resistance in *S. typhimurium*. The relative expression levels of efflux pump-related genes (*acrA*, *acrB*, and *tolC*) and outer membrane-related genes (*ompC*, *ompD*, and *ompF*) were decreased at all treatments. This study provides useful information for designing new antibiotic therapy to control antibiotic-resistant bacteria. ISSN: 12267708

Kim, J.N.

Genetics of CRISPR arrays in Salmonella Typhimurium 14028 associated with foreign DNA decay (2018) Genes and Genomics, 40 (8), pp. 865-872.

ABSTRACT: Clustered regularly interspaced short palindromic repeats (CRISPRs) are a genetic locus of prokaryotes and contain highly conserved direct repeats, spacers, and CRISPR-associated genes. Spacers in CRISPRs are known as adaptive immune markers and reveal what types of phage or foreign DNA have been introduced in the past. The primary objective of this study was to analyze spacer sequences in CRISPR arrays of 15 *Salmonella enterica* subspecies and to determine if *Salmonella* CRISPRs are indeed involved in resistance to foreign DNAs. Using a bioinformatics algorithm, the CRISPR arrays of 15 subspecies of *S. enterica* were predicted. The transformation efficiencies of the wild-type and mutant strains lacking a spacer were determined using the plasmid harboring the same sequences with the spacer. Analysis of the CRISPR arrays indicated that

S. Typhimurium encoded three possible CRISPR regions in the genome. Notably, 48 or 55 spacers were predicted in the genomes of *S. Typhimurium* 14028 and LT2 strains, respectively, and 39 were precisely identical. To confirm this prediction, the predicted CRISPR regions of *S. Typhimurium* 14028 were sequenced using the specific primers. Interestingly, a homology search of individual spacers found that the 2nd spacer of CRISPR 2 was nearly identical to a partial genome region of phage FSL SP-016. The mutant strain showed two to threefold increased transformation efficiency compared to that of the wild-type strain. These results demonstrate that the spacer sequence is dependent on genetic relations, especially for adaptive immunity against phage or foreign DNAs.
ISSN: 19769571

Donachie, A., Melillo, T., Bubba, L., Hartman, H., Borg, M.-L.

National outbreak of Salmonella Give linked to a local food manufacturer in Malta, October 2016

(2018) Epidemiology and Infection, 146 (11), pp. 1425-1432.

ABSTRACT: *Salmonella Give* is a rare serotype across Europe. In October 2016, a national outbreak of *S. Give* occurred in Malta. We describe the epidemiological, environmental, microbiological and veterinary investigations. Whole-genome sequencing (WGS) was performed on human, food, environmental and veterinary isolates. Thirty-six human cases were reported between October and November 2016, 10 (28%) of whom required hospitalisation. Twenty-six (72%) cases were linked to four restaurants. *S. Give* was isolated from ready-to-eat antipasti served by three restaurants which were all supplied by the same local food manufacturer. Food-trace-back investigations identified *S. Give* in packaged bean dips, ham, pork and an asymptomatic food handler at the manufacturer; inspections found inadequate separation between raw and ready-to-eat food during processing. WGS indicated two genetically distinguishable strains of *S. Give* with two distinct clusters identified; one cluster linked to the local food manufacturer and a second linked to veterinary samples. Epidemiological, environmental and WGS evidence pointed towards cross-contamination of raw and ready-to-eat foods at the local manufacturer as the likely source of one cluster. Severity of illness indicates a high virulence of this specific serotype. To prevent future cases and outbreaks, adherence to food safety practices at manufacturing level need to be reinforced. ISSN: 09502688

Vincent, C., Usongo, V., Berry, C., Tremblay, D.M., Moineau, S., Yousfi, K., Doualla-Bell, F., Fournier, E., Nadon, C., Goodridge, L., Bekal, S.

Comparison of advanced whole genome sequence-based methods to distinguish strains of Salmonella enterica serovar Heidelberg involved in foodborne outbreaks in Québec
(2018) Food Microbiology, 73, pp. 99-110.

ABSTRACT: *Salmonella enterica* serovar Heidelberg (*S. Heidelberg*) is one of the top serovars causing human salmonellosis. This serovar ranks second and third among serovars that cause human infections in Québec and Canada, respectively, and has been associated with severe infections. Traditional typing methods such as PFGE do not display adequate discrimination required to resolve outbreak investigations due to the low level of genetic diversity of isolates belonging to this serovar. This study evaluates the ability of four whole genome sequence (WGS)-based typing methods to differentiate among 145 *S. Heidelberg* strains involved in four distinct outbreak events and sporadic cases of salmonellosis that occurred in Québec between 2007 and 2016. Isolates from all outbreaks were indistinguishable by PFGE. The core genome single nucleotide variant (SNV), core genome multilocus sequence typing (MLST) and whole genome MLST approaches were highly discriminatory and separated outbreak strains into four distinct phylogenetic clusters that were concordant with the epidemiological data. The clustered regularly interspaced short palindromic repeats (CRISPR) typing method was less discriminatory. However, CRISPR typing may be used as a secondary method to differentiate isolates of *S. Heidelberg* that are genetically similar but epidemiologically unrelated to outbreak events. WGS-based typing methods provide a highly discriminatory alternative to PFGE for the laboratory investigation of foodborne outbreaks. ISSN: 07400020

Bennett, S.D., Sodha, S.V., Ayers, T.L., Lynch, M.F., Gould, L.H., Tauxe, R.V.

Produce-associated foodborne disease outbreaks, USA, 1998-2013

(2018) Epidemiology and Infection, 146 (11), pp. 1397-1406.

ABSTRACT: The US Food Safety Modernization Act (FSMA) gives food safety regulators increased authority to require implementation of safety measures to reduce the contamination of produce. To evaluate the future impact of FSMA on food safety, a better understanding is needed regarding outbreaks attributed to the consumption of raw produce. Data reported to the US Centers for Disease Control and Prevention's Foodborne Disease Outbreak Surveillance System during 1998-2013 were analysed. During 1998-

2013, there were 972 raw produce outbreaks reported resulting in 34 674 outbreak-associated illnesses, 2315 hospitalisations, and 72 deaths. Overall, the total number of foodborne outbreaks reported decreased by 38% during the study period and the number of raw produce outbreaks decreased 19% during the same period; however, the percentage of outbreaks attributed to raw produce among outbreaks with a food reported increased from 8% during 1998-2001 to 16% during 2010-2013. Raw produce outbreaks were most commonly attributed to vegetable row crops (38% of outbreaks), fruits (35%) and seeded vegetables (11%). The most common aetiologic agents identified were norovirus (54% of outbreaks), *Salmonella enterica* (21%) and Shiga toxin-producing *Escherichia coli* (10%). Food-handling errors were reported in 39% of outbreaks. The proportion of all foodborne outbreaks attributable to raw produce has been increasing. Evaluation of safety measures to address the contamination on farms, during processing and food preparation, should take into account the trends occurring before FSMA implementation. ISSN: 09502688

Venglovsky, J., Sasakova, N., Gregova, G., Papajova, I., Toth, F., Szaboova, T.
Devitalisation of pathogens in stored pig slurry and potential risk related to its application to agricultural soil

(2018) *Environmental Science and Pollution Research*, 25 (22), pp. 21412-21419.

ABSTRACT: The study investigated the risks arising from application of pig slurry to soil in relation to viability of *Salmonella typhimurium*, *Escherichia coli*, total coliforms, faecal enterococci and eggs of *Ascaris suum* at different temperatures. Potential effect of changes in physico-chemical parameters, particularly dry matter (DM), pH and ammonia, were also investigated. Examination showed that *S. typhimurium* was devitalised after storage in the slurry for 115 days at 4 °C and after 90 days at 20 and 42 °C. Devitalization of *E. coli* and faecal enterococci required more than 115 at temperature of 4 °C and faecal enterococci were recovered from slurry after 115 days of storage even at temperature of 20 °C. Total coliforms survived for 115 days at all investigated temperatures. Complete devitalization of *A. suum* eggs was not achieved even after 115 days at 42 °C. Our investigations indicated potential microbial and parasitic risk related to application of pig slurry to soil even after 115 days of storage. ISSN: 09441344

Müller, A., Jansen, W., Grabowski, N.T., Kehrenberg, C.

Characterization of Salmonella enterica serovars recovered from meat products legally and illegally imported into the EU reveals the presence of multiresistant and AmpC-producing isolates

(2018) *Gut Pathogens*, 10 (1), art. no. 40, .

ABSTRACT: Background: Food products of animal origin brought into the EU from third countries, both legally and illegally, can harbor foodborne pathogens such as *Salmonella enterica*. In this study, we examined five *S. enterica* isolates recovered either from legally imported chicken meat (n = 3) or from meat products confiscated from air travel passengers arriving in Germany (n = 2). The isolates were serotyped and further characterized by antimicrobial susceptibility testing, PCR-detection and sequencing of genes associated with antimicrobial resistances, and macrorestriction analysis. Transferability of resistance to third-generation cephalosporins was assessed by conjugation experiments and the plasmids tested for their incompatibility groups. Results: The three isolates from legal imports were identified as *S. Heidelberg* or as non-flagellated. All three isolates were identified as AmpC producers carrying bla CMY-2 and as non-susceptible to ciprofloxacin. They were additionally resistant to tetracycline and sulfamethoxazole. The bla CMY-2-carrying plasmids were transferable by conjugation and belonged to incompatibility groups Inc11 or IncA/C. The two isolates from illegally imported meat belonged to the serovars *Infantis* or *Weltevreden*. The former was phenotypically resistant to five classes of antimicrobial agents while the *S. Weltevreden* isolate was fully susceptible to all agents tested. Conclusion: The results of this study demonstrate that meat products imported from third countries, both legally and illegally, can harbor multiresistant *Salmonella enterica*. Consequently, these imports could constitute a source for the dissemination of antimicrobial resistant isolates, including those resistant to third-generation cephalosporins and fluoroquinolones. ISSN: 17574749

Marin, C., Torres, C., Marco-Jiménez, F., Cerdà-Cuéllar, M., Sevilla, S., Ayats, T., Vega, S.

Supplementary feeding stations for conservation of vultures could be an important source of monophasic Salmonella typhimurium 1,4,[5],12:i:-

(2018) *Science of the Total Environment*, 636, pp. 449-455.

ABSTRACT: Vultures are nature's most successful scavengers, feeding on the carcasses of dead animals present in the field. Availability of domestic carrion has been unstable due to

rapidly changing agro-grazing economies and increasing sanitary regulations that may require burial or burning of livestock carcasses. Thus, several griffon vulture (*Gyps fulvus*) recoveries are based on European legislation that guarantees the animals' welfare, avoids intense persecution of the vultures and allows the feeding of threatened wildlife in supplementary feeding stations (SFS). However, in recent years, many studies have speculated on the likelihood that avian scavengers may be infected by feeding on pig carcasses at SFS from intensive livestock. In this context, the present study evaluated whether free-living griffon vultures and pig farms share zoonotic *Salmonella* strains to test the hypothesis that vulture are infected during consumption of carcasses provided at SFS. Here, the occurrence, serotypes and genomic DNA fingerprinting (phage typing and pulsed-field gel electrophoresis) of isolated strains were carried out in griffon vultures and pig farms authorised to provided carcasses at SFS in Castellón province (eastern Spain). The bacteriological analyses revealed that 21.1% of vultures and 14.5% for pig farms samples tested were *Salmonella*-positive. Monophasic *S. typhimurium* 1,4,[5],12:i:- was the most frequently isolated serovar. Comparison of *Salmonella* strains isolated from vultures and pig farms revealed that monophasic *S. typhimurium* 1,4,[5],12:i:-, *S. Derby* and *S. Rissen* strains were highly genetically homogeneous (similar DNA fingerprint). In conclusion, the current study indicates that free-living griffon vultures and pig farms that provide the carcasses at SFS share several zoonotic *Salmonella* strains. On this basis, and although transmission could be bidirectional, our result seems to corroborate the pig carcasses-to-vulture transmission and cross-infection at SFS. As an immediate *Salmonella* control strategy in wild avian scavengers, we suggest the implementation of a programme to guarantee that solely pig carcasses from *Salmonella*-free farms arrive at SFS.
ISSN: 00489697

Wang, Y., Jia, B., Xu, X., Zhang, L., Wei, C., Ou, H., Cui, Y., Shi, C., Shi, X.

Comparative genomic analysis and characterization of two Salmonella enterica serovar enteritidis isolates from poultry with notably different survival abilities in egg whites (2018) Frontiers in Microbiology, 9 (SEP), art. no. 2111, .

ABSTRACT: *Salmonella enterica* serovar Enteritidis (*Salmonella* Enteritidis) is a globally important foodborne pathogen, and the contaminated chicken eggs are the major source of salmonellosis in humans. *Salmonella* Enteritidis strains are differentially susceptible to the hostile environment of egg whites. Strains with superior survival ability in egg whites are more likely to contaminate eggs and consequently infect humans. However, the genetic basis for this phenotype is unclear. We characterized two *Salmonella* Enteritidis strains isolated from chicken meat that had similar genetic backgrounds but large differences in survival ability in egg whites. Although genome comparisons indicated that the gene content and genomic synteny were highly conserved, variations including six insertions or deletions (INDELs) and 70 single nucleotide polymorphisms (SNPs) were observed between the two genomes. Of these, 38 variations including four INDELs and 34 non-synonymous SNPs (nsSNP) were annotated to result in amino acid substitutions or INDELs in coding proteins. These variations were located in 38 genes involved in lysozyme inhibition, vitamin biosynthesis, cell division and DNA damage response, osmotic and oxidative protection, iron-related functions, cell envelope maintenance, amino acid and carbohydrate metabolism, antimicrobial resistance, and type III secretion system. We carried out allelic replacements for two nsSNPs in *bioC* (biotin synthesis) and *pliC* (lysozyme inhibition), and two INDELs in *ftsK* and *yqjJ* (DNA damage response) by homologous recombination, and these replacements did not alter the bacterial survival ability in egg whites. However, the bacterial survival ability in egg whites was reduced when deletion mutation of the genes *bioC* and *pliC* occurred. This study provides initial correlations between observed genotypes and phenotypes and serves as an important caveat for further functional studies. ISSN: 1664302X

De Abrew Abeyesundara, P., Dhowlaghar, N., Nannapaneni, R., Schilling, M.W., Mahmoud, B., Sharma, C.S., Ma, D.-P.

Salmonella enterica growth and biofilm formation in flesh and peel cantaloupe extracts on four food-contact surfaces

(2018) *International Journal of Food Microbiology*, 280, pp. 17-26.

ABSTRACT: *Salmonella enterica* is responsible for the highest number of foodborne disease outbreaks pertaining to cantaloupe industry. The objective of this study was to examine the growth and biofilm formation by outbreak strains of *S. enterica* ser. Poona (*S. Poona*), *S. enterica* ser. Stanley (*S. Stanley*) and *S. enterica* ser. Montevideo (*S. Montevideo*) on different food-contact processing surfaces in cantaloupe flesh and peel extracts at 22 °C and 10 °C. The generation time of all *S. enterica* strains tested was shorter in the high concentration (50 mg/ml) of cantaloupe extract and high temperature. In 50 mg/ml of cantaloupe flesh or peel extract, the populations of *S. enterica* were increased by 5 log

CFU/ml in 24 h at 22 °C and 1 log CFU/ml in 72 h at 10 °C. In 2 mg/ml of cantaloupe flesh or peel extracts, the populations of *S. enterica* were increased by 3.5 log CFU/ml in 56 h at 22 °C, but there were no changes in 72 h at 10 °C. The biofilm production of *S. enterica* was greater at 50 mg/ml of cantaloupe extract and 22 °C, but no major differences ($P \geq 0.05$) were found among the strains tested. In 50 mg/ml cantaloupe extract, *S. enterica* produced 5–6 log CFU/cm² biofilm in 4–7 d at 22 °C and approximately 3.5–4 log CFU/cm² in 7 d at 10 °C. In 2 mg/ml of cantaloupe extract, *S. enterica* produced 4–4.5 log CFU/cm² biofilms in 4–7 d at 22 °C and 3 log CFU/cm² in 7 d at 10 °C. Biofilm formation by *S. Poona* (O1A4754) was lowest on buna-n rubber compared to stainless steel, polyethylene and polyurethane surfaces under the majority of conditions tested. Overall, these findings show that *S. enterica* strains can grow rapidly and form biofilms on different cantaloupe processing surfaces in the presence of low concentrations of cantaloupe flesh or peel extracts. ISSN: 01681605

Elkenany, R.M., Eladi, A.H., El-Shafei, R.A.

Genetic characterisation of class 1 integrons among multidrug-resistant Salmonella serotypes in broiler chicken farms

(2018) *Journal of Global Antimicrobial Resistance*, 14, pp. 202-208.

ABSTRACT: Objectives: Antimicrobial resistance in *Salmonella* serotypes has been reported. Integrons play an important role in the dissemination of antimicrobial resistance genes in bacteria. Scarce literature is available on the identification of integrons in *Salmonella* isolated from broiler chickens. In this study, antimicrobial susceptibility testing and characterisation of class 1 integrons among multidrug-resistant (MDR) *Salmonella enterica* serotypes in broiler chicken farms in Egypt were performed. Methods: Antimicrobial susceptibility was determined by the disk diffusion method. PCR was performed to detect antimicrobial resistance genes and class 1 integrons in the tested *Salmonella* serotypes. Gene sequencing of the variable region of a class 1 integron was performed. Results: *Salmonella* spp. were detected in 26 (13.5%) of 192 broiler samples, with *Salmonella* Enteritidis being the most frequently detected serotype, followed by *Salmonella* Kentucky and *Salmonella* Typhimurium and other serotypes. A very high resistance rate was observed to trimethoprim/sulfamethoxazole (100%), whilst a low resistance rate was observed to cefuroxime (57.7%). MDR *S. enterica* isolates displayed resistance to ciprofloxacin and azithromycin. Class 1 integrons were detected in 20 (76.9%) of the 26 *Salmonella* isolates. A high prevalence of class 1 integrons, as the first recorded percentage in the literature, associated with MDR *Salmonella* isolates was observed. Conclusions: Antimicrobial resistance rates in *Salmonella* serotypes from broiler chicken farms were alarming, especially for ciprofloxacin and azithromycin. Thus, another therapeutic strategy other than antimicrobials is recommended to prevent outbreaks of MDR *Salmonella*. ISSN: 22137165

Sexton, T., Geornaras, I., Belk, K.E., Bunning, M., Martin, J.N.

Salmonella contamination in broiler synovial fluid: Are we missing a potential reservoir? (2018) *Journal of Food Protection*, 81 (9), pp. 1425-1431.

ABSTRACT: The objective of this study was to assess the presence and characteristics of *Salmonella enterica* found in the synovial fluid of broiler carcasses. The synovial fluid of three individual joints from 500 broiler carcasses was individually sampled from five broiler processing facilities located in the Southeast and West regions of the United States (1,500 total samples). The external surface of broiler carcass was decontaminated before sampling of the shoulder, coxofemoral, and tibiofemoral joints. Individual samples were enriched, composited, and subjected to rapid PCR-based detection of *Salmonella*. Individual samples from any positive composites were also enriched before determination of *Salmonella* presence in the same manner. Positive individual samples were subjected to secondary enrichment before plating onto selective agar for isolation of *Salmonella*. *Salmonella* isolates were serotyped before determination of antimicrobial susceptibility. Overall, 1.00% (5 of 500 broiler carcasses) of composite samples and 0.47% (7 of 1,500 samples) of individual samples were positive for *Salmonella*. Five of the seven isolates were susceptible to all drugs tested and determined to be *Salmonella* Enteritidis. The remaining two isolates, identified as *Salmonella* Typhimurium, were resistant to streptomycin. To our knowledge, no previous assessments of *Salmonella* in the synovial fluid of broilers has been reported; however, results of the present study suggested that the synovial fluid may be a reservoir for *Salmonella* in broilers. Although the prevalence of *Salmonella* is low, this information provides valuable insight into potential poultry contamination pathways and warrants further exploration. ISSN: 0362028X

Dev Kumar, G., Williams, R.C., Sriranganathan, N., Boyer, R.R., Eifert, J.D.

Survival of tomato outbreak associated salmonella serotypes in soil and water and the role of biofilms in abiotic surface attachment

(2018) *Foodborne Pathogens and Disease*, 15 (9), pp. 548-553.

ABSTRACT: *Salmonella* serotypes linked to tomato-associated outbreaks were evaluated for survival in soil and water over a 40-day period. *Salmonella enterica* serotypes Anatum, Baildon, Braenderup, Montevideo, Newport, and Javiana were inoculated separately into sterile soil and water, followed by plating onto TSAYE and XLT4 at 10-day intervals. Biofilm production by *Salmonella* serotypes was measured on both quartz particles (soil surrogate) and glass coverslips, and was evaluated using a crystal violet dye assay. *Salmonella* populations in soil and water over 40 days indicated no significant differences between *Salmonella* serotypes tested ($p > 0.05$). Over a 40-day period, there was a 1.84 ± 0.22 log CFU/g and 1.56 ± 0.54 CFU/mL decrease in populations of *Salmonella* in soil and water, respectively. Enumeration indicated that *Salmonella* population fluctuated in water but decreased linearly in soil. All serotypes tested produced the "red dry and rough" morphotype on Congo Red agar. Biofilm produced by all the *Salmonella* serotypes tested was significantly different on quartz particles than on glass coverslips ($p < 0.0001$), indicating that material and surface characteristics could affect biofilm development. The ability of *Salmonella* serotypes to persist in soil or water and attach to abiotic surfaces through biofilm formation affirms that contact surfaces, soil, water, and sediment should be considered as possible sources of cross-contamination in the farm environment.

ISSN: 15353141

Beshiru, A., Igbinosa, I.H., Igbinosa, E.O.

Biofilm formation and potential virulence factors of Salmonella strains isolated from ready-to-eat shrimps

(2018) *PLoS ONE*, 13 (9), art. no. e0204345, .

ABSTRACT: *Salmonella* species is an important foodborne pathogen with the non-typhoidal serovars such as Enteritidis and Typhimurium as the most predominant strains. This study examines the biofilm formation, phenotypic virulence factors and cell surface characteristics of *Salmonella* strains from ready-to-eat shrimps. The ready-to-eat shrimps were obtained from open markets between November 2016 and October 2017 in Edo and Delta States, Nigeria. The occurrence of *Salmonella* strains in this study was 210/1440 (14.58%) of the ready-to-eat shrimp's samples. The identified strains comprise of *Salmonella* Enteritidis 11, *Salmonella* Typhimurium 14 and other *Salmonella* spp. 20. The 45 identified *Salmonella* strains revealed the following virulence properties: swimming and swarming motility 45(100%); Slayer 39(86.67%); haemolytic activity 40(88.89%); lipase activity 43(95.56%); protease activity 43(95.56%); gelatinase production 43(95.56%); and DNA degrading activity 41 (91.11%). The variation in the formation of biofilm-based on the diversity of *Salmonella* species was observed with higher percentage of *Salmonella* Typhimurium strains as strong biofilms producers under different environmental conditions. For surface hydrophobicity using bacterial adherence to hydrocarbons, 25(55.56%) were hydrophilic while 20(44.44%) were moderately hydrophobic from the 45 *Salmonella* isolates. Using salting aggregation test for surface hydrophobicity, all selected isolates 45(100%) was hydrophilic. Autoaggregation index for the 12 selected *Salmonella* isolates ranged from 15.2–47.2%, while the autoaggregation index for the 12 selected test bacteria ranged from 26.2–71.3%. Coaggregation between the 12 selected test bacteria and 12 *Salmonella* isolates ranged from 12.5–81.0%. The occurrence of pathogenic species of *Salmonella* from ready-to-eat shrimps could be detrimental to the consumers. Findings on the physiological conditions of biofilms formed by the foodborne pathogenic *Salmonella* and the cell surface characteristics therein are crucial for the advancement of methods for controlling *Salmonella* from ready-to-eat foods. ISSN: 19326203

Yuqiao, J.I.N., Pickens, S.R., Hildebrandt, I.M., Burbick, S.J., Grasso-Kelley, E.M., Keller, S.E., Anderson, N.M.

Thermal inactivation of salmonella agona in Low-Water activity foods: Predictive models for the combined effect of temperature, water activity, and food component

(2018) *Journal of Food Protection*, 81 (9), pp. 1411-1417.

ABSTRACT: *Salmonella* can survive in low-moisture, high-protein, and high-fat foods for several years. Despite nationwide outbreaks and recalls due to the presence of *Salmonella* in low-moisture foods, information on thermal inactivation of *Salmonella* in these products is limited. This project evaluated the impact of water activity (a_w), temperature, and food composition on thermal inactivation of *Salmonella enterica* serovar Agona in defined high-protein and high-fat model food matrices. Each matrix was inoculated with *Salmonella* Agona and adjusted to obtain a target a_w , ranging from 0.50 to 0.98. Samples were packed into aluminum test cells and heated (52 to 90°C) under isothermal conditions. Survival of *Salmonella* Agona was detected on tryptic soy agar with 0.6% yeast extract.

Complex influences by food composition, aw, and temperature resulted in significantly different ($P, 0.05$) thermal resistance of Salmonella for the conditions tested. It was estimated that the same point temperatures at which the D-values of the two matrices at each aw (0.63, 0.73, 0.81, and 0.90) were identical were 79.48, 71.28, 69.62, and 38.428C, respectively. Above these temperatures, the D-values in high-protein matrices were larger than the D-values in high-fat matrices at each aw. Below these temperatures, the inverse relationship was observed. A correlation between temperature and aw existed on the basis of the level of fat or protein in the food, showing that these compositional factors must be accounted for when predicating thermal inactivation of Salmonella in foods. ISSN: 0362028X

Alzwghaibi, A.B., Yahyaraeyat, R., Fasaee, B.N., Langeroudi, A.G., Salehi, T.Z.
Rapid molecular identification and differentiation of common Salmonella serovars isolated from poultry, domestic animals and foodstuff using multiplex PCR assay (2018) Archives of Microbiology, 200 (7), pp. 1009-1016.

ABSTRACT: Salmonella is widely distributed throughout the world and can be found in poultry industry, animal breeding centers, food and feedstuffs of all geographical regions. This study was conducted to determine and identify Salmonella serovars isolated from poultry, calves and foodstuffs (poultry and animals products such as egg and meat). A total of one hundred isolates of Salmonella serovars including Salmonella Typhimurium, Salmonella Enteritidis, Salmonella Infantis, Salmonella Gallinarum and Salmonella Pullorum consecutively were subjected to the conventional culture, biochemical and serological assays. The utility of molecular multiplex PCR was investigated to identify and differentiate among five Salmonella serovars which were identified according to the presence of rfbJ, fljB, invA, and fliC genes in *S. Typhimurium*, sefA, invA and spv genes in Salmonella Enteritidis, fljB, fliC and invA genes in Salmonella Infantis, hut and slgC genes in both Salmonella Gallinarum and Salmonella Pullorum and speC gene specifically in Salmonella Gallinarum. Biochemical assays and serotyping are complicated to directly differentiate between Salmonella Gallinarum and Salmonella Pullorum because of their antigenic similarity. According to the results, Multiplex PCR can be considered as simple, rapid, accurate and useful test to identify and differentiate among Salmonella serovars. ISSN: 03028933

Ed-Dra, A., Karraouan, B., Allaoui, A.E., Khayatti, M., Ossmani, H.E., Filali, F.R., EIMdaghri, N., Bouchrif, B.

Antimicrobial resistance and genetic diversity of Salmonella Infantis isolated from foods and human samples in Morocco (2018) Journal of Global Antimicrobial Resistance, 14, pp. 297-301.

ABSTRACT: Objectives: Genotyping of Salmonella strains is an important molecular tool to discriminate isolates and to improve epidemiological studies when an outbreak occurs. Among the DNA-based genotyping methods, pulsed-field gel electrophoresis (PFGE) is currently used to subtype Salmonella isolates. In this study, the feasibility of genotyping Salmonella enterica serotype Infantis strains using XbaI restriction enzyme was evaluated. Separation of restricted fragments was performed by PFGE. Methods: To test the possibility of applying this methodology to epidemiological investigation, a collection of 26 Salmonella Infantis strains were tested for their susceptibility to 14 antimicrobial agents and were analysed by XbaI macrorestriction followed by PFGE. Detection of class 1 integrons as well as intI1 and blaTEM genes in resistant strains was also studied. Results: Antimicrobial susceptibility testing showed that 84.6% (22/26) of Salmonella Infantis isolates were susceptible to all of the antimicrobials tested, whereas 7.7% (2/26) had low-level resistance to β -lactams and harboured the blaTEM gene. A class 1 integron (0.8 kb) and the intI1 gene (898 bp) were detected in one Salmonella Infantis strain. However, five different PFGE profiles were defined by XbaI macrorestriction. Conclusions: The PFGE method demonstrated adequate typing ability and represents a powerful tool to discriminate the serotype Salmonella Infantis. ISSN: 22137165

Cevallos-Almeida, M., Houdayer, C., Rose, V., Bailly, Y., Paboeuf, F., Fablet, C., Denis, M., Kerouanton, A.

Colonization of pigs experimentally infected with a monophasic variant of salmonella typhimurium (2018) Foodborne Pathogens and Disease, 15 (9), pp. 576-582.

ABSTRACT: The monophasic variant of Salmonella Typhimurium is highly prevalent in human and in pork. However, little is known about colonization dynamics and serology in pigs. We orally inoculated 24 seven-week-old piglets with 10⁹ CFU/pig of a porcine strain of monophasic Salmonella Typhimurium in an experimental trial. Three groups of eight piglets were orally inoculated and monitored for 21, 49, or 84 days post-inoculation until

necropsied. From 3 days post-inoculation to necropsy, individual feces were sampled twice weekly and blood once weekly. At necropsy, the tonsils, mesenteric lymph nodes, and the contents of the duodenum, jejunum, ileum, and cecum were collected from each pig. We determined the number of CFU/g in all the samples and measured also *Salmonella* antibodies in OD% in all blood samples. At different times during the trial, we tested by MLVA (Multilocus Variable Number Tandem Repeat Analysis) the genomic stability of the strain after passing through the intestinal tract. *Salmonella* was continuously excreted by pigs, ranging from 1.4 to 5.8 log₁₀ CFU/g. At necropsy, *Salmonella* was present in all samples, but the tonsils were particularly infected. *Salmonella* antibodies were detected in five pigs 7 days post-inoculation. At 49 days post-inoculation, all the pigs were seropositive. We observed new MLVA types for 3.3% of the isolates tested over the trial. Our study allowed us to show the serovar's ability to persist in pigs after infection up to 84 days post-inoculation. We demonstrated that *Salmonella* seroconversion appeared earlier than in naturally infected pigs and that the strain's genome can evolve after passing through the digestive tract of pigs. ISSN: 15353141

Cervelin, V., Fongaro, G., Pastore, J.B., Engel, F., Reimers, M.A., Viancelli, A.
Enterobacteria associated with houseflies (Musca domestica) as an infection risk indicator in swine production farms
(2018) *Acta Tropica*, 185, pp. 13-17.

ABSTRACT: Houseflies (*Musca domestica*) spend part of their life development on animal or human manure. Manure is high in pathogenic microbes; thus, houseflies have been known as a mechanical vector for various important zoonotic diseases. Therefore, the present study showcases captured houseflies from intensive swine production regions (which are areas of high manure concentration) in Southern Brazil, and analyses their bodies' to the presence of *Escherichia coli* and *Salmonella* sp. and the sensitivity of these bacteria to various antibiotics. Additionally, Quantitative Microbiology Risk Assessment was performed simulating the contamination of lettuce by flies' bacteria and subsequent lettuce consumption by an adult human being. Houseflies were captured in swine buildings and farm houses from five farms. *E. coli* quantification values ranged from 10⁴ to 10⁶ CFU/20 flies, and all sampling sites had positive results from bacteria presence in the collected houseflies. On the other hand, *Salmonella* sp. presence was observed in only three farms, where the quantification ranged from 10² to 10⁵ CFU/20 flies. The bacteria showed to be resistant to at least two from the four tested antibiotics (ampicillin, Cefalotin, Ciprofloxacin and Norfloxacin) antibiotics used in human or veterinary medicine. Infection probability analyses showed risk of human infection by *E. coli*, indicating possible transmission of zoonotic pathogens through flies. In this context, it was possible to conclude that there is a need for flies control, especially in swine farms where zoonotic pathogens can be abundant, to minimize the health impact of the vectorization of enteric bacteria. ISSN: 0001706X

Yuan, C., Krull, A., Wang, C., Erdman, M., Fedorka-Cray, P.J., Logue, C.M., O'Connor, A.M.

Changes in the prevalence of Salmonella serovars associated swine production and correlations of avian, bovine and swine-associated serovars with human-associated serovars in the United States (1997–2015)
(2018) *Zoonoses and Public Health*, 65 (6), pp. 648-661.

ABSTRACT: As *Salmonella enterica* is an important pathogen of food animals, surveillance programmes for *S. enterica* serovars have existed for many years in the United States. Surveillance programmes serve many purposes, one of which is to evaluate alterations in the prevalence of serovars that may signal changes in the ecology of the target organism. The primary aim of this study was to evaluate changes in the proportion of *S. enterica* serovars isolated from swine over a near 20-year observation period (1997–2015) using four longitudinal data sets from different food animal species. The secondary aim was to evaluate correlations between changes in *S. enterica* serovars frequently recovered from food animals and changes in *S. enterica* serovars associated with disease in humans. We found decreasing proportions of *S. enterica* serovar Typhimurium, serovar Derby and serovar Heidelberg and increasing proportions of *S. enterica* serovar 4,[5],12:i:-, serovar Infantis and serovar Johannesburg in swine over time. We also found positive correlations for the yearly changes in *S. enterica* serovar 4,[5],12:i:-, serovar Anatum and serovar Johannesburg between swine and human data; in *S. enterica* Worthington between avian and human data; and in *S. enterica* serovar 4,[5],12:i:- between bovine and human data. We found negative correlations for the yearly changes in *S. enterica* serovar 4,[5],12:i:- and serovar Johannesburg between avian and human data. ISSN: 18631959

Ruggeri, J., Foresti, F., Pavesi, R., Terrini, A., Giudici, F., Padoan, D., Corradi, A., Ossiprandi, M.C., Pasquali, P., Alborali, G.L.

The synergistic effect of organic acids, phytochemicals and a permeabilizing complex reduces Salmonella Typhimurium 1,4,[5],12:i-shedding in pigs (2018) Veterinary Research Communications, 42 (3), pp. 209-217.

ABSTRACT: Salmonella Typhimurium (including S.Typhimurium 1,4,[5],12:i-) and other enteric pathogens cause acute infection in pigs during the weaning stage, often evolving into chronic infections responsible for the introduction of zoonotic bacteria into the slaughterhouse and thus determining carcass contamination. In addition to being zoonotic hazards, these pathogens are responsible for economic losses in affected farms. Traditionally, antibiotic treatments have been largely administered in order to reduce the infection burden but it favored, as a direct consequence, an increase in the number of multi-drug resistance strains. In order to overcome antibiotic-resistance concerns, new alternative control strategies should be developed. In this context, a blend of organic acids, phytochemicals and a permeabilizing complex, administered in feed (Group A - 459 piglets) or water (Group B - 458 piglets), was tested in field conditions for its capability of reducing Salmonella-infection in weaned piglets of an endemic farm. Data recorded were compared to results of a control group (Group C - 456 piglets). Zootechnical parameters were recorded in all animals, while microbiological, serological and PCR analyses were conducted in 15 piglets for each group. Results demonstrated that additive administered in feed improved animal weight gain (better average daily gain [A.D.G.] and increment), and rapidly reduced Salmonella-shedding in feces. Administration of additive in feed gave better results than in water. ISSN: 01657380

Djordjević, J., Bošković, M., Starčević, M., Ivanović, J., Karabasil, N., Dimitrijević, M., Lazić, I.B., Baltić, M.Ž.

Survival of Salmonella spp. in minced meat packaged under vacuum and modified atmosphere (2018) Brazilian Journal of Microbiology, 49 (3), pp. 607-613.

ABSTRACT: The effect of different modified atmosphere packaging regimes on the behavior of Salmonella spp. on minced meat was studied. Minced meat was experimentally contaminated with a Salmonella spp. cocktail (S. Enteritidis, S. Typhimurium, S. Infantis and S. Arizonae), packaged under vacuum or modified atmosphere with initial headspaces containing 20%O₂/50%CO₂/30%N₂ and 20%O₂/30%CO₂/50%N₂) and stored at 3 ± 1 °C for 12 days. Samples were analyzed for Salmonella spp., viable and lactic acid bacteria count every third day. Salmonella spp. counts decreased during storage in all packaging types, with reductions of about 1.5 log CFU/g. A significant difference (p < 0.01) was noted between Salmonella spp. counts in meat packaged in vacuum and modified atmospheres, although there was no significant difference in Salmonella spp. count between meat packaged in 50%CO₂, and meat packaged in 30%CO₂. At the end of the study, there were significant differences (p < 0.01; p < 0.05) in total viable and lactic acid bacterial counts between meat packaged in vacuum and modified atmosphere, and the lowest counts were noted in meat packaged in modified atmosphere with 50%CO₂. ISSN: 15178382