NEWSLETTER

European Union Reference Laboratory for *Salmonella*

Vol. 26 No. 1
March 2020

ISSN 2211-6877

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

Produced by

European Union Reference Laboratory for *Salmonella*

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Editorial Note

Bilthoven, 6 April 2020

Dear colleague,

Currently we live in strange times. The **COVID-19 pandemic** has hit us all very hard. It influences our daily live, our work and, most of all, our health. I sincerely hope that you are all healthy and that these difficult times come to an end soon. I wish you all much strength to cope with all difficulties due to this pandemic.

In relation to the COVID-19 pandemic, and upon request of EC DG SANTE, we made a short inventory among the NRLs-*Salmonella* on possible difficulties that the NRLs and/or local laboratories may currently encounter to conduct the *Salmonella* testing on poultry. I want to express my gratitude for the fact that so many of you could send me a reply within 1,5 (working) days! Very helpful! From the replies it was clear that even in these difficult times the NRLs-*Salmonella* and/or the local official laboratories do the outmost to ensure the continuity of the important *Salmonella* analyses.

Concerning our own activities, I can inform you that, like many of you, the EURL-*Salmonella* staff is currently mainly working at home, trying to continue with the different activities as much as possible. An important part of our activities concerns the organisation of the **EURL-*Salmonella* Proficiency Tests (PTs)**. You may understand that we are not yet sure if we can organise all PTs planned for 2020 and/or whether we have to change dates or formats. This not just depends on the situation at the EURL, but of course also on the situation at the NRLs. We will review the situation case by case and for sure keep you informed.

In January/February 2020, the evaluation of the serotyping results of the **PT on typing of *Salmonella* 2019** was performed. By the end of February 2020 the participants received their own results as well as the interim summary report containing the results of all participants. This interim summary report is also available at the EURL-*Salmonella* website: https://www.eurlsalmonella.eu/publications/interlaboratory-comparison-study-reports. Two participants did not meet the level of good performance at the first stage of the study and a follow-up study and/or training for these laboratories may be organised later this year. 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 was free of choice (e.g. PFGE, MLVA, WGS). We were very happy to see that as many as 18 NRLs-*Salmonella* actively participated in this pilot study. The results of the molecular sub-typing part of the study are currently under analysis.

In March 2020 we had planned to organise the first **PT on detection of *Salmonella* in bivalve molluscs (mussels)**. Initially we had some difficulties with sending the reference materials on dry ice for this PT. By the time we solved this problem and had sent all samples to the participants, we (and you) were facing the problems due to the COVID-19 pandemic. Although all parcels arrived safely and on time at the participants, several NRLs indicated not to be able to perform the analysis of this PT due to restrictions of the laboratory activities. We fully understand that under these exceptional conditions not all NRLs were able to perform the analysis. We advised these laboratories to store the reference materials

(RMs) at approx. -70 °C so that perhaps at a later date these RMs can be used in a second round of this PT (if feasible).

Related to the **analysis of bivalve molluscs**, I want to inform you that we published a document at the EURL-*Salmonella* website to give some background information on the detection of *Salmonella* in live bivalve molluscs (LBM), and especially on the transport and handling of the samples. This document can be found at the following link: https://www.eurlsalmonella.eu/documenten/salmonella-detection-in-bivalve-molluscs

Earlier this year we started with the registrations for the **EURL-*Salmonella* workshop of 2020**, originally planned on 28 and 29 May. The list of participants was almost complete, but due to the COVID-19 pandemic we had to cancel the workshop for the original dates. We now plan to organise the workshop on 17 and 18 September 2020. The location will be the same as planned for the workshop in May: Zaandam, the Netherlands. In the coming month(s) we will send again a link for (re)registration to the workshop so that we can make an updated list of participants.

By mid-March 2020 we informed you that, on behalf of EFSA and ECDC, the EURL-*Salmonella* is monitoring the incidence of *Salmonella* Mikawasima in food (products), animals, animal feed or the environment. The aim of the monitoring is to follow cases throughout the year, as there seems to be a yearly trend with peaks in human cases across EU/EEA member states in autumn each year. By monitoring the events during the year, EFSA and ECDC could be prepared to react more rapidly when outbreaks are reported. Throughout the whole year, you can report your findings of *Salmonella* Mikawasima through the link sent to you by e-mail and also available at the EURL-*Salmonella* website (https://www.eurlsalmonella.eu/about-eurl).

In the previous Newsletter we informed you about the organisation of a **conference entitled 'Modern technologies to enable response to crises: Next Generation Sequencing to tackle food-borne diseases in the EU'**. This conference was planned to take place at the Instituto Superiore di Sanità in Rome on 10 March 2020. However, as you may understand, this conference has also been postponed. The new date will be Friday 25 September 2020. The registration already done for the event on 10 March could not be maintained and all people interested in the conference should (again) register online. Very recently the link for the new registration to the conference became available, so that you can now register (again) at: https://w3.iss.it/site/SANVevent.

For your information, Amendment 1 of ISO 6579-1 has been published early March 2020, called **ISO 6579-1:2017/Amd.1:2020** 'Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of *Salmonella* - Part 1: Detection of *Salmonella* spp. - Amendment 1: Broader range of incubation temperatures, amendment to the status of Annex D, and correction of the composition of MSRV and SC'. The main amendments to EN ISO 6579-1 are the following:

- The temperature range for incubation of selective media has been extended from 37 °C ± 1 °C to 34 °C to 38 °C without further tolerance (like it already was for non-selective media). This amendment also resulted in an update of the flow schemes in Annex A.
- In Annex B (B.4) the composition of Modified semi-solid Rappaport-Vassiliadis (MSRV) agar was corrected when preparing it from individual ingredients. In the composition described in EN ISO 6579-1 the final concentration of $MgCl_2$ in MSRV agar was not correct.
- The status of Annex D on detection of *Salmonella* Typhi and *Salmonella* Paratyphi was changed from normative to informative.

- A few corrections were included in Annex D, especially concerning the composition of selenite cystine medium (broth) in Annex D.3. Currently it is indicated that 100 ml L-cystine solution (D.3.1.2) should be added to 1000 ml base medium, but this has to be only 10 ml.

Best wishes,
Kirsten Mooijman
Coordinator EURL-*Salmonella*

From the Literature

Salmonella-related Literature from Scopus: January – March 2020

Naberhaus, S.A., Krull, A.C., Arruda, B.L., Arruda, P., Sahin, O., Schwartz, K.J., Burrough, E.R., Magstadt, D.R., Matias Ferreyra, F., Gatto, I.R.H., Meiroz de Souza Almeida, H., Wang, C., Kreuder, A.J.
Pathogenicity and Competitive Fitness of Salmonella enterica Serovar 4,[5],12:i:- Compared to Salmonella Typhimurium and Salmonella Derby in Swine
(2020) Frontiers in Veterinary Science, 6, art. no. 502, .
ABSTRACT: Since 2014, Salmonella 4,[5],12:i:- has emerged as the most common serovar of Salmonella enterica identified from swine samples submitted to veterinary diagnostic laboratories in the United States. To compare the pathogenicity of S. 4,[5],12:i:- in swine to the known pathogenic Salmonella Typhimurium and lesser pathogenic Salmonella Derby, 72 pigs (20 per Salmonella serovar treatment and 12 controls) were inoculated with either S. Typhimurium, S. 4,[5],12:i:-, S. Derby, or sham-inoculated and followed for up to 28 days thereafter via rectal temperature, fecal scoring, and fecal culture. Animals were euthanized on days 2, 4, or 28 to determine the gross and histopathologic signs of disease and tissue colonization. The results clearly demonstrate that for the isolates selected, serovar 4,[5],12:i:- possesses similar ability as serovar Typhimurium to cause clinical disease, colonize the tonsils and ileocecal lymph nodes, and be shed in the feces of infected swine past resolution of clinical disease. To compare the competitive fitness of S. 4,[5],12:i:- to S. Typhimurium in swine when co-infected, 12 pigs were co-inoculated with equal concentrations of both S. Typhimurium and S. 4,[5],12:i and followed for up to 10 days thereafter. When co-inoculated, serovar 4,[5],12:i:- was consistently detected in the feces of a higher percentage of pigs and at higher concentrations than serovar Typhimurium, suggesting an increased competitive fitness of 4,[5],12:i:- relative to serovar Typhimurium when inoculated simultaneously into naïve pigs. Whole genome sequencing analysis of the isolates used in these studies revealed similar virulence factor presence in all S. 4,[5],12:i:- and S. Typhimurium isolates, but not S. Derby, providing additional evidence for similar pathogenicity potential between serovars 4,[5],12:i:- and Typhimurium. Altogether, this data strongly supports the hypothesis that S. 4,[5],12:i:- is a pathogen of swine and suggests a mechanism through increased competitive fitness for the increasing identification of Salmonella 4,[5],12:i:- in swine diagnostic samples over the past several years. ISSN: 22971769

Redweik, G.A.J., Daniels, K., Severin, A.J., Lyte, M., Mellata, M.
Oral Treatments With Probiotics and Live Salmonella Vaccine Induce Unique Changes in Gut Neurochemicals and Microbiome in Chickens
(2020) Frontiers in Microbiology, 10, art. no. 3064, .
ABSTRACT: Cross-talk between the gut microbiota and neurochemicals affects health and well-being of animals. However, little is known about this interaction in chickens despite their importance in food production. Probiotics and live Salmonella vaccines are microbial products commonly given orally to layer pullets to improve health and ensure food safety. This study's objective was to determine how these oral treatments, individually or in combination, would impact the gut environment of chickens. White Leghorn chicks were either non-treated (CON) or orally given probiotics (PRO), a recombinant attenuated Salmonella vaccine (RASV; VAX), or both (P+V). Birds were fed with probiotics daily beginning at 1-day-old and orally immunized with RASV at 4-days-old and boosted 2 weeks post-primary vaccination. At 5 weeks, ceca content, ceca tissues, and small intestinal scrapings (SISs) were collected from ten birds/group post-euthanasia for analyses. Catecholamine, but not serotonergic, metabolism was affected by treatments. Dopamine metabolism, indicated by L-DOPA and DOPAC levels, were increased in P+V birds versus CON and PRO birds. Based on 16S sequencing, beta diversity was more similar among vaccinated birds versus birds given probiotics, suggesting live Salmonella vaccination has a major selective pressure on microbial diversity. Abundances of Akkermansia muciniphila and Enterobacteriaceae positively correlated with levels of tyrosine and norepinephrine, respectively. Both enumeration and 16S sequencing, determined that PRO exhibited the greatest levels of Enterobacteriaceae in the ceca and feces, which was associated with greater IgA production against E. coli virulence factors as tested by ELISA. In summary, we demonstrate that using probiotics alone versus in combination with a live vaccine has major implications in catecholamine production and the

microbiota of layer pullets. Additionally, unique correlations between changes in some neurochemicals and specific bacteria have been shown. ISSN: 1664302X

Du, J., Wu, S., Niu, L., Li, J., Zhao, D., Bai, Y.
A gold nanoparticles-assisted multiplex PCR assay for simultaneous detection of: Salmonella typhimurium, Listeria monocytogenes and Escherichia coli O157:H7 (2020) Analytical Methods, 12 (2), pp. 212-217.
ABSTRACT: Foodborne pathogens are a major cause of foodborne illness, leading to a growing food safety problem in public health. This work aims to develop a novel gold nanoparticles (AuNPs)-assisted multiplex PCR assay for rapid, simple and simultaneous detection of Salmonella typhimurium (S. typhimurium), Listeria monocytogenes (L. monocytogenes) and Escherichia coli O157:H7 (E. coli O157:H7), which are the top three foodborne pathogenic bacteria. Flower-shaped AuNPs (F-AuNPs) were used as a colorimetric sensor in this assay, based on PCR product that can help improve stability of the F-AuNPs in a certain concentration of salt solution. Detection of PCR product can be directly achieved by mixing it with F-AuNPs and NaCl, and the result is visible to the naked eye. Results showed that the optimal annealing temperature was 53.1 °C to amplify the three target pathogenic strains in multiplex PCR assay, and the optimal concentrations of the primer pairs were 0.4 μM for each of L. monocytogenes and E. coli O157:H7, and 0.2 μM for S. typhimurium. The colorimetric detection limit of PCR products by F-AuNPs was 3.125 ng μL-1, and the detection time was approximately 10 min. Simultaneous detection limit of the multiplex PCR method was 10 pg μL-1 for L. monocytogenes and S. typhimurium, and 50 pg μL-1 for E. coli O157:H7. Compared with conventional multiplex PCR assay, the F-AuNPs-assisted assay is a convenient, rapid and simple visual detection method. The excellent performance of the colorimetric sensor shows potential application in on-site detection of foodborne pathogenic strains in food samples. © ISSN: 17599660

Phungamngoen, C., Rittisak, S.
Surface Characteristics of Leafy Vegetables and Their Effects on Salmonella Attachment (2020) E3S Web of Conferences, 141, art. no. 03002, .
ABSTRACT: Leafy vegetables exhibit non-uniform surfaces and are structured with interconnected networks of veinlets or wrinkle characteristics, making the quantification of the changes rather difficult. In this study, attempt was made to quantify the surface topographical features of leafy vegetable. Image analysis was used to determine the characteristic of vegetable surface. In term of surface area (A), the results were compared with those correlated with the data obtained by a conventional measurement method. It was also performed to determine fractal dimension (FD) and roughness value (R) to describe the behavior of bacteria attached on the vegetable surface. The results showed that different leafy vegetable (basil, lemon basil, peppermint and cabbage) did not have a significant effect on Salmonella attached on surface. Dorsal side (upper side) of leaves exhibited higher R and lower FD than ventral side (lower side). It led to number of Salmonella attached on upper side of leaves showed higher than their lower side. From Pearson's correlation, FD could relate well with the number of Salmonella attached on surface of vegetable. FD showed the highest correlation (-0.78-(-0.97)) follow by A (0.77-0.86) and R (0.61-0.87), respectively. Therefore, the parameters from image analysis were found to be good indicator to describe the physical characteristics of leafy vegetable. ISSN: 25550403

Bjelland, A.M., Sandvik, L.M., Skarstein, M.M., Svendal, L., Debenham, J.J.
Prevalence of Salmonella serovars isolated from reptiles in Norwegian zoos (2020) Acta Veterinaria Scandinavica, 62 (1), art. no. 3, .
ABSTRACT: Background: Reptiles are known to be asymptomatic carriers of Salmonella spp. in their gastrointestinal mucosa and a variety of Salmonella serovars including exotic serovars mainly associated with reptiles as well as human pathogenic serovars have been isolated. There are many case reports of reptile-associated Salmonella infections worldwide, including one case in Norway in 2000. In August 2017, there was a legislative change in Norway that allowed more permissive reptile ownership and legalized the keeping of 19 different reptile species by private persons. There has been a concern that this new legislation will lead to an increase in reptile-associated salmonellosis in Norway, however knowledge is lacking on the occurrence of Salmonella spp. in Norwegian reptiles. The aim of this study was therefore to investigate the prevalence of Salmonella spp. in captive reptile species in Norway, identify the serovars and evaluate their zoonotic potential. Thus, cloacal swabs were taken from 53 snakes, 15 lizards and 35 chelonians from three Norwegian zoos, and assessed for the presence of Salmonella spp. by culture, biochemical testing and serotyping. Results: In total, 43% of the reptiles were shedding Salmonella spp., with a prevalence of 62%, 67% and 3% in snakes, lizards and

chelonians, respectively. A total of 26 different serovars were found, including Salmonella enterica spp. enterica (40%) and S. enterica spp. arizonae (4%), both of which are considered to have a high zoonotic potential. S. enterica spp. diarizonae, salamae and houtenae were also identified, however these serovars are considered to have a lower zoonotic potential. Conclusions: The current study demonstrates that captive Norwegian reptiles are carriers of potentially zoonotic Salmonella spp. Given the increasing popularity of reptiles as pets and the legislative change, reptile-associated salmonellosis could become an increasingly important public health concern in Norway. Adequate public information about the risk of Salmonella infection as well as preventive measures to avoid Salmonella transmission from reptiles to humans is needed. The risk of Salmonella infection is considered low when recommended precautions are taken and good hygiene exhibited. ISSN: 0044605X

Oblessuc, P.R., Matiolli, C.C., Melotto, M.
Novel molecular components involved in callose-mediated Arabidopsis defense against Salmonella enterica and Escherichia coli O157:H7
(2020) BMC Plant Biology, 20 (1), art. no. 16, .
ABSTRACT: Background: Food contamination with Salmonella enterica and enterohemorrhagic Escherichia coli is among the leading causes of foodborne illnesses worldwide and crop plants are associated with > 50% of the disease outbreaks. However, the mechanisms underlying the interaction of these human pathogens with plants remain elusive. In this study, we have explored plant resistance mechanisms against these enterobacteria and the plant pathogen Pseudomonas syringae pv. tomato (Pst) DC3118, as an opportunity to improve food safety. Results: We found that S. enterica serovar Typhimurium (STm) transcriptionally modulates stress responses in Arabidopsis leaves, including induction of two hallmark processes of plant defense: ROS burst and cell wall modifications. Analyses of plants with a mutation in the potentially STm-induced gene EXO70H4 revealed that its encoded protein is required for stomatal defense against STm and E. coli O157:H7, but not against Pst DC3118. In the apoplast however, EXO70H4 is required for defense against STm and Pst DC3118, but not against E. coli O157:H7. Moreover, EXO70H4 is required for callose deposition, but had no function in ROS burst, triggered by all three bacteria. The salicylic acid (SA) signaling and biosynthesis proteins NPR1 and ICS1, respectively, were involved in stomatal and apoplastic defense, as well as callose deposition, against human and plant pathogens. Conclusions: The results show that EXO70H4 is involved in stomatal and apoplastic defenses in Arabidopsis and suggest that EXO70H4-mediated defense play a distinct role in guard cells and leaf mesophyll cells in a bacteria-dependent manner. Nonetheless, EXO70H4 contributes to callose deposition in response to both human and plant pathogens. NPR1 and ICS1, two proteins involved in the SA signaling pathway, are important to inhibit leaf internalization and apoplastic persistence of enterobacteria and proliferation of phytopathogens. These findings highlight the existence of unique and shared plant genetic components to fight off diverse bacterial pathogens providing specific targets for the prevention of foodborne diseases.
ISSN: 14712229

Romeu, M.J., Rodrigues, D., Azeredo, J.
Effect of sub-lethal chemical disinfection on the biofilm forming ability, resistance to antibiotics and expression of virulence genes of Salmonella Enteritidis biofilm-surviving cells
(2020) Biofouling, 36 (1), pp. 101-112.
ABSTRACT: Although disinfection procedures are widely implemented in food environments, bacteria can survive and present increased virulence/resistance. Since little is known about these phenomena regarding biofilms, this study aimed to investigate the effect of chemical disinfection on biofilm-derived cells of Salmonella Enteritidis. Using a reference strain (NCTC 13349) and a food isolate (350), biofilm susceptibility to benzalkonium chloride (BAC), sodium hypochlorite (SH) and hydrogen peroxide (HP) was evaluated and biofilms were exposed to sub-lethal concentrations of each disinfectant. Biofilm-derived cells were characterized for their biofilm forming ability, antibiotic resistance and expression of virulence-associated genes. Except for a few instances, disinfectant exposure did not alter antibiotic susceptibility. However, SH and HP exposure enhanced the biofilm forming ability of Salmonella Enteritidis NCTC 13349. After BAC and HP exposure, biofilm-derived cells presented a down-regulation of rpoS. Exposure to BAC also revealed an up-regulation of invA, avrA and csgD on Salmonella Enteritidis NCTC 13349. The results obtained suggest that biofilm-derived cells that survive disinfection may represent an increased health risk. ISSN: 08927014

Fabà, L., Litjens, R., Allaart, J., Van Den Hil, P.R.

Feed additive blends fed to nursery pigs challenged with Salmonella
(2020) Journal of Animal Science, 98 (1), art. no. skz382, .
ABSTRACT: Salmonella in pigs is a concern for human foodborne salmonellosis. Dietary fungal fermented products, coated butyrate, and organic acids (OAs) may be promising control strategies. The objectives of this study were (i) to evaluate in vitro binding affinity of Salmonella enterica serovar Typhimurium (S. Typh) and Enteritidis (S. Ent), and enterotoxigenic Escherichia coli (ETEC) F4 or F18 to mannan-rich hydrolyzed copra meal (MCM) and fermented rye (FR) with Agaricus subrufescens; and (ii) to assess MCM and FR efficacy to control in vivo S. Typh shedding when combined with OAs and compared with coated butyrate strategy. A 31-d study included 32 pigs [6.29 ± 0.76 kg BW] individually housed and distributed into four dietary treatments: control diet; OA.BU, 4 kg/t OA plus 6 kg/t coated butyrate; OA.MCM, 4 kg/t OA plus 1 kg/t MCM; and OA.FR, 4 kg/t OA plus 2 kg/t FR. All pigs were challenged for 7 d with 1 mL S. Typh (109 colony forming units daily) at 10 d postweaning. Temperature and fecal samples were collected before and after challenge, and fecal Salmonella shedding quantified. Diarrhea scores were monitored daily and growth performance was evaluated weekly. In vitro, culture with MCM and FR showed significant (P < 0.01) binding affinity for both S. Typh and S. Ent, but not for ETEC F4 and F18. In vivo, pigs fed OA.MCM and OA.FR had lower (P < 0.05) shedding and day 3 peak shedding of S. Typh after infections than pigs fed control and OA.BU diets. Pigs fed OA.FR diet tended to have an 18% increase (P = 0.068) in BW on day 14 post first inoculation compared with control and OA.BU, and 19% increased (P = 0.093) final BW at day 21 compared with control. Diarrhea frequency post infection was overall lower (P = 0.006) for OA.FR (18.9%) than OA.BU (44.8%) and OA.MCM (41.7%) while control (28.7%) was not different. In conclusion, FR and MCM show in vitro-binding affinity to Salmonella enterica serovars Typh and Ent. Feeding FR or MCM combined with OA to nursery pigs reduces the peak and averages S. Typh shedding compared with control. Fermented rye with OA tends to improve pig performance after S. Typh challenge.
ISSN: 00218812

Yang, K., Wang, A., Fu, M., Wang, A., Chen, K., Jia, Q., Huang, Z.
Investigation of incidents and trends of antimicrobial resistance in foodborne pathogens in eight countries from historical sample data
(2020) International Journal of Environmental Research and Public Health, 17 (2), art. no. 472, .
ABSTRACT: Antimicrobial resistance (AMR) causes millions of illnesses every year, threatening the success of lifesaving antibiotic therapy and, thus, public health. To examine the rise and spread of antimicrobial resistance around the world, our study performs a multivariate statistical analysis of antimicrobial resistance gene data from eight different countries: the US, the UK, China, Brazil, Mexico, Canada, Australia, and South Africa. Multi-dimensional data points were projected onto a two-dimensional plane using principal component analysis and organized into a dendrogram utilizing hierarchical clustering to identify significant AMR genes and pathogens. Outlier genes/pathogens were typically involved in high occurrences of antimicrobial resistance, and they were able to indicate the trend of antimicrobial resistance in the future. Statistical analysis of the data identified: (1) tet(A), aph(3'')-Ib, aph(6)-Id, blaEC, blaTEM-1, qacEdelta1, sul1, sul2, and aadA1 as the nine most common AMR genes among the studied countries; (2) Salmonella enterica and E. coli and Shigella as the most common AMR foodborne pathogens; and (3) chicken as the most prevalent meat carrier of antimicrobial resistance. Our study shows that the overall number of reported antimicrobial resistance cases in foodborne pathogens is generally rising. One potential contributing factor for this is the increasing antimicrobial usage in the growing livestock industry. ISSN: 16617827

Kim, W.-I., Choi, S.Y., Han, I., Cho, S.K., Lee, Y., Kim, S., Kang, B., Choi, O., Kim, J.
Inhibition of Salmonella enterica growth by competitive exclusion during early alfalfa sprout development using a seed-dwelling Erwinia persicina strain EUS78
(2020) International Journal of Food Microbiology, 312, art. no. 108374, .
ABSTRACT: Salmonella enterica outbreaks in sprouts originate from contaminated seeds; conventional prevention technologies have been reported from many research institutes. In this study, we applied a biological control approach to inhibit S. enterica growth using the seed-dwelling non-antagonistic bacteria. We isolated non-antibacterial seed-dwelling bacteria from vegetable sprouts. A total of 206 bacteria exhibiting non-antibacterial activity against S. enterica were subjected to alfalfa sprout development tests. Eight isolates exhibiting no deleterious effect on the growth of alfalfa sprouts were tested for S. enterica growth inhibition on alfalfa seeds and sprouts, and an isolate EUS78 was finally selected for further investigation. Based on 16S rRNA, gyrB, and rpoB gene sequence analyses, strain EUS78 was identified as Erwinia persicina. In population competition, the

S. enterica population increased by >3 log CFU/g after 6 days of alfalfa sprout growth, whereas S. enterica growth was significantly inhibited by treatment with EUS78 (P < .05). This effect of S. enterica growth inhibition by EUS78 was sustained until the end of the alfalfa sprout harvest. Overall, bacterial strain EUS78 significantly reduced S. enterica growth on alfalfa sprouts in a manner consistent with competitive exclusion. These findings led us to monitor EUS78 behavior on seeds during early sprout development using fluorescence and scanning electron microscopy. Strain EUS78 initially colonized alfalfa sprout seed coat edges, cotyledons, and finally root surfaces during early sprout germination. As alfalfa sprouts grew, EUS78 bacterial cells established colonies on newly emerged plant tissues such as root tips. The results of this study suggest that strain EUS78 has potential as a biological control agent to inhibit S. enterica contamination in the sprout food industry. ISSN: 01681605

Song, J., Li, Q., Everaert, N., Liu, R., Zheng, M., Zhao, G., Wen, J.
Effects of inulin supplementation on intestinal barrier function and immunity in specific pathogen-free chickens with Salmonella infection
(2020) Journal of Animal Science, 98 (1), art. no. skz396, .
ABSTRACT: We investigated the effects of inulin on intestinal barrier function and mucosal immunity in Salmonella enterica serovar Enteritidis (SE)-infected specific pathogen-free (SPF) chickens. SPF chickens (n = 240, 1-d-old) were divided into 4 groups (6 replicates per group, 10 chickens per replicate): a control group (CON) fed a basal diet without inulin supplementation and 3 SE-infected groups fed a basal diet supplemented with inulin 0% (SE group), 0.5% (0.5% InSE group), and 1% (1% InSE group), respectively. At 28 d of age, the chickens in SE-infected groups were orally infected with SE and in CON group were administrated with phosphated-buffered saline (PBS). Intestinal morphology, mucosal immunity, and intestinal barrier function-related gene expression were analyzed at 1-and 3-d post-infection (dpi). SE challenge significantly increased the mucosal gene expression, such as interleukin-1β (IL-1β), lipopolysaccharide-induced tumor necrosis factor factor (LITAF), interferon-γ(IFN-γ), and interleukin-6 (IL-6), and increased serum IFN-γ, secretory IgA (sIgA), and IgG concentration, and significantly decreased the gene expression levels of mucin 2 (MUC2) and claudin-1 at 3 dpi compared with the CON group (P < 0.05). Inulin supplementation improved the expression levels of these immunity-and intestinal barrier function-related genes, increased villus height (VH), and decreased crypt depth (CD) in the duodenum, jejunum, and ileum at 1 and 3 dpi within the SE-challenged groups (P < 0.05). SE challenge significantly increased ileal Toll-like receptor 4 (TLR4) mRNA at 1 and 3 dpi, suppressor of cytokine signaling 3 (SOCS3) mRNA at 1 dpi, and phospho-signal transducer and activator of transcription 3 (p-STAT3) and Janus kinase1 (JAK1) protein expression at 3 dpi compared with the CON group (P < 0.05). Inulin supplementation suppressed p-STAT3 and JAK1 protein expression and promoted ileal TLR4 and SOCS3 mRNA expression at 3 dpi compared with SE group (P < 0.05). In conclusion, inulin alleviated SE-induced gut injury by decreasing the proinflammatory response and enhancing mucosal immunity in chickens.
ISSN: 00218812

Buch, J.-M., Visscher, C., Zu Sundern, A.S., Schulte-Wülwer, J., Deermann, A., Holling, C.
Prevalence of salmonella by serological and direct detection methods in piglets from inconspicuous, conspicuous, and vaccinated sow herds
(2020) Animals, 10 (1), art. no. 29, .
ABSTRACT: Due to the zoonotic potential of Salmonella, the high prevalence of Salmonella on pig farms deserves particular attention. Because there is limited precise data on piglet-producing farms, this survey evaluated the Salmonella status of 24 different pig farms that had previously been divided into 12 Salmonella-conspicuous (SC) and 12 Salmonella-inconspicuous (SI) farms on the basis of the serological status of their piglets (25 kg). The evaluation was based on 498 environmental samples and 2641 blood samples, as well as on a biosecurity screening. SC farms were subdivided into farms with sow vaccination against Salmonella (n = 3) and those without vaccination (n = 9). In accordance with the previous classification, both the highest Salmonella prevalence in the environment and the highest antibody titers of the examined piglets were determined on SC farms at both defined time points. Piglets from vaccinated sows showed the highest OD% values, before and after vaccination. On SC farms, most Salmonella-positive samples could be obtained in rearing areas (2017: 40.8%, 2019: 26.0%). The results of this study indicate that sow vaccination alone cannot influence Salmonella prevalence at the farm level. Above all, general infection pressure seems to play a major role for Salmonella prevalence in the environment and for high OD% values of related pigs. ISSN: 20762615

Wang, H., Ryser, E.T.
Quantitative transfer and sanitizer inactivation of Salmonella during simulated commercial dicing and conveying of tomatoes
(2020) Food Control, 107, art. no. 106762, .
ABSTRACT: Diced tomatoes have been linked to outbreaks of salmonellosis in the United States. Compared to slicing, commercial production of diced tomatoes is more complex and includes mechanical dicing as well as washing, dewatering, conveying, and packing. Consequently, this study aimed to 1) quantify Salmonella transfer during pilot-scale dicing of tomatoes, 2) assess the efficacy of three sanitizer treatments against Salmonella during flume tank washing, and 3) assess the efficacy of four sanitizers against Salmonella during conveyance of diced tomatoes. One 0.9 kg batch of Salmonella Typhimurium LT2-inoculated Roma tomatoes (~5 log CFU/g) was mechanically diced, followed by ten batches of uninoculated tomatoes. All uninoculated tomatoes yielded Salmonella with populations decreasing from 3.3 to 1.1 log CFU/g during dicing. Flume tank washing in sanitizer-free water or water containing 80 ppm of peroxyacetic acid, mixed peracid, or total chlorine decreased Salmonella populations in diced tomatoes 1.3 ± 0.2, 2.3 ± 0.4, 2.35 ± 0.4, and 2.4 ± 0.1 log CFU/g, respectively. After processing, Salmonella populations in flume water containing a sanitizer were always below the limit of detection (−1.0 log CFU/ml) and were significantly lower ($P \leq 0.05$) than for the sanitizer-free control (1.5 ± 0.3 log CFU/ml). When the same three sanitizer treatments as well as electrolyzed water (80 ppm chlorine) were applied to smooth and interlocking belts on a dual track conveyor, Salmonella reductions were greater using mixed peracid (6.49 and 6.76 log) and peroxyacetic acid (5.95 and 6.10 log) as compared to chlorine (3.72 and 5.70 log) and electrolyzed water (3.50 and 4.53 log), respectively. All four sanitizer treatments were more effective than water alone in reducing Salmonella on conveyor belt surfaces. These findings should help provide some practical guidelines for the industry and aide in the development of improved risk assessments. ISSN: 09567135

Yanagimoto, K., Yamagami, T., Uematsu, K., Haramoto, E.
Characterization of Salmonella isolates from wastewater treatment plant influents to estimate unreported cases and infection sources of salmonellosis
(2020) Pathogens, 9 (1), art. no. 52, .
ABSTRACT: Salmonella enterica is a major cause of gastroenteritis usually caused by animal-based contaminated foods. Since the current passive surveillance is not sufficient to detect all infections and infection sources, we determined the prevalence of Salmonella isolated from sewage influent of wastewater treatment plants (WWTPs) and compared the characteristics of human and food isolates to identify the infection sources. Sewage influent samples were collected monthly from two WWTPs located in the Yamanashi Prefecture, Japan, for three years. Serotypes, antimicrobial resistances, isolation periods, isolated areas, and pulsed-field gel electrophoresis patterns of six isolates belonging to five serotypes were consistent with those of the isolates from patients. Real-time PCR for Salmonella indicated that sewage influents reflect cases of patients infected with Salmonella, including unreported cases. Serovars Schwarzengrund and Anatum were predominant in sewage, but not in humans, and their characteristics were closely related or identical to those isolated from poultry heart and liver, respectively. These results suggest that sewage influent contains Salmonella isolates from humans and that some originated from unreported human cases infected by poultry-associated products. Therefore, it is necessary to take countermeasures against Salmonella infection based on the unreported cases, which would be disclosed by analysis of sewage influent.
ISSN: 20760817

El-Shall, N.A., Awad, A.M., El-Hack, M.E.A., Naiel, M.A.E., Othman, S.I., Allam, A.A., Sedeik, M.E.
The simultaneous administration of a probiotic or prebiotic with live Salmonella vaccine improves growth performance and reduces fecal shedding of the bacterium in Salmonella-challenged broilers
(2020) Animals, 10 (1), art. no. 70, .
ABSTRACT: Salmonellosis is one of the most important bacterial diseases in poultry, causing heavy economic losses, increased mortality and reduced production. The aim of this study was the comparative efficacy of a commercial probiotic and/or prebiotic with a live attenuated Salmonella Enteritidis (SE) vaccine on the protection of broiler chickens from SE challenge. The efficacy of probiotic or prebiotic products, as well as a live Salmonella Enteritidis (SE) vaccine at the 7th day of age, administered via drinking water, were evaluated for clinical protection and effects on growth performance of broiler chickens experimentally challenged with SE at the 28th day of age. The use of probiotic or prebiotic simultaneously with the live Salmonella vaccine can diminish the negative effect of live

vaccine growth performance, reducing mortality rate, fecal shedding, and re-isolation of SE from liver, spleen, heart and cecum. The use of probiotic or prebiotic simultaneously with the application of the live Salmonella vaccine is a good practice to diminish the negative effect of the harmful bacteria and improve the growth performance of broilers. Thus, further studies may be carried out with layers and breeders. ISSN: 20762615

Khan, S., Chousalkar, K.K.
Short-term feeding of probiotics and synbiotics modulates caecal microbiota during Salmonella Typhimurium infection but does not reduce shedding and invasion in chickens (2020) Applied Microbiology and Biotechnology, 104 (1), pp. 319-334.
ABSTRACT: Positive modulation of gut microbiota in laying chickens may offer a strategy for reduction of Salmonella Typhimurium shedding and production of safer poultry products. In the current study, the caecal luminal microbiota of laying chicks was studied using 16S rRNA amplicon sequencing on DNA obtained from the chicks that were offered supplementation with commercial probiotics, synbiotics and/or Salmonella Typhimurium challenge. The load of Salmonella Typhimurium in various organs was quantified. Irrespective of the probiotics and synbiotics supplementation and Salmonella Typhimurium challenge, caecal microbiota was dominated by 22 distinct bacterial genera and 14 families that clustered into Actinobacteria, Proteobacteria and Firmicutes at phylum level. Taken together, probiotics and synbiotics supplementation increased (false discovery rate; FDR < 0.05) the abundance of Ruminococcus, Trabulsiella, Bifidobacterium, Holdemania and Oscillospira, indicating their role in maintaining gut health through lowering luminal pH and digestion of complex polysaccharides. Salmonella Typhimurium challenge decreased the abundance of Trabulsiella, Oscillospira, Holdemania, Coprococcus, Bifidobacterium and Lactobacillus and increased Klebsiella and Escherichia, indicating its role in caecal dysbiosis. Although probiotics and synbiotics supplementation positively modulated the caecal microbiota, they were not effective in significantly (P > 0.05) reducing Salmonella Typhimurium load in caecal tissue and invasion into vital organs such as liver and spleen. The early colonisation of laying chick caeca by probiotics and synbiotics had the potential to positively influence luminal microbiota; however, the microbial abundance and diversity were not sufficient to significantly reduce the shedding of Salmonella Typhimurium in faeces or invasion into internal organs during this study. ISSN: 01757598

Krishnasamy, V.P., Marshall, K., Dewey-Mattia, D., Wise, M.
Outbreak Characteristics and Epidemic Curves for Multistate Outbreaks of Salmonella Infections Associated with Produce: United States, 2009-2015
(2020) Foodborne Pathogens and Disease, 17 (1), pp. 15-22.
ABSTRACT: Produce is recognized as a source of Salmonella-related foodborne outbreaks in the United States. Identifying produce as a source of foodborne outbreaks is challenging given short product shelf lives and durations of many produce-associated outbreaks. Investigators consider produce a plausible source when illnesses occur over a short time period and disproportionately affect middle-aged or female individuals. We reviewed characteristics of past Salmonella produce outbreaks and their consistency with principles used by epidemiologists when generating hypotheses about an outbreak source. We queried the Foodborne Disease Outbreak Surveillance System for multistate, produce-associated Salmonella outbreaks reported to the Centers for Disease Control and Prevention from 2009 to 2015. All produce-associated outbreaks were classified as fruit outbreaks or vegetable outbreaks using an established classification scheme. We then compared fruit and vegetable outbreaks by characteristics of size, gender, age, age groups, geographic spread, duration, and velocity measures using Wilcoxon rank-sum tests. Epidemic curves were created to display visual representations of outbreak duration and velocity. We identified 14 fruit outbreaks and 24 vegetable outbreaks. The median number of illnesses for all produce-associated outbreaks was 30 and a high median percentage of illnesses were in females (61.9%). Median age was 34 years, with a median of 53.2% of illnesses affecting the 18-59 age group. For all outbreaks, median duration was 77 d and median time to the 50th percentile of illnesses was 32.5 d. Fruit and vegetable outbreaks differed only in the age groups affected. We used outbreak data to verify common indicators of produce-associated Salmonella outbreaks. Outbreaks affected females and middle-aged individuals more commonly, while fruit and vegetable outbreaks impacted different age groups. Although median outbreak duration was less than 12 weeks for both fruit and vegetable outbreaks, there was considerable variation, decreasing its utility as an indicator of produce as a source of the outbreak. ISSN: 15353141

Lucca, V., Apellanis Borges, K., Quedi Furian, T., Borsoi, A., Pippi Salle, C.T., de Souza Moraes, H.L., Pinheiro do Nascimento, V.

Influence of the norepinephrine and medium acidification in the growth and adhesion of Salmonella Heidelberg isolated from poultry
(2020) Microbial Pathogenesis, 138, art. no. 103799, .
ABSTRACT: Salmonella spp. are among the leading pathogens responsible for foodborne illnesses worldwide. Bacterial communities use a quorum sensing (QS) system to control biofilm formation. QS is a cell-to-cell signaling mechanism involving compounds called auto-inducers (AI). Norepinephrine utilizes the same bacterial signaling of AI-3 and serves as a signal of QS. Acid stress is a challenge encountered by microorganisms in food processing environments and in the gastrointestinal tracts of hosts. Thus, adaptation to acidic environments may increase the pathogenicity of the strain. The aim of this study was to evaluate the influence of two concentrations of norepinephrine (100 μM and 250 μM) and acidification (pH 3.0) of the medium on the growth and adhesion of Salmonella Heidelberg strains isolated from poultry sources at 12 °C and 25 °C. Furthermore, three genes associated with the biofilm formation process were detected (adrA, csgD, and sidA). Norepinephrine stimulation did not influence the growth or adhesion of Salmonella Heidelberg strains, regardless of the catecholamine concentration and temperature. On the other hand, the use of acidified medium (pH 3.0) resulted in a significant reduction of growth and a significant increase of S. Heidelberg adhesion at both temperatures, indicating that the acidified medium favors the biofilm formation process. The adrA and sidA genes showed higher detection frequencies than csgD. Experiments analyzing the biofilm production process by S. Heidelberg strains are not common, and further studies are necessary to understand this complex process. ISSN: 08824010

Huang, X., Hu, M., Zhou, X., Liu, Y., Shi, C., Shi, X.
Role of yoaE gene regulated by CpxR in the survival of Salmonella enterica serovar Enteritidis in antibacterial egg white
(2020) mSphere, 5 (1), art. no. e00638, .
ABSTRACT: The survival ability of Salmonella enterica serovar Enteritidis in antibacterial egg white is an important factor leading to Salmonella outbreaks through eggs and egg products. In this study, the role of the gene yoaE, encoding an inner membrane protein, in the survival of Salmonella Enteritidis in egg white, and its transcriptional regulation by CpxR were investigated. Quantitative reverse transcription-PCR (RT-qPCR) results showed that the yoaE gene expression was upregulated 35-fold after exposure to egg white for 4 h compared to that in M9FeS medium, and the deletion of yoaE (ΔyoaE) dramatically decreased the survival rate of bacteria in egg white to less than 1% of the wild type (WT) and the complementary strain at both 37 and 20°C, indicating that yoaE was essential for bacteria to survive in egg white. Furthermore, the ΔyoaE strain was sensitive to a 3-kDa ultrafiltration matrix of egg white because of its high pH and antimicrobial peptide components. Putative conserved binding sites for the envelope stress response regulator CpxR were found in the yoaE promoter region. In vivo, the RT-qPCR assay results showed that the upregulation of yoaE in a ΔcpxR strain in egg white was 1/5 that of the WT. In vitro, results from DNase I footprinting and electrophoretic mobility shift assays further demonstrated that CpxR could directly bind to the yoaE promoter region, and a specific CpxR binding sequence was identified. In conclusion, it was shown for the first time that CpxR positively regulated the transcription of yoaE, which was indispensable for survival of Salmonella Enteritidis in egg white. IMPORTANCE Salmonella enterica serovar Enteritidis is the predominant Salmonella serotype that causes human salmonellosis mainly through contaminated chicken eggs or egg products and has been a global public health threat. The spread and frequent outbreaks of this serotype through eggs correlate significantly with its exceptional survival in eggs, despite the antibacterial properties of egg white. Research on the survival mechanisms of S. Enteritidis in egg white will help develop effective strategies to control the contamination of eggs by this Salmonella serotype and help further elucidate the complex antibacterial mechanisms of egg white. This study revealed the importance of yoaE, a gene with unknown function, on the survival of S. Enteritidis in egg white, as well as its transcriptional regulation by CpxR. Our work provides the basis to reveal the mechanisms of survival of S. Enteritidis in egg white and the specific function of the yoaE gene. ISSN: 23795042

Cohen, E., Davidovich, M., Rokney, A., Valinsky, L., Rahav, G., Gal-Mor, O.
Emergence of new variants of antibiotic resistance genomic islands among multidrug-resistant Salmonella enterica in poultry
(2020) Environmental Microbiology, 22 (1), pp. 413-432.
ABSTRACT: Non-typhoidal Salmonella enterica (NTS) are diverse and important bacterial pathogens consisting of more than 2600 different serovars, with varying host-specificity. Here, we characterized the poultry-associated serovars in Israel, analysed their resistome and illuminated the molecular mechanisms underlying common multidrug resistance (MDR)

patterns. We show that at least four serovars including Infantis, Muenchen, Newport and Virchow present a strong epidemiological association between their temporal trends in poultry and humans. Worrisomely, 60% from all of the poultry isolates tested (n = 188) were multidrug resistant, mediated by chromosomal SNPs and different mobile genetics elements. A novel streptomycin-azithromycin resistance island and previously uncharacterized versions of the mobilized Salmonella genomic island 1 (SGI1) were identified and characterized in S. Blockley and S. Kentucky isolates respectively. Moreover, we demonstrate that the acquisition of SGI1 does not impose fitness cost during growth under nutrient-limited conditions or in the context of Salmonella infection in the mouse model. Overall, our data emphasize the role of the poultry production as a pool of specific epidemic MDR strains and autonomous genetic elements, which confer resistance to heavy metals and medically relevant antibiotics. These are likely to disseminate to humans via the food chain and fuel the increasing global antibiotic resistance crisis. ISSN: 14622912

Sirsat, S.A.
The persistence of foodborne pathogens on produce box cartons
(2020) Journal of Environmental Health, 82 (6), pp. 16-21.
ABSTRACT: Previous studies have shown that a majority of vendors at farmers markets reuse cardboard cartons to store and transport produce to and from farmers markets, rendering the cartons a potential source of microbial contamination. This study investigated the ability of foodborne pathogens to persist on cardboard cartons over 44 days. Briefly, a mixture of Listeria monocytogenes, Salmonella spp., and E. coli O157:H7 were inoculated onto cardboard coupons and pathogen viability was quantified for up to 44 days. The results demonstrated that while E. coli O157:H7 survived for no longer than 2 days, L. monocytogenes and Salmonella spp. were recovered up to 32 and 44 days, respectively. These results highlight key challenges associated with reusing cardboard containers and the potential of microbial contamination transfer onto produce. The results of this study emphasize the need for science-based food safety training for vendors and managers at farmers markets to ensure that only containers that can be easily cleaned and sanitized are used to transport and store produce. ISSN: 00220892

Fagre, A.C., Pabilonia, K.L., Johnston, M.S., Morley, P.S., Burgess, B.A.
Comparison of detection methods for Salmonella enterica shedding among reptilian patients at a veterinary teaching hospital
(2020) Journal of Veterinary Diagnostic Investigation, 32 (1), pp. 118-123.
ABSTRACT: In the United States, ~1.4 million sporadic human Salmonella enterica infections occur annually, with an estimated 6% attributable to reptile exposure. Detection of Salmonella in reptiles can be challenging given the limitations among detection methods. We evaluated sampling and detection methods for S. enterica in a cross-sectional study of reptilian patients (n = 45) over the course of 13 mo. Two sampling methods (cloacal swabs, electrostatic cloth body-feet samples) and 3 detection methods (enriched culture, lateral flow immunoassay [LFI], real-time PCR) were compared using McNemar and Fisher exact tests. Results varied by species, sample type, and detection method. In total, 14 of 45 (33%) patients were positive by culture, 10 of 45 (22%), and/or 13 of 45 (29%) by rtPCR. Among rtPCR-positive results, cloacal swabs (12 of 45 [27%]) resulted in a higher detection than body-feet wipes (4 of 45 [9%]; p = 0.01). Among culture-positive results, shedding was most commonly detected after additional incubation at room temperature when testing cloacal swabs (9 of 45 [20%]). However, there was significant disagreement between sampling methods (cloacal vs. body-feet; p = 0.03). No samples were positive by LFI. In general, cloacal swabs yielded the highest test-positive rates, irrespective of testing method. Our study highlights the importance of using detection methods optimized for the sample being tested. ISSN: 10406387

Lei, C.-W., Zhang, Y., Kang, Z.-Z., Kong, L.-H., Tang, Y.-Z., Zhang, A.-Y., Yang, X., Wang, H.-N.
Vertical transmission of Salmonella Enteritidis with heterogeneous antimicrobial resistance from breeding chickens to commercial chickens in China
(2020) Veterinary Microbiology, 240, art. no. 108538, .
ABSTRACT: Human salmonellosis caused by the consumption of eggs and chicken meat contaminated with Salmonella Enteritidis has become a continuing public health concern worldwide. In this study we adopted whole genome sequencing (WGS) to determine the genetic relationship and antimicrobial resistance of S. enterica strains isolated from a poultry breeding enterprise that consists of one breeding chicken farm, one egg hatchery and one commercial chicken farm. A total of 148 S. enterica including 147 S. Enteritidis strains were isolated from 2100 fecal swab samples, with 16 (5.3 %, 16/300) from breeding chicken farm, 38 (4.2 %, 38/900) from egg hatchery and 94 (10.4 %, 94/900)

from commercial chicken farm. WGS revealed that all 147 S. Enteritidis strains belonged to ST11, and further divided into 4 different ribosomal STs and 64 core genome STs. Single nucleotide polymorphism typing suggested the presence of the vertical transmission of S. Enteritidis from breeding chicken to commercial chicken. Three different antimicrobial-resistant plasmids including one blaCTX-M-14-carrying plasmid and two virulence-resistance plasmids were characterized, resulting in the heterogeneous antimicrobial resistance of clonally related S. Enteritidis strains. Routine surveillance in breeding chicken farms is conducive to the control of S. Enteritidis from farm to fork. ISSN: 03781135

Alvarez, J., Lopez, G., Muellner, P., de Frutos, C., Ahlstrom, C., Serrano, T., Moreno, M.A., Duran, M., Saez, J.L., Dominguez, L., Ugarte-Ruiz, M.
Identifying emerging trends in antimicrobial resistance using Salmonella surveillance data in poultry in Spain
(2020) Transboundary and Emerging Diseases, 67 (1), pp. 250-262.
ABSTRACT: Despite of controls and preventive measures implemented along the food chain, infection with non-typhoidal Salmonella (NTS) remains one of the major causes of foodborne disease worldwide. Poultry is considered one of the major sources of NTS. This has led to the implementation of monitoring and control programmes in many countries (including Spain) to ensure that in poultry flocks infection is kept to a minimum and to allow the identification and monitoring of circulating NTS strains and their antimicrobial resistance (AMR) phenotypes. Here, we investigated the information from the monitoring programme for AMR in Salmonella from poultry in Spain in 2011–2017 to assess the diversity in phenotypic resistance and to evaluate the programme's ability to detect multi-resistance patterns and emerging strains in the animal reservoir. Data on serotype and AMR to nine antimicrobials obtained from 3,047 NTS isolates from laying hens (n = 1,060), broiler (n = 765) and turkey (n = 1,222) recovered during controls performed by the official veterinary services and food business operators were analysed using univariate and multivariate methods in order to describe host and serotype-specific profiles. Diversity and prevalence of phenotypic resistance to all but one of the antimicrobials (colistin) were higher in NTS from broiler and turkey compared with laying hen isolates. Certain combinations of serotype and AMR pattern (resistotype) were particularly linked with certain hosts (e.g. susceptible Enteritidis with laying hens, multi-drug resistant (MDR) Derby in turkey, MDR Kentucky in turkey and broiler). The widespread presence of certain serotype-resistotype combinations in certain hosts/years suggested the possible expansion of MDR strains in the animal reservoir. This study demonstrates the usefulness of the analysis of data from monitoring programmes at the isolate level to detect emerging threats and suggests aspects that should be subjected to further research to identify the forces driving the expansion/dominance of certain strains in the food chain.
ISSN: 18651674

Collineau, L., Phillips, C., Chapman, B., Agunos, A., Carson, C., Fazil, A., Reid-Smith, R.J., Smith, B.A.
A within-flock model of Salmonella Heidelberg transmission in broiler chickens
(2020) Preventive Veterinary Medicine, 174, art. no. 104823, .
ABSTRACT: As part of the development of a quantitative microbial risk assessment (QMRA) model of third-generation cephalosporins (3GC)-resistant Salmonella Heidelberg, a compartmental (SEIR) model for S. Heidelberg transmission within a typical Canadian commercial broiler chicken flock was developed. The model was constructed to estimate the within-flock prevalence and the bacterial concentration in the barn environment at pre-harvest, and to assess the effect of selected control measures. The baseline scenario predicted an average within-flock prevalence of 23.5 % (95 % tolerance interval: 15.7–31.4) and an average bacterial concentration of 3.579 (0–4.294) log CFU/g of feces in the barn environment at pre-harvest (on the day the flock is sent to slaughter). Because vertical introduction of S. Heidelberg into the barn was already uncommon in the baseline scenario, vaccination of broiler parent flocks appeared to have a negligible effect, while vaccination of broiler chicken flocks substantially reduced the bacterial concentration at pre-harvest. Cleaning and disinfection between batches markedly reduced the within-flock prevalence at pre-harvest, but the effect on bacterial concentration was limited outside of the beginning of the production period. Extending downtime between batches by 7 days had little effect on within-flock prevalence or bacterial concentration of S. Heidelberg when compared to the baseline scenario. This study provides a basis to describe S. Heidelberg dynamics within a broiler chicken flock and to predict the within-flock prevalence and bacterial concentration at pre-harvest, and includes a description of the limitations and data gaps. The results of these analyses and associated uncertainties are critical information for populating QMRA models of the downstream impacts on public health from on-farm and other food-chain practices. Specifically, the study findings will be integrated

into a broader farm-to-fork QMRA model to support the risk-based control of S. Heidelberg resistant to 3GC in broiler chicken in Canada. ISSN: 01675877

Correia-Gomes, C., Sparks, N.
Exploring the attitudes of backyard poultry keepers to health and biosecurity
(2020) Preventive Veterinary Medicine, 174, art. no. 104812, .
ABSTRACT: Backyard poultry producers have been associated with outbreaks of exotic (e.g. avian influenza) and endemic (e.g. Salmonella) disease all over the world. Currently in the UK the registration of small flocks (less than 50 birds) with local authorities is voluntary therefore there is not an accurate record of how many keepers and birds there are or where they are located. This lack of information (e.g. how many birds they keep, what type of birds, biosecurity measures they implement, etc.) may compromise contingency planning in an outbreak. A questionnaire was designed and implemented to gather information that will allow some of the knowledge gaps to be filled. The questionnaire comprised a total of 63 questions divided into seven sections (characterisation of the keeper, location of the enterprise and interest in poultry, poultry husbandry, transport of poultry, details about the poultry enterprise, marketing of poultry products, and poultry health/biosecurity). The questionnaire was implemented through an online survey, which was promoted through web links in smallholders' websites, Facebook pages, the SRUC network, a course about poultry welfare, and leaflets at smallholders' festivals. The survey was open from 24th October 2016 to 10th April 2017 and 176 questionnaires were completed by target respondents. Overall, our results suggest that the level of disease identified by backyard poultry keepers is low but the majority of the backyard poultry keepers also keep other livestock species, with an associated increased risk for disease transmission between species. Almost all respondents reported implementing at least one biosecurity measure, although in the majority of cases the measures taken were not comprehensive. A lack of knowledge about the legislation concerning poultry-keeping activities was evidenced by the answers given to some questions, such as the feeding of kitchen scraps and how to dispose of dead stocks. This investigation fills gaps in knowledge which will allow industry stakeholders and policy makers to adapt their current disease programmes and contingency plans to the reality of the health and biosecurity status of backyard poultry. It also highlights that government could play a more active role in engaging with backyard poultry keepers and in finding ways to disseminate reliable information generally and about disease outbreaks specifically, to these keepers. ISSN: 01675877

Gu, D., Wang, Z., Tian, Y., Kang, X., Meng, C., Chen, X., Pan, Z., Jiao, X.
Prevalence of Salmonella Isolates and Their Distribution Based on Whole-Genome Sequence in a Chicken Slaughterhouse in Jiangsu, China
(2020) Frontiers in Veterinary Science, 7, art. no. 29, .
ABSTRACT: Salmonella has been known as the most important foodborne pathogen, which can infect humans via consuming contaminated food. Chicken meat has been known as an important vehicle to transmit Salmonella by the food supply chain. This study determined the prevalence, antimicrobial resistance, and genetic characteristics of Salmonella at different chicken slaughtering stages in East China. In total, 114 out of 200 (57%) samples were Salmonella positive, while Salmonella contamination was gradually increasing from the scalding and unhairing stage (17.5%) to the subdividing stage (70%) throughout the slaughtering. Whole-genome sequencing (WGS) was then performed to analyze the serotype, antimicrobial resistance gene profiles, and genetic relationship of all Salmonella isolates. The most common serotypes were S. Kentucky (51/114, 44.7%) and S. Enteritidis (37/114, 32.5%), which were distributed throughout the four slaughtering stages, and were also identified in the corresponding environments. The multilocus sequence typing (MLST) analysis revealed that seven sequence types (STs) were occupied by six different serotypes, respectively. Only S. Kentucky had two STs, ST314 was the predominant ST shared by 50 isolates, while the ST198 has 1 isolate. The antimicrobial resistance gene analysis demonstrated that most of the strains belonging to S. Kentucky (39/51, 76.5%) and S. Indiana (15, 100%) contained over five groups of antimicrobial resistance genes. Based on the core genome analysis, 50 S. Kentucky isolates were genetically identical, indicating that one S. Kentucky strain with the same genetic background was prevalent in the chicken slaughtering line. Although 37 S. Enteritidis isolates only had three different antimicrobial resistance gene profiles, the core genome sequence analysis subtyped these S. Enteritidis isolates into five different clusters, which revealed the diverse genetic background of S. Enteritidis in the slaughterhouse. The antimicrobial resistance phenotypes were consistent with the presence of the corresponding resistance genes of S. Kentucky and S. Enteritidis, including tetA, floR, blaTEM-1B, strA/B, sul1/sul2, and gyrA (D87Y). Our study observed a high prevalence of

Salmonella in the chicken slaughter line and identified the slaughtering environment as a main source of causing Salmonella cross-contamination during chicken slaughtering. Further studies will be needed to limit the transmission of Salmonella in the slaughterhouse. ISSN: 22971769

Uelze, L., Borowiak, M., Deneke, C., Szabó, I., Fischer, J., Tausch, S.H., Malorny, B.
Performance and Accuracy of Four Open-Source Tools for In Silico Serotyping of Salmonella spp. Based on Whole-Genome Short-Read Sequencing Data
(2020) Applied and environmental microbiology, 86 (5), .
ABSTRACT: We compared the performance of four open-source in silico Salmonella typing tools (SeqSero, SeqSero2, Salmonella In Silico Typing Resource [SISTR], and Metric Oriented Sequence Typer [MOST]) to assess their potential for replacing laboratory serological testing with serovar predictions from whole-genome sequencing data. We conducted a retrospective analysis of 1,624 Salmonella isolates of 72 serovars submitted to the German National Salmonella Reference Laboratory between 1999 and 2019. All isolates are derived from animal and foodstuff origins. We conducted Illumina short-read sequencing and compared the in silico serovar prediction results with the results of routine laboratory serotyping. We found the best-performing in silico serovar prediction tool to be SISTR, with 94% correctly typed isolates, followed by SeqSero2 (87%), SeqSero (81%), and MOST (79%). Furthermore, we found that mapping-based tools like SeqSero and SeqSero2 (allele mode) were more reliable for the prediction of monophasic variants, while sequence type and cluster-based methods like MOST and SISTR (core-genome multilocus sequence type [cgMLST]), showed greater resilience when confronted with GC-biased sequencing data. We showed that the choice of library preparation kit could substantially affect O antigen detection, due to the low GC content of the wzx and wzy genes. Although the accuracy of computational serovar predictions is still not quite on par with traditional serotyping by Salmonella reference laboratories, the command-line tools investigated in this study perform a rapid, efficient, inexpensive, and reproducible analysis, which can be integrated into in-house characterization pipelines. Based on our results, we find SISTR most suitable for automated, routine serotyping for public health surveillance of SalmonellaIMPORTANCESalmonella spp. are important foodborne pathogens. To reduce the number of infected patients, it is essential to understand which subtypes of the bacteria cause disease outbreaks. Traditionally, characterization of Salmonella requires serological testing, a laboratory method by which Salmonella isolates can be classified into over 2,600 distinct subtypes, called serovars. Due to recent advances in whole-genome sequencing, many tools have been developed to replace traditional testing methods with computational analysis of genome sequences. It is crucial to validate that these tools, many already in use for routine surveillance, deliver accurate and reliable serovar information. In this study, we set out to compare which of the currently available open-source command-line tools is most suitable to replace serological testing. A thorough evaluation of the differing computational approaches is highly important to ensure the backward compatibility of serotyping data and to maintain comparability between laboratories. ISSN: 10985336

Marin, C., Chinillac, M.C., Cerdà-Cuéllar, M., Montoro-Dasi, L., Sevilla-Navarro, S., Ayats, T., Marco-Jimenez, F., Vega, S.
Contamination of pig carcass with Salmonella enterica serovar Typhimurium monophasic variant 1,4[5],12:i:- originates mainly in live animals
(2020) Science of the Total Environment, 703, art. no. 134609, .
ABSTRACT: Pork is considered a major source of Salmonella Typhimurium infection in humans in the EU, including monophasic strains (mST). Widespread distribution of virulent serotypes such as monophasic variants of S. Typhimurium have emerged as a public health threat. Despite the current situation, within the EU there is no mandatory programme for the control of Salmonella at pork production level. In this context, the aims of this study were: to examine the presence of Salmonella in the swine production system from arrival at the slaughterhouse until the end of processing, and investigate the genetic relationship among serovars. A total of 21 pig herds were intensively sampled during processing at the slaughterhouse. ERIC-PCR followed by PFGE were performed among isolates recovered at the different steps in the slaughterhouse to assess their genetic relationship. The results showed a high level of Salmonella pork batch contamination upon arrival at the slaughterhouse (71.4%) and at the end of the slaughtering process (66.7%), with mST the main serovar isolated from both origins (53.1% and 38.2%, respectively). Similarly, this study shows that 14.3% of the strains isolated from carcasses have the same XbaI-PFGE profile as those previously recovered in the slaughterhouse environment, but not in the live animals from that same batch. In conclusion, there is a high level of Salmonella swine batch contamination upon arrival at the slaughterhouse and at the end of

the slaughtering process, mST being the most frequently isolated serovar. Moreover, a strong genetic relationship has been observed between strains isolated from the batch on arrival at the slaughterhouse, the processing environment and pork carcass contamination. In this sense, it would be necessary to implement a control programme to reduce the bacterium from pork farms and raise the awareness of biosecurity measures.
ISSN: 00489697

Porter, S., Strain, S.A.J., Bagdonaite, G., McDowell, S.W., Bronckaers, T., Sherrey, M., Devine, P., Pascual-Linaza, A.V., Spence, N., Porter, R., Guelbenzu-Gonzalo, M., Davies, R.H., Lahuerta-Marin, A.
Trends in Salmonella serovars and antimicrobial resistance in pigs and poultry in Northern Ireland between 1997 and 2016
(2020) Veterinary Record, 186 (5), p. 156.
ABSTRACT: In the EU, salmonellosis is the second most commonly reported zoonosis. This pattern is reflected in Northern Ireland. Historically, foodborne salmonellosis has largely been attributed to the consumption of poultry products, and as such a number of legislative measures have been introduced by the EC. These policies focus mainly on five target Salmonella serovars. Methods Here the authors present a descriptive analysis of 20 years of data from the Northern Ireland National Reference Laboratory for Salmonella. Results The study's results show, for poultry submissions, a large decrease in the detection of four of the five targeted Salmonella serovars over the study period, with the fifth serovar undetected throughout the study. Additionally, there was an increase in the detection of a number of other non-regulated serovars. In pigs, S Typhimurium, which is among the most common causes of human salmonellosis, was the most commonly isolated serovar. When comparing levels of antimicrobial resistance in S Typhimurium between livestock groups, the authors found a decrease over time in poultry, but an increase in pigs, highlighting the potential significance of pigs in addressing public health concerns. Conclusion The authors conclude that continued surveillance is important in the assessment of control measures at a national and transnational scale. ISSN: 00424900

Sarengaowa, Hu, W., Feng, K., Jiang, A., Xiu, Z., Lao, Y., Li, Y., Long, Y.
An in situ-Synthesized Gene Chip for the Detection of Food-Borne Pathogens on Fresh-Cut Cantaloupe and Lettuce
(2020) Frontiers in Microbiology, 10, art. no. 3089, .
ABSTRACT: Fresh foods are vulnerable to foodborne pathogens which cause foodborne illness and endanger people’s life and safety. The rapid detection of foodborne pathogens is crucial for food safety surveillance. An in situ-synthesized gene chip for the detection of foodborne pathogens on fresh-cut fruits and vegetables was developed. The target genes were identified and screened by comparing the specific sequences of Salmonella Typhimurium, Vibrio parahemolyticus, Staphylococcus aureus, Listeria monocytogenes, and Escherichia coli O157:H7 from the National Center for Biotechnology Information database. Tiling array probes were designed to target selected genes in an optimized hybridization system. A total of 141 specific probes were selected from 3,227 hybridization probes, comprising 26 L. monocytogenes, 24 S. aureus, 25 E. coli O157:H7, 20 Salmonella Typhimurium, and 46 V. parahemolyticus probes that are unique to this study. The optimized assay had strong amplification signals and high accuracy. The detection limit for the five target pathogens on fresh-cut cantaloupe and lettuce was approximately 3 log cfu/g without culturing and with a detection time of 24 h. The detection technology established in this study can rapidly detect and monitor the foodborne pathogens on fresh-cut fruits and vegetables throughout the logistical distribution chain, i.e., processing, cleaning, fresh-cutting, packaging, storage, transport, and sale, and represents a valuable technology that support the safety of fresh agricultural products. ISSN: 1664302X

Borowiak, M., Baumann, B., Fischer, J., Thomas, K., Deneke, C., Hammerl, J.A., Szabo, I., Malorny, B.
Development of a Novel mcr-6 to mcr-9 Multiplex PCR and Assessment of mcr-1 to mcr-9 Occurrence in Colistin-Resistant Salmonella enterica Isolates From Environment, Feed, Animals and Food (2011–2018) in Germany
(2020) Frontiers in Microbiology, 11, art. no. 80, .
ABSTRACT: The polymyxin antibiotic colistin has been used in decades for treatment and prevention of infectious diseases in livestock. Nowadays, it is even considered as last-line treatment option for severe human infections caused by multidrug- and carbapenem-resistant Gram-negative bacteria. Therefore, the discovery of plasmid-mediated mobile colistin resistance (mcr) genes raised major public health concern. The aim of our study was to analyze colistin-resistant Salmonella enterica strains from animals, food, feed and the environment collected at the National Reference Laboratory for Salmonella in Germany

on the presence of mcr-1 to mcr-9 genes. Altogether 407 colistin-resistant (MIC >2 mg/L) Salmonella isolates received between 2011 and 2018 were selected and screened by PCR using a published mcr-1 to mcr-5 as well as a newly developed mcr-6 to mcr-9 multiplex PCR protocol. 254 of 407 (62.4%) isolates harbored either mcr-1 (n = 175), mcr-4 (n = 53), mcr-5 (n = 18) or mcr-1 and mcr-9 (n = 8). The number of mcr-positive isolates ranged from 19 (2017) to 64 (2012) per year. WGS revealed that none of our isolates harbored the mcr-9.1 gene. Instead, two novel mcr-9 variants were observed, which both were affected by frameshift mutations and are probably non-functional. The mcr-harboring isolates were mainly derived from animals (77.2%) or food (20.1%) and could be assigned to ten different Salmonella serovars. Many of the isolates were multidrug-resistant. Co-occurrence of mcr-1 and AmpC or ESBL genes was observed in eight isolates. Our findings suggest that mcr genes are widely spread among colistin-resistant Salmonella isolates from livestock and food in Germany. Potential transfer of mcr-harboring isolates along the food chain has to be considered critically. ISSN: 1664302X

Kubo, I., Kajiya, M., Aramaki, N., Furutani, S.
Detection of salmonella enterica in egg yolk by PCR on a microfluidic disc device using immunomagnetic beads
(2020) Sensors (Switzerland), 20 (4), art. no. 1060, .
ABSTRACT: Salmonella enterica is a pathogenic bacterium that causes foodborne illness. One of the vehicle foods of S. enterica are chicken eggs. Efficient collection of the bacterium is necessary to detect it specifically. We developed a method to detect S. enterica by PCR on a microfluidic disc device using a fluorescent probe. Salmonella enterica cells were isolated in the microchambers on the device, followed by thermal lysis and PCR targeting with the invA gene, a gene specific to S. enterica, were observed by measurement of the fluorescent signal that resulted from gene amplification. However, the developed method was unable to discriminate viable cells from dead cells. Consequently, in this study, magnetic beads modified with anti-Salmonella antibody were utilized to detect viable Salmonella cells from egg yolk prior to PCR on the device. While using the antibody-modified beads, egg yolk components, which inhibit PCR, were removed. The collected cells were subsequently detected by PCR of the invA gene on a microfluidic disc device. This method enabled the detection of viable cells without the inhibition of PCR by any egg component. S. enterica was detected at 5.0×104 cells mL−1 or at a higher concentration of egg yolk within 6 h including the sampling time. ISSN: 14248220

Ortiz-Solà, J., Viñas, I., Colás-Medà, P., Anguera, M., Abadias, M.
Occurrence of selected viral and bacterial pathogens and microbiological quality of fresh and frozen strawberries sold in Spain
(2020) International Journal of Food Microbiology, 314, art. no. 108392, .
ABSTRACT: Strawberry production and exports have been increasing in Spain in recent decades. However, little information is available about their microbiological quality. Due to the growing concern about the microbial safety of these fruits, the objective of this investigation was to study the microbiological quality and the prevalence of the main foodborne pathogens on strawberries sold in Spain. Fresh (n = 152) and frozen (n = 31) samples were obtained from marketplaces and fields in 2017 and 2018. The samples were assayed for total aerobic mesophilic microorganisms (TAM), moulds and yeasts (M&Y), total coliforms (TC), Escherichia coli, Salmonella spp., Listeria monocytogenes as well as Norovirus (NoV) GI and GII. The microbiological counts ranged from <1.70 (detection limit, dl) – 5.89 log10 CFU/g (mean 3.78 log10 CFU/g) for TAM; 2.10–5.86 log10 CFU/g (mean 3.80 log10 CFU/g) for M&Y; and <0.70 (dl) – 4.91 log10 CFU/g (mean 2.15 log10 CFU/g) for TC in fresh strawberries. In frozen strawberries, the counts were <1.70 (dl) – 3.66 log10 CFU/g (mean 2.30 log10 CFU/g) for TAM; <1.70 (dl) – 2.76 log10 CFU/g (mean 1.82 log10 CFU/g) for M&Y; and <0.70(dl) – 1.74 log10 CFU/g (mean 0.77 log10 CFU/g) for TC. All the samples in this study tested negative for Salmonella spp., L. monocytogenes. E. coli and NoV GI and GII genome. A global overview of all the data was executed using Principal Component Analysis (PCA), and the results showed that the scores and loadings according to principal components 1 (PC1) and 2 (PC2) accounted for 75.9% of the total variance, allowing a distinction between fresh and frozen samples. The presence of moulds was significantly higher in the supermarket samples whereas the presence of total coliforms was significantly higher in the field samples (p < 0.05). Although pathogenic microorganisms were not found, preventative measures and prerequisites in the strawberry production chain must be considered in order to avoid possible foodborne diseases related to the microbiological quality of the fruit. ISSN: 01681605

Sevilla-Navarro, S., Catalá-Gregori, P., García, C., Cortés, V., Marin, C.

Salmonella Infantis and Salmonella Enteritidis specific bacteriophages isolated form poultry faeces as a complementary tool for cleaning and disinfection against Salmonella
(2020) Comparative Immunology, Microbiology and Infectious Diseases, 68, art. no. 101405, .
ABSTRACT: Salmonellosis represents an important public health concern. Several authors point out the inefficiency of the cleaning and disinfection protocols to remove the bacteria from the field. For this reason, innovative techniques, as bacteriophages, could be implemented to control the bacteria. The main objectives of this study were to assess the effect of bacteriophages against Salmonella Infantis and Salmonella Enteritidis on farm surfaces, and to evaluate bacteriophage procedure application as sanitiser against Salmonella in field conditions. Thus, most prevalent serovars in poultry production were selected (Salmonella Infantis and Salmonella Enteritidis) to contaminate farm facilities. Then, two specific bacteriophages isolated from poultry faeces were applied against them. Results showed Salmonella Infantis and Salmonella Enteritidis decreased of 4.55 log10CFU/mL and 3.85 log10CFU/mL, respectively; the maximum reduction in Salmonella was the 5th day, after 108 PFU/mL and 103 PFU/mL bacteriophage application. These results highlight bacteriophages as a promising tool together with cleaning and disinfection. ISSN: 01479571

Trinetta, V., Magossi, G., Allard, M.W., Tallent, S.M., Brown, E.W., Lomonaco, S.
Characterization of Salmonella enterica Isolates from Selected U.S. Swine Feed Mills by Whole-Genome Sequencing
(2020) Foodborne Pathogens and Disease, 17 (2), pp. 126-136.
ABSTRACT: Every year salmonellosis is responsible for $2.3 billion in costs to the U.S. food industry, with nearly 6% of the reported cases associated with pork and/or pork products. Several studies have demonstrated the role of pigs as Salmonella reservoirs. Furthermore, this pathogen has been identified as a potential biological hazard in many livestock feeds. The overall objective of this research was to characterize Salmonella enterica isolates in selected U.S. swine feed mills by whole-genome sequencing (WGS) and evaluate isolates in association with the season and feed production stages. Salmonella isolates were collected from 11 facilities during a previous study. Samples were analyzed for Salmonella prevalence following the U.S. Department of Agriculture guidelines and confirmed by PCR. WGS was carried out on either the MiSeq or NextSeq sequencer. De novo genome assemblies were obtained with the Shovill pipeline, version 0.9. ResFinder and SPIFinder were used to identify antibiotic resistance genes and pathogenicity islands. Finally, their phylogenetic relationship and diversity were determined by core genome multilocus sequence typing. Overall, our analysis showed the presence of S. enterica in the feed mill environment. Isolates belonged to 16 different serotypes. Salmonella Agona, Salmonella Mbandaka, Salmonella Senfenberg, and Salmonella Scharzengrund were the most frequently found, and 18 single-nucleotide polymorphism clusters were identified. In silico analysis showed that 40% of the strains carried at least one antimicrobial resistance gene. All isolates in this study could be considered of public health concern and pathogenic potential. Our findings underscore the potential role of the feed mill environment as the pathogen entry route into the human food value chain. ISSN: 15353141

Kasturi, K.N.
A real-time PCR for rapid identification of Salmonella enterica Gaminara serovar
(2020) Journal of Microbiological Methods, 169, art. no. 105729, .
ABSTRACT: Salmonella is one of the leading causes of foodborne illnesses in the USA. When a Salmonella outbreak occurs, rapid identification of the causative serovar is important for tracing the source of contamination and for preventing the further spread of the illness. Each serovar is characterized by the presence of a group-specific somatic O-antigen(s) and an assortment of flagellar phase-1 and phase-2 antigens. As the traditional serotyping protocol is time consuming, labor intensive, and expensive, faster and less expensive molecular diagnostic methods are needed. This report outlines the development of a rapid multiplex real-time PCR procedure that facilitates the identification of Salmonella serogroup I and the serovars of the group. Using Salmonella Gaminara serovar (O16:d:1,7) as an example, first the gene(s) responsible for expression of the somatic O antigen, O16, and the nucleotide sequence of the variable-region of genes encoding the flagellar phase-1 (d) and phase-2 (1,7) antigens were identified. Then, a multiplex real-time PCR was designed that incorporated primers and probes specific for the three target genes and confirmed the specificity. The assay had 100% inclusivity for all three gene targets, detecting 2 genomic DNA copies of O16 and 1,7 gene targets and 10 copies of d gene target. Importance: Rapid molecular methods to identify Salmonella serovars should increase the precision of routine surveillance of clinically important serovars and promote public health. ISSN: 01677012

Luvsansharav, U.O., Vieira, A., Bennett, S., Huang, J., Healy, J.M., Hoekstra, R.M., Bruce, B.B., Cole, D.
Salmonella Serotypes: A Novel Measure of Association with Foodborne Transmission (2020) Foodborne Pathogens and Disease, 17 (2), pp. 151-155.
ABSTRACT: Most nontyphoidal Salmonella (NTS) illnesses in the United States are thought to be foodborne. However, transmission routes likely vary among the different serotypes. We developed a relative ranking of NTS serotypes according to the strength of their association with foodborne transmission. We used Laboratory-based Enteric Disease Surveillance data to estimate the proportion of infections for each Salmonella serotype reported from 1998 to 2015 and Foodborne Disease Outbreak Surveillance System data to calculate the proportion of foodborne outbreak-associated Salmonella illnesses caused by each serotype. We calculated the ratios of these proportions to create a foodborne relatedness (FBR) measure for each serotype. Of the top 20 serotypes, Saintpaul (2.14), Heidelberg (1.61), and Berta (1.48) had the highest FBR measures; Mississippi (0.01), Bareilly (0.13), and Paratyphi B variant L(+) tartrate(+) (0.20) had the lowest. The FBRs for the three most prevalent serotypes were 1.22 for Enteritidis, 0.77 for Typhimurium, and 1.16 for Newport. This method provides a quantitative approach to estimating the relative differences in the likelihood that an illness caused by a particular serotype was transmitted by food, which may aid in tailoring strategies to prevent Salmonella illnesses and guide future research into serotype-specific source attribution. ISSN: 15353141

Sun, H., Wan, Y., Du, P., Bai, L.
The Epidemiology of Monophasic Salmonella Typhimurium (2020) Foodborne Pathogens and Disease, 17 (2), pp. 87-97.
ABSTRACT: Salmonella enterica remains an important foodborne pathogen in all regions of the world, with Typhimurium as one of the most frequent serotypes causing foodborne disease. However, the past two decades have seen a rapid worldwide emergence of a new Salmonella serotype, namely monophasic variant of S. Typhimurium, whose antigenic formula is 1,4,[5],12:i:-. It has become one of the 2-5 most common Salmonella serotypes responsible for animal and human infections in different regions. The global epidemic of monophasic S. 1,4,[5],12:i:-has mainly been characterized by an increase in multidrug-resistant S. 1,4,[5],12:i:-isolated in Europe since 1997. The unexpected link to swine has escalated monophasic S. Typhimurium infections to the status of a global public health emergency. The large-scale application of whole genome sequencing (WGS) in the last 10 years has revealed the phylogenetic associations of the bacterium and its antimicrobial resistance (AMR) genes. Local and global transmission reconstructed by WGS have shown that different clones have emerged following multiple independent events worldwide, and have elucidated the role of this zoonotic pathogen in the spread of AMR. This article discusses our current knowledge of the global ecology, epidemiology, transmission, bacterial adaptation, and evolution of this emerging Salmonella serotype. ISSN: 15353141

Lobacz, A., Kowalik, J., Zulewska, J.
Determination of the survival kinetics of Salmonella spp. on the surface of ripened raw milk cheese during storage at different temperatures (2020) International Journal of Food Science and Technology, 55 (2), pp. 610-618.
ABSTRACT: The aim of this study was to determine the survival kinetics of Salmonella enterica subsp. enterica in ripened raw milk cheese. Cheese samples inoculated with S. enterica subsp. enterica were stored at 5, 15 and 25 °C and analysed in terms of physico-chemical and microbiological characteristics. Three primary models were used to estimate the kinetic parameters of S. enterica subsp. enterica. The secondary Arrhenius model was used to establish the relationship between temperature and parameter α of the Weibull model. Additionally, prediction of S. enterica subsp. enterica survival as a function of storage temperature was made. S. enterica subsp. enterica growth was inhibited during storage, and bacteria survived for an extensive period of time at high number (60 day at 5 °C, 26 day at 25 °C). The storage temperature significantly influenced the inactivation rate of Salmonella in raw milk ripened cheese and proceeded faster at 25 °C compared to remaining storage temperature. Obtained results suggest that contamination by Salmonella in raw milk cheese might result in residual risk. ISSN: 09505423

Perez-Sancho, M., García-Seco, T., Porrero, C., García, N., Gomez-Barrero, S., Cámara, J.M., Domínguez, L., Álvarez, J.
A ten-year-surveillance program of zoonotic pathogens in feral pigeons in the City of Madrid (2005–2014): The importance of a systematic pest control

(2020) Research in Veterinary Science, 128, pp. 293-298.
ABSTRACT: Feral pigeons have increased in urban settings worldwide becoming a potential health risk for humans and other animals. Control and surveillance programs are essential to prevent the possible transmission of zoonotic pathogens carried by pigeons. A surveillance program was carried out in Madrid City (Spain) during 2005–2014 to determine the role of urban pigeons as carriers of zoonotic agents comparing these results with studies performed elsewhere in the last fifteen years. A total of 1372 pigeons were randomly captured and tested for detection of Antimicrobial susceptibility and genetic heterogeneity of Campylobacter and Salmonella isolates were determined. During the first phase (August 2005–July 2010), 428 animals were analyzed individually, while in the second period (August 2010–December 2014), 944 pigeons were analyzed in pools (n = 2–3 in 2010 and n = 5–6 in 2013 and 2014). The most prevalent pathogen during the first phase was Campylobacter spp., (6.57%, 95% confidence interval 3.05–12.10%) followed by Salmonella spp. (4.41%, 95% CI: 2.30–7.58%) and C. psittaci (2.56%, 95% CI: 0.70–6.53%)]. The PCR techniques, used during the 2010–2014 phase of the study, confirmed the presence of Campylobacter spp. (prevalence of 0–14.83%) and C. psittaci (0–12,94%) among pigeons of Madrid. Antimicrobial susceptibility testing suggested low levels of resistance. Presence of zoonotic agents in feral pigeons highlights the importance of surveillance programs on this species, although the relative low prevalence found suggests a limited risk to Public and Animal Health in Madrid. ISSN: 00345288

Flink, C., Nyberg, K.
Occurrence of Campylobacter spp., Salmonella spp. and shiga toxin-producing Escherichia coli in inline milk filters from Swedish dairy farms
(2020) Journal of Food Safety, 40 (1), art. no. e12726, .
ABSTRACT: This study investigated the occurrence of shiga toxin-producing Escherichia coli (STEC), thermotolerant Campylobacter spp. and Salmonella spp. in Swedish dairy milk. A total of 302 inline milk filters were analyzed. Salmonella was not isolated from any filters. Polymerase chain reaction screening detected thermotolerant Campylobacter in 30.5% of the milk filters analyzed and it was isolated from 12.6% of filters. The stx genes (stx1, stx2, or both) were screened from 71% of the filters and STEC was isolated from 14% of these. Of the STEC isolates, 21 contained the stx1 gene, 19 the stx2 gene, and five a combination of both stx1 and stx2 genes. Whole genome sequence typing on 34 of the 45 STEC showed that they belonged to 21 different serotypes, of which STEC O145:H28 was the most common (2%). STEC O157:H7 was only found from one (0.3%) of the filters. A combination of stx2 and eae genes was found from 0.7% of the total number of inline milk filters analyzed, while stx2a was found in 24% of the whole genome-sequenced isolates. There was a significant positive correlations between number of animals per farm and presence of pathogens on milk filters. ISSN: 01496085

Lorenzo, F., Sanz-Puig, M., Bertó, R., Orihuel, E.
Assessment of performance of two rapid methods for on-site control of microbial and biofilm contamination
(2020) Applied Sciences (Switzerland), 10 (3), art. no. 744, .
ABSTRACT: (1) Background: The validation of hygiene procedures in food industries is paramount to ensure that food contact surfaces are properly decontaminated before production. Rapid, sensitive and reliable tools are needed for routine hygiene validation in order to increase food safety levels. Two novel tools for biofilm detection (TBF 300) and detection of low levels of microbial contamination (FreshCheck) have been assessed. (2) Methods: Biofilms of relevant food pathogens: Listeria monocytogenes and Salmonella spp. were grown for 3 and 10 days to assess the performance of the biofilm detection product. Surfaces were inoculated with different levels of L. monocytogenes to determine the limit of detection of FreshCheck. (3) Results: TBF 300 visibly stained 3 days-old biofilms of both pathogens, containing 5.0-5.4 log CFU/cm2. FreshCheck showed a positive reaction with contamination levels as low as 10 CFU/cm2 for L. monocytogenes. (4) Conclusions: Assessment of the hygienic status of food contact surfaces before production can be greatly improved with the use of the two novel tools evaluated in this study. The detection of microorganisms' presence at very low levels of contamination as well as identification of biofilm growth spots is available in a rapid and easy way, with a big potential contribution to food safety. ISSN: 20763417

Torres, M.A., Terraf, M.C.L., Minahk, C.J., Delgado, M.A.
Stability of the Salmonella Typhimurium rcsC11 mutant under different stress conditions
(2020) Microbiology (Reading, England), 166 (2), pp. 157-168.
ABSTRACT: The virulence genes of Salmonella are modulated during infection by several regulatory systems, and the RcsCDB system is one of the most important of these. The S.

Typhimurium EG14873 (rcsC11) strain harbours the rcsC11 point mutation, displaying a constitutive activation of this system, which is characterized by mucoid colonies and attenuated virulence phenotypes. In this work, the stability of the rcsC11 mutation was analysed under stress conditions. Under acid and anaerobic stresses, we observed the appearance of small and non-mucoid colonies of the rcsC11 strain. The sequencing of the rcsC gene from these colonies showed that the mutation is conserved. Moreover, we found that small colonies were also generated when the wild-type strain grew in acid and anaerobic conditions. It is worth noting that the transition from normal to atypical colonies of both strains only took place after several days of incubation and was not observed during eukaryotic cell infection. Therefore, the appearance of these atypical colonies is a characteristic feature of S. Typhimurium strains under stressful situations and does not involve a reversion of the rcsC11 allele and nor does it imply any risk to mammalian cells. Therefore, we propose that the S. Typhimurium rcsC11 strain is a good candidate for the development of attenuated vaccines. ISSN: 14652080

Kudirkiene, E., Sørensen, G., Torpdahl, M., de Knegt, L.V., Nielsen, L.R., Rattenborg, E., Ahmed, S., Olsen, J.E.
Epidemiology of salmonella enterica serovar Dublin in Cattle and humans in Denmark, 1996 to 2016: A retrospective whole-genome-based study
(2020) Applied and Environmental Microbiology, 86 (3), art. no. e01894, .
ABSTRACT: Salmonella enterica serovar Dublin is a cattle-adapted S. enterica serovar causing both intestinal and systemic infection in its bovine host, and it is also a serious threat to human health. The present study aimed to determine the population structure of S. Dublin isolates obtained from Danish cattle herds and to investigate how cattle isolates relate to Danish human isolates, as well as to non-Danish human and bovine isolates. Phylogenetic analysis of 197 Danish cattle isolates from 1996 to 2016 identified three major clades corresponding to distinct geographical regions of cattle herds. Persistence of closely related isolates within the same herd and their circulation between epidemiologically linked herds for a period of more than 20 years were demonstrated. These findings suggest that a lack of internal biosecurity and, to some extent, also a lack of external biosecurity in the herds have played an important role in the long-term persistence of S. Dublin in Danish cattle herds in the period investigated. Global population analysis revealed that Danish cattle isolates clustered separately from bovine isolates from other countries, whereas human isolates were geographically spread. Resistance genes were not commonly demonstrated in Danish bovine isolates; only the isolates within one Danish clade were found to often harbor two plasmids of IncFII/IncFIB and IncN types, the latter plasmid carrying blaTEM-1, tetA, strA, and strB antibiotic resistance genes. IMPORTANCE S. Dublin causes economic losses in cattle production, and the bacterium is a public health concern. A surveillance and control program has been in place in Denmark since 2002 with the ultimate goal to eradicate S. Dublin from Danish cattle herds; however, a small proportion of herds have remained positive for many years. In this study, we demonstrate that herds with persistent infection often were infected with the same strain for many years, indicating that internal biosecurity has to be improved to curb the infection. Further, domestic cases of S. Dublin infection in humans were found to be caused both by Danish cattle isolates and by isolates acquired abroad. This study shows the strength of whole-genome sequencing to obtain detailed information on epidemiology of S. Dublin and allows us to suggest internal biosecurity as a main way to control this bacterium in Danish cattle herds. ISSN: 00992240

Mutz, Y.D.S., Rosario, D.K.A., Paschoalin, V.M.F., Conte-Junior, C.A.
Salmonella enterica: A hidden risk for dry-cured meat consumption?
(2020) Critical Reviews in Food Science and Nutrition, 60 (6), pp. 976-990.
ABSTRACT: Meat curing, fermentation, and drying are both preservation technologies, and traditional manufacturing practices. Despite being considered a safe food, due to the several hurdles that prevent pathogen growth, dry-cured meat manufacturing may not ensure complete pathogen elimination. Besides, the final products are still susceptible to microbial contamination. Salmonella enterica is noteworthy among the pathogenic microorganisms that can contaminate these products. To survive hypertonic/hyperosmotic, acid, and low aw/desiccation stresses intrinsically associated with dry-curing, Salmonella has evolved with highly sophisticated mechanisms, comprising sensors/receptors, signaling cascade systems, and enzymes/transcription factors that ensure their tolerance and survival despite many harsh environmental conditions. Links between osmotic and acid stresses, such as the dissociable sigma factor of RNA polymerase, which regulates gene transcription, and mutual membrane receptors like the two-component system EnvZ/OmpR, which senses abiotic conditions, lead to stress cross-protection. Furthermore, virulence gene expression seems to be triggered by sublethal stresses on pre-adapted

Salmonella cells, increasing their adherence and invasiveness of host cells. These are evidence that the ability to tolerate stresses enhances Salmonella pathogenicity and compromises the safety of dry-cured meats, by sheltering the pre-exposed and, subsequently, more virulent, stressed bacterial cells. ISSN: 10408398

Michael, M., Acuff, J., Lopez, K., Vega, D., Phebus, R., Thippareddi, H., Channaiah, L.H.
Comparison of survival and heat resistance of Escherichia coli O121 and Salmonella in muffins
(2020) International Journal of Food Microbiology, 317, art. no. 108422, .
ABSTRACT: This study was conducted to validate a simulated commercial baking process for plain muffins against E. coli O121 (isolated from the recent illness outbreak associated with flour), and compare the thermal inactivation parameters (D- and z-values) of cocktails of four isolates of E. coli O121 and three serovars of Salmonella (Newport, Typhimurium, and Senftenberg) in muffin batter. Flour samples were spray inoculated with the E. coli O121 or Salmonella cocktails, dried back to the pre-inoculation weight to achieve ~7 log10 CFU/g, and used to prepare muffin batter. For the muffin baking validation study using E. coli O121, muffin batter was baked at 375 °F (190.6 °C) oven temperature for 21 min followed by 30 min of ambient cooling. The E. coli O121 population decreased by >7 log10 CFU/g in muffins by 17 min of baking, and was completely eradicated after 21 min of baking and ambient cooling. The D-values of E. coli O121 and Salmonella cocktails in muffin batter at 60, 65 and 70 °C were 42.0 and 38.4, 7.5 and 7.2, and 0.4 and 0.5 min, respectively; whereas the z-values of E. coli O121 and Salmonella were 5.0 and 5.2 °C, respectively. ISSN: 01681605

Henke, K.A., Alter, T., Doherr, M.G., Merle, R.
Comparison of consumer knowledge about Campylobacter, Salmonella and Toxoplasma and their transmissibility via meat: results of a consumer study in Germany
(2020) BMC public health, 20 (1), p. 336.
ABSTRACT: BACKGROUND: Campylobacter is the most commonly reported causative agent of foodborne bacterial infection in Germany, and contaminated chicken meat is an important source of this zoonotic agent. The aim of this study was to determine the knowledge of consumers in Germany about Campylobacter, Salmonella and Toxoplasma and their transmissibility via meat. In addition, we investigated the level of knowledge between selected consumer groups and whether the results coincided with those of international studies. METHODS: We conducted a cross-sectional survey of 1008 consumers in Germany via an online panel to record, analyse and evaluate the state of knowledge about Campylobacter, Salmonella and Toxoplasma. The participants were selected according to age, gender and federal states to be representative of the German population. RESULTS: Overall, 68.3% of the respondents had never heard of Campylobacter, 20.2% had heard of Campylobacter but did not know how to protect themselves, and only 11.5% knew how to protect themselves from Campylobacter infections. Slightly more than half (52.2%) of the respondents who had at least heard of Campylobacter knew that Campylobacter was transmissible via meat. Knowledge increased significantly with age. Participants over 60 years old knew about Campylobacter almost three times as often as the 16- to 19-year-old comparison group (OR = 2.982). Consumers who had at least a secondary school certificate were almost twice as likely to know about Campylobacter as those who had no school certificate or a lower secondary school certificate (OR = 1.899). Participants who were not actors in the food chain were significantly less frequently informed about Campylobacter than were those who were actors in the food chain. Consumer knowledge of Toxoplasma was better than that of Campylobacter. Consumers have the most knowledge about Salmonella. CONCLUSIONS: Consumers in Germany are predominantly poorly informed about Campylobacter and the transmission route via meat. General knowledge of Toxoplasma is better than that of Campylobacter. Among the three pathogens, consumers are best informed about Salmonella. This finding highlights the importance of making existing information materials more accessible to consumers in the future to increase their knowledge, with the objective of reducing the incidence of Campylobacter infections. ISSN: 14712458

Mastrorilli, E., Petrin, S., Orsini, M., Longo, A., Cozza, D., Luzzi, I., Ricci, A., Barco, L., Losasso, C.
Comparative genomic analysis reveals high intra-serovar plasticity within Salmonella Napoli isolated in 2005-2017
(2020) BMC Genomics, 21 (1), art. no. 202, .
ABSTRACT: Background: Salmonella enterica subsp. enterica serovar Napoli (S. Napoli) is among the top serovars causing human infections in Italy, although it is relatively

uncommon in other European countries; it is mainly isolated from humans and the environment, but neither the reservoir nor its route of infection are clearly defined. This serovar is characterized by high genomic diversity, and molecular evidences revealed important similarities with typhoidal serovars. Results: 179 S. Napoli genomes as well as 239 genomes of typhoidal and non-typhoidal serovars were analyzed in a comparative genomic study. Phylogenetic analysis and draft genome characterization in terms of Multi Locus Sequence Typing (MLST), plasmid replicons, Salmonella Pathogenicity Islands (SPIs), antimicrobial resistance genes (ARGs), phages, biocide and metal-tolerance genes confirm the high genetic variability of S. Napoli, also revealing a within-serovar phylogenetic structure more complex than previously known. Our work also confirms genomic similarity of S. Napoli to typhoidal serovars (S. Typhi and S. Paratyphi A), with S. Napoli samples clustering primarily according to ST, each being characterized by specific genomic traits. Moreover, two major subclades of S. Napoli can be clearly identified, with ST-474 being biphyletic. All STs span among isolation sources and years of isolation, highlighting the challenge this serovar poses to define its epidemiology and evolution. Altogether, S. Napoli strains carry less SPIs and less ARGs than other non-typhoidal serovars and seldom acquire plasmids. However, we here report the second case of an extended-spectrum β-lactamases (ESBLs) producing S. Napoli strain and the first cases of multidrug resistant (MDR) S. Napoli strains, all isolated from humans. Conclusions: Our results provide evidence of genomic plasticity of S. Napoli, highlighting genomic similarity with typhoidal serovars and genomic features typical of non-typhoidal serovars, supporting the possibility of survival in different niches, both enteric and non-enteric. Presence of horizontally acquired ARGs and MDR profiles rises concerns regarding possible selective pressure exerted by human environment on this pathogen. ISSN: 14712164

Barrere, V., Tompkins, E., Armstrong, M., Bird, P., Bastin, B., Goodridge, L.
Optimization of Salmonella detection in garlic, onion, cinnamon, red chili pepper powders and green tea
(2020) International Journal of Food Microbiology, 316, art. no. 108440, .
ABSTRACT: Salmonella is the causative agent of many outbreaks related to spice consumption. However, because of the antimicrobial properties of various spices which hinders recovery and detection, Salmonella detection in spices remains a challenge. The objective of this study was to optimize an enrichment broth for Salmonella growth in different spices and tea, in order to maintain an adequate pH and decrease the antimicrobial effects of spices during Salmonella enrichment and subsequent detection. Salmonella contaminated spice and tea dried samples were prepared and the detection of Salmonella was assessed using the developed broth and automated DNA extraction and RT-PCR. Double strength Buffered Peptone Water (BPW) was used to maintain pH, and L-cysteine and DL-serine were added to the broth to reduce the effects of antimicrobial compounds in spices. The modified enrichment broth allowed the growth of Salmonella from each spice sample. Sample to broth ratios varied from 1:9 (garlic powder, chili peppers and tea), to 1:20 (cinnamon). The pH value of each enrichment varied but remained above 4.8. The addition of L-cysteine (30 mmol/L) allowed Salmonella recovery and growth in garlic and onion samples and the addition of DL-serine (11.23 mmol/L) allowed the recovery and growth in cinnamon. The results indicated that Salmonella detection was achieved in <24 h in the modified (BPW + L-cysteine and DL-serine) enrichment broth followed by detection by RT-PCR. This protocol could allow for a more rapid, robust, and sensitive enrichment method for Salmonella in spices. ISSN: 01681605

Todd-Searle, J., Friedrich, L.M., Oni, R.A., Shenge, K., LeJeune, J.T., Micallef, S.A., Danyluk, M.D., Schaffner, D.W.
Quantification of Salmonella enterica transfer between tomatoes, soil, and plastic mulch
(2020) International Journal of Food Microbiology, 316, art. no. 108480, .
ABSTRACT: Tomatoes have been linked to Salmonella outbreaks in the United States (US). Plasticulture systems, that combine raised beds, plastic mulch, drip irrigation and fumigation, are common in commercial staked fresh tomato production in the US. The US FDA Produce Safety Rule prohibits the distribution of any produce covered by the rule (including fresh market tomatoes) that drops to the ground before harvest. This research was undertaken to better characterize the risks posed by tomatoes that touch plastic mulch or soil immediately before or during harvest. Research was conducted in three states (Florida, Maryland, and Ohio). Each state utilized tomatoes from their state at the point of harvest maturity most common in that state. Each state used indigenous soil and plastic mulch for transfer scenarios. New plastic mulch obtained directly from the application roll and used plastic mulch that had been present on beds for a growing season were evaluated. A five-strain cocktail of Salmonella enterica isolates obtained from tomato outbreaks was used. Mulch (new or used), soil, or tomatoes were spot inoculated with 100

µl of inoculum to obtain a final population of ~6 log CFU/surface. Items were either touched to each other immediately (1–2 s) after inoculation (wet contact) or allowed to dry at ambient temperature for 1 h or 24 h (dry contact). All surfaces remained in brief (1–5 s) or extended (24 h) contact at ambient temperature. Transfer of Salmonella between a tomato and plastic mulch or soil is dependent on contact time, dryness of the inoculum, type of soil, and contact surface. Transfer of Salmonella to and from the mulch and tomatoes for wet and 1 h dry inocula were similar with mean log % transfers varying from 0.7 ± 0.2 to 1.9 ± 0.1. The transfer of Salmonella between soil or plastic mulch to and from tomatoes was dependent on moisture with wet and 1 h dry inocula generally yielding significantly ($p < 0.05$) higher transfer than the 24 h dry inoculum. Results indicate that harvesting dry tomatoes significantly ($p < 0.05$) reduces the risk of contamination from soil or mulch contact. Transfer to tomatoes was generally significantly greater ($p < 0.05$) from new and used plastic mulch than from soil. If contamination and moisture levels are equivalent and contact times are equal to or <24 h before harvest, significantly ($p < 0.05$) more Salmonella transfers to tomatoes from mulch than from soil. Our findings support that harvesting tomatoes from soil has similar or lower risk than harvesting from plastic mulch. ISSN: 01681605

Yang, X., Wu, Q., Huang, J., Wu, S., Zhang, J., Chen, L., Wei, X., Ye, Y., Li, Y., Wang, J., Lei, T., Xue, L., Pang, R., Zhang, Y.
Prevalence and characterization of Salmonella isolated from raw vegetables in China (2020) Food Control, 109, art. no. 106915, .
ABSTRACT: Raw vegetables have been associated with numerous foodborne Salmonella outbreaks; however, there is little epidemiological or molecular data on Salmonella contaminants of raw vegetables in China. Here, we investigated the prevalence and molecular characteristics of Salmonella isolates from raw vegetables in China. In total, 406 raw vegetable samples were collected covering most provincial capitals in China. The overall prevalence of Salmonella was 3.4% (14/406), with contamination levels of <1 MPN/g. Coriander (7.8%, n = 90) and lettuce (6.0%, n = 83) showed the highest contamination rates. Among the 31 Salmonella isolates recovered from the 14 positive samples, 14 different serovars and 15 multilocus sequence typing patterns were identified. All of the identified serovars have previously caused infections in humans, with several also linked to raw vegetable-associated disease outbreaks. Of the 15 non-duplicate isolates, 7 (46.7%) were resistant to at least one class of antibiotics and 4 (26.7%) were multidrug-resistant. The highest rate of resistance was observed for nalidixic acid (33.3%). This study provides a systematical surveillance on prevalence of Salmonella in Chinese raw vegetables, and suggests that the Salmonella contamination of fresh vegetables is a potential risk to public health when they are eaten raw. ISSN: 09567135

Hong, H., Sun, C., Wei, S., Sun, X., Mutukumira, A., Wu, X.
Development of a real-time recombinase polymerase amplification assay for rapid detection of Salmonella in powdered infant formula
(2020) International Dairy Journal, 102, art. no. 104579, .
ABSTRACT: Salmonella is a foodborne pathogen that may cause serious neonatal disease. In this study, an isothermal real-time recombinase polymerase amplification (RPA) was established to detect Salmonella at 37 °C within 20 min, with the detection sensitivity of 103 cfu mL−1 in pure culture. In food applications using powdered infant formula (PIF), the detection limit of Salmonella using this assay was 2 × 103 cfu mL−1 without a pre-enrichment procedure. When PIF was spiked with Salmonella at 0.1, 1 and 10 cfu mL−1 and enriched at 37 °C, results showed that this assay can detect Salmonella at initial inoculation level of 0.1 cfu mL−1 in PIF after 8 h pre-enrichment. This real-time RPA assay was considerably faster than qPCR and exhibited no losses in detection sensitivity and specificity, therefore it was suitable for on-site detection, especially in resource-poor environments. ISSN: 09586946

Iannetti, L., Neri, D., Santarelli, G.A., Cotturone, G., Podaliri Vulpiani, M., Salini, R., Antoci, S., Di Serafino, G., Di Giannatale, E., Pomilio, F., Messori, S.
Animal welfare and microbiological safety of poultry meat: Impact of different at-farm animal welfare levels on at-slaughterhouse Campylobacter and Salmonella contamination (2020) Food Control, 109, art. no. 106921, .
ABSTRACT: Stress factors and poor animal welfare can increase the susceptibility of food-producing animals to diseases, posing microbial risks to consumers. Animal welfare levels, objectively measured with the application of the Welfare Quality® protocol, were assessed in thirteen broiler flocks, including organic ones, to evaluate the presence of statistically significant differences in relation to Campylobacter and Salmonella faecal shedding and consequent microbiological contamination of broiler carcases at slaughterhouse. Each flock

underwent animal welfare evaluation the day before slaughtering, followed by Campylobacter and Salmonella detection in faeces (caecal content) and neck skin at slaughterhouse. A total of 1040 samples (520 caecal contents; 520 neck skins) were included in the study. Campylobacter enumeration and Salmonella serotyping were also carried out. The highest welfare scores were reported in organic flocks. Significantly lower Campylobacter concentrations both in caecal content and neck skins ($P < 0.05$) were reported in organic batches, compared to high welfare conventional batches. Low-welfare batches showed higher prevalence of Salmonella both in neck skins and caecal content, with a statistically significant difference compared to high-welfare batches (43.6% versus 2.9% in neck skins; 19.3% versus 0% in caecal content; $P < 0.00001$). Salmonella Infantis and Salmonella Bredeney were the most common serotypes, while Campylobacter jejuni and Campylobacter coli were the detected species. This study provides new evidence that high animal welfare standards at farms, other than an ethical issue and a value-add for the end product, could also improve the microbiological safety of poultry meat, ultimately contributing to the protection of consumers. ISSN: 09567135

Dantas, S.T.A., Camargo, C.H., Tiba-Casas, M.R., Vivian, R.C., Pinto, J.P.A.N., Pantoja, J.C.F., Hernandes, R.T., Fernandes Júnior, A., Rall, V.L.M.
Environmental persistence and virulence of Salmonella spp. Isolated from a poultry slaughterhouse
(2020) Food Research International, 129, art. no. 108835, .
ABSTRACT: Salmonella spp. is responsible for severe foodborne disease, and is one of the main agents involved in foodborne outbreaks worldwide. Contamination occurs mainly as a result of poultry and egg consumption since they can carry some serotypes pathogenic to humans. The aim of the study was to evaluate the persistence and pathogenic potential of Salmonella spp. (n = 40) isolated from poultry slaughterhouse mats, using adhesion and invasion assays, antimicrobial susceptibility by disc diffusion, and biofilm production as phenotypic tests and genotypic analyses. Polystyrene mats presented 3.2 times greater chance of isolating Salmonella than canvas mats. Besides, we observed resistance to tetracycline (17.5%), ampicillin (10%), cefotaxime (7.5%), trimethoprim-sulfamethoxazole (5%), and chloramphenicol (2.5%). All strains possessed the invA, sipB, sipD, ssaR, sifA, sitC, iroN, tolC, flgK, fljB, and flgL genes. The genes sopB and sipA were both present in 92.5% of the isolates, while sopD and spvB were observed in 90% and 32.5% of strains, respectively. All strains adhered to and invaded HeLa cells. Regarding biofilm production, 31 (77.5%) strains were able to produce biofilm on polystyrene microplates. Using PFGE, we detected the persistence of clones in the environment for up to 18 fromthe 20 weeks. The ability of these strains to produce a biofilm and thus persist in the environment and disperse through contact surfaces in the processing plant favors the contamination of food, aggravated by the pathogenic potential of these isolates demonstrated by their adhesion capacity, invasion and resistance to various antibiotic agents. ISSN: 09639969

Friker, B., Morach, M., Püntener, S., Cernela, N., Horlbog, J., Stephan, R.
Assessing the microbiological quality of raw goats' and ewes' tank milk samples in Switzerland
(2020) International Dairy Journal, 102, art. no. 104609, .
ABSTRACT: In recent years, popularity of raw milk has increased in many industrialised countries. This study (i) enumerated total viable counts (TVC) and Escherichia coli counts, (ii) assessed prevalence of Staphylococcus (S.) aureus, Salmonella spp. and STEC, (iii) screened for methicillin resistant S. aureus (MRSA) and extended-spectrum β-lactamases (ESBL)-producing Enterobacteriaceae in sheep and goat tank milk samples collected throughout Switzerland and (iv) provided further strain characteristics on isolated pathogens and MRSA. One hundred and twenty-three tank milk samples from 116 farms were analysed. The median TVC was 3.8 log cfu mL−1. E. coli was detected in 16 (13.0%) and S. aureus in 18 (14.6%) samples. Polymerase chain reaction for stx genes was positive in 14 (11.4%) samples. MRSA were isolated from 4 (3.3%) samples. Salmonella spp. and ESBL-producing Enterobacteriaceae were not isolated. ISSN: 09586946

Hatamzade Isfahani, N., Rahimi, S., Rasaee, M.J., Karimi Torshizi, M.A., Zahraei Salehi, T., Grimes, J.L.
The effect of capsulated and noncapsulated egg-yolk–specific antibody to reduce colonization in the intestine of Salmonella enterica ssp. enterica serovar Infantis–challenged broiler chickens
(2020) Poultry Science, 99 (3), pp. 1387-1394.

ABSTRACT: The antibacterial properties of egg yolk antibodies have been known for many years. Enhanced antibiotic resistance has resulted in increased need for using these antibodies as an alternative. In the present study, generation, capsulation, and inhibition growth properties of IgY directed against Salmonella enterica subsp. enterica serovar Infantis (SI) were evaluated. White Leghorn layer hens were immunized using whole cell of inactivated SI. Salmonella Infantis–specific antibody activities in sera and egg yolk were determined by ELISA. A total of 480 one-day-old male "Cobb 500" chicks were randomly divided into 8 groups, with 6 replications of 10 birds kept for 21 D. All birds from 7 challenged groups were orally inoculated with 1 mL of SI suspension (1 × 107 CFU/mL) at 3 and 4 D of age. Two groups were dietary supplemented with 5 g/kg immune powdered yolk or nonimmune powdered yolk. One group was dietary supplemented with 12.8 g/kg capsulated immune yolk (CIY). Two groups were given 8.3 mL/L of immune water-soluble yolk or nonimmune water-soluble yolk fraction in drinking water. In the antibiotic group, 1 mL/L Enrofloxacin 10% was added to drinking water. All supplements except for the antibiotic (on Day 4 for 10 D) were added on day one and continued during the experiment. Negative and positive control groups received no supplements. During the experiment, among the challenged groups, the minimum SI cecal colonization and the lowest isolation of SI from the liver (P < 0.01) was observed in the antibiotic group. Following antibiotic group, in the group receiving CIY, colonization of bacteria in ceca and liver was significantly reduced during the second and third weeks of the experiment (P < 0.01). According to the results, capsulated specific IgY has a beneficial effect in reducing the colonization of Salmonella under the conditions of this study in comparison with other forms of IgY antibody. ISSN: 00325791

Cox, N.A., Oladeinde, A.A., Cook, K.L., Zock, G.S., Berrang, M.E., Ritz, C.W., Hinton, A.
Research Note: Evaluation of several inoculation procedures for colonization of day-old broiler chicks with Salmonella Heidelberg
(2020) Poultry Science, 99 (3), pp. 1615-1617.
ABSTRACT: Before starting a study with many birds, it helps to know the method of chick inoculation. The objective was to compare 3 methods of Salmonella challenge (oral gavage [OR], intracloacal inoculation [IC], and seeder bird [SB]). Day-old broiler chicks (n = 100) were inoculated with 106 colony forming units (CFU) per chick of a marker strain of Salmonella Heidelberg (SH) with each route of inoculation. Chicks (n = 25) inoculated by each route were placed in floor pens on fresh pine shavings litter. For the seeder batch, 5 colonized chicks, each orally gavaged with 106 CFUs, were placed with 20 pen mates. Two weeks after inoculation, 10 birds from each pen and the 5 inoculated seeder birds were euthanized, the ceca were aseptically removed and macerated with a rubber mallet and weighed, and 3 times (w/v) buffered peptone was added and stomached for 60 s. Serial dilutions were made and plated onto Brilliant Green Sulfa plates containing 200 ppm nalidixic acid. Plates were incubated along with the stomached ceca for 24 h at 37°C. If no colonies appeared on the plates, an additional plate was streaked from the preenriched bag and incubated for 24 h at 37°C. In addition to all seeder birds being positive, the number of SH-positive birds out of 20 sampled in each group was 13, 17, and 7 for OR, IC, and SB, respectively. The level of SH per g of ceca and cecal contents was log (SE) 3.0 (0.7), 2.0 (0.4), and 2.6 (0.4) for OR, IC, and SB, respectively. After enrichment, the number of colonized birds out of 20 was 18, 20, and 10 for OR, IC, and SB, respectively. In conclusion, this study suggests that IC is the method to use to ensure most of the challenged birds are colonized. However, if you prefer to have a smaller percentage of the birds colonized with higher levels, then OR might be better. ISSN: 00325791

Morelli, G., Catellani, P., Miotti Scapin, R., Bastianello, S., Conficoni, D., Contiero, B., Ricci, R.
Evaluation of microbial contamination and effects of storage in raw meat-based dog foods purchased online
(2020) Journal of Animal Physiology and Animal Nutrition, 104 (2), pp. 690-697.
ABSTRACT: Feeding raw-meat-based diets to companion animals has become a widespread practice, and many owners are now accustomed to buying frozen ingredients online. The goals of this study were to assess the microbiological quality of raw-meat dog foods obtained from specialized websites and to evaluate the effects of storage at different temperatures for a few days. Twenty-nine raw dog food products were processed for quantitative bacteriology (i.e. total viable count, TVC; Escherichia coli; faecal coliforms, FC) and sulphite-reducing clostridia, and analysed for the presence of Salmonella spp., Listeria monocytogenes, Yersinia enterocolitica and Clostridium difficile. Every sample was examined right after the delivery (T0), after 24 to 48 hr and after 72 hr, both at 2°C and 7°C. At T0, the mean score for the TVC was 5.9 × 106 cfu/g (SD = 4.8 × 107 cfu/g), while

those for E. coli and FC were 1.1 × 104 cfu/g (SD = 2.5 × 105 cfu/g) and 3.3 × 103 cfu/g (SD = 6.5 × 104 cfu/g) respectively. The samples stored at 2°C had a significant increase of all parameters (TVC: p <.01; E. coli: p =.03; FC: p =.04) through time. Noteworthy differences between the analyses performed at 2°C and 7°C were found for TVC (p <.01), being the samples considerably more contaminated at higher temperatures. No sample tested positive for Salmonella spp., while L. monocytogenes was isolated from 19 products, Y. enterocolitica from three products and Clostridium perfringens and C. difficile from four and six products respectively. The microbiological quality of raw-meat dog foods sold online appears to be poor, carrying considerable amounts of potentially zoonotic bacteria and reaching greater levels of bacterial contaminations if not kept at proper refrigeration temperatures and fed soon after defrosting. ISSN: 09312439

European Food Safety Authority, European Centre for Disease Prevention and Control
The European Union Summary Report on Antimicrobial Resistance in zoonotic and indicator bacteria from humans, animals and food in 2017/2018
(2020) EFSA Journal, 18 (3), art. no. e06007, .
ABSTRACT: Data on antimicrobial resistance (AMR) in zoonotic and indicator bacteria from humans, animals and food are collected annually by the EU Member States (MSs), jointly analysed by EFSA and ECDC and reported in a yearly EU Summary Report. The annual monitoring of AMR in animals and food within the EU is targeted at selected animal species corresponding to the reporting year. The 2017 monitoring specifically focussed on pigs and calves under 1 year of age, as well as their derived carcases/meat, while the monitoring performed in 2018 specifically focussed on poultry and their derived carcases/meat. Monitoring and reporting of AMR in 2017/2018 included data regarding Salmonella, Campylobacter and indicator Escherichia coli isolates, as well as data obtained from the specific monitoring of ESBL-/AmpC-/carbapenemase-producing E. coli isolates. Additionally, some MSs reported voluntary data on the occurrence of meticillin-resistant Staphylococcus aureus in animals and food, with some countries also providing data on antimicrobial susceptibility. This report provides, for the first time, an overview of the main findings of the 2017/2018 harmonised AMR monitoring in the main food-producing animal populations monitored, in related carcase/meat samples and in humans. Where available, data monitoring obtained from pigs, calves/cattle, broilers, laying hens and turkeys, as well as from carcase/meat samples and humans were combined and compared at the EU level, with particular emphasis on multiple drug resistance, complete susceptibility and combined resistance patterns to critically important antimicrobials, as well as Salmonella and E. coli isolates exhibiting presumptive ESBL-/AmpC-/carbapenemase-producing phenotypes. The outcome indicators for AMR in food-producing animals, such as complete susceptibility to the harmonised panel of antimicrobials in E. coli and the prevalence of ESBL-/AmpC-producing E. coli have been also specifically analysed over the period 2014–2018. ISSN: 18314732

Rusiñol, M., Martínez-Puchol, S., Timoneda, N., Fernández-Cassi, X., Pérez-Cataluña, A., Fernández-Bravo, A., Moreno-Mesonero, L., Moreno, Y., Alonso, J.L., Figueras, M.J., Abril, J.F., Bofill-Mas, S., Girones, R.
Metagenomic analysis of viruses, bacteria and protozoa in irrigation water
(2020) International Journal of Hygiene and Environmental Health, 224, art. no. 113440, .
ABSTRACT: Viruses (e.g., noroviruses and hepatitis A and E virus), bacteria (e.g., Salmonella spp. and pathogenic Escherichia coli) and protozoa (e.g., Cryptosporidium parvum and Giardia intestinalis) are well-known contributors to food-borne illnesses linked to contaminated fresh produce. As agricultural irrigation increases the total amount of water used annually, reclaimed water is a good alternative to reduce dependency on conventional irrigation water sources. European guidelines have established acceptable concentrations of certain pathogens and/or indicators in irrigation water, depending on the irrigation system used and the irrigated crop. However, the incidences of food-borne infections are known to be underestimated and all the different pathogens contributing to these infections are not known. Next-generation sequencing (NGS) enables the determination of the viral, bacterial and protozoan populations present in a water sample, providing an opportunity to detect emerging pathogens and develop improved tools for monitoring the quality of irrigation water. This is a descriptive study of the virome, bacteriome and parasitome present in different irrigation water sources. We applied the same concentration method for all the studied samples and specific metagenomic approaches to characterize both DNA and RNA viruses, bacteria and protozoa. In general, most of the known viral species corresponded to plant viruses and bacteriophages. Viral diversity in river water varied over the year, with higher bacteriophage prevalences during the autumn and winter. Reservoir water contained Enterobacter cloacae, an opportunistic

human pathogen and an indicator of fecal contamination, as well as Naegleria australiensis and Naegleria clarki. Hepatitis E virus and Naegleria fowleri, emerging human pathogens, were detected in groundwater. Reclaimed water produced in a constructed wetland system presented a virome and bacteriome that resembled those of freshwater samples (river and reservoir water). Viral, bacterial and protozoan pathogens were occasionally detected in the different irrigation water sources included in this study, justifying the use of improved NGS techniques to get a comprehensive evaluation of microbial species and potential environmental health hazards associated to irrigation water. ISSN: 14384639

Soares, K., Moura, A.T., García-Díez, J., Oliveira, I., Esteves, A., Saraiva, C.
Evaluation of Hygienic Quality of Food Served in Universities Canteens of Northem Portugal (2020) Indian Journal of Microbiology, 60 (1), pp. 107-114.
ABSTRACT: Mass catering services have increased in the last years since people need to eat out mainly by work or study reasons. Microbiological quality of foodstuffs (n = 156) was evaluated in 20 food establishment (cafes and canteens) of two universities of northern Portugal. Overall, data revealed a high level of microbiological quality of foods served. No safety risks for consumers were detected since Clostridium spp., Listeria monocytogenes and Salmonella spp. were not detected. Among food types, hot meals displayed better microbiological results than cold foods ($p < 0.05$) as expected. Regarding hot meals, no differences were observed among different types ($p > 0.05$). Among cold meals, salads displayed the highest microbiological counts for hygiene indicators as well for food foodborne pathogens such as Staphylococcus aureus, Escherichia coli and Bacillus cereus. Although the risk of foodborne disease is scarce since counts were low. In cafes' meals, higher counts were observed than in canteens' meals which indicates that monitoring measures should be improved to avoid potential foodborne outbreaks related to the ready-to-eat products (salads, sandwiches and pastry). Results could be used as microbiological guidelines for canteens. Results indicated that proper food handling and adequate conservation of fresh foods along the food chain is essential in mass catering services to guarantee the food safety. ISSN: 00468991

Banach, J.L., Hoek-van den Hil, E.F., van der Fels-Klerx, H.J.
Food safety hazards in the European seaweed chain
(2020) Comprehensive Reviews in Food Science and Food Safety, 19 (2), pp. 332-364.
ABSTRACT: Seaweed is a source of protein that can help overcome the anticipated challenges of a growing world population and the current challenges for finding alternatives for animal proteins in the Western diet. Thus far, data on the safety of seaweed for feed and food purposes in the Western world are scattered. This study aimed to review the available knowledge on the presence of food safety hazards in seaweed, including factors influencing their presence, and to prioritize the hazards that may pose a risk to human health. Given current knowledge from the literature, data from the Rapid Alert System for Food and Feed, and results from a stakeholder survey, 22 food safety hazards were ranked into major (4), moderate (5), and minor (13) hazards. Arsenic, cadmium, iodine, and Salmonella were identified as major hazards. Hazards, where data gaps exist, should be carefully assessed. These include pesticide residues, dioxins and polychlorinated biphenyls, brominated flame retardants, polycyclic aromatic hydrocarbons, pharmaceuticals, marine biotoxins, allergens, micro- and nanoplastics, other pathogenic bacteria, norovirus, and hepatitis E virus. It is recommended to collect more data on these hazards in future studies. Many factors can affect the presence of hazards including seaweed type, physiology, season, harvest and cultivation environment, geography including the location of cultivation, alongside further processing. Moreover, when seaweed is cultivated near industrialized or anthropogenic activities, these activities may negatively influence water quality, which can increase the likelihood of hazards in seaweed. Results of the ranking prioritized hazards can be used to prioritize monitoring programs and adjusted given future additional knowledge covering the data gaps. ISSN: 15414337