



Reply

Reply to Comment on “The Occurrence of Shiga Toxin-Producing *E. coli* in Aquaponic and Hydroponic Systems”

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Recently, the Aquaponic Association (AA) published a statement through multiple outlets in response to our article entitled “The Occurrence of Shiga Toxin-Producing *E. coli* in Aquaponic and Hydroponic Systems.” In this paper, we reported that Shiga toxin-producing *Escherichia coli* (STEC) was found in fish feces, in the water of both aquaponic and hydroponic systems, and on the surface of the roots of lettuce, basil, and tomato regardless of the system, but not in the edible portions of the plants. Our results demonstrated that the presence of STEC in aquaponics was due to the introduction of contaminated fish that were brought into the system and that cross-contamination might have occurred in the adjacent hydroponic systems during handling events.

In this reply, we would like to re-emphasize that STEC was accidentally introduced to our aquaponic systems from the handling of the fish. Tilapia do not produce STEC, but it may have been contaminated on the surface from outside the system or from eating algae/plants that were contaminated. Despite the accidental introduction, STEC did not contaminate the edible portions of the plants. Our study highlights the importance of using clean fish stocks and proper management practices to avoid the potential for food safety risks associated with fresh produce grown in soilless production systems.

The AA raised questions and made comments on our approaches. We have generated a list of criteria to provide point-by-point responses to specific comments in their statement, hoping to clarify some aspects.

1. Responses to the AA’s Statement

1. The AA questioned fish feces as the source of contamination.

The AA wrote in the statement that “*Blaming fish feces as the contaminating source seems incredibly misleading when so many other options exist, and no traceback proved that as the source. The contents of the fish intestines were tested for the presence of *E. coli*, and none was found (Kim personal communications). It seems that if the fish does not have STEC *E. coli* inside its gut, then it is more likely the fish feces being positive would be related to the contaminated water that the feces was floating in.*” To clarify, Kim did not communicate this with the AA.

As such, the AA contended that we tested both the fish feces and fish intestines and found no STEC in the intestines, and therefore the source of contamination was from elsewhere. As described in our paper, we tested the fish feces and obtained positive results for STEC, but did not test the fish intestines.

The fact that STEC was found in the fish feces (but not the other inputs into the system) means that the fish were contaminated externally (on the surface) or internally with STEC during handling.

Based on our scientific investigation and inductive reasoning, we conclude that STEC can be detected in fish feces as a result of the introduction of the fish into an aquaponic system. Tilapia are considered filter feeders and efficiently harvest filamentous and planktonic algae [1]. Therefore, if a fish ingests a piece of filamentous algae that has a colony of STEC, it is likely that the STEC will then be detected in the tilapia feces (M.B. Timmons, personal communication, 9 September 2020).

We are seriously concerned that the above false and misleading information has been communicated across the media and confused readers into believing that our approaches are not scientifically valid and our conclusions are groundless. Further, the misinformation might lead industry participants not to take important safety precautions and eventually lead to a foodborne illness that might devastate the industry economically.

As the AA pointed out, there are several potential sources of contamination in aquaponic systems, which include water, seeds, fish feces (or fish), and handlers. While this aspect was discussed in our paper, we made the following list of possible introductions of STEC to aquaponic systems and explained our reasoning for further clarification.

- Reverse osmosis water for water supply (not the contamination source):

We tested reverse osmosis (RO) water (the source water was municipal water) used to fill and refill the systems and found that it was not the source of STEC. Since this information was not included in our original paper, we made corrections to clarify this aspect.

- Seeds (not the contamination source):

We obtained the seeds from a commercial source. While we did not test the seeds for STEC, we found none of the plants grown from the seeds to be contaminated with STEC (Table 3 in our previous paper). The results indicated that the seeds were not the source of contamination.

- Fish:

The fish were originally cultured in a commercial source and transported and raised in the Purdue Animal Sciences Research and Education Center. We have been operating the recirculating systems since 2015. Each time we receive fish from the center, the fish are transported in tubs for over 10 miles and housed temporarily in fish tanks before being introduced to the lab-scale aquaponic systems. Although we do not know when, where, and how the fish were contaminated with STEC, it is speculated that it happened during these procedures.

- Fish feces:

In our investigation on tilapia-based aquaponic systems, we found that STEC associated with the fish feces was present in the aquaponic solution, indicating that fish feces were likely the major source of contamination in the aquaponic systems due to the handling of the fish. The results were confirmed by presumptive positive colonies on selective media (colorless on CT-SMAC plates) followed by polymerase chain reaction (PCR) detection for the presence of the *stx1* gene [2,3]. In addition, we directly tested the fish feces which were collected from a separate system (this method was described in the paper) and verified that the STEC was associated with fish feces. PCR is a common method to confirm presumptive positive colonies from selective media. Therefore, we believe that we provided evidence to support our conclusions.

- Handling:

Handling practices could vary greatly even among the commercial growers, depending on their production scale, climate, location, and resources. Although we performed the best practices for our indoor lab-scale operation systems guided by our world-class greenhouse crew members, it appears that STEC was accidentally introduced into the systems. Therefore, the introduction of clean fish stocks into aquaponics is essential. Again, it is important to note that the bacteria were not transferred to the edible portion of the plants, therefore highlighting the importance of good handling practices in soilless systems to avoid splash as much as possible during production and harvest.

2. *The AA claimed that further research must be performed to prove that cold-blooded, non-mammal aquatic species such as tilapia can harbor STEC and that a wide group of studies, university professors and industry professionals currently refute the possibility that tilapia can harbor this strain.*

Certainly, STEC did not originate from cold-blooded vertebrates like tilapia, but there are several supporting articles that, if contaminated, fish can be a potential carrier of pathogenic *E. coli*.

According to the descriptions provided by the EFSA BIOHAZ Panel (2020) [4], *Escherichia coli* is a facultative anaerobic, Gram-negative, non-spore forming bacterium of the Enterobacteriaceae family. It is part of the normal gastrointestinal flora of humans and of many warm-blooded animals, often present as a harmless commensal. On the other hand, pathogenic *E. coli* include variants causing enteric illnesses, and Shiga toxin-producing *E. coli* (STEC) is one of the six *E. coli* pathotypes [5]. Pathogenic *E. coli* may be present in aquatic environments following the release of fecal material into water bodies from the natural hosts.

The EFSA BIOHAZ Panel (2020) reported that pathogenic *E. coli* (mainly STEC, but also ETEC and EPEC) have been detected in fresh fish at landing or at markets [6–13], in fishing ponds [14], and in aquaculture farms [15,16]. When fish were reared in ponds where the concentration of coliforms was low, a small number of *E. coli* O157:H7 cells were recovered from the intestines of tilapia, common carp, silver carp, and another four species of freshwater fish [17,18]. Transient colonization of the bacterial pathogens has been demonstrated in fish intestines when the fish were exposed to the contaminated water [19,20]. Foodborne pathogens can be carried in fish intestines for up to 7 days [21]. If a contaminated fish is introduced to an aquaponic system, fish feces can be a potential source of contamination of pathogens for fresh produce [22], because the bacterial intestinal flora of fish can survive for up to 84 days in water at 20–30 °C [23,24]. Although refrigeration temperatures are non-permissive for pathogenic *E. coli*, with the minimum growth temperature being 7–8 °C [25], outbreaks associated with fish consumption have been occasionally reported [26–28,4].

3. *The AA claimed that the lack of traceability is a concern.*

The purpose of this study was to determine the occurrence of foodborne pathogens in greenhouse-based aquaponic and hydroponic systems. We examined fish feces as one of the potential contamination sources because it was evident that RO water and seeds were not the contamination sources. Tracing the origin of contamination was not our major interest as it was beyond the scope of this study and requires dedicated personnel and funding. While there are many studies reporting foodborne pathogens in fresh produce, not all studies have traced the origin [29–33].

4. *The AA suspected that a two-month-old system in a controlled environment lab could have been so quickly contaminated.*

Our systems were built in 2015, and we have used these systems for several research projects [34–38]. In this particular study, we sanitized the systems one-month before the start of the study by filling the systems with RO water, we added the fish back to the systems, and introduced and grew new plants for 2-months in the systems. Many of the details, including these procedures and timelines, cannot be fully described in the paper due to the space limitation. The lack of detailed information in the paper might have caused the AA to think it is a two-month-old system. Since this study was conducted between December 2017 and February 2018; the systems were two- to three-years-old at that time. It is also important to note that both the hydroponic and aquaponic systems are housed in the same greenhouse bay at Purdue.

5. *The AA claimed that if hydroponics used synthetic nutrients, there would be very little chance for the *E. coli* to survive without a biological host or continuous contamination source being present.*

In a study evaluating the survival of *E. coli* O157:H7, non-O157 Shiga toxin-producing *E. coli*, and *Salmonella* in hydroponic fertilizer solutions, Shaw et al. (2016) found that the foodborne pathogens survived for 24 h in a fertilizer solution, and populations grew more rapidly in these

solutions than in untreated water [39]. The authors addressed that human pathogens accidentally introduced in hydroponic systems can rapidly propagate and spread throughout the system and potentially contaminate the entire crop. Our study points out that such accidental introduction could occur, and therefore, the likelihood of an accidental introduction should be paid attention to and pointed out to avoid food safety issues in indoor systems.

6. *The AA claimed that the lack of third party or peer university test verification is a concern.*

The microbiology technique used in the study is a routinely and widely used method in the field of food safety. We are confident in our results because the results were confirmed from over 250 samples for STEC (18 samples per each treatment). It is common in academic research when using their own facilities to not use a third-party lab or to verify the results through other labs. This is especially true when the researcher's lab has a strong capacity to handle samples properly.

7. *The AA expressed concerns about our handling and management practices and the safety of our students and staff.*

Our aquaponic and hydroponic systems are dedicated to scientific research. The fish and plants in our aquaponic systems are not for sale and used only for research purposes. Appropriate personal protective equipment (PPE) is provided to ensure occupational health and safety of the students and staff working in the greenhouse. After each experiment, we strictly follow the protocol to handle and dispose of diseased/dead fish. All plant materials grown from the systems are disposed of or processed for data collection. Our lab-scale aquaponic systems are situated in the greenhouse section where the access of other personnel is limited. After each experiment, the systems are sanitized for the next experiment.

8. *The AA pointed out that our recommendation on sterilization is inaccurate and could be detrimental to proper food safety practices.*

We recommended that *"Our results indicated that contamination with bacterial pathogens could likely be reduced in aquaponic and hydroponic systems if the entire system—except biofilters—were thoroughly sanitized before each use and pathogen-free fish were used for the operation."*

We appreciate the AA for their suggestions and concerns regarding our recommendation on sanitation. It would have been described more accurately by including "except biofilters" in the sentence, as shown above. However, we do not agree that the impact of sanitation could be detrimental to proper food safety practices. Because "thorough sanitation" can negatively affect the microbiome, some reduction in the initial growth of fish and plants is expected, but the growth of fish and plants can recover as soon as the microbiome is re-established.

2. Conclusions

Our results and conclusions were based on the scientific data and scholarly articles published by others. Due diligence was made to ensure that STEC was correctly identified according to the methods stated in the paper. Based on scientific reasoning, we concluded that fish feces were one of the significant sources of STEC in the aquaponic systems due to the handling of the fish. There may be a need to conduct future research focusing on identifying contamination sources in soilless systems.

In closing, we believe that the advancement of scientific knowledge is promoted by accumulated research and should be pursued by all scientists. We hope our response addressed all the questions raised by the AA and clarified some of the confusion over the manuscript.

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References

1. Popma, T.; Masser, M. *Tilapia: Life History and Biology*; SRAC Publication No. 283, Southern Regional Aquaculture Center, USA, 1999.
2. Bettelheim, K.A.; Beutin, L. Rapid laboratory identification and characterization of verocytotoxigenic (Shiga toxin producing) *Escherichia coli* (VTEC/STEC). *J. Appl. Microbiol.* **2003**, *95*, 205–217, doi:10.1046/j.1365-2672.2003.02031.x.
3. Rocha, L.B.; Piazza, R.M.F. Production of Shiga toxin by Shiga toxin-expressing *Escherichia coli* (STEC) in broth media: From divergence to definition. *Lett. Appl. Microbiol.* **2007**, *45*, 411–417, doi:10.1111/j.1472-765X.2007.02214.x.
4. Koutsoumanis, K.; Allende, A.; Alvarez-Ordóñez, A.; Bolton, D.; Chemaly, M.; Davies, R.; De Cesare, A.; Herman, L.; Hilbert, F.; Lindqvist, R.; et al. The use of the so-called ‘tubs’ for transporting and storing fresh fishery products. *EFSA J.* **2020**, *18*, e06091, doi:10.2903/j.efsa.2020.6091.
5. Centers for Disease Control and Prevention (CDC). *E. coli (Escherichia coli): Questions and Answers*. National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of Foodborne, Waterborne, and Environmental Diseases (DFWED). 2014. Available online: <https://www.cdc.gov/ecoli/general/index.html> (accessed on 1 June 2020).
6. Kumar, H.S.; Otta, S.K.; Karunasagar, I.; Karunasagar, I. Detection of Shiga-toxigenic *Escherichia coli* (STEC) in fresh seafood and meat marketed in Mangalore, India by PCR. *Lett. Appl. Microbiol.* **2001**, *33*, 334–338, doi:10.1046/j.1472-765X.2001.01007.x.
7. Teophilo, G.N.D.; dos Fernandes Vieira, R.H.S.; dos Prazeres Rodrigues, D.; Menezes, F.G.R. *Escherichia coli* isolated from seafood: Toxicity and plasmid profiles. *Int. Microbiol.* **2002**, *5*, 11–14, doi:10.1007/s10123-002-0052-5.
8. Cardozo, M.V.; Borges, C.A.; Beraldo, L.G.; de Oliveira, F.E.; de Avila, F.A. Prevalence and characterization of shigatoxigenic (STEC) and enteropathogenic (EPEC) *Escherichia coli* strains from fishes for human consumption isolated by Polymerase Chain Reaction (PCR). *FEBS J.* **2012**, *279*, 530–530.
9. Koo, H.-J.; Kwak, H.-S.; Yoon, S.-H.; Woo, G.-J. Phylogenetic group distribution and prevalence of virulence genes in *Escherichia coli* isolates from food samples in South Korea. *World J. Microbiol. Biotechnol.* **2012**, *28*, 1813–1816, doi:10.1007/s11274-011-0954-5.
10. Murugadas, V. Distribution of Pathotypes of *E. coli* in seafood from retail markets of Kerala (India). *Indian J. Fish.* **2016**, *63*, 152–155.
11. Cardozo, M.V.; Borges, C.A.; Beraldo, L.G.; Maluta, R.P.; Pollo, A.S.; Borzi, M.M.; dos Santos, L.F.; Kariyawasam, S.; de Avila, F.A. Shigatoxigenic and atypical enteropathogenic *Escherichia coli* in fish for human consumption. *Braz. J. Microbiol.* **2018**, *49*, 936–941, doi:10.1016/j.bjm.2018.02.013.
12. Leila Dib, A.; Agabou, A.; Chahed, A.; Kurekci, C.; Moreno, E.; Espigares, M.; Espigares, E. Isolation, molecular characterization and antimicrobial resistance of enterobacteriaceae isolated from fish and seafood. *Food Control* **2018**, *88*, 54–60, doi:10.1016/j.foodcont.2018.01.005.
13. Hussein, M.A.; Merwad, A.M.A.; Elabbasy, M.T.; Suelam, I.I.A.; Abdelwahab, A.M.; Taha, M.A. Prevalence of enterotoxigenic *Staphylococcus aureus* and Shiga toxin producing *Escherichia coli* in fish in Egypt: Quality parameters and public health hazard. *Vector-Borne Zoonotic Dis.* **2019**, *19*, 255–264, doi:10.1089/vbz.2018.2346.
14. Ribeiro, L.F.; Barbosa, M.M.C.; de Rezende Pinto, F.; Guariz, C.S.L.; Maluta, R.P.; Rossi, J.R.; Rossi, G.A.M.; Lemos, M.V.F.; do Amaral, L.A. Shiga toxigenic and enteropathogenic *Escherichia coli* in water and fish from pay-to-fish ponds. *Lett. Appl. Microbiol.* **2016**, *62*, 216–220, doi:10.1111/lam.12536.
15. Alagarsamy, S.; Thampuran, N.; Joseph, T.C. Virulence genes, serobiotypes and antibiotic resistance profile of *Escherichia coli* strains isolated from aquaculture and other sources. *Aquac. Res.* **2010**, *41*, 1003–1014, doi:10.1111/j.1365-2109.2009.02384.x.

16. Siddh Nath, K.; Majumdar, R.K.; Parhi, J.; Sharma, S.; Mehta, N.K.; Laishram, M. Detection and characterization of Shiga toxin-producing *Escherichia coli* from carps from integrated aquaculture system. *Aquaculture* **2018**, *487*, 97–101, doi:10.1016/j.aquaculture.2018.01.008.
17. Buras, N.; Duek, L.; Niv, S.; Hephher, B.; Sandbank, E. Microbiological aspects of fish grown in treated wastewater. *Water Res.* **1987**, *21*, 1–10, doi:10.1016/0043-1354(87)90092-3.
18. Apun, K.; Yusof, A.M.; Jugang, K. Distribution of bacteria in tropical freshwater fish and ponds. *Int. J. Environ. Health Res.* **1999**, *9*, 285–292, doi:10.1080/09603129973083.
19. WHO. *Health Guidelines for the Use of Wastewater in Agriculture and Aquaculture: Report of a World Health Organization (WHO) Scientific Group*; WHO Technical Report Series No. 778; WHO: Geneva, Switzerland, 1989.
20. Pillay, T.V.R. *Aquaculture and the Environment*, 2nd ed.; Blackwell Pub.: Oxford, UK; Malden, MA, USA, 2004.
21. Geldreich, E.E.; Clarke, N.A. Bacterial pollution indicators in intestinal tract of freshwater fish. *Appl. Microbiol.* **1966**, *14*, 429–437, doi:10.1128/aem.14.3.429-437.1966.
22. Wang, Y.-J.; Deering, A.J.; Kim, H.J. The Occurrence of Shiga toxin-producing *E. coli* in aquaponic and hydroponic systems. *Horticulturae* **2020**, *6*, 1, doi:10.3390/horticulturae6010001.
23. Wang, G.D.; Doyle, M.P. Survival of enterohemorrhagic *Escherichia coli* O157 : H7 in water. *J. Food Prot.* **1998**, *61*, 662–667, doi:10.4315/0362-028x-61.6.662.
24. Youssef, H.; Eltimawy, A.K.; Ahmed, S. Role of aerobic intestinal pathogens of fresh-water fish in transmission of human-diseases. *J. Food Prot.* **1992**, *55*, 739–740, doi:10.4315/0362-028x-55.9.739.
25. International Commission on Microbial Specifications for Food. *Microorganisms in Foods. 5: Microbiological Specifications of Food Pathogens*; Springer US: New York, NY, USA, 1996; p. 513.
26. Dewey-Mattia, D.; Kisselburgh, H.; Manikonda, K.; Silver, R.; Subramhanya, S.; Sundararaman, P.; Whitham, H.; Crowe, S.J. *Surveillance for Foodborne Disease Outbreaks*; Annual Report 2017; Centers for Disease Control and Prevention (CDC): Atlanta, GA, USA, 2017. Available online: https://www.cdc.gov/fdoss/pdf/2017_FoodBorneOutbreaks_508.pdf (accessed on 10 August 2019).
27. Richardson, L.C.; Bazaco, M.C.; Parker, C.C.; Dewey-Mattia, D.; Golden, N.; Jones, K.; Klontz, K.; Travis, C.; Kufel, J.Z.; Cole, D. An updated scheme for categorizing foods implicated in foodborne disease outbreaks: A tri-agency collaboration. *Foodborne Pathog. Dis.* **2017**, *14*, 701–710, doi:10.1089/fpd.2017.2324.
28. Dewey-Mattia, D.; Manikonda, K.; Hall, A.J.; Wise, M.E.; Crowe, S.J. Surveillance for foodborne disease outbreaks—United States, 2009–2015. *MMWR Surveill. Summ.* **2018**, *67*, 1–11, doi:10.15585/mmwr.ss6710a1.
29. Sivapalasingam, S.; Friedman, C.R.; Cohen, L.; Tauxe, R.V. Fresh Produce: A Growing Cause of Outbreaks of Foodborne Illness in the United States, 1973 through 1997. *J. Food Prot.* **2004**, *67*, 2342–2353, doi:10.4315/0362-028X-67.10.2342.
30. Hanning, I.B.; Nutt, J.D.; Ricke, S.C. Salmonellosis Outbreaks in the United States Due to Fresh Produce: Sources and Potential Intervention Measures. *Foodborne Pathog. Dis.* **2009**, *6*, 635–648, doi:10.1089/fpd.2008.0232.
31. Crowe, S.J.; Mahon, B.E.; Vieira, A.R.; Gould, L.H. Vital Signs: Multistate Foodborne Outbreaks—United States, 2010–2014. *MMWR-Morb. Mortal. Wkly. Rep.* **2015**, *64*, 1221–1225, doi:10.15585/mmwr.mm6443a4.
32. Herman, K.M.; Hall, A.J.; Gould, L.H. Outbreaks attributed to fresh leafy vegetables, United States, 1973–2012. *Epidemiol. Infect.* **2015**, *143*, 3011–3021, doi:10.1017/s0950268815000047.
33. Pattillo, A.; Shaw, A.M.; Currey, C.J.; Xie, K.; Rosentrater, K.A. Efficacy of UV-sterilization in reducing food-borne pathogens in an aquaponics system. **2015**, https://southcenters.osu.edu/sites/southc/files/site-library/site-documents/abc/aquaponics_workshop/AquaponicsFoodSafetyandHumanHealthAllenPatillo.pdf (accessed on 20 January 2020).
34. Yang, T.; Kim, H.J. Nutrient management regime affects water quality, crop growth, and nitrogen use efficiency of aquaponic systems. *Sci. Hortic.* **2019**, *256*, 108619.
35. Yang, T.; Kim, H.J. Effects of hydraulic loading rate on spatial and temporal water quality characteristics and crop growth and yield in aquaponic systems. *Horticulturae* **2020**, *6*, 9.
36. Yang, T.; Kim, H.J. Characterizing nutrient composition and concentration in tomato-, basil-, and lettuce-based aquaponic and hydroponic systems. *Water* **2020**, *12*, 1259.
37. Yang, T.; Kim, H.J. Comparisons of nitrogen and phosphorus mass balance for tomato-, basil-, and lettuce-based aquaponic and hydroponic systems. *J. Clean. Prod.* **2020**, *274*, 122619.

38. Chen, P.; Zhu, G.; Kim, H.J.; Brown, P.; Huang, J.Y. Comparative life cycle assessment of aquaponics and hydroponics in the midwestern United States. *J. Clean. Prod.* **2020**, *275*, 122888.
39. Shaw, A.; Helterbran, K.; Evans, M.R.; Currey, C. Growth of *Escherichia coli* O157:H7, Non-O157 Shiga Toxin–Producing *Escherichia coli*, and Salmonella in Water and Hydroponic Fertilizer Solutions. *J. Food Prot.* **2016**, *79*, 2179–2183, doi:10.4315/0362-028X.JFP-16-073.

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