Would my sampling plan have detected contamination levels that resulted in an outbreak?

A Thought Experiment

There is general acceptance that preharvest sampling and testing may provide insight into trends over time, but is not an effective tool for lot acceptance, since contamination is often at such low levels that the likelihood of finding a "needle in a haystack" is very low. When produce-related outbreaks trace back to the growing environment, there is speculation around whether preharvest sampling could have detected contamination and thus prevented or limited the size of the outbreak.

An expert workgroup[1] facilitated by United Fresh Produce Association sought to further examine this issue using data from an actual outbreak as a case study. This technical bulletin explains the thought process, assumptions, and calculations deliberated by the group.

Key takeaways include:

- This thought experiment can be used as an example of the role testing could have played in reducing the probability of an outbreak.
- Assuming limited clustering (pathogens are disperse enough to be viewed as independent contaminations) of pathogen cells enabled the group to reverse engineer an effective sampling plan; it would be challenging to propose a sampling plan to detect the same overall level of cells which are concentrated in extremely localized clusters (pathogens cannot be considered independent).
- The definition of a field "lot" for the purpose of production may not correspond with the breadth of contamination. The "sampling lot" may encompass several production lots, and a single positive result within the sampling lot must trigger action for the entire sampling lot, including for production lots that tested negative.
    - In other words, in order to "pass", **all** samples must test negative.
    - The accompanying slides illustrate this point.
- The calculations assume that samples are collected and tested correctly and that the method is sensitive enough to detect any pathogen cells present.

Background

Here are the details of the scenario, provided by FDA, that were used by the workgroup:

> *An outbreak of* E. coli *O157:H7 traced back to 44.93 acres dedicated to romaine production caused 62 confirmed illnesses.*

The question the group sought to address was, "What field sampling plan would have had a high likelihood (90-95% chance) of rejecting the ~45 acres, preventing the outbreak"?

[1] 2020 United Fresh Workgroup members include: Jim Brennan, SmartWash Solutions; Don Schaffner, Rutgers University; Matt Stasiewicz, University of Illinois; Lourdes Tamborello, Del Monte Fresh; Eric Wilhelmsen, ATP Consultants; Joan Rosen, JC Rosen Resources

Methodology and underlying assumptions

- For every confirmed case, there are many more cases that are not confirmed. Specifically, we assumed that 62 confirmed cases equated to 1618 actual cases (using the Scallan et al.[2] multiplier of 26.1)
- The 44.93 acres can be rounded to 45 acres
- The total yield of romaine is 38,000 lb/acre
- Using the following two different approaches we arrived at roughly the same conclusion:
    - One approach using dose response curves assuming 1 cfu ingested per serving and 4 servings/head and calculating the probability of illness from one serving
    - The other approach multiplying the actual number of cases by an average infectious dose to determine the total number of *E. coli* O157:H7 present in the 45 acres
- Detailed calculations and associated assumptions can be found in the associated spreadsheet

Results of case study calculations

The accompanying slide deck helps illustrate the following points:

- To be at least 90% confident that the sampling plan would result in rejection of the 45 acres (considered as one sampling lot), nine (9) test samples of 150 g each would need to be tested for a total 1350 g tested.
    - If **any one** of the 9 samples tested positive, the **entire** 45 acres would have to be implicated and rejected. In other words, all 9 samples need to test negative.
    - If the 45 acres were divided into 9, 5-acre "sub-lots" and 150 g of romaine was tested from each sub-lot, a positive test result would need to result in the destruction of ***all*** sub-lots (all 45 acres), not just the one that tested positive. In this scenario, the other 8 sub-lots would have negative test results, but would also be implicated and would not be harvested.
    - In order to "accept" any of the 5-acre sublots, one would need 9 negative results (of 150g each) representing the 5 acres, not just one negative result.
- To be 95% confident, twelve (12) samples would need to be taken to represent the 45 acres, for a total of 1800g.
- N60 sampling using 25 g per sample results in 1500 g of product tested, which is similar to both of these results.
    - This assumes you either test *all* 60 individual samples, or test *all* 4 composites of 375 g using a validated enrichment method
    - Statistical tables show that an N60 sampling plan provides 95% confidence in detecting product when the contamination rate is 5%; if the contamination rate is only 2%, then N60 sampling only provides 70% confidence (e.g., 30% of the time all 60 samples will test negative and the lot will be accepted, even though there actually is contamination present).

[2] Scallan, E et al. 2011. Foodborne illness acquired in the United States--major pathogens. *Emerging infectious diseases*, 17 (1): 7-15. doi:10.3201/eid1701.p11101

- More explanation is provided in the 2010 United Fresh publication, Microbiological Testing of Fresh Produce[3].

Additional assumptions

- The level of contaminant was unchanged between the point of harvest and consumption (e.g., no growth or reduction in pathogen concentration)
- One of the two approaches (the dose-response approach) also assumes homogeneity of distribution in the growing environment (as opposed to sporadic contamination, including contamination of additional product through cross contamination, during post-harvest handling, etc.). The average infectious dose approach does not require this assumption.
- The samples are collected correctly, and the test methods can detect the presence of any pathogens in a sample.

Conclusions

- In the example provided by FDA of a traceback of a highly contaminated production lot of 44.93 acres that resulted in 62 confirmed illnesses, preharvest testing using sampling plans as described above and with the associated assumptions (e.g., no pathogen growth or reduction) would, with significant confidence, have prevented this outbreak.
    - Had the in-field contamination level differed, a different sampling plan would be needed to have the same degree of confidence in the result
- The industry does not currently implement a standardized sampling plan for leafy greens[4]. The variables that were important in this exercise included:
    - Conducting sampling and testing (versus not).
    - Testing all sampled material (e.g., 9 to 12 individual samples, totaling 1350-1800 g) versus collecting a composite of 1500 g but only analyzing 375 g or any amount less than the amount collected.
    - Applying a positive result from any one test to the entire area of inquiry (e.g., all 45 acres implicated as a result of the positive result), versus considering only the sub-lot associated with a positive test to be “positive” and assuming negative test results in the other sub-lots are due to the absence of the pathogen.

[3] https://www.unitedfresh.org/content/uploads/2014/07/FST_MicroWhite-Paper.pdf

[4] LGMA Commodity Specific Food Safety Guidelines for the Lettuce and Leafy Greens Supply Chain [Appendix C: Product Testing Protocol