

Virginia's RRT Eggcellent Response to a Restaurant Associated Foodborne Outbreak

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Overview

The Virginia Department of Health, Office of Epidemiology's Division of Surveillance and Investigation (VDH EPI), was notified of a PFGE cluster of *Salmonella* Braenderup by the Division of Consolidated Laboratory Services (DCLS). The two new 2019 cases had the same PFGE pattern as the 2018 S. Braenderup outbreak involving shell egg consumption. Case interviews determined that a common exposure among the outbreak cases was the consumption of foods at two Virginia locations of a national chain restaurant (Firm A). These locations of Firm A were also identified by a patient in the previous S. Braenderup outbreak.

A total of 45 patients in 10 states were identified as part of the 2018 S. Braenderup 1802MLJBP-1 outbreak, with Virginia having the second highest case count with eight patients. The food history surveys reported 5 out of the 8 Virginia cases consumed eggs, with four reporting egg consumption at one of the Firm A locations. All cases were highly genetically related to positive environmental samples taken at one of the Firm A locations (0-23 alleles, most comparisons were 0-12 alleles different).

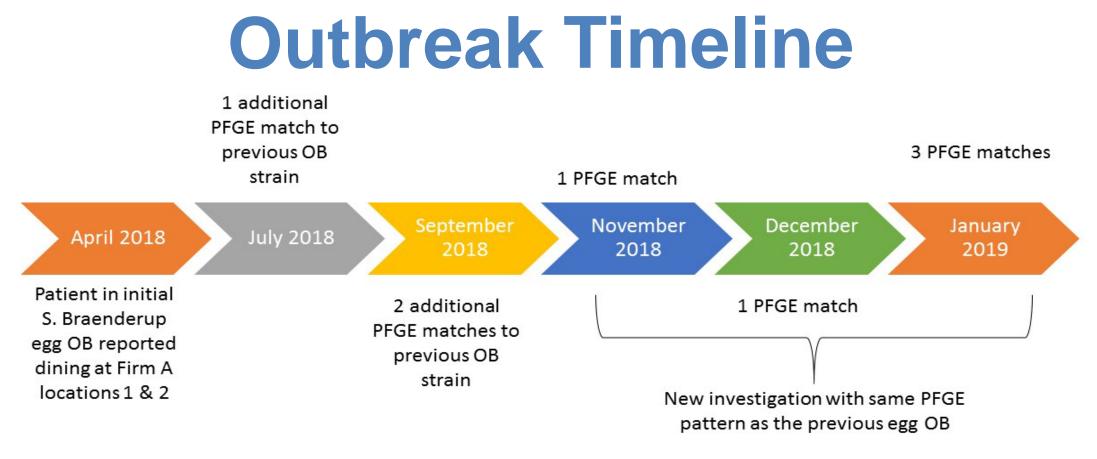
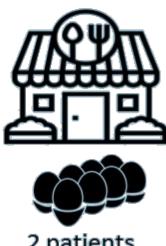


Figure 1. A timeline of the outbreak as it occurred in Virginia.



Location 1

2 patients consumed eggs from Firm A-



consumed food from Firm A – Location 2



consumed eggs from Firm A – Locations 1 & 2



1 patient consumed eggs from Firm A – Location 3

Figure 2. Virginia patients' exposure at Firm A locations.

Environmental Assessment

The Virginia RRT was activated. A team which included representatives from the local health district, VDH Environmental Health, and Virginia Department of Agriculture & Consumer Services (VDACS) was assembled to conduct an Environmental Assessment and Traceback Investigation at location 1, Firm A.



Figure 3. Environmental swabbing of Firm A. Plate storage and grill area, drain, egg cooler exterior and interior. All locations pictured tested positive for Salmonella

Environmental Assessment

The Environmental Assessment team's findings included:

- Eggs were delivered by a single source provider one day/week. Eggs were placed directly into a designated location in the walk-in cooler.
- > Cooks covered hands with disposable mitts when they cracked eggs onto grill. Mitts were removed by shaking mitts off into a garbage can.
- Final cook temperature of eggs (and other foods) were not taken. Eggs are offered undercooked with a Consumer Advisory on the menu.
- Plates stored under the shelf in front of the grill were exposed to potential contamination from eggs cracked and whisked on the shelf.
- The facility was cleaned at night when the establishment was closed. Floors were cleaned by spraying with a hose. Some equipment and the floor were not easily cleanable and were not clean to sight.

The VA RRT investigation team collected invoices, five environmental swabs (total 32 subsamples), and 90 fresh eggs. The eggs were from a single source, Source B, same firm as 2018 outbreak but from a different farm and all eggs were supplied by Distributor A.

VDH EH communicated findings with Firm A corporate office and requested a remediation plan.

Upon learning that the environmental samples were positive for *Salmonella* Braenderup and matched the clinical specimens, Firm A responded by introducing better engineering controls to facilitate cleaning and reduce the risk of future environmental contamination. They replaced porous grout in floors with epoxy grout, replaced damaged equipment, strengthened sanitation programs, updated employee practices, and retrained all employees on SOPs. They also increased the audit frequency by both Firm A auditors and third party auditors and initiated their own environmental sampling program.

Figure 4. Follow-up environmental samples of the floor drain tested positive for the outbreak strain.



Clinical Laboratory Testing

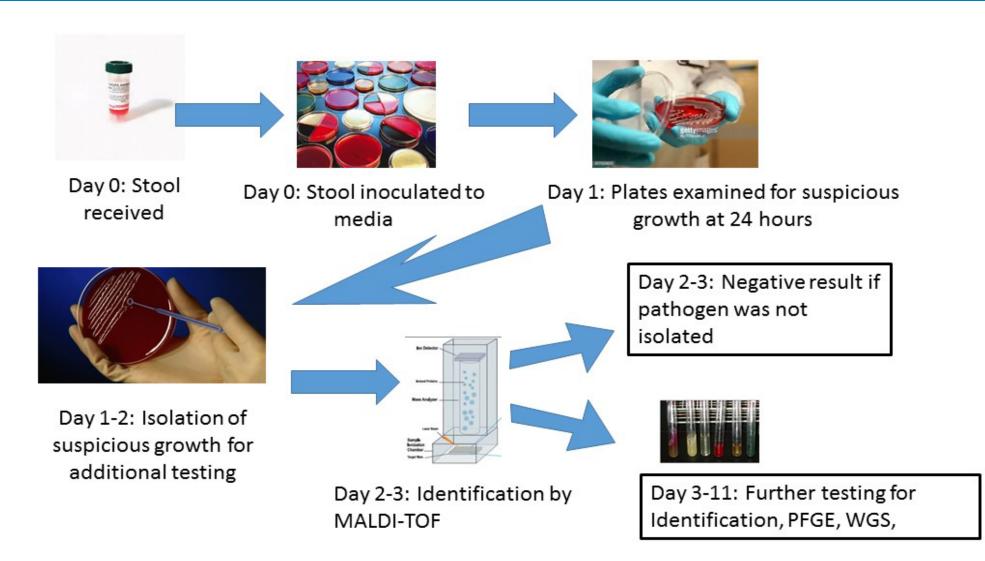


Figure 5. DCLS testing algorithm for stool specimens received in the laboratory. In this outbreak, out of all patients positive for Salmonella, one patient had a culture-independent diagnostic test result indicating the presence of Salmonella. DCLS Enteric Bacteriology used standard microbiology procedures to recovery, isolate and identify the *Salmonella* pathogen from this specimen.

Laboratory Testing and Analysis

Eggs were tested by the Division of Consolidated Laboratory Services (DCLS) on January 31, 2019 along with a total of 32 environmental swabs. Swabs were taken from various locations in the kitchen and dishwashing room and 3 lots of shell eggs were received from Firm A, location 1. All samples were processed for Salmonella testing using DCLS ISO 17025 accredited method which references FDA BAM Chapter 5. Testing was completed on February 8, 2019 with 7 out of the 32 samples testing positive for *Salmonella* spp. All shell egg samples tested negative. On February 21, 2019, the PA Department of Agriculture reached out to the FDA FERN office for assistance in testing shell eggs from Firm A location in PA that was involved in the multistate investigation. DCLS provided testing for the shell eggs. All tested negative for *Salmonella*.





Figure 6. DCLS testing environmental swabs from Firm A, location 1. Enrichment media is used to encourage growth of pathogens found during environmental swabbing. Seven of 32 swabs were positive for Salmonella spp.



Figure 7. (A) DCLS Testing shell eggs for *Salmonella* from Firm A, location 1. The ISO accredited methodologies for regulatory testing require aseptic sampling of the internal contents of the eggs followed by enrichment. (B) A shell analysis was performed with guidance from FDA SMEs. All egg and shell analysis were negative for Salmonella.

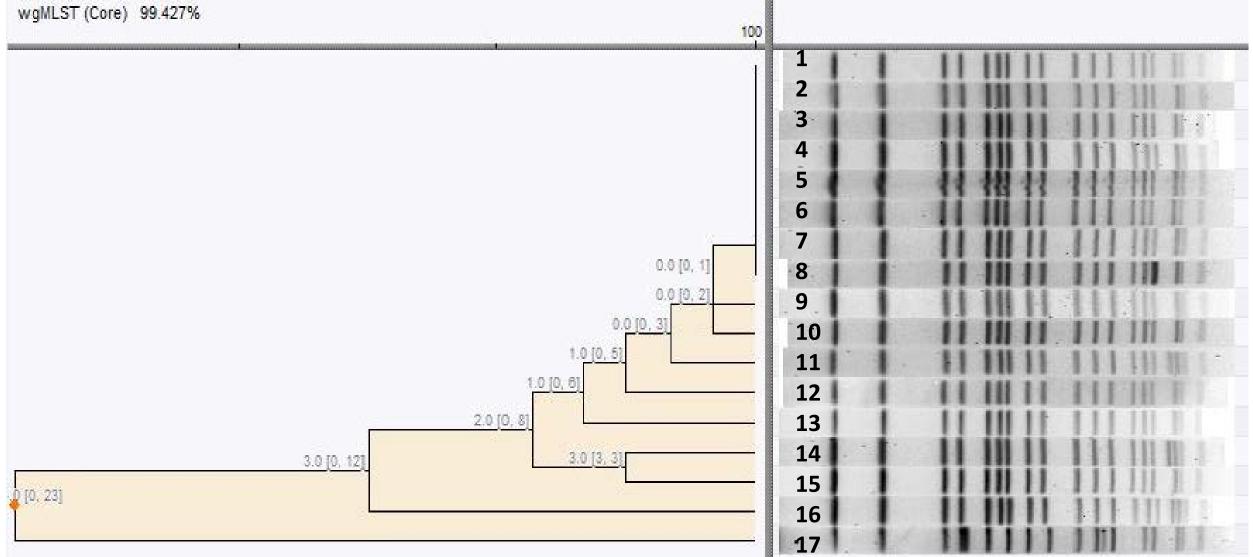




For each *Salmonella* positive sample, a minimum of two isolates were further characterized by PFGE with minor modifications to the standardized PulseNet procedure. Genomic DNA (gDNA) from isolates was restricted by Xbal, electrophoresed per CDC PulseNet run parameters and the resulting DNA fingerprint patterns were compared in BioNumerics v. 6.6 (Applied Maths) with PulseNet scripts. DNA fingerprint results were submitted to the CDC PulseNet Salmonella National Database for pattern naming and national surveillance.

Isolates with unique DNA fingerprint patterns underwent further characterization by WGS. Purified gDNA was sequenced by 2 x 250 paired end sequencing on the Illumina MiSeq platform using the Nextera XT procedure with minor modifications. Sequencing reads were assembled and compared between clinical and environmental isolates by core genome multi-locus sequence typing (cgMLST)

analysis in BioNumerics v. 7.6.3 (Applied Maths), with comparison of ~ 3,000 genetic loci across the different isolates.



| ID Number | Whole Genome Sequencing ID | Source Type | Year/Location | ID Number | Whole Genome Sequencing ID | Source Type | Year/Location | |
|--------------|-------------------------------|---------------|----------------------------|--------------|-------------------------------|---------------|---------------------------------|--|
| 1 | PNUSAS065159 | Human | 2019 | 9 | VA-WGS-19002 | Environmental | Egg Cooler | |
| 2 | PNUSAS067058 | Human | 2019 | 10 | PNUSAS05486 | Human | 2018 | |
| 3 | PNUSAS069114 | Human | 2019 | 11 | PNUSAS065175 | Human | 2019 | |
| | | | | 12 | PNUSAS058520 | Human | 2018 | |
| 4 | VA-WGS-19001 | Environmental | Hose | 13 | PNUSAS040439 | Human | 2018 | |
| 5 | VA-WGS-19003 | Environmental | Egg Cooler | 14 | VA-WGS-19007 | Environmental | Walk-in Cooler | |
| 6 | VA-WGS-19004 | Environmental | Egg Cooler | 15 | VA-WGS-19020 | Environmental | Drain (2 nd Sampling | |
| 7 | VA-WGS-19005 | Environmental | Plate Guard/Ticket Area | 10 | | | Event) | |
| | | | | 16 | VA-WGS-19006 | Environmental | Drain | |
| 8 | PNUSAS058485 | Human | 2018 | 17 | PNUSAS067650 | Human | 2019 | |
| | | | | | | | | |

Table 1. Clinical cases and environmental isolates from location 1 were similar by PFGE and highly genetically related by WGS. Clinical cases from 2018 outbreak and 2019 outbreak are also similar by PFGE and highly genetically related by WGS.

Conclusion

The VA RRT activation maintained coordination and communication between all partners during the investigation. The response demonstrated that normal sanitation practices may not effectively remove pathogens after handling (Class 1 Recall) contaminated product. These pathogens may be present in the environment for extended periods and require additional sanitation procedures to ensure the pathogen is eliminated. Verifying the effectiveness of the sanitation procedures in place could prevent future outbreaks. Additionally, including a Consumer Advisory statement on the menu informs the consumer of the risks of eating undercooked eggs.

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