CPS Symposium Session 4 – Executive Summary

Session 4 of the 2021 Center for Produce Safety (CPS) Research Symposium was conducted on July 6th and featured four presentations from leading research scientists on critical food safety topics for the produce industry. You might recall that the 2021 CPS Symposium kicked off 4 weeks ago with a session on *Cyclospora* [Recording of Session 1 on *Cyclospora* and Executive Summary]. Session 4 expanded on that session with a report on *Cyclospora* in water from the mid-Atlantic region of the U.S. and detection challenges involved when sampling microbially complex agricultural waters. This was followed by research findings on: genetic mutation rates among pathogens in important growing regions and what these mutation rates can tell us about modes of contamination; strategies for managing *Salmonella* contamination in peaches; and a study on *Listeria* in commercial distribution facilities and considerations for hazard analysis and risk assessments in these operations. An executive summary of session 4 is included below and the recording can be found on the CPS website.

1. **Parasitic protozoan species like *Cyclospora cayetanensis* are found in water samples from the mid-Atlantic region of the eastern U.S.** Kalmia Kniel from the University of Delaware began Session 4 with a presentation of her project, *"Analysis of the presence of Cyclospora in waters of the mid-Atlantic states and evaluation of removal and inactivation by filtration"* [Kniel 2019]. Dr. Kniel's team collected surface water, recycled vegetable processing water and treated wastewater samples for concentration and analysis of protozoan parasites from June 2017 to October 2018. 28 of 72 (39%) samples were found by PCR (using FDA 19b *Cyclospora cayetanensis* DNA primers) to be presumptive *C. cayetanensis* positives, which were then subjected to additional molecular analysis. The research team also evaluated filtration as a method for removal of oocysts from irrigation water. The Kniel group has previously reported on CPS-funded projects using zero valent iron (ZVI) and sand to remove bacterial pathogens from water [Kniel 2009 and Kniel 2013]. Some learnings to consider are:
   - ***Complex microbial environments are difficult to elucidate***. With surface water samples that contain complex mixtures of closely related microorganisms, precise identification of species can be exceedingly difficult. Many of our advanced DNA-based tools require detailed knowledge of the target organism's genome and with *C. cayetanensis* and some of its close cousins that knowledge base is currently limited. Nested PCR (FDA 19a:9 method) using smaller *C. cayetanensis* genetic sequences to improve specificity was employed to further study the 28 presumptive *Cyclospora* positive water samples. Only one of the 28 presumptive positive samples showed similarity to *C. cayetanensis* through this analysis. Other samples were subsequently identified as phylogenetically related species, e.g., *Eimeria*, *Isospora, Caryospora*, etc. This work confirms the high degree of genetic similarity between protozoan species of the Apicomplexan family and highlights the importance of understanding the methodological limitations and nuances that must be considered to authenticate *Cyclospora* identity when produce safety decisions are riding on the results. It must be noted that the water samples were taken for this project before FDA's new 19c method for sampling water for *Cyclospora* detection was published. Dr. Kniel also indicated that she has communicated the results to FDA, and they continue to share information so that the detection methodology can continue to improve.
   - ***ZVI/sand filtration may be useful to ensure the microbiological quality of agricultural water***. Oocysts of varying sizes can be removed from water by sand filtration and by filtration incorporating ZVI at the laboratory scale. Using more readily available *Cryptosporidium* oocysts as surrogates for *Cyclospora* oocysts, sand filtration alone achieved a 1.82 log reduction of oocysts in the effluent while a sand/ZVI filter resulted in >4-log reduction. Oocysts recovered in the effluent were found to be infective. Larger-sized *Eimeria* oocysts were also employed as surrogates for *Cyclospora* oocysts and passed through a sand-only filter achieving a 2.3-log reduction in the effluent. A 6-log reduction was realized when ZVI was added. Oocysts interactions with ZVI result in inactivation; the degree of which is under continuing study as is modifications required for commercial scale.

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2. **Genetic mutation rates in pathogens in production environments provide insights into outbreaks.** Kerry Cooper from the University of Arizona discussed his team's project "Illuminating the role of whole genome sequencing in produce safety" [Cooper 2018]. With the advent of whole genome sequencing and its applications to more specifically identifying pathogens involved in illness outbreaks and their movement through production environments there has been a question about the significance of the degree of genetic variation sometimes detected between isolates both within an outbreak and between outbreaks over time in specific growing regions. Generally, DNA mutations or changes can only occur when the organism is replicating. This project measures the rate of mutation in outbreak strains of *E. coli* O157:H7, *Salmonella typhimurium* and *Listeria monocytogenes* in soil and irrigation waters as an indicator of whether pathogens merely survive (low mutation rates) or grow (higher mutation rates), using lab conditions that replicate the Salinas and Yuma growing regions. Some key learnings from this project:
   - ***The survivability of pathogens in the Salinas and Yuma model environments is very different***. Microcosms (incubators with temperature, light and humidity controls) programed to approximate growing conditions in Salinas, CA and Yuma, AZ were used to monitor pathogen survival. In the Yuma, AZ conditions, pathogens in soil or irrigation water sourced from that region did not survive the hot, dry conditions of the summer months but showed variable and low levels of survival when cycled (moved from soil to water, soil to soil, water to water) from December to mid-June conditions. In Salinas where conditions are more temperate, pathogens in the soil or irrigation waters survived from January to July while survivability became variable in the August through mid-October conditions.
   - ***Pathogens persist and not grow in long-term microcosms***. No mutations were detected in long-term microcosms suggesting pathogens are not actively replicating in these environments but merely persisting. This situation reflects the genomic analysis and behavior of the *E. coli* O157:H7 strain responsible for reoccurring illness outbreaks linked through romaine to the Salinas area. This strain has undergone only limited genetic mutations over an extend period indicating it is persisting in the environment, presumably in a somewhat dormant state.
   - ***When pathogens cycle between environments there are opportunities for growth which can result in mutations***. Reoccurring strains that develop mutations (as evidenced by single nucleotide polymorphisms (SNPs)) are likely cycling through different hosts and environments. When this is observed it means that there may be multiple sources of the pathogen and routes of contamination. This condition likely represents the *E. coli* O157:H7 strain associated with the 2018 outbreak tracked to romaine sourced from the Yuma region. This strain has been detected in petting zoos, recreational water, in swans and cattle across several states in different timeframes. Genome sequencing has revealed multiple SNPs indicating it is mutating while moving through multiple hosts in these different environments.

3. **Postharvest treatment of fresh peaches with sanitizers holds promise for managing *Salmonella* contamination risks.** Kim-Yen Phan Thien from the University of Sidney shared the results of her work, "*Investigation of potential pre-harvest and post-harvest treatments targeting Salmonella spp. Risk reduction on peach in Australia*" [Phan-Thien 2020]. This CPS rapid response project was initiated in response to a 2020 *Salmonella* outbreak associated with fresh peaches in the US and employed contra-seasonal production in Australia to accelerate efforts to explore *Salmonella* pre- and post-harvest mitigation measures. Preharvest measures included testing antimicrobials in-field via spray applications to evaluate effcaciousconcentrations that did not pose phytotoxicity or fruit quality risks. Postharvest mitigations focused on the use of antimicrobials in wash systems. Some of the key learnings from this project follow:
   - ***Preharvest, in-field spray treatments were generally ineffective against Salmonella***. Copper chelate and zinc sulphate did not demonstrate antimicrobial activity in the lab while Peroxy Treat (hydrogen

peroxide plus PAA) showed some lab efficacy against *Salmonella* that did not carry through significantly to the field-level experiments. This result was likely due to variables in field production and difficulties achieving fruit coverage.

- ***Postharvest treatments of fruit with sanitizers may be optimized to control Salmonella***. In laboratory challenge studies against *Salmonella*, sodium hypochlorite (2-log reduction), lactic acid (3-log reduction) and Nylate® or bromo-chloro-dimethyl-hydantoin (6-log reduction) were most effective. In on-site packinghouse trials, lactic acid, Nylate® and Tsunami were comparable to sodium hypochlorite against *E. coli* (since *Salmonella* could not be used in a packinghouse facility). Work on this project will now shift back to the U.S. to examine treatment optimization to control *Salmonella* postharvest.

4. ***Listeria* can be found in commercial distribution facilities (DC's).** Laurel Dunn from the University of Georgia reviewed her project, "*Environmental microbial risk associated with vented produce distribution centers"* [Dunn 2019]. Historically, there has been a deficit of data on *Listeria* prevalence and contamination risks originating in commercial distribution centers (DC's). This knowledge gap has become a point of focus owing to the FDA requirement to assess the risk of *Listeria* contamination in ready to eat foods exposed to the environment, which includes vented packaging used for several fresh produce commodities. The team sampled for *Listeria spp.* and collected current cleaning and sanitation documentation (e.g., cleaning schedules, SSOPs, SOPs, environmental sampling, etc.) from 18 cooperating commercial DC's that handle fresh produce and other types of foods. Some critical learnings were:
   - ***Listeria spp. were present in 12 of 18 (66%) of DCs sampled.*** Nearly 1,000 samples were taken from 18 DC's and while two-thirds of the DC's had at least one positive sample the total *Listeria* spp. prevalence was around 5% (49/982 samples). Prevalence ranged from 0-33% when examined by facility. Though detection of generic *Listeria* is not the same as detection of the human pathogen*, L. montocytogenes*, the results here indicate that operators need to be aware of the potential risk and use data-driven risk assessments to assess their operations and the potential for transference to products via circulating air, water (used in cleaning, splash from puddles, ice melt through iced products over pallets, etc.), equipment or human interactions.
   - ***Certain operational areas and materials yield more frequent positive Listeria samples than others***. Within DC's, shipping and receiving areas are most likely to test positive for *Listeria* (13%) followed by storage areas for cleaning equipment (10.6%). Not surprisingly, floors were the most likely location for *Listeria* detection (8.75%) with truck trailers, cleaning equipment, pallets, bins, shelving and walls also yielding positive samples. Surface materials also appear to play a role in *Listeria* harborage in DC's. Epoxy (13%), fabric (7.7%), rubber, concrete, wood, and plastics yielded *Listeria* positive samples. These facility locations and more porous materials have long been associated with being difficult to clean and sanitize in processing, packing and shipping operations, so the data are consistent. In addition, places where water "puddled" in facilities also yielded positive samples (12.5%).
   - ***Change to facilities or equipment should signal a need to update your risk assessment***. The DC with the highest *Listeria* prevalence in this study had just undergone a renovation. Changes in design, construction materials, product flow and equipment use can impact harborage potentials for *Listeria* and impact cleaning and limit access for sanitation efforts. Any operational change should precipitate a review of the operations hazard analysis and risk assessment and within that a reappraisal of environmental sampling.
   - ***ATP bioluminescence testing is a poor predictor of Listeria spp. presence*.** One of the pillars of an effective environmental monitoring program (EMP) is microbial testing to verify cleaning and sanitation practices are effective in eliminating pathogens. ATP bioluminescence testing is commonly employed in processing and packing facilities as an easy to execute, low-cost method to demonstrate reduction of biological cells (including plant tissue) that contain ATP. However, while bioluminescence testing has value in verifying cleaning procedures, this study reveals that it does not predict the presence or

absence of *Listeria* species. It is important to include generic *Listeria* testing as part of any EMP if the operational hazard analysis and risk assessment points to *Listeria* as a foreseeable risk in a facility.

Acknowledgements*: The Center for Produce Safety would like to thank session 4 presenters and moderators for their work and dedication to produce safety. More detail on the research projects can be found at www.centerforproducesafety.org. This Executive Summary is meant to inform and provoke thought with an eye towards inspiring readers to examine their own produce safety programs and to use the research to make improvements. It is not meant as a directive on what must be done to produce safe food. If you have additional questions, please feel free to contact Bonnie Fernandez-Fenaroli [info@centerforproducesafety.org]. Thank you.*