NEWSLETTER

European Union Reference Laboratory for *Salmonella*

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Editorial Note

Bilthoven, 9 January 2020

Dear colleague,

Hopefully your Christmas break was nice and relaxing, with a good start in the New Year. On behalf of the EURL-*Salmonella* staff, I would like to wish you **all the best for 2020!**

In the last quarter of 2019, we organised two Proficiency Tests (PTs) and are currently preparing the next PT to be organised in Spring 2020.

In September/October 2019 the **PT on detection of *Salmonella* in samples from the primary production stage** (chicken faeces) was organised. The results of this PT (interim summary and NRLs' own results) were reported to the participants shortly before the Christmas break.

In November 2019 the **PT on typing of *Salmonella*** was organised. In addition to the obligatory serotyping part, this study included a new, voluntary part for molecular sub-typing to determine whether some strains concern common types (cluster analysis). The (molecular) method used is free of choice (e.g. PFGE, MLVA, WGS). We were very happy to notice that the number of applications for this part of the study exceeded largely the minimum number of applications (>7 participants). This additional part of the study is also new for us and we consider it as a pilot to learn if such a set-up works well. The performance of the NRLs-*Salmonella* will only be judged on the serotyping of *Salmonella* and not on the outcome of the molecular additional part of the study. The deadline for submission of the serotyping results was shortly before the Christmas break and the deadline for the cluster analysis is 31 January 2020.

By mid-October 2019 we made an 'inventory' to collect information on the organisations which act as **NRL for the detection of *Salmonella* in bivalve molluscs**, since the EURL for live bivalve molluscs ceased to exist (per 1-1-2019). By the end of November 2019 we had collected a list of almost 30 organisations indicating to be the (new) NRL for the detection of *Salmonella* in bivalve molluscs. In fall 2019 we started the preparatory work for organising an **PT on detection of *Salmonella* in mussels,** to be organised in March 2020. A tentative timetable for this PT was sent to the list of (new) NRLs for the detection of *Salmonella* in bivalve molluscs, in December 2019. This timetable is also included in this Newsletter. If your country did not yet submit the information of the NRL-*Salmonella* bivalve molluscs (e.g. because the information of the relevant NRL was not yet available by November 2019), but still want to do so, please contact Kirsten Mooijman (Kirsten.mooijman@rivm.nl).

Shortly before the Christmas break, information was sent to the NRLs about a **conference entitled 'Modern technologies to enable response to crises: Next Generation Sequencing to tackle food-borne diseases in the EU'**. This conference will take place at the Instituto Superiore di Sanità in Rome on 10 March 2020. It aims to discuss the vision and challenges of the NGS technology in its adoption at the EU level as well as in its use for multi country outbreaks. This conference is organised by the working group on Next Generation Sequencing (NGS) of the EURLs biorisks (Anti-Microbial Resistance, *Campylobacter, E. coli,* Foodborne viruses, *Listeria*, Parasites, *Salmonella* and Staphylococci) coordinated by Stefano Morabito (EURL *E. coli*, ISS, Rome, Italy) with the support of the Med-Vet-Net association. There is no fee for participation in this conference, but the costs for travel and stay in Rome are for your own

(organisation) budget. The flyer for this conference was sent to you earlier and is also included in this Newsletter.

In the last quarter of 2019, we also started with the preparation of the **EURL-*Salmonella* workshop of 2020**. Again this workshop will be organised in Zaandam in the Netherlands, but in another hotel than before. In December 2019 we have sent you the first announcement of the workshop, which is planned on 28 and 29 May 2020. More information, as well as a registration form will be sent to the NRLs-*Salmonella* later in January.

For your information, the following reports about EURL-*Salmonella* activities were published in the last quarter of 2019:

Diddens, R.E. and Mooijman, K.A, 2019. EURL-*Salmonella* Proficiency Test food-feed 2019. Detection of *Salmonella* in flaxseed. National Institute for Public Health and the Environment, Bilthoven, the Netherlands. RIVM Report no.: 2019-0134. https://www.rivm.nl/bibliotheek/rapporten/2019-0134.pdf

Mooijman, K.A. The 24th EURL-*Salmonella* workshop – 28 and 29 May 2019, Amersfoort, the Netherlands. National Institute for Public Health and the Environment, Bilthoven, the Netherlands. RIVM Report no.: 2019-0135. https://www.rivm.nl/bibliotheek/rapporten/2019-0135.pdf

Best wishes,
Kirsten Mooijman
Coordinator EURL-*Salmonella*

Contribution of the EURL-*Salmonella*

Tentative timetable
EURL - *Salmonella* Proficiency Test Live Bivalve Molluscs 2020
Detection of *Salmonella* in mussels

Week	Date	Subject
	Week(s) before start PT Live Bivalve Molluscs 2020	Mailing of *Salmonella* reference material to the NRLs as Biological Substance Cat. B (UN3373) by door-to-door courier service Mailing of the protocol and instructions for the result form to the NRLs by e-mail Sending the link for the result form to the participants by e-mail Preparation of media by the NRLs
12	16 March	Mailing of parcels with samples to the NRLs by door-to-door courier service
12	18 March	Performance of the Proficiency Test
16	Before 11 April	Deadline for completing the electronic submission of results: **10 April 2020** (23:59h CET) After this deadline the result form will be closed

For Information

'Science meets Policy' conference:
Modern technologies to enable response to crises: Next Generation Sequencing to tackle food-borne diseases in the EU

March 10, 2020. Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy

Over the last decade, the rapid development of Next Generation Sequencing (NGS) for the characterization and typing of foodborne pathogens through whole genome analysis has provided many laboratories involved in testing food, animal and clinical samples with fit-for-purpose application and a realistic alternative to the classical methods. Especially in terms of precise sequences for source tracking, antibiotic resistance, virulence factors and toxicity. The acceptable costs, reasonable speed of execution and potential automation are major breakthroughs.
The EURL Working Group on NGS launches a workshop with support from the Med-Vet-Net Association.
The workshop aims to inform Member States about the benefits of introducing NGS for typing food-borne pathogens and to start a general discussion on the infrastructure needed to enable the competent authorities to take measures based on evidences from the application of NGS, including the need for a legal framework.

The workshop structure
The workshop will consist of presentations from representatives of the European Commission, EFSA and ECDC about the vision and the challenges of the adoption of the NGS technology. Further, the practical application of the NGS technique in multi country outbreaks will be described. Finally, a presentation on the legal aspects will be included providing a base for the following general discussion.
In the second session of the workshop all participants will have the opportunity to discuss all the topics presented with a panel of experts.
Highlighting possible hindrances at country level in endorsing the NGS technique and discussing possible solutions will also represent a goal of the initiative.

If you are one of the following stakeholders you should attend this workshop

- National Reference Laboratories belonging to the EURLs networks for the biological hazards.
- Relevant Competent Authorities of the EU Member States (MS).
- Scientists that actively work on building the knowledge on the use of NGS for food safety.
- Anyone involved in the official control of food in the EU MS.

Link for registration: https://w3.iss.it/site/sanvevent

DRAFT AGENDA 'Science meets Policy' conference

	Tuesday 10 March 2020	
09:00	**Welcome and Introduction (10')**	**Silvio Brusaferro** **ISS President**
09:15	**Greetings (10')**	**Stefano Morabito** **Chair of the organization committee**
	Session 1: Building the capacity in Europe	
09:30	Vision on NGS (20')	**Pamina Suzuki,** **European Commission G4**
09:50	EFSA State of play and future perspectives (20')	**Valentina Rizzi, Mirko Rossi** **European Food Safety Authority**
10:10	ECDC State of play and future perspectives (20')	**Johanna Takkinen,** **Eurpean Center for Diseases Control and prevention**
10:30	The inter EURL WG on NGS Work program: State of play (20')	**TBD** **Inter EURL WG on NGS**
11:00	**Coffee Break (30')**	
	Session 2: Policy and Science	
11:30	Need for a legal framework for NGS data supporting action by the CAs (30')	**George Haringhuizen** **RIVM**
12:00	Use of NGS to face crises (Provisional title) (20')	**Eelco Franz** **RIVM**
12:20	Toward an extended use of whole genome sequencing for the investigation of multi-EU country *Listeria monocytogenes* outbreaks (20')	**Benjamin FELIX** **EURL Listeria**
12.40	Activities of the one-health EJP on the implementation and harmonization of NGS across Europe (20')	**Annemarie Kaesbohrer** **BfR**
13:00	**Lunch (1h)**	
14:00	**Open discussion** (2h, 15') Chair: Stefano Morabito Speakers: Valentina Rizzi, Johanna Takkinen, Pamina Suzuki, George Haringhuizen, Eelco Franz, Annemarie Kaesbohrer, Benjamin FELIX	
16:15	**Concluding remarks and closure**	

From the Literature

Salmonella-related Literature from Scopus: October – December 2019

Xu, W., Gao, J., Zheng, H., Yuan, C., Hou, J., Zhang, L., Wang, G.
Establishment and Application of Polymerase Spiral Reaction Amplification for Salmonella Detection in Food
(2019) Journal of microbiology and biotechnology, 29 (10), pp. 1543-1552.
ABSTRACT: Salmonella is a common zoonotic and foodborne pathogen that causes high morbidity and mortality in developing countries. In this study, we established and validated a polymerase spiral reaction (PSR) assay which targeted the conserved invasion gene (invA) of Salmonella by SYBR Green I indicator methods. Subsequently, assays for determination of the optimal conditions for optimal specificity and sensitivity of PSR were performed. We performed comprehensive evaluations using loop-mediated isothermal amplification (LAMP) and realtime PCR. A total number of 532 samples of daily food were analyzed by PSR. Twenty-seven bacterial strains were tested in the specificity assay, from which positive results were obtained only for 14-Salmonella strains. However, none of the 13 non-Salmonella strains was amplified. Similarly with LAMP and real-time PCR, the detection limit of the PSR assay was 50 CFU/ml. The PSR method was also successfully applied to evaluate the contamination with Salmonella in 532 samples of daily food, corroborating traditional culture method data. The novel PSR method is simple, sensitive, and rapid and provides new insights into the prevention and detection of foodborne diseases. ISSN: 17388872

Marin, C., D'Auria, G., Martínez-Priego, L., Marco-Jiménez, F.
Draft genome sequences of 12 monophasic Salmonella enterica subsp. enterica serotype typhimurium 1,4,[5], 12:I: Strains isolated from wild Griffon vultures in eastern Spain
(2019) Microbiology Resource Announcements, 8 (42), art. no. e00570-19, .
ABSTRACT: Monophasic Salmonella enterica subsp. enterica serovar Typhimurium is one of the most common zoonotic pathogens. Salmonella species reside in a wide variety of hosts, including wild animals. Thus, we report here the genome sequences of 12 monophasic S. Typhimurium strains isolated from healthy wild vultures to gain better insight into their epidemiology and host-pathogen interactions. ISSN: 2576098X

Paudyal, N., Pan, H., Elbediwi, M., Zhou, X., Peng, X., Li, X., Fang, W., Yue, M.
Characterization of Salmonella Dublin isolated from bovine and human hosts
(2019) BMC Microbiology, 19 (1), art. no. 226, .
ABSTRACT: Background: Salmonella enterica subsp. enterica serovar Dublin (S. Dublin), a cattle adapted serovar causes enteritis, and systemic disease in bovines. The invasive index of this serovar far exceeds that of the other serovars and human infections often present as fatal or highly resistant infections. In this, observational study, phenotypic properties of human and bovine-derived isolates of S. Dublin along with antibiogram of common antimicrobials were evaluated. The multiplex PCR confirmed isolates were genotyped using 7-gene legacy MLST. MIC assay was done by broth microdilution method. Previously published protocols were used to assess the motility, biofilm formation and morphotype. Vi antigen was agglutinated using commercial antiserum. Caenorhabditis elegans infection model was used to evaluate the virulence potiential. Phenotyping experiments were done in duplicates while virulence assay was done in triplicates. Whole-genome sequencing was used to predict the genes responsible for acquired resistance and a genotype-phenotype comparison was made. Results: We evaluated 96 bovine and 10 human isolates in this study. All the isolates belonged to ST10 in eBG53 and were negative for Vi-antigen. The swarming motility, biofilm formation and morphotype were variable in the isolates of both groups. Resistance to sulfamethoxazole, ampicillin, chloramphenicol, tetracycline was > 90% in animal isolates whereas resistance to sulfamethoxazole was > 70% in human isolates. MDR was also higher in animal isolates. Human isolates were significantly ($P < 0.0001$) more virulent than animal isolates on C. elegans infection model. The genomic comparison based on the core SNPs showed a high degree of homogeneity between the isolates. The carriage of IncA/C2 plasmid was seen as a typical feature of isolates from the bovine hosts. Conclusion: Human isolates showed more diversity in the phenotypic assays. Animal isolates showed a higher degree of antimicrobial resistance with greater MDR but human isolates formed more biofilm and had greater swarming motility as well as increased virulence to the nematode C. elegans. The carriage of IncA/C2 plasmid could contribute to the distinguishing feature of the bovine isolates. The tandem use of

genotypic-phenotypic assays improves the understanding of diversity and differential behaviour of the same serovar from unrelated host sources. ISSN: 14712180

Thomson, R.M., Henderson, H.J., Smith-Palmer, A.
An outbreak of Salmonella Saintpaul in a Scottish childcare facility: The influence of parental under-reporting
(2019) BMC Infectious Diseases, 19 (1), art. no. 847, .
ABSTRACT: Background: Salmonella outbreaks in childcare facilities are relatively rare, most often occurring secondary to contaminated food products or poor infection control practices. We report an outbreak of Salmonella Saintpaul at a pre-school facility in Ayrshire, Scotland with atypical clinical and epidemiological features. Methods: Following notification of the initial two cases, the multi-disciplinary Incident Management Team initiated enhanced active case finding and two environmental inspections of the site, including food preparation areas. Parent and staff interviews were conducted by the Public Health department covering attendance, symptomatology and risk factors for all probable and confirmed cases. Microbiological testing of stool samples and the facility water tank was conducted. Whole Genome Sequencing (WGS) was performed for positive stool samples at the national reference laboratory. Infection control measures were introduced iteratively due to the atypical progression of the outbreak. Results: There were 15 confirmed cases and 3 children admitted to hospital during the outbreak. However, 35.7% of cases reported extremely mild symptoms. The attack rate was 15.2%, and age of affected children ranged from 18 to 58 months (mean 35 months). All cases were the same Multilocus Sequence Type (MLST50). Epidemiological investigation strongly suggested person-to-person spread within the facility. Existing infection control practices were found to be of a high standard, but introduction of additional evidence-based control measures was inadequate in halting transmission. Facility staff reported concerns about lack of parental disclosure of gastrointestinal symptoms, particularly where these were mild, with 50.0% of cases having attended while symptomatic against public health advice. Voluntary two-week closure of the facility was implemented to halt transmission, following which there were no new cases. WGS results were unavailable until after the decision was taken to close the facility. Conclusions: This is the first reported instance of a Salmonella Saintpaul outbreak at a childcare facility, or where person-to-person transmission is indicated. Clinicians should consider the influence of parental under-reporting on gastrointestinal outbreaks in childcare settings, particularly where perceived severity is low and financial or social pressures to attend work may reduce compliance. WGS cannot yet replace conventional microbiological techniques during short, localised outbreaks due to delays receiving results. ISSN: 14712334

Sloan-Gardner, T.S., Waters, N., Marmor, A., Mude, W.
Free range eggs does not mean safe eggs: an outbreak of Salmonella Typhimurium linked to free range eggs
(2019) Communicable diseases intelligence (2018), 43, .
ABSTRACT: An outbreak of Salmonella enterica serovar Typhimurium with closely related Multiple Locus Variable-number Tandem Repeat Analysis (MLVA) patterns was detected by routine surveillance by the Australian Capital Territory Health Protection Service in May 2018. The outbreak consisted of three cases in 2018 (MLVA 03-10-10-09-496) and one in 2016 (MLVA 03-10-09-09-496), who reported eating home-cooked eggs from the same local producer. Environmental investigations found significant problems with egg cleaning, hand hygiene and documentation of food safety procedures on farm. Environmental samples collected from the farm were found to have the same MLVA pattern as the 2018 cases. Although poor farm practices most likely led to contamination of the eggs, this outbreak highlights the need for consumer education about safe handling of eggs in the home. ISSN: 22096051

Harfield, S., Beazley, R., Denehy, E., Centofanti, A., Dowsett, P., Housen, T., Flood, L.
An outbreak and case-control study of Salmonella Havana linked to alfalfa sprouts in South Australia, 2018
(2019) Communicable diseases intelligence (2018), 43, .
ABSTRACT: An epidemiological investigation and a retrospective case-control study were conducted into an outbreak of Salmonella Havana in alfalfa sprouts, in Adelaide, Australia. In total, 31 cases of S. Havana were notified during June and July 2018 and linked to the outbreak. Eighteen cases and 54 unmatched controls were included in a case-control study. Results from the case-control study indicated an increased risk of illness linked to the consumption of alfalfa sprouts; this was supported by trace-back, sampling and environmental investigations. This outbreak of S. Havana was caused by consumption of

alfalfa sprouts from one local sprouts producer. It is unclear as to when in the production of alfalfa sprouts the contamination occurred. However, contaminated seeds and poor pest control are the most likely causes. This investigation highlights the importance of ensuring that producers take appropriate action to minimise the likelihood of contamination and to comply with legislation and standards for primary production and food safety.
ISSN: 22096051

Dmitric, M., Vidanovic, D., Matovic, K., Saric, L.J., Karabasil, N.
Real-time PCR methods for detecting Salmonella spp. in food after different DNA extraction procedures
(2019) IOP Conference Series: Earth and Environmental Science, 333 (1), art. no. 012041.
ABSTRACT: The aim of this paper was to evaluate two real-time PCR (qPCR) protocols for the detection of Salmonella spp. in minced meat and chicken neck skin, after DNA extraction using the InstaTM Gene matrix (BioRad, USA) and DNA extraction based on thermal cell lysis. The applied molecular methods were sensitive and specific for the rapid detection of Salmonella spp. in minced meat and chicken neck skin. The qualitative results were identical regardless of the applied DNA extraction or qPCR protocols. Lower Cq values were achieved after DNA extraction using the InstaTM Gene matrix. ISSN: 17551307

Betic, N., Baltic, T., Ciric, J., Bajcic, A., Raseta, M., Mrdovic, B., Karabasil, N.
Process hygiene of pig carcasses in one large-scale slaughterhouse in the west of Serbia, during 48 months
(2019) IOP Conference Series: Earth and Environmental Science, 333 (1), art. no. 012046.
ABSTRACT: This study was conducted to determine microbial contamination of pig carcasses during four years in one slaughterhouse. The numbers of total viable counts and Enterobacteriaceae and the presence/absence of Salmonella spp. are the process hygiene criteria for pig carcasses. We collected 240 samples from April of 2015 to April of 2019, with swabs being continually taken from the carcasses of pigs every month for 48 months in slaughterhouse in the west of Serbia. Over 48 consecutive months of testing, Salmonella spp. presence was detected on 1.67% of the pig carcasses, while the determined mean numbers of Enterobacteriaceae were 0.18±0.37 log CFU/cm2, and the mean total viable count of aerobic bacteria was 1.88±0.85 log CFU/cm2. The process hygiene criteria results for the tested pig carcasses showed that for total viable count of aerobic bacteria, 95.35% of carcasses fell into the satisfactory process hygiene group, while 4.17% belonged to the acceptable group. Enterobacteriaceae numbers showed 97.90% of the tested pig carcasses belonged to the satisfactory process hygiene group, and 2.10% of carcasses belonged to the acceptable group. ISSN: 17551307

Mrdovic, B., Nastasijevic, I., Brankovic Lazic, I., Jovanovic, J., Nikolic, A., Petrovic, Z., Raseta, M.
Examination of meat preparations in order to control process hygiene in retail
(2019) IOP Conference Series: Earth and Environmental Science, 333 (1), art. no. 012083.
ABSTRACT: The production and trade of meat preparations (minced meat and semi-finished meat products, including fresh sausages) are registering significant annual increases in Serbia. There is an increasing number of specialized plants, as well as suppliers who directly supply consumers with this type of meat preparation. The aim of this paper is to determine the microbiological risks in the meat preparations production process by taking samples from retail facilities in order to verify HACCP compliance. HACCP systems and good hygiene practices as their pre-requisite programs, require food business operators to identify potential hazards that threaten product safety in order to eliminate or control them. Over 27 months, 297 samples of meat preparations were taken from nine retail stores. Escherichia coli was detected in 5% (16/297) of the meat preparations, and Salmonella spp. were found in 1.6% (5/297). The results obtained are signals for initiating corrective measures in the production processes and improving current sanitary procedures. ISSN: 17551307

Raharjo, D., Yulistiani, R., Setyarini, W., Arizandy, R.Y., Prayoga, W., Shirakawa, T.
Rapid identification of Salmonella enterica serovars Typhi and Salmonella enterica serovars Paratyphi A from chicken meat
(2019) IOP Conference Series: Materials Science and Engineering, 633 (1), art. no. 012003.
ABSTRACT: Rapid diagnosis of pathogenic bacteria in food is closely related to safety. We modified a Culture-based methods for Salmonella sp. which rapidly identified Salmonella enterica serovars Typhi and Salmonella enterica serovars Paratyphi A from chicken meat samples. The World Health Organization method consists of six steps of the test,

sequentially pre-enrichment, selective enrichment, selective diagnostic isolation, pick presumptive Salmonella colonies, biochemical and serological confirmation. Modifications made at three stages of pick presumptive Salmonella colonies, biochemical confirmation and serological confirmation into a single stage of molecular testing to detect genes rfbE and, using tyv and prt primer. In summary, from 120 samples, the modified technique successfully identified 39 (32.5%) Salmonella enterica serovars Typhi and one Salmonella enterica serovars Paratyphi A (0, 83%) compare with 13 (10.8%) and negative result with conventional protocol. The modified were faster and more sensitive than conventional techniques. SSN: 17578981

Ritter, A.C., Tondo, E.C., Siqueira, F.M., Soggiu, A., Varela, A.P.M., Mayer, F.Q., Brandelli, A.
Genome analysis reveals insights into high-resistance and virulence of Salmonella Enteritidis involved in foodborne outbreaks
(2019) International Journal of Food Microbiology, 306, art. no. 108269, .
ABSTRACT: Salmonella enterica serovar Enteritidis strain SE86 has been associated with several foodborne diseases occurring in Southern Brazil, becoming and important causative agent of human salmonellosis. In this work, the complete genome of the bacterium Salmonella Enteritidis SE86 was sequenced using the Illumina MiSeq platform. An in silico analysis of the SE86 genome was performed in order to compare it with different Salmonella strains as well as to investigate the presence of stress-resistance and virulence genes. This strain showed a variety of genes that can be involved in antimicrobial and biocide resistance, acid and thermal resistance as well as virulence and adhesion. These genetic features could explain its increased resistance and the prevalence of this strain in foodborne outbreaks in Southern Brazil. ISSN: 01681605

Portela, J.B., Coimbra, P.T., Cappato, L.P., Alvarenga, V.O., Oliveira, R.B.A., Pereira, K.S., Azeredo, D.R.P., Sant'Ana, A.S., Nascimento, J.S., Cruz, A.G.
Predictive model for inactivation of salmonella in infant formula during microwave heating processing
(2019) Food Control, 104, pp. 308-312.
ABSTRACT: This study aimed to study the behavior of Salmonella submitted to domestic microwave through the use of predictive microbiology. The results showed reductions of 9.22, 9.59, 8.23, and 8.57 log CFU/mL in Salmonella counts after exposure to microwave heating at 20 W (750 s), 40 W (90 s), 60 W (120 s), and 80 W (120 s), respectively, with a maximum temperature rise of 110.2 °C. For the primary inactivation model, a biphasic profile was initially observed, obtaining a linear log behavior with the increase in power values. Otherwise, the square root model was used for the secondary modeling, resulting in the equation: √ kmax = 0.0055 (P + 9.98). From the validation of the secondary model, the MSE and R 2 presented a good fit for the model of Salmonella spp inactivation in infant formulas by microwave heating. Overall, the models demonstrated efficacy to ensure the safety of infant formulas, preventing Salmonella contamination and should be considered considering a practical point of view. ISSN: 09567135

Deen, B., Diez-Gonzalez, F.
Assessment of Pediococcus acidilactici ATCC 8042 as potential Salmonella surrogate for thermal treatments of toasted oats cereal and peanut butter
(2019) Food Microbiology, 83, pp. 187-192.
ABSTRACT: The control of Salmonella in low water activity foods poses a challenge for the food industry because of its thermal resistance. The use of surrogate bacteria in a food plant is considered a critical component to validate processing steps. The objective of this study was to evaluate the use of Pediococcus acidilactici ATCC 8042, a generally recognized as safe bacterium (GRAS), as potential surrogate for Salmonella in commercial toasted oats cereal (TOC) and peanut butter. P. acidilactici was compared to a five-serovar cocktail of Salmonella and Enterococcus faecium NRRL-B2354, separately. Cultures were inoculated into TOC and thermal kinetic parameters (δ, β) were determined at 80, 85, 90, and 95 °C using the Weibull model. In peanut butter, δ and β parameters were obtained at 63, 68, 73, and 77 °C. In TOC, the δ values (initial decimal reduction time) of P. acidilactici were 63 and 7 min at 80 and 95 °C, respectively, and at all four temperatures they were not significantly different from δ values of E. faecium. The δ value of Salmonella at 80 °C (139 min) was two-fold greater than the other two bacteria's values ($p < 0.05$). In peanut butter, δ values of P. acidilactici ranged from 31 min at 63 °C to 2.6 min at 77 °C, and at all temperatures they were not significantly different from E. faecium's δ values. In peanut butter, all Salmonella cocktail's δ values were significantly smaller than P. acidilactici's with values of 2 min at 63 °C and 0.4 min at 77 °C. These results indicated that P. acidilactici was as heat tolerant as E. faecium in these food matrices. However, the thermal

inactivation kinetic parameters suggested that P. acidilactici can only be considered a Salmonella surrogate in TOC at temperatures above 85 °C. Because of its greater thermal tolerance in peanut butter, P. acidilactici may be used as Salmonella surrogate if an additional safety factor is recommended. ISSN: 07400020

Mei, X., Zhai, X., Lei, C., Ye, X., Kang, Z., Wu, X., Xiang, R., Wang, Y., Wang, H.
Development and application of a visual loop-mediated isothermal amplification combined with lateral flow dipstick (LAMP-LFD)method for rapid detection of Salmonella strains in food samples
(2019) Food Control, 104, pp. 9-19.
ABSTRACT: Salmonella strains are major foodborne pathogens for the animals and humans, presenting a significant threat to food safety and public health worldwide. The rapid and accurate diagnosis of Salmonella infection is required for effective control and management of disease epidemics. In this study, we developed a rapid and efficient assay that combines loop-mediated isothermal amplification with lateral flow dipstick (LAMP-LFD)for detection of the Salmonella hilA gene. Compared with PCR and real-time PCR methods, the LAMP-LFD assay has the same specificity and higher sensitivity, and required only 40 min (10 min for LFD detection)at 65 °C. All 52 strains of Salmonella yielded positive results using the LAMP-LFD assay and showed no cross reaction with 37 tested non-Salmonella strains. The detection limit of the LAMP-LFD assay was 13.5 fg/μl (genomic DNA)and 6.7 CFU/mL (cell), which was 1000-fold more sensitive than conventional PCR and 100-fold more sensitive than real-time PCR. Additionally, the LAMP-LFD method could detect Salmonella in artificially contaminated food samples (milk, pork, beef and chicken)when present as low as 144 CFU/mL or CFU/g and without the use of an enrichment step. Nevertheless, the sensitivity was increased to 1.44 CFU/mL or CFU/g after culturing at 37 °C for 6 h. Fifty food samples (chicken meat)were used to test the practicality of the LAMP-LFD assay. After an enrichment step at 37 °C for 6 h, the results showed 100% accuracy compared to the standard culture-based method (ISO 6579:2002)in which 17 out of 50 food samples gave positive results. Overall, the results demonstrated that the developed LAMP-LFD method targeting the hilA gene is rapid, specific, sensitive and allows ease of operation for Salmonella detection, suggesting the potential for this assay to be used as an alternative to traditional testing methods and could applied in low-resource settings. ISSN: 09567135

Wilkinson, V., Fernandez, J.R.-R., Núñez, A., Macgregor, S.K., John, S.K., Dallman, T.J., Cunningham, A.A., de Pinna, E.M., Lawson, B.
Novel salmonella variant associated with mortality in two great spotted woodpeckers (Dendrocopos major)
(2019) Journal of Wildlife Diseases, 55 (4), pp. 874-878.
ABSTRACT: Two adult Great Spotted Woodpeckers (Dendrocopos major) from separate sites in Great Britain were examined postmortem in 2013 and 2016. A Salmonella sp. was isolated from multiple tissues in both birds. Histopathology and immunohistochemistry confirmed disseminated salmonellosis. Whole-genome sequencing and biochemical analyses putatively identified both isolates as a novel variant of Salmonella enterica subsp. enterica serovar Hessarek (S. Hessarek). Salmonellosis has seldom been reported in Piciformes, and never before in association with S. Hessarek infection. These findings, therefore, add to current knowledge regarding the range of wild bird species susceptible to this Salmonella serovar, and our understanding of the pathogens affecting Great Spotted Woodpeckers, in particular. ISSN: 00903558

Antonelli, P., Belluco, S., Mancin, M., Losasso, C., Ricci, A.
Genes conferring resistance to critically important antimicrobials in Salmonella enterica isolated from animals and food: A systematic review of the literature, 2013–2017
(2019) Research in Veterinary Science, 126, pp. 59-67.
ABSTRACT: Antimicrobial resistance is a major public health concern, and food systems are a crucial point in the epidemiology of these resistances. Among antimicrobials, critically important ones are therapeutic drugs that should be primarily safeguarded to allow successful outcomes against important bacterial infections in humans. The most important source of antimicrobial resistance has been recognized in the inappropriate use of antimicrobials in human and animal medicine, with farming being a critical stage. Products of animal origin are the link between animal and humans and can contribute to the spread of antimicrobial resistance, in particular through bacteria such as Enterobacteriaceae, commonly present in both animals' gut and food. Salmonella is an important member of this bacterial family due to its pathogenicity, its noteworthy prevalence and the frequent detection of resistance genes in different isolates. In the present systematic review, the distribution of antimicrobial resistance determinants among Salmonella enterica serovars

in pigs, cattle and poultry production was investigated in the European context. A comprehensive literature search was carried out in three different databases, and 7955 papers were identified as relevant. After the different steps of the review process, 31 papers were considered eligible for data extraction to gain insight about sources and reservoirs for such genes. Results suggest that despite the increasing attention directed toward antimicrobial resistance in animal production, a wide plethora of genes still exist and further actions should be undertaken to face this challenge. ISSN: 00345288

New, C.Y., Abdul Rahman, R., Mohammed, A.S., Ubong, A., Chang, W.S., Thung, T.Y., Tan, C.W., Lee, E., Tang, J.-Y.-H., Son, R.
Influence of food composition type on the microwave heating time in relation to the inactivation of salmonella enterica serovar enteritidis and shiga-toxigenic escherichia coli (STEC) O157
(2019) Food Research, 3 (5), pp. 597-603.
ABSTRACT: The interaction of microwaves with the food molecules creates different volumetric heating effect. This research was aimed to study the influence of food composition type on the microwave heating time in relation to the inactivation of Salmonella enterica serovar Enteritidis and Shiga-toxigenic Escherichia coli (STEC) O157. Different food composition types, i.e. carbohydrate, protein and fats, were inoculated with 106 CFU/mL of bacteria cocktail and microwave heated. Food sampling was performed to enumerate the remaining surviving bacteria. The outcome of the research showed that fat food material had the shortest thermal inactivation time (<50s) compared to carbohydrate and protein food materials due to low specific heat of fat. In addition, S. enterica serovar Enteritidis exhibit a consistent inactivation profile compared to STEC O157. Both foodborne pathogens showed no signs of thermal resistance towards microwave heating as they were fully thermal inactivated at 60s of microwave heating for all food compositions. This study could provide an insight for further studies on the interaction of microwaves with mixture food compositions and other foodborne pathogens microwave heating inactivation profile in relation to microwave heating time.
ISSN: 25502166

Gast, R.K., Regmi, P., Guraya, R., Jones, D.R., Anderson, K.E., Karcher, D.M.
Contamination of eggs by Salmonella Enteritidis in experimentally infected laying hens of four commercial genetic lines in conventional cages and enriched colony housing
(2019) Poultry Science, 98 (10), pp. 5023-5027.
ABSTRACT: Human illness caused by the consumption of eggs contaminated with Salmonella Enteritidis is a continuing international public health concern. This pathogen is deposited inside the edible contents of eggs as a consequence of its ability to colonize reproductive tissues in infected hens. Conditions in the housing environment can influence the persistence and transmission of avian Salmonella infections, but the food safety ramifications of different poultry management systems are not entirely clear. The present study assessed the deposition of S. Enteritidis inside eggs laid by groups of experimentally infected laying hens of 4 commercial genetic lines (designated as white egg lines W1 and W2 and brown egg lines B1 and B2). Groups of hens from each line were housed at 555 cm2 of floor space per bird in both conventional cages and colony units enriched with access to perches and nesting areas. All hens were orally inoculated with 5.75 × 107 cfu of a 2-strain S. Enteritidis mixture, and the internal contents of eggs laid 5 to 24 D post-inoculation were cultured to detect the pathogen. No significant differences in egg contamination frequencies were found between the 2 housing systems for any of the hen lines. Contaminated eggs were laid between 7 and 21 D post-inoculation at an overall frequency of 2.47%, ranging from 0.25 to 4.38% for the 4 hen lines. The frequency of S. Enteritidis recovery from egg samples was significantly (P < 0.05) lower for line B2 than for any of the other lines, and the egg contamination frequency for line W1 was significantly greater than for line W2. The overall incidence of contamination among white eggs (3.38%) was significantly higher than among brown eggs (1.56%). These results demonstrate that S. Enteritidis deposition inside eggs can vary between genetic lines of infected laying hens, but housing these hens in 2 different systems did not affect the production of contaminated eggs. ISSN: 00325791

Oscar, T.P.
Process risk model for Salmonella and ground chicken
(2019) Journal of Applied Microbiology, 127 (4), pp. 1236-1245.
ABSTRACT: Aims: To develop a process risk model (PRM) for evaluating the safety of individual lots of ground chicken (GC) contaminated with Salmonella (Salm). Methods and Results: Data for prevalence, number and serotype of Salm were collected with 25 g samples of GC using a combination of methods (whole sample enrichment, quantitative

polymerase chain reaction, cultural isolation and serotyping). These data were used to develop a predictive model for Salm contamination of GC as a function of serving size from 25 to 300 g. This model was combined with a model for thermal inactivation of Salm in GC and a dose–response model for Salm to develop a PRM in Excel that was simulated with NeuralTools and @Risk. Of 100, 25 g samples of GC examined, 19 tested positive for Salm. Three serotypes were isolated: Infantis (n = 13), Enteritidis (n = 5) and Typhimurium (n = 1). The number of Salm ranged from 0 to 2·56 log with a median of 0·93 log per 25 g of GC. The PRM predicted that Salm prevalence would increase (P < 0·05) from 19 to 57% to 82 to 93% as serving size increased from 25 to 100 g to 200 to 300 g. However, the total number of Salm in a 100-kg lot of GC and total severity of illness (TSI) were not affected (P> 0·05) by serving size. The PRM was also used to evaluate effects of serving size distribution, cooking, food consumption behaviour, consumer demographics and Salmonella virulence on TSI. Conclusions: How a lot of GC is partitioned and consumed does not affect TSI. Scenario analysis demonstrated that the PRM can integrate prevalence, number and serotype data for Salm with consumer handling, consumption and demographics data to identify safe and unsafe lots of GC for improved food safety and public health. Significance and Impact of the Study: Process-risk models like the one developed in this study represent a new, holistic approach to food safety that holds great promise for improving public health and reducing food recalls. ISSN: 13645072

Vohra, P., Vrettou, C., Hope, J.C., Hopkins, J., Stevens, M.P.
Nature and consequences of interactions between Salmonella enterica serovar Dublin and host cells in cattle
(2019) Veterinary Research, 50 (1), art. no. 99, .
ABSTRACT: Salmonella enterica is a veterinary and zoonotic pathogen of global importance. While murine and cell-based models of infection have provided considerable knowledge about the molecular basis of virulence of Salmonella, relatively little is known about salmonellosis in naturally-affected large animal hosts such as cattle, which are a reservoir of human salmonellosis. As in humans, Salmonella causes bovine disease ranging from self-limiting enteritis to systemic typhoid-like disease and exerts significant economic and welfare costs. Understanding the nature and consequences of Salmonella interactions with bovine cells will inform the design of effective vaccines and interventions to control animal and zoonotic infections. In calves challenged orally with S. Dublin expressing green fluorescent protein (GFP) we observed that the bacteria were predominantly extracellular in the distal ileal mucosa and within gut-associated lymph nodes 48 h post-infection. Intracellular bacteria, identified by flow cytometry using the GFP signal, were predominantly within MHCII+ macrophage-like cells. In contrast to observations from murine models, these S. Dublin-infected cells had elevated levels of MHCII and CD40 compared to both uninfected cells from the same tissue and cells from the cognate tissue of uninfected animals. Moreover, no gross changes of the architecture of infected lymph nodes were observed as was described previously in a mouse model. In order to further investigate Salmonella-macrophage interactions, net replication of S. enterica serovars that differ in virulence in cattle was measured in bovine blood-derived macrophages by enumeration of gentamicin-protected bacteria and fluorescence dilution, but did not correlate with host-specificity. ISSN: 09284249

Müller, J., Spriewald, S., Stecher, B., Stadler, E., Fuchs, T.M.
Evolutionary Stability of Salmonella Competition with the Gut Microbiota: How the Environment Fosters Heterogeneity in Exploitative and Interference Competition
(2019) Journal of Molecular Biology, 431 (23), pp. 4732-4748.
ABSTRACT: Following ingestion, gastrointestinal pathogens compete against the gastrointestinal microbiota and overcome host immune defenses in order to cause infections. Besides employing direct killing mechanisms, the commensal microbiota occupies metabolic niches to outcompete invading pathogens. Salmonella enterica serovar Typhimurium (S. Typhimurium) uses several strategies to successfully colonize the gut and establish infection, of which an increasing number is based on phenotypic heterogeneity within the S. Typhimurium population. The utilization of myo-inositol (MI) and the production of colicin confer a selective advantage over the microbiota in terms of exploitative and interference competition, respectively. In this review, we summarize the genetic basis underlying bistability of MI catabolism and colicin production. As demonstrated by single-cell analyses, a stochastic switch in the expression of the genes responsible for colicin production and MI degradation constitutes the heterogeneity of the two phenotypes. Both genetic systems are tightly regulated to avoid their expression under non-appropriate conditions and possible detrimental effects on bacterial fitness. Moreover, evolutionary mechanisms underlying formation and stability of these phenotypes in S. Typhimurium are discussed. We propose that both MI catabolism and

colicin production create a bet-hedging strategy, which provides an adaptive benefit for S. Typhimurium in the fluctuating environment of the mammalian gut. ISSN: 00222836

Chattaway, M.A., Dallman, T.J., Larkin, L., Nair, S., McCormick, J., Mikhail, A., Hartman, H., Godbole, G., Powell, D., Day, M., Smith, R., Grant, K.
The Transformation of Reference Microbiology Methods and Surveillance for Salmonella With the Use of Whole Genome Sequencing in England and Wales
(2019) Frontiers in Public Health, 7, art. no. 317, .
ABSTRACT: The use of whole genome sequencing (WGS) as a method for supporting outbreak investigations, studying Salmonella microbial populations and improving understanding of pathogenicity has been well-described (1–3). However, performing WGS on a discrete dataset does not pose the same challenges as implementing WGS as a routine, reference microbiology service for public health surveillance. Challenges include translating WGS data into a useable format for laboratory reporting, clinical case management, Salmonella surveillance, and outbreak investigation as well as meeting the requirement to communicate that information in an understandable and universal language for clinical and public health action. Public Health England have been routinely sequencing all referred presumptive Salmonella isolates since 2014 which has transformed our approach to reference microbiology and surveillance. Here we describe an overview of the integrated methods for cross-disciplinary working, describe the challenges and provide a perspective on how WGS has impacted the laboratory and surveillance processes in England and Wales. ISSN: 22962565

Gymoese, P., Kiil, K., Torpdahl, M., Østerlund, M.T., Sørensen, G., Olsen, J.E., Nielsen, E.M., Litrup, E.
WGS based study of the population structure of Salmonella enterica serovar Infantis
(2019) BMC Genomics, 20 (1), art. no. 870, .
ABSTRACT: Background: Salmonella Infantis (S. Infantis) is one of the most frequent Salmonella serovars isolated from human cases of salmonellosis and the most detected serovar from animal and food sources in Europe. The serovar is commonly associated with poultry and there is increasing concern over multidrug resistant clones spreading worldwide, as the dominating clones are characterized by presence of large plasmids carrying multiple resistance genes. Increasing the knowledge of the S. Infantis population and evolution is important for understanding and preventing further spread. In this study, we analysed a collection of strains representing different decades, sources and geographic locations. We analysed the population structure and the accessory genome, in particular we identified prophages with a view to understand the role of prophages in relation to the evolution of this serovar. Results: We sequenced a global collection of 100 S. Infantis strains. A core-genome SNP analysis separated five strains in e-Burst Group (eBG) 297 with a long branch. The remaining strains, all in eBG31, were divided into three lineages that were estimated to have separated approximately 150 years ago. One lineage contained the vast majority of strains. In five of six clusters, no obvious correlation with source or geographical locations was seen. However, one cluster contained mostly strains from human and avian sources, indicating a clone with preference for these sources. The majority of strains within this cluster harboured a pESI-like plasmid with multiple resistance genes. Another lineage contained three genetic clusters with more rarely isolated strains of mainly animal origin, possibly less sampled or less infectious clones. Conserved prophages were identified in all strains, likely representing bacteriophages which integrated into the chromosome of a common ancestor to S. Infantis. We also saw that some prophages were specific to clusters and were probably introduced when the clusters were formed. Conclusions: This study analysed a global S. Infantis population and described its genetic structure. We hypothesize that the population has evolved in three separate lineages, with one more successfully emerging lineage. We furthermore detected conserved prophages present in the entire population and cluster specific prophages, which probably shaped the population structure. ISSN: 14712164

Diep, B., Barretto, C., Portmann, A.-C., Fournier, C., Karczmarek, A., Voets, G., Li, S., Deng, X., Klijn, A.
Salmonella Serotyping; Comparison of the Traditional Method to a Microarray-Based Method and an in silico Platform Using Whole Genome Sequencing Data
(2019) Frontiers in Microbiology, 10, art. no. 2554, .
ABSTRACT: Salmonella is one of the most common causes of food-borne diseases worldwide. While Salmonella molecular subtyping by Whole Genome Sequencing (WGS) is increasingly used for outbreak and source tracking investigations, serotyping remains as a first-line characterization of Salmonella isolates. The traditional phenotypic method for serotyping is logistically challenging, as it requires the use of more than 150 specific

antisera and well trained personnel to interpret the results. Consequently, it is not a routine method for the majority of laboratories. Several rapid molecular methods targeting O and H loci or surrogate genomic markers have been developed as alternative solutions. With the expansion of WGS, in silico Salmonella serotype prediction using WGS data is available. Here, we compared a microarray method using molecular markers, the Check and Trace Salmonella assay (CTS) and a WGS-based serotype prediction tool that targets molecular determinants of serotype (SeqSero) to the traditional phenotypic method using 100 strains representing 45 common and uncommon serotypes. Compared to the traditional method, the CTS assay correctly serotyped 97% of the strains, four strains gave a double serotype prediction. Among the inconclusive data, one strain was not predicted and two strains were incorrectly identified. SeqSero was evaluated with two versions (SeqSero 1 and the alpha test version of SeqSero 2). The correct antigenic formula was predicted by SeqSero 1 for 96 and 95% of strains using raw reads and assembly, respectively. However, 34 and 33% of these predictions included multiple serotypes by raw reads and assembly. With raw reads, one strain was not identified and three strains were discordant with phenotypic serotyping result. With assembly, three strains were not predicted and two strains were incorrectly predicted. While still under development, SeqSero 2 maintained the accuracy of antigenic formula prediction at 98% and reduced multiple serotype prediction rate to 13%. One strain had no prediction and one strain was incorrectly predicted. Our study indicates that the CTS assay is a good alternative for routine laboratories as it is an easy to use method with a short turn-around-time. SeqSero is a reliable replacement for phenotypic serotyping if WGS is routinely implemented. ISSN: 1664302X

Iwu, C.D., Okoh, A.I.
Preharvest transmission routes of fresh produce associated bacterial pathogens with outbreak potentials: A review
(2019) International Journal of Environmental Research and Public Health, 16 (22), art. no. 4407, .
ABSTRACT: Disease outbreaks caused by the ingestion of contaminated vegetables and fruits pose a significant problem to human health. The sources of contamination of these food products at the preharvest level of agricultural production, most importantly, agricultural soil and irrigation water, serve as potential reservoirs of some clinically significant foodborne pathogenic bacteria. These clinically important bacteria include: Klebsiella spp., Salmonella spp., Citrobacter spp., Shigella spp., Enterobacter spp., Listeria monocytogenes and pathogenic E. coli (and E. coli O157:H7) all of which have the potential to cause disease outbreaks. Most of these pathogens acquire antimicrobial resistance (AR) determinants due to AR selective pressure within the agroecosystem and become resistant against most available treatment options, further aggravating risks to human and environmental health, and food safety. This review critically outlines the following issues with regards to fresh produce; the global burden of fresh produce-related foodborne diseases, contamination between the continuum of farm to table, preharvest transmission routes, AR profiles, and possible interventions to minimize the preharvest contamination of fresh produce. This review reveals that the primary production niches of the agro-ecosystem play a significant role in the transmission of fresh produce associated pathogens as well as their resistant variants, thus detrimental to food safety and public health. ISSN: 16617827

Zarkani, A.A., Schierstaedt, J., Becker, M., Krumwiede, J., Grimm, M., Grosch, R., Jechalke, S., Schikora, A.
Salmonella adapts to plants and their environment during colonization of tomatoes
(2019) FEMS microbiology ecology, 95 (11), .
ABSTRACT: Humans and animals are considered typical hosts for Salmonella, however, also plants can be colonized. Tomatoes were linked to salmonellosis outbreaks already on several occasions. The aim of this study was, therefore, to establish a comprehensive view on the interaction between Salmonella enterica and tomatoes, and to test the hypothesis that colonization of plants is an interactive process. We assessed the persistence of Salmonella in agricultural soil, the colonization pattern in and on tomatoes, as well as the reciprocal responses of tomatoes to different Salmonella strains and Salmonella to root exudates and tomato-related media. This study revealed that Salmonella can persist in the soil and inside the tomato plant. Additionally, we show that Salmonella strains have particular colonization pattern, although the persistence inside the plant differs between the tested strains. Furthermore, the transcriptome response of tomato showed an up-regulation of several defense-related genes. Salmonella transcriptome analysis in response to the plant-based media showed differentially regulated genes related to amino acid and fatty acid synthesis and stress response, while the response to root exudates revealed

regulation of the glyoxylate cycle. Our results indicate that both organisms actively engage in the interaction and that Salmonella adapts to the plant environment. ISSN: 15746941

Naberhaus, S.A., Krull, A.C., Bradner, L.K., Harmon, K.M., Arruda, P., Arruda, B.L., Sahin, O., Burrough, E.R., Schwartz, K.J., Kreuder, A.J.
Emergence of Salmonella enterica serovar 4,[5],12:i:- as the primary serovar identified from swine clinical samples and development of a multiplex real-time PCR for improved Salmonella serovar-level identification
(2019) Journal of Veterinary Diagnostic Investigation, 31 (6), pp. 818-827.
ABSTRACT: Rapid identification of the infecting Salmonella serovar from porcine diagnostic samples is vital to allow implementation of appropriate on-farm treatment and management decisions. Although identification at the serogroup level can be rapidly achieved at most veterinary diagnostic laboratories, final Salmonella serovar identification often takes several weeks because of the limited number of reference laboratories performing the complex task of serotyping. Salmonella serogroup B, currently the dominant serogroup identified from swine clinical samples in the United States, contains serovars that vary from highly pathogenic to minimally pathogenic in swine. We determined the frequency of detection of individual group B serovars at the Iowa State Veterinary Diagnostic Laboratory from 2008 to 2017, and validated a multiplex real-time PCR (rtPCR) to distinguish pathogenic serogroup B serovars from those of lesser pathogenicity. Our results indicate that, since 2014, Salmonella enterica ssp. enterica serovar 4,[5],12:i:- has been the dominant serovar identified from swine clinical samples at the ISU-VDL, with S. Typhimurium now the second most common serovar identified. We developed a rtPCR to allow rapid differentiation of samples containing S. 4,[5],12:i:- and S. Typhimurium from samples containing serovars believed to be of less pathogenicity, such as S. Agona and S. Derby. When combined with enrichment culture, this rtPCR has the ability to significantly improve the time to final serovar identification of the 2 most commonly identified pathogenic Salmonella serovars in swine, and allows rapid implementation of serovar-specific intervention strategies. ISSN: 10406387

Longo, A., Losasso, C., Vitulano, F., Mastrorilli, E., Turchetto, S., Petrin, S., Mantovani, C., Dalla Pozza, M.C., Ramon, E., Conedera, G., Citterio, C.V., Ricci, A., Barco, L., Lettini, A.A.
Insight into an outbreak of Salmonella Choleraesuis var. Kunzendorf in wild boars
(2019) Veterinary Microbiology, 238, art. no. 108423, .
ABSTRACT: An unusual mortality of wild boars occurred in Italy from 2012 to 2015 due to Salmonella Choleraesuis infection. In order to confirm the occurrence of an outbreak of S. Choleraesuis in wild boars and to epidemically characterise the unique S. Choleraesuis biovar, a collection of isolates belonging to wild boars was investigated from the phenotypic, molecular and genomic points of view (PFGE and WGS). Moreover, the possibility of transmission to domestic pigs and humans, temporally and geographically close to the wild boar epidemic, was tested by also including in the panel isolates from infected domestic pigs and from one human case of infection. Wild boar isolates displayed a high genetic correlation, thus suggesting they are part of the same outbreak, with a common invasiveness potential. Conversely, no correlation between pig isolates and those from the other sources (wild boars and human) was found. However, the phylogenetic and PFGE analyses suggest a high degree of similarity between the human and the investigated wild boar outbreak isolates, implying the potential for the spread of Salmonella Choleraesuis among these species. ISSN: 03781135

Colombe, S., Jernberg, C., Löf, E., Angervall, A.L., Mellström-Dahlgren, H., Dotevall, L., Bengnér, M., Hall, I., Sundqvist, L., Kühlmann-Berenzon, S., Galanis, I., Lindblad, M., Hansen, A., Rehn, M.
Outbreak of unusual H2S-negative monophasic Salmonella Typhimurium strain likely associated with small tomatoes, Sweden, August to October 2019
(2019) Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin, 24 (47), .
ABSTRACT: Sweden is investigating an outbreak of monophasic Salmonella Typhimurium. Eighty-two nationally-distributed cases have been confirmed, with date of symptom onset between 28 August and 29 October. Cases were 51 years of age on average (range: 0-89) and the majority of cases were female (62%). A case-control study was conducted and suggested small tomatoes as source of the outbreak (adjusted odds ratio (OR): 10.8, 95% confidence interval (CI): 4.15-112.68, p value < 0.001), and a trace-back investigation led to a single, non-Swedish producer in Europe. Both the Salmonella strain and the source of the outbreak are rarely encountered in Europe. Results from investigation at the producer are pending. ISSN: 15607917

Proroga, Y.T.R., Mancusi, A., Peruzy, M.F., Carullo, M.R., Montone, A.M.I., Fulgione, A., Capuano, F.
Characterization of Salmonella Typhimurium and its monophasic variant 1,4, [5],12:i:- isolated from different sources
(2019) Folia Microbiologica, 64 (6), pp. 711-718.
ABSTRACT: In order to characterize the most commonly detected Salmonella serotypes, we tested 124 isolates of S. Typhimurium and 89 isolates of the monophasic variant of S. Typhimurium (S. 1,4, [5],12:i:-) for their antimicrobial susceptibility by means of the Kirby–Bauer disk-diffusion method, and for the detection of 19 genes (four Phage Markers (g13, Sieb, eat, g8), ten prophage-related virulence genes (gipA, gtgB, nanH, gogB, grvA, sopE, sspH1, sspH2, sodC1, gtgE), and five plasmid-borne virulence genes (spvC, pefA, mig5, rcK, srgA)) by means of PCR-based assays. A total of 213 strains were analyzed from, humans (n = 122), animals (n = 25), food (n = 46), and irrigation water (n = 20). S. Typhimurium isolates showed higher variability, in both their resistance profiles and molecular typing, than S. 1,4, [5],12:i:-. Strains from irrigation water displayed significantly higher susceptibility to antibiotics than those from the other sources. Resistance to ampicillin, streptomycin, sulfonamide, and tetracycline was the most commonly detected resistance profile (R-type), being in serovar S. 1,4, [5],12:i:-, frequently associated to resistance to other antimicrobials. Significant differences in genetic profiles in the two abovementioned Salmonella serotypes were found. None of the plasmid-borne virulence genes investigated were detected in S. 1,4, [5],12:i:- isolates, while those genes, characterized 37.9% of the S. Typhimurium strains. Differences in the prevalence of some molecular targets between the two Salmonella serotypes deserve further study. Importantly, the grvA gene was found exclusively in S. Typhimurium strains, whereas sopE, sodC, gtgB, and gipA were mainly detected, with a statistically significant difference, in S. 1,4, [5],12:i:- isolates. ISSN: 00155632

Kubota, K.A., Wolfgang, W.J., Baker, D.J., Boxrud, D., Turner, L., Trees, E., Carleton, H.A., Gerner-Smidt, P.
PulseNet and the Changing Paradigm of Laboratory-Based Surveillance for Foodborne Diseases
(2019) Public Health Reports, 134 (2_suppl), pp. 22S-28S.
ABSTRACT: PulseNet, the National Molecular Subtyping Network for Foodborne Disease Surveillance, was established in 1996 through a collaboration with the Centers for Disease Control and Prevention; the US Department of Agriculture, Food Safety and Inspection Service; the US Food and Drug Administration; 4 state public health laboratories; and the Association of Public Health Laboratories. The network has since expanded to include 83 state, local, and food regulatory public health laboratories. In 2016, PulseNet was estimated to be helping prevent an estimated 270 000 foodborne illnesses annually. PulseNet is undergoing a transformation toward whole-genome sequencing (WGS), which provides better discriminatory power and precision than pulsed-field gel electrophoresis (PFGE). WGS improves the detection of outbreak clusters and could replace many traditional reference identification and characterization methods. This article highlights the contributions made by public health laboratories in transforming PulseNet's surveillance and describes how the transformation is changing local and national surveillance practices. Our data show that WGS is better at identifying clusters than PFGE, especially for clonal organisms such as Salmonella Enteritidis. The need to develop prioritization schemes for cluster follow-up and additional resources for both public health laboratory and epidemiology departments will be critical as PulseNet implements WGS for foodborne disease surveillance in the United States. ISSN: 00333549

Machado-Moreira, B., Richards, K., Brennan, F., Abram, F., Burgess, C.M.
Microbial Contamination of Fresh Produce: What, Where, and How?
(2019) Comprehensive Reviews in Food Science and Food Safety, 18 (6), pp. 1727-1750.
ABSTRACT: Promotion of healthier lifestyles has led to an increase in consumption of fresh produce. Such foodstuffs may expose consumers to increased risk of foodborne disease, as often they are not subjected to processing steps to ensure effective removal or inactivation of pathogenic microorganisms before consumption. Consequently, reports of ready-to-eat fruit and vegetable related disease outbreak occurrences have increased substantially in recent years, and information regarding these events is often not readily available. Identifying the nature and source of microbial contamination of these foodstuffs is critical for developing appropriate mitigation measures to be implemented by food producers. This review aimed to identify the foodstuffs most susceptible to microbial contamination and the microorganisms responsible for disease outbreaks from information available in peer-reviewed scientific publications. A total of 571 outbreaks were identified from 1980 to

2016, accounting for 72,855 infections and 173 deaths. Contaminated leafy green vegetables were responsible for 51.7% of reported outbreaks. Contaminated soft fruits caused 27.8% of infections. Pathogenic strains of Escherichia coli and Salmonella, norovirus, and hepatitis A accounted for the majority of cases. Large outbreaks resulted in particular biases such as the observation that contaminated sprouted plants caused 31.8% of deaths. Where known, contamination mainly occurred via contaminated seeds, water, and contaminated food handlers. There is a critical need for standardized datasets regarding all aspects of disease outbreaks, including how foodstuffs are contaminated with pathogenic microorganisms. Providing food business operators with this knowledge will allow them to implement better strategies to improve safety and quality of fresh produce. ISSN: 15414337

Doster, E., Rovira, P., Noyes, N.R., Burgess, B.A., Yang, X., Weinroth, M.D., Linke, L., Magnuson, R., Boucher, C., Belk, K.E., Morley, P.S.
A Cautionary Report for Pathogen Identification Using Shotgun Metagenomics; A Comparison to Aerobic Culture and Polymerase Chain Reaction for Salmonella enterica Identification
(2019) Frontiers in Microbiology, 10, art. no. 2499, .
ABSTRACT: This study was conducted to compare aerobic culture, polymerase chain reaction (PCR), lateral flow immunoassay (LFI), and shotgun metagenomics for identification of Salmonella enterica in feces collected from feedlot cattle. Samples were analyzed in parallel using all four tests. Results from aerobic culture and PCR were 100% concordant and indicated low S. enterica prevalence (3/60 samples positive). Although low S. enterica prevalence restricted formal statistical comparisons, LFI and deep metagenomic sequencing results were discordant with these results. Specifically, metagenomic analysis using k-mer-based classification against the RefSeq database indicated that 11/60 of samples contained sequence reads that matched to the S. enterica genome and uniquely identified this species of bacteria within the sample. However, further examination revealed that plasmid sequences were often included with bacterial genomic sequence data submitted to NCBI, which can lead to incorrect taxonomic classification. To circumvent this classification problem, we separated all plasmid sequences included in bacterial RefSeq genomes and reassigned them to a unique taxon so that they would not be uniquely associated with specific bacterial species such as S. enterica. Using this revised database and taxonomic structure, we found that only 6/60 samples contained sequences specific for S. enterica, suggesting increased relative specificity. Reads identified as S. enterica in these six samples were further evaluated using BLAST and NCBI's nr/nt database, which identified that only 2/60 samples contained reads exclusive to S. enterica chromosomal genomes. These two samples were culture- and PCR-negative, suggesting that even deep metagenomic sequencing suffers from lower sensitivity and specificity in comparison to more traditional pathogen detection methods. Additionally, no sample reads were taxonomically classified as S. enterica with two other metagenomic tools, Metagenomic Intra-species Diversity Analysis System (MIDAS) and Metagenomic Phylogenetic Analysis 2 (MetaPhlAn2). This study re-affirmed that the traditional techniques of aerobic culture and PCR provide similar results for S. enterica identification in cattle feces. On the other hand, metagenomic results are highly influenced by the classification method and reference database employed. These results highlight the nuances of computational detection of species-level sequences within short-read metagenomic sequence data, and emphasize the need for cautious interpretation of such results. ISSN: 1664302X

Bose, T., Venkatesh, K.V., Mande, S.S.
Investigating host-bacterial interactions among enteric pathogens
(2019) BMC genomics, 20 (1), p. 1022.
ABSTRACT: BACKGROUND: In 2017, World Health Organization (WHO) published a catalogue of 12 families of antibiotic-resistant "priority pathogens" that are posing the greatest threats to human health. Six of these dreaded pathogens are known to infect the human gastrointestinal system. In addition to causing gastrointestinal and systemic infections, these pathogens can also affect the composition of other microbes constituting the healthy gut microbiome. Such aberrations in gut microbiome can significantly affect human physiology and immunity. Identifying the virulence mechanisms of these enteric pathogens are likely to help in developing newer therapeutic strategies to counter them. RESULTS: Using our previously published in silico approach, we have evaluated (and compared) Host-Pathogen Protein-Protein Interaction (HPI) profiles of four groups of enteric pathogens, namely, different species of Escherichia, Shigella, Salmonella and Vibrio. Results indicate that in spite of genus/ species specific variations, most enteric pathogens possess a common repertoire of HPIs. This core set of HPIs are probably responsible for the survival of these pathogen in the harsh nutrient-limiting environment

within the gut. Certain genus/ species specific HPIs were also observed. CONSLUSIONS: The identified bacterial proteins involved in the core set of HPIs are expected to be helpful in understanding the pathogenesis of these dreaded gut pathogens in greater detail. Possible role of genus/ species specific variations in the HPI profiles in the virulence of these pathogens are also discussed. The obtained results are likely to provide an opportunity for development of novel therapeutic strategies against the most dreaded gut pathogens. ISSN: 14712164

Atkinson, B.M., Bearson, B.L., Loving, C.L., Zimmerman, J.J., Kich, J.D., Bearson, S.M.D.
Detection of Salmonella-specific antibody in swine oral fluids
(2019) Porcine Health Management, 5 (1), art. no. 29, .
ABSTRACT: Salmonella is a leading cause of bacterial foodborne-related illness and pork products are a food-Associated source. With > 50% of U.S. swine herds testing positive for Salmonella, asymptomatic carrier pigs that shed Salmonella in their feces are a food safety and environmental contamination issue. Herd level surveillance of Salmonella shedding status is useful, but collection of feces and culture methods for Salmonella detection are laborious and time-consuming. Surveillance for Salmonella-exposure through detection of Salmonella-specific serum antibody is a reliable method, but presents labor and animal-welfare issues. Oral fluids are a reliable, antemortem sample with proven utility for surveillance in the swine industry. We tested oral fluid samples as a potential non-invasive, repeatable sample type for the presence of Salmonella-specific antibodies. An indirect enzyme-linked immunosorbent assay (ELISA) detected anti-Salmonella IgG, IgM, and predominantly IgA in oral fluids from Salmonella enterica serovar Typhimurium-exposed pigs. Furthermore, with minor modifications, a commercial ELISA-based kit also detected Salmonella-specific antibodies in oral fluids. Collectively, oral fluids may serve as a prospective surveillance tool for herd level monitoring of Salmonella exposure.
ISSN: 20555660

Thung, T.Y., Lee, E., Wai, G.Y., Pui, C.F., Kuan, C.H., Premarathne, J.M.K.J.K., Nurzafirah, M., Tan, C.W., Malcolm, T.T.H., Ramzi, O.S.B., Wendy, D.R., New, C.Y., Son, R.
A review of culture-dependent and molecular methods for detection of salmonella in food safety
(2019) Food Research, 3 (6), pp. 622-627.
ABSTRACT: Salmonella is the well-recognized foodborne pathogen leading the most research and surveillance attention especially from government agencies as well as in food industry. In Malaysia, Salmonella is one of the main bacteria which monitored by the National Laboratory Surveillance System. Previously, standard culture methods have always been employed by many laboratories for Salmonella detection in Food Surveillance Programs. However, more advanced detection methods will be needed to improve the sensitivity and specificity of Salmonella identification. In this review, Salmonella detection methods including conventional and recent advances in molecular-based methods will be discussed. ISSN: 25502166

Pradhan, D., Devi Negi, V.
Stress-induced adaptations in Salmonella: A ground for shaping its pathogenesis
(2019) Microbiological Research, 229, art. no. 126311, .
ABSTRACT: Microorganisms are able to adapt to multiple adverse environmental conditions that facilitate their survival. These microorganisms including bacteria, viruses, algae, fungi, and protozoans are exposed to different abiotic and biotic challenges throughout their life. Adaptations help these organisms to overcome the challenges and evolve as successful pathogens which at the same time might lead to severe disease outcome. The intracellular gram-negative pathogen Salmonella, the causative agent of typhoid fever has evolved into a successful pathogen and shows increasing host mortality and morbidity every year across the globe. Salmonella adapts itself in the different extreme host and non-host environments both at genetic and phenotypic level leading to their better survival and propagation. The uncontrolled and improper use of antibiotics against several Salmonella serovars has not only given rise to various multidrug resistance strains but also the emergence of hyper-infectious Salmonella strains adds to the severity of disease manifestation and treatment. Besides, several disadvantages in the existing Salmonella vaccines stand against the current therapeutic interventions against the bug. This review deals with the wide array of stresses that Salmonella encounter in its life cycle and outlines the adaptations occurring in Salmonella upon exposure to such stresses as well as how adaptations help the pathogen to withstand such extreme conditions. Insights in these

aspects will help to understand Salmonella pathogenesis and associated consequences which might help in the development of new strategies in combating Salmonella infection. ISSN: 09445013

de Oliveira Elias, S., Noronha, T.B., Tondo, E.C.
Salmonella spp. and Escherichia coli O157:H7 prevalence and levels on lettuce: A systematic review and meta-analysis
(2019) Food Microbiology, 84, art. no. 103217, .
ABSTRACT: Lettuce (Lactuca sativa), one of the most consumed leafy vegetables in the world, is frequently implicated with foodborne disease (FBD) outbreaks, with Salmonella spp. and Escherichia coli O157:H7 being the most common bacteria to cause this illness. Estimates of prevalence and levels of these pathogens on lettuce are scarce in developed or in developing countries, which hinders risk assessment attempts. In here, we present a systematic review and meta-analysis of reported prevalence and levels of Salmonella spp. and E. coli O157:H7 on lettuce using the worldwide available data. Literature was reviewed and examined the results for inclusion of articles in the meta-analysis. Data (prevalence and/or concentration of Salmonella spp. and E. coli O157:H7 on lettuce, sample characteristic, country of origin, and Salmonella identified serovars) were extracted, and meta-analysis was performed using Open Meta-Analyst, Task Order # 2 software. Although only one work reported the presence of E coli O157:H7 on lettuce, several reports indicated the presence of other, distinct enterohemorrhagic E. coli (EHEC) strains, with a mean prevalence of 0.041 (95% CI: 0.005–0.078) and concentration varying from <3.0 MPN/g to >1100 MPN/g. Furthermore, the mean prevalence of Salmonella spp. on lettuce was 0.041 (95% CI: 0.030–0.052), with reported concentrations varying between 0.054 ± 0.058 CFU/g to 218.78 MPN/g. In addition, subgroup analysis of the presence of Salmonella spp. in lettuce revealed a mean prevalence of the bacteria of 0.028 (95% CI: 0.014–0.042) in developed nations and 0.064 (0.041–0.087) in developing nations, with reports varying from 0.001 in Japan to 0.5 in Burkina Faso. Despite a relatively low prevalence, consumption of lettuce is inherently risky because it usually is eaten raw, without thermal treatment to inactivate pathogens. This potential risk further supports performance of quantitative risk assessments to quantify the probability of FBD caused by Salmonella spp. and E. coli O157:H7 transmitted to lettuce. ISSN: 07400020

Sobur, A., Hasan, M., Haque, E., Mridul, A.I., Noreddin, A., El Zowalaty, M.E., Rahman, T.
Molecular detection and antibiotyping of multidrug-resistant Salmonella isolated from houseflies in a fish market
(2019) Pathogens, 8 (4), art. no. 191, .
ABSTRACT: Houseflies (Musca domestica) are well-known mechanical vectors for spreading multidrug-resistant bacteria. Fish sold in open markets are exposed to houseflies. The present study investigated the prevalence and antibiotypes of multidrug-resistant (MDR) Salmonella spp. in houseflies captured from a fish market. Direct interviews with fish vendors and consumers were also performed to draw their perceptions about the role of flies in spreading antibiotic-resistant bacteria. A total of 60 houseflies were captured from a local fish market in Bangladesh. The presence of Salmonella spp. was confirmed using PCR method. Antibiogram was determined by the disk diffusion method, followed by the detection of tetA, tetB, and qnrA resistance genes by PCR. From the interview, it was found that most of the consumers and vendors were not aware of antibiotic resistance, but reported that flies can carry pathogens. Salmonella spp. were identified from the surface of 34 (56.7%) houseflies, of which 31 (91.2%) were found to be MDR. This study revealed 25 antibiotypes among the isolated Salmonella spp. All tested isolates were found to be resistant to tetracycline. tetA and tetB were detected in 100% and 47.1% of the isolates, respectively. Among the 10 isolates phenotypically found resistant to ciprofloxacin, six (60%) were found to be positive for qnrA gene. As far as we know, this is the first study from Bangladesh to report and describe the molecular detection of multidrug-resistant Salmonella spp. in houseflies in a fish market facility. The occurrence of a high level of MDR Salmonella in houseflies in the fish market is of great public health concerns. ISSN: 20760817

Kingsbury, J.M., Thom, K., Soboleva, T.
Effect of Storage Temperature on the Survival of New Zealand Egg-Associated Salmonella Isolates in and on Eggs
(2019) Journal of food protection, 82 (12), pp. 2161-2168.
ABSTRACT: The influence of egg storage temperature on Salmonella contamination of eggs is a key consideration in determining storage and shelf life recommendations for eggs. In this study, experiments assessed the survival of Salmonella isolates on and in eggs at

storage temperatures (15 and 22°C) currently used in New Zealand. Eggshell surfaces were inoculated with a cocktail of 10 Salmonella isolates comprising five serotypes, at a concentration of ~106 CFU per egg (for determining shell surface survival) or ~103 CFU per egg (for determining internalization). Additionally, a subset of eggs was artificially contaminated with sterile chicken feces prior to Salmonella inoculation. Inoculated eggs were incubated at 15 and 22°C. At 0, 21, and 35 days of incubation, eggshells were enumerated for Salmonella, and egg contents were tested for Salmonella presence or absence (yolk) or most probable number (albumen). Higher levels of Salmonella were recovered from eggshells following incubation at 15°C (31% relative humidity [RH]) compared with 22°C (45% RH) after both 21 and 35 days of incubation. Recoverable numbers of Salmonella from visibly clean eggshell surfaces declined over time at both storage temperatures and were at, or below, the limit of detection from eggs stored at 22°C and 45% RH for 35 days. A substantially higher concentration of viable Salmonella was recovered from eggshells that were experimentally contaminated with chicken feces compared with those without, particularly from eggs stored at 15°C and 31% RH for 35 days (2.38 log higher CFU from eggs containing feces). No Salmonella was detected in egg contents (albumen or yolk) at any incubation temperature or time point, regardless of the presence of feces. Findings emphasize the importance of current regulations that require eggs sold at retail to be visibly clean and will inform risk management decisions regarding egg storage times and temperatures with respect to Salmonella control in and on New Zealand eggs at retail. ISSN: 19449097

Lee, D., Tertuliano, M., Harris, C., Vellidis, G., Levy, K., Coolong, T.
Salmonella Survival in Soil and Transfer onto Produce via Splash Events
(2019) Journal of food protection, 82 (12), pp. 2023-2037.
ABSTRACT: Nearly one-half of foodborne illnesses in the United States can be attributed to fresh produce consumption. The preharvest stage of production presents a critical opportunity to prevent produce contamination in the field from contaminating postharvest operations and exposing consumers to foodborne pathogens. One produce-contamination route that is not often explored is the transfer of pathogens in the soil to edible portions of crops via splash water. We report here on the results from multiple field and microcosm experiments examining the potential for Salmonella contamination of produce crops via splash water, and the effect of soil moisture content on Salmonella survival in soil and concentration in splash water. In field and microcosm experiments, we detected Salmonella for up to 8 to 10 days after inoculation in soil and on produce. Salmonella and suspended solids were detected in splash water at heights of up to 80 cm from the soil surface. Soil-moisture conditions before the splash event influenced the detection of Salmonella on crops after the splash events-Salmonella concentrations on produce after rainfall were significantly higher in wet plots than in dry plots (geometric mean difference = 0.43 CFU/g; P = 0.03). Similarly, concentrations of Salmonella in splash water in wet plots trended higher than concentrations from dry plots (geometric mean difference = 0.67 CFU/100 mL; P = 0.04). These results indicate that splash transfer of Salmonella from soil onto crops can occur and that antecedent soil-moisture content may mediate the efficiency of microbial transfer. Splash transfer of Salmonella may, therefore, pose a hazard to produce safety. The potential for the risk of splash should be further explored in agricultural regions in which Salmonella and other pathogens are present in soil. These results will help inform the assessment of produce safety risk and the development of management practices for the mitigation of produce contamination. ISSN: 19449097

Wotzka, S.Y., Kreuzer, M., Maier, L., Arnoldini, M., Nguyen, B.D., Brachmann, A.O., Berthold, D.L., Zünd, M., Hausmann, A., Bakkeren, E., Hoces, D., Gül, E., Beutler, M., Dolowschiak, T., Zimmermann, M., Fuhrer, T., Moor, K., Sauer, U., Typas, A., Piel, J., Diard, M., Macpherson, A.J., Stecher, B., Sunagawa, S., Slack, E., Hardt, W.-D.
Escherichia coli limits Salmonella Typhimurium infections after diet shifts and fat-mediated microbiota perturbation in mice
(2019) Nature Microbiology, 4 (12), pp. 2164-2174.
ABSTRACT: The microbiota confers colonization resistance, which blocks Salmonella gut colonization1. As diet affects microbiota composition, we studied whether food composition shifts enhance susceptibility to infection. Shifting mice to diets with reduced fibre or elevated fat content for 24 h boosted Salmonella Typhimurium or Escherichia coli gut colonization and plasmid transfer. Here, we studied the effect of dietary fat. Colonization resistance was restored within 48 h of return to maintenance diet. Salmonella gut colonization was also boosted by two oral doses of oleic acid or bile salts. These pathogen blooms required Salmonella's AcrAB/TolC-dependent bile resistance. Our data indicate that fat-elicited bile promoted Salmonella gut colonization. Both E. coli and Salmonella show

much higher bile resistance than the microbiota. Correspondingly, competitive E. coli can be protective in the fat-challenged gut. Diet shifts and fat-elicited bile promote S. Typhimurium gut infections in mice lacking E. coli in their microbiota. This mouse model may be useful for studying pathogen–microbiota–host interactions, the protective effect of E. coli, to analyse the spread of resistance plasmids and assess the impact of food components on the infection process. ISSN: 20585276

Bridier, A., Le Grandois, P., Moreau, M.-H., Prénom, C., Le Roux, A., Feurer, C., Soumet, C.
Impact of cleaning and disinfection procedures on microbial ecology and Salmonella antimicrobial resistance in a pig slaughterhouse
(2019) Scientific Reports, 9 (1), art. no. 12947, .
ABSTRACT: To guarantee food safety, a better deciphering of ecology and adaptation strategies of bacterial pathogens such as Salmonella in food environments is crucial. The role of food processing conditions such as cleaning and disinfection procedures on antimicrobial resistance emergence should especially be investigated. In this work, the prevalence and antimicrobial resistance of Salmonella and the microbial ecology of associated surfaces communities were investigated in a pig slaughterhouse before and after cleaning and disinfection procedures. Salmonella were detected in 67% of samples and isolates characterization revealed the presence of 15 PFGE-patterns belonging to five serotypes: S.4,5,12:i:-, Rissen, Typhimurium, Infantis and Derby. Resistance to ampicillin, sulfamethoxazole, tetracycline and/or chloramphenicol was detected depending on serotypes. 16S rRNA-based bacterial diversity analyses showed that Salmonella surface associated communities were highly dominated by the Moraxellaceae family with a clear site-specific composition suggesting a persistent colonization of the pig slaughterhouse. Cleaning and disinfection procedures did not lead to a modification of Salmonella susceptibility to antimicrobials in this short-term study but they tended to significantly reduce bacterial diversity and favored some genera such as Rothia and Psychrobacter. Such data participate to the construction of a comprehensive view of Salmonella ecology and antimicrobial resistance emergence in food environments in relation with cleaning and disinfection procedures. ISSN: 20452322

Hyeon, J.-Y., Mann, D.A., Wang, J., Kim, W.K., Deng, X.
Rapid detection of Salmonella in poultry environmental samples using real-time PCR coupled with immunomagnetic separation and whole genome amplification
(2019) Poultry science, 98 (12), pp. 6973-6979.
ABSTRACT: We evaluated the combination of immunomagnetic separation (IMS), multiple displacement amplification (MDA), and real-time PCR to detect Salmonella from poultry environmental samples. The limits of detection (LODs) of IMS-MDA real-time PCR with different culture enrichment hours (0, 4, 6, and 8 h) were determined in artificially inoculated litter samples from a specific pathogen-free (SPF) poultry farm. In addition, Salmonella detection rate of IMS-MDA real-time PCR with 8-h culture enrichment was compared with that of conventional real-time PCR and culture-based detection by analyzing 174 poultry environmental samples (boot swabs, drag swabs, and litter), and the levels of Salmonella in the samples were quantified using the most probably number method. The LODs of IMS-MDA real-time PCR with 0, 4 to 6, and 8-h enrichment were 10, 1, and 0.1 CFU/g, respectively. Salmonella was detected in 25 of the 174 environmental samples (14.4%) by IMS-MDA real-time PCR, compared with 24 (13.8%) by conventional real-time PCR and 19 (10.9%) by culturing. Cohen's kappa index indicated strong concordance (0.79) between IMS-MDA real-time PCR and culture detection. We demonstrated the potential of the IMS-MDA real-time PCR assay as a faster and more sensitive alternative to culture-based Salmonella detection from poultry environmental samples. ISSN: 15253171

European Food Safety Authority and European Centre for Disease Prevention and Control (EFSA and ECDC)
The European Union One Health 2018 Zoonoses Report
(2019) EFSA Journal, 17 (12), art. no. e05926, .
ABSTRACT: This report of the European Food Safety Authority and the European Centre for Disease Prevention and Control presents the results of zoonoses monitoring activities carried out in 2018 in 36 European countries (28 Member States (MS) and 8 non-MS). The first and second most commonly reported zoonoses in humans were campylobacteriosis and salmonellosis, respectively. The European Union (EU) trend for confirmed human cases of these two diseases was stable during 2014–2018. The proportion of human salmonellosis cases due to Salmonella Enteritidis was at the same level in 2018 as in 2017. Of the 27 reporting MS, 16 met all Salmonella reduction targets for poultry, whereas 11

MS failed meeting at least one. The EU flock prevalence of target Salmonella serovars in breeding hens, laying hens, broilers and fattening turkeys decreased during recent years but stalled in breeding turkeys. Salmonella results from Competent Authorities for pig carcasses and for poultry tested through National Control Programmes were more frequently positive compared with food business operators. Shiga toxin-producing Escherichia coli (STEC) infections in humans were the third most commonly reported zoonosis in the EU and increased from 2014 to 2018. Yersiniosis was the fourth most frequently reported zoonosis in humans in 2018 with a stable trend in 2014–2018. The number of reported confirmed listeriosis cases further increased in 2018, despite Listeria rarely exceeding the EU food safety limit tested in ready-to-eat food. In total, 5,146 food- and waterborne outbreaks were reported. Salmonella was the most commonly detected agent with S. Enteritidis causing one in five outbreaks. Salmonella in eggs and egg products was the highest risk agent/food pair. A large increase of human West Nile virus infections was reported in 2018. The report further updates on bovine tuberculosis, Brucella, Trichinella, Echinococcus, Toxoplasma, rabies, Coxiella burnetii (Q fever) and tularaemia. ISSN: 18314732

Sedeik, M.E., El-shall, N.A., Awad, A.M., Elfeky, S.M., Abd El-Hack, M.E., Hussein, E.O.S., Alowaimer, A.N., Swelum, A.A.
Isolation, conventional and molecular characterization of Salmonella spp. from newly hatched broiler chicks
(2019) AMB Express, 9 (1), art. no. 136, .
ABSTRACT: Salmonella is an important pathogen for poultry production as well as for human due to zoonotic importance. It has more than 2600 identified serovars despite of this identification and classification of Salmonella isolates into different serovars is critical for study of incidence and surveillance. This study investigates the epidemiology and molecular characterization of Salmonella isolates in broiler chicks during 1st week of life. A total of (n = 1000) samples including liver, intestine, yolk sac, spleen and heart blood were collected from El-Gharbia, El-Behera, Kafr-Elshikh, Alexandria, Marsamatroh Provinces in Egypt and tested through bacteriological, biochemical, serological and molecular examinations. Incidence of Salmonella was demonstrated on 75 positive samples from 1000 samples and the predominance of Salmonella that isolated from internal organs of newly hatched chicks was highest from yolk sacs (10%), liver and intestines (9%) followed by the spleen (7.5%) then heart blood (2%). Serotyping of the isolated strains using slide agglutination test revealed that 24 isolates belonging to S. enteritidis (1,9,12 g.m 1,7), while, 14 isolates belonging to S. virchow (6,7 r 1,2), in addition to, 12 isolates belonging to S. typhimurium (1,4,5,12.i.1,2) and 8 isolates belonging to S. kentucky (6,8.I,z). Enterobacterial Repetitive Intergenic Consensus (ERIC) PCR revealed that two S. enteriditis isolates were identical and one isolate differ by 40%, while two S. typhimurium isolates were identical by 80% and one isolate was similar by 20% to the other two isolates, in addition, two S. virchow isolates were identical by 80% and the two S. kentucky isolates were different. ISSN: 21910855

Grattarola, C., Gallina, S., Giorda, F., Pautasso, A., Ballardini, M., Iulini, B., Varello, K., Goria, M., Peletto, S., Masoero, L., Serracca, L., Romano, A., Dondo, A., Zoppi, S., Garibaldi, F., Scaglione, F.E., Marsili, L., Di Guardo, G., Lettini, A.A., Mignone, W., Fernandez, A., Casalone, C.
First report of Salmonella 1,4,[5],12:i:- in free-ranging striped dolphins (Stenella coeruleoalba), Italy
(2019) Scientific Reports, 9 (1), art. no. 6061, .
ABSTRACT: Between 2015 and the beginning of 2018 (January-March), 30 cetaceans were found stranded along the Ligurian Sea coast of Italy. Necropsies were performed in 22 cases and infectious diseases resulted the most common cause of death. Three striped dolphins, showed a severe coinfection involving the monophasic variant of Salmonella Typhimurium (Salmonella 1,4,[5],12:i:-). The isolates were characterized based on antimicrobial resistance, Multiple-Locus Variable-number tandem-repeat Analysis (MLVA) and whole-genome sequencing (WGS). All isolates demonstrated the same multidrug resistant genotype (ASSuT isolates), showed three different MLVA profiles, two of which closely related, and were identified as Sequence Type 34. Moreover, Single nucleotide polymorphisms (SNP) analysis confirmed strong correlations between two out of the three isolates. To our knowledge, S. 1,4,[5],12:i:-, one of the most common serovars in cases of human infection and food sources worldwide, has not previously been described in marine mammals, and reports of Salmonella-associated disease in free-ranging cetaceans are rare. These results highlight the role of cetaceans as sentinel species for zoonotic and terrestrial pathogens in the marine environment, suggest a potential risk for cetaceans and

public health along the North Western Italian coastline and indicate cetaceans as a novel potential reservoir for one of the most widespread Salmonella serovars. ISSN: 20452322

Keerthirathne, T.P., Ross, K., Fallowfield, H., Whiley, H.
The combined effect of pH and temperature on the survival of salmonella enterica serovar typhimurium and implications for the preparation of raw egg mayonnaise
(2019) Pathogens, 8 (4), art. no. 218, .
ABSTRACT: Raw egg products are often associated with salmonellosis. The Australian guidelines recommend raw egg mayonnaise to be prepared and stored under 5◦C and adjusted to a pH less than 4.6 or 4.2. Despite these guidelines, a significant amount of salmonellosis outbreaks are recorded annually in Australia. The aim of this study was to investigate the effect of pH and temperature on the survival of Salmonella Typhimurium (ST) in peptone water (PW) and mayonnaise. The pH of PW and mayonnaise was adjusted to 4.2, 4.4 and 4.6 using acetic acid and vinegar, respectively. The PW and mayonnaise were inoculated with ST and incubated at 37◦C, 23◦C, and 4◦C. The survival of Salmonella was determined using the drop plate method. Survival was significantly (p < 0.05) improved at 4◦C. In both mayonnaise and PW, following 24 h, there was no ST growth at pH 4.2. Resuscitation of ST was rapidly observed at 4◦C while complete inactivation was observed at 37◦C at pH 4.2, 4.4, and 4.6 in both PW and mayonnaise. Lower temperatures protected ST from the bactericidal effect of low pH. "The preparation of mayonnaise at pH 4.2 or less and incubating it at room temperature for at least 24 h could reduce the incidence of salmonellosis". ISSN: 20760817

Crabb, H.K., Allen, J.L., Devlin, J.M., Firestone, S.M., Stevenson, M., Wilks, C.R., *Gilkerson, J.R.*
Traditional Salmonella Typhimurium typing tools (phage typing and MLVA) are sufficient to resolve well-defined outbreak events only.
(2019) Food Microbiology, 84, art. no. 103237, .
ABSTRACT: Between 1991 and 2014 the per capita notification rate of salmonellosis in Australia increased from 31.9 to 69.7 cases per 100,000 people. Salmonella Typhimurium accounted for nearly half the human cases until the end of 2014. In this study, we used cluster analysis tools to compare S. Typhimurium isolates from a chicken-meat study with those reported to the National Enteric Pathogen Surveillance System (NEPSS) from the coincident human and non-human populations. There was limited phage type diversity within all populations and a lack of specificity of MLVA profiling within phage types. The chicken-meat study isolates were not significantly clustered with the human cases and at least 7 non-human sources, based on typing profiles (PT/MLVA combination), could be implicated as a source of human cases during the same period. In the absence of a strong surveillance system representative of all putative sources, MLVA and phage typing alone or in combination are insufficient to identify the source of human cases. ISSN: 07400020

Kim, C., Alrefaei, R., Bushlaibi, M., Ndegwa, E., Kaseloo, P., Wynn, C.
Influence of growth temperature on thermal tolerance of leading foodborne pathogens
(2019) Food Science and Nutrition, 7 (12), pp. 4027-4036.
ABSTRACT: Accurate prediction of the thermal destruction rate of foodborne pathogens is important for food processors to ensure proper food safety. When bacteria are subjected to thermal stress during storage, sublethal stresses and/or thermal acclimation may lead to differences in their subsequent tolerance to thermal treatment. The aim of the current study was to evaluate the thermal tolerance of Escherichia coli O157:H7, Listeria monocytogenes, Salmonella enterica, and Staphylococcus aureus that are incubated during overnight growth in tryptic soy broth at four temperatures (15, 25, 35, and 45°C). Following incubation, the bacteria were subjected to thermal treatments at 55, 60, and 65°C. At the end of each treatment time, bacterial survival was quantified and further calculated for the thermal death decimal reduction time (D-value) and thermal destruction temperature (z-value) using a linear model for thermal treatment time (min) vs. microbial population (Log CFU/ml) and thermal treatment temperature (°C) vs. D-value, respectively, for each bacterium. Among the four bacterial species, E. coli generally had longer D-values and lower z-values than did other bacteria. Increasing patterns of D- and z-values in Listeria were obtained with the increment of incubation temperatures from 15 to 45°C. The z-values of Staphylococcus (6.19°C), Salmonella (6.73°C), Listeria (7.10°C), and Listeria (7.26°C) were the highest at 15, 25, 35, and 45°C, respectively. Although further research is needed to validate the findings on food matrix, findings in this study clearly affirm that adaptation of bacteria to certain stresses may reduce the effectiveness of preservation hurdles applied during later stages of food processing and storage. ISSN: 20487177

Lupolova, N., Lycett, S.J., Gally, D.L.
A guide to machine learning for bacterial host attribution using genome sequence data (2019) Microbial genomics, 5 (12), .
ABSTRACT: With the ever-expanding number of available sequences from bacterial genomes, and the expectation that this data type will be the primary one generated from both diagnostic and research laboratories for the foreseeable future, then there is both an opportunity and a need to evaluate how effectively computational approaches can be used within bacterial genomics to predict and understand complex phenotypes, such as pathogenic potential and host source. This article applied various quantitative methods such as diversity indexes, pangenome-wide association studies (GWAS) and dimensionality reduction techniques to better understand the data and then compared how well unsupervised and supervised machine learning (ML) methods could predict the source host of the isolates. The study uses the example of the pangenomes of 1203 Salmonella enterica serovar Typhimurium isolates in order to predict 'host of isolation' using these different methods. The article is aimed as a review of recent applications of ML in infection biology, but also, by working through this specific dataset, it allows discussion of the advantages and drawbacks of the different techniques. As with all such sub-population studies, the biological relevance will be dependent on the quality and diversity of the input data. Given this major caveat, we show that supervised ML has the potential to add real value to interpretation of bacterial genomic data, as it can provide probabilistic outcomes for important phenotypes, something that is very difficult to achieve with the other methods. ISSN: 20575858