Outbreak of *E.coli* 0103 Associated with Clover Sprouts Sold at Jimmy John's
Iowa, December 2019

I. Summary/Abstract

In December, 2019, 22 Iowans became ill with *Escherichia coli (E.coli)* O103 after consuming clover sprouts produced by Sprouts Unlimited Wholesale Foods, Inc (Sprouts Unlimited) and sold on sandwiches at Jimmy John's restaurants. The Iowa Department of Public Health (IDPH) first identified this outbreak through routine surveillance on December 18, 2019 when four individuals reported consuming sandwiches sold at various Jimmy John's locations.

Iowa's food retail and manufacturing regulatory agency, the Iowa Department of Inspections and Appeals (DIA), notified Jimmy John's district office of the findings. Based on the epidemiological information, Sprouts Unlimited voluntarily ceased further distribution of clover sprouts pending additional information.

On February 21, 2020, the Food and Drug Administration (FDA) issued a warning letter to Jimmy John's with evidence that demonstrates Jimmy John's engaged in a pattern of receiving and offering for sale adulterated fresh produce, specifically clover sprouts and cucumbers.

The FDA also issued a warning letter to Sprouts Unlimited on February 25, 2020 for supplying sprouts to Jimmy John's which lead to this outbreak and for several violations observed during FDA's inspection of Sprouts Unlimited on December 31, 2019.

II. Introduction

The outbreak was identified through routine disease surveillance. On December 18, 2019, two local public health agencies (LPHA), Linn and Black Hawk counties, notified IDPH field epidemiologist of four cases of *E.coli* O103 that reported eating at a Jimmy John's restaurant in the 10 days prior to their onset of illness. That evening, IDPH reviewed all *E.coli* cases (including those pending serotype) reported in the past two months and identified six more cases that reported eating at Jimmy John's within their incubation period.

E.coli is a bacteria that normally lives in the intestines of people and animals. Most *E.coli* are harmless and are an important part of a healthy human intestinal tract. However, some *E.coli* are pathogenic, and can cause illness. The types of *E.coli* that can cause diarrhea can be transmitted through contaminated water or food, or through contact with animals or persons (1). *E.coli* serotype O103 is a type of *E.coli* that can cause disease by making a toxin called Shiga toxin. The bacteria that make these toxin are called *Shiga toxin-producing E.coli*, or STEC.

The incubation period for *STEC* bacteria is usually from 3 - 4 days after the exposure, but may be as short as 1 day or as long as 10 days. Symptoms of STEC infections vary for each person but often include severe stomach cramps, diarrhea (often bloody), and vomiting. Some infections are very mild, but others are severe or even life-threatening.

III. Background

Agencies involved in this investigation included IDPH, DIA, Iowa's State Hygienic Laboratory (SHL), 10 Local Public Health Agencies (LPHA), the Center for Disease Prevention and Control (CDC) and the FDA.

Jimmy John's Franchise, LLC is an American franchised sandwich fast food restaurant chain owned by Inspire Brands.

Sprouts Unlimited is a wholesale fresh produce supplier located in Marion, Iowa.

IV. Methods

a. Epidemiological Investigation

A cohort study was conducted. Interviews were performed via phone by IDPH's epidemiologists, student interview team, and LPHA partners using IDPH's routine Shiga toxin-producing *E.coli* case investigation form and an outbreak-specific supplemental questionnaire. Data collected included demographic information, occupation, clinical symptoms, travel history, grocery stores where food was purchased, and detailed food history

On December 19, 2019, a meeting was held at IDPH to review identified cases with exposures to Jimmy John's. In addition, all Shiga toxin-producing *E.coli* cases reported to IDPH from November, 2019 – December, 2019 were reviewed for exposure to Jimmy John's. IDPH notified SHL of findings and requested prioritization of all *E.coli* cases submitted for serotyping and DNA fingerprinting. In addition, IDPH reached out to neighboring state health departments to see if they had identified an increase in cases of *E.coli* O103 with exposure to Jimmy John's.

Also on December 19, 2019, IDPH created an outbreak-specific supplemental questionnaire, which specifically asked cases if they had consumed food at Jimmy John's and other similar establishments in the 10 days prior to their onset of illness. IDPH staff and LPHA began attempting to re-interview cases using the outbreak specific supplemental questionnaire. This questionnaire was also used to interview newly reported Shiga toxin-producing *E.coli* cases with exposure to Jimmy John's.

On December 20, 2019, both CDC and FDA were notified of the ongoing investigation. Online order receipts were requested from Jimmy John's.

Data collected was entered into the Iowa Disease Surveillance System and exported into Microsoft Excel for analysis. The 2006 – 2007 CDC FoodNet Population Survey on consumption rates was used to evaluate the expected consumption frequency of specific types of produce. Maps were created using ArcGIS ArcMap 10.

b. Environmental Investigation

On December 19, 2019, IDPH notified DIA that sandwiches sold at Jimmy John's restaurants were a suspected source of illness for some Iowa *E.coli* O103 cases and multiple locations had been identified. DIA communicated this information to the Jimmy John's district office and determined the locations where *E.coli* O103 cases ate were owned and operated by four separate franchisees. On December 20, 2019, DIA contacted all four franchisees to request produce supplier information for each location.

During initial phone conversations, all four franchisees stated verbally that Sprouts Unlimited was their primary produce supplier, and, on occasion, Supplier A was used when product was not available from Sprouts Unlimited. DIA requested produce delivery invoices for each Jimmy John's location.

December 22, 2019, DIA notified Sprouts Unlimited operator of the outbreak. DIA requested that Sprouts Unlimited provide the following information:

1. Clover sprout distribution records
2. Clover seed supplier records
3. Spent irrigation water testing results
4. Clover sprout production records

Sprouts Unlimited collected two spent irrigation water (SIW) samples (from bean tanks 1 & 2 and sprout racks 1 & 2) and clover sprouts (16 packages/subsamples from sprout rack 1) on December 22, 2029 and submitted to SHL for testing on December 23, 2019.

On December 31, 2019, DIA and FDA performed a week-long inspection at Sprouts Unlimited reviewing planting and packaging procedures.

c. Laboratory Investigation

Stool specimens from culture independent diagnostic tests and bacterial isolates from clinical testing were sent to SHL for serotyping and DNA fingerprinting.

SIW and 16 packages of clover sprout samples were collected and sent to SHL for testing. Enrichment procedures recommended for *E.coli* O157 testing in this complex matrix were used.

V. Results

a. Epidemiological Results

A total of 23 individuals were identified and interviewed as part of this investigation. Of these individuals, 22 were laboratory confirmed and one probable case was epidemiologically linked to a laboratory confirmed case.

A case was defined as a person with *E.coli* O103 infection, whose clinical isolate was related within 0-2 single-nucleotide polymorphisms (SNPs), and with specimen collection dates between November 26, 2019 and December 21, 2019. A total of 22 (96%) individuals met this case definition from 10 Iowa counties (Figure 1). Of these, 14 (64%) were female. The median age was 29 years (range 18 – 50 years) [Table 1].

Figure 1. Map of confirmed cases, by county of residence (N=22)

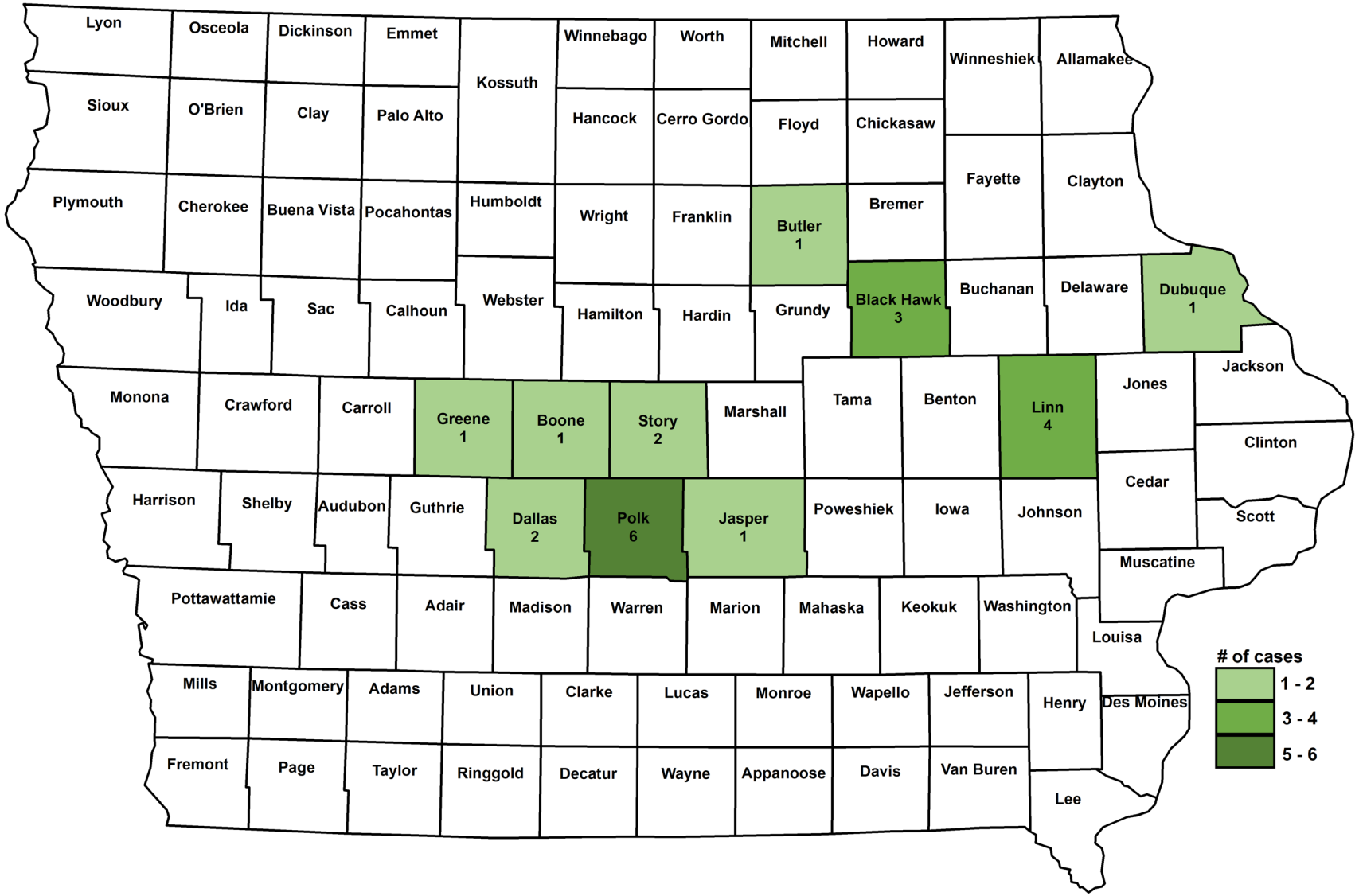
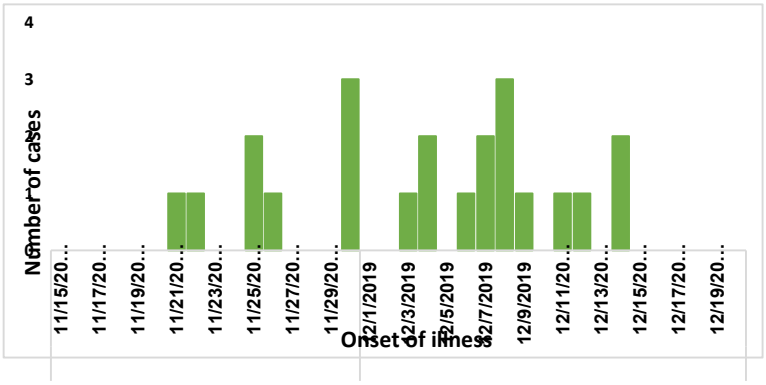


Table 1. Confirmed case demographics in *E.coli* O103 Investigation

Demographics	
Age range (median), (N=22)	18-50 (29)
Age categories in years, (N=22)	N (%)
0-17	0 (0)
18-40	18 (82)
41-60	4 (18)
61-80	0 (0)
81+	0 (0)
Gender, (N=22)	N (%)
Female	14 (64)
Male	8 (36)

Onset of symptoms was known for all 22 confirmed cases (Figure 2). One (5%) individual was hospitalized and no deaths were reported (Table 2).

Figure 2. Epidemiologic curve of confirmed cases, by date of illness onset



Of the 22 identified cases, 21 were interviewed using IDPH's standard Shiga toxin-producing *E.coli* questionnaire and an outbreak-specific supplemental questionnaire. One case completed the standard questionnaire but was not responsive to multiple attempts to complete the outbreak-specific supplemental questionnaire. During the standard interview, this case did not report consuming food from a Jimmy John's restaurant in the 10 days prior to onset of illness, though this individual would not have been specifically asked about Jimmy John's during the standard interview.

Of the 21 cases interviewed using both the standard *E.coli* questionnaire and an outbreak-specific supplemental questionnaire:

- One (5%) case denied eating food from a Jimmy John's restaurant in the 10 days prior to onset of illness.
- 20 (95%) cases reported eating food from a Jimmy John's restaurant in the 10 days prior to onset of illness.
  - The 20 cases reported consuming food from 15 different Jimmy John's restaurant locations. These 15 restaurants were located in seven different central and eastern Iowa counties (Table 3).

Table 2: of Jimmy John's locations where complainants purchased food

# of cases	Location	County	Produce Supplier	Franchisee
1	Store 1	Black Hawk	Sprouts Unlimited and Supplier A	Franchise A
1	Store 2	Black Hawk	Sprouts Unlimited and Supplier A	Franchise B
1	Store 3	Black Hawk	Sprouts Unlimited and Supplier A	Franchise B
1	Store 4	Dubuque	Sprouts Unlimited and Supplier A	Franchise C
1	Store 5	Jasper	Sprouts Unlimited	Franchise D
1	Store 6	Linn	Sprouts Unlimited and Supplier A	Franchise A
1	Store 7	Marion	Sprouts Unlimited and Supplier A	Franchise C
1	Store 8	Polk	Sprouts Unlimited and Supplier A	Franchise D
1	Store 9	Polk	Sprouts Unlimited	Franchise D
1	Store 10	Story	Sprouts Unlimited/Sprouts Unlimited	Franchise D
1	Store 11	Story	Supplier information not obtained.	Franchise D
2	Store 12	Polk	Sprouts Unlimited	Franchise D
2	Store 13	Polk	Sprouts Unlimited and Supplier A	Franchise D
2	Store 14	Polk	Supplier information not obtained.	Franchise D
3	Store 15	Story	Sprouts Unlimited	Franchise D

Cases reported consuming the following produce items on their sandwich (Table 4). To better understand the expected frequency of consumption of specific types of produce, the 2006 – 2007 CDC FoodNet Population Survey consumption rates were used.

Table 3: Rate of cases reporting consumption of produce in comparison to FoodNet Survey

Produce type	Number of Iowa cases reporting consumption of specific produce type	2006-2007 CDC FoodNet Population Survey- estimated consumption rate by produce type
Sprouts	9/20 (45%)	3.3%
Tomatoes	13/20 (65%)	60%
Onion	7/20 (35%)	71%
Avocado	3/20 (15%)	27%
Lettuce	18/20 (90%)	41.1%
Cucumber	4/19 (21%)	46.9%
Hot Pepper	4/20 (20%)	N/A

Forty-five percent of cases in this outbreak reported consumption of sprouts, which is much higher than the FoodNet Survey expected rate of 3.3%. The consumption rate of lettuce is also significantly higher for cases at 90% compared to the FoodNet Survey expected rate of 41.1%. IDPH did not focus on lettuce based on the fact that lettuce served at the implicated Jimmy John's locations were supplied by multiple suppliers.

In addition, to better understand the consumption rate of spouts among Jimmy John's patrons, IDPH analyzed 182 random online order receipts from 11 of the implicated Jimmy John's locations. Only five percent of these patrons consumed sprouts on their sandwich. This is significantly lower than the 45% of the cases that reported consuming sprouts on their sandwich (Table 5).

Table 4: Rate of patrons reporting consumption of produce

Produce	Number of Iowa cases reporting consumption of specific produce type
Sprouts	10 (5%)
Tomatoes	107 (59%)
Onion	52 (29%)
Avocado	28 (15%)
Lettuce	147 (81%)
Cucumber	31 (17%)
Hot Pepper	18 (10%)

Neighboring state health departments had not identified an increase in cases of *E.coli* O103 with exposure to Jimmy John's. No other common exposures were identified.

b. Environmental Results

DIA's traceback investigation focused on clover sprouts based on epidemiological information provided by IDPH and localization of the outbreak to one restaurant chain within Iowa.

During the initial phone conversation on December 22, 2019, Sprouts Unlimited stated that approximately 98% of their clover sprouts were distributed to Iowa based Jimmy John's locations. The remaining clover sprouts were distributed to a few Hy-Vee locations and Fareway distribution center. Sprouts Unlimited stated that they did not distribute clover sprouts outside of Iowa. Based on the epidemiological information, Sprouts Unlimited voluntarily ceased distribution of clover sprouts pending additional information.

On December 23, 2019, Sprouts Unlimited provided all of the requested information and submitted SIW samples and sprout samples to SHL for STEC testing. DIA reviewed distribution records and confirmed the verbal statements provided by Sprouts Unlimited. Sprouts Unlimited also supplied distribution records for an additional location, Grocery A in Fairfield, IA. DIA shared all records with FDA Half West 2. Sprouts Unlimited voluntarily recalled clover sprouts and decided not to distribute any additional clover sprouts or other sprout varieties grown on the same line as the clover sprouts. The following facilities were included in the recall notification:

- All Jimmy John's stores within IA
- Hy-Vee Food Stores
- Fareway Grocery Stores
- Grocery A (single store located in Fairfield, IA)

On December 23, 2019, DIA contacted Jimmy John's parent company, Inspire Brands, to inform them of the outbreak. Based on available epidemiological information, Inspire Brands, voluntarily removed sprouts from all Iowa Jimmy Johns' menus immediately and implemented a cleaning and sanitizing protocol at each store.

On December 23, 2019, Sprouts Unlimited agreed to a voluntary recall and that they would not distribute any additional clover sprouts. A joint recall was issued by FDA and Sprouts Unlimited on December 27, 2019.

From December 31, 2019 through January 9, 2020, DIA inspector and FDA conducted a joint inspection at Sprouts Unlimited reviewing planting and packaging procedures. Violations observed during this inspection lead FDA to issue a warning letter to Sprouts Unlimited for supplying clover sprouts to Jimmy John's which lead to this outbreak on February 25, 2020.

On February 21, 2020, the Food and Drug Administration (FDA) issued a warning letter to Jimmy John's with evidence that demonstrated Jimmy John's engaged in a pattern of receiving and offering for sale adulterated fresh produce, specifically clover sprouts and cucumbers.

DIA conducted a physical recheck inspection at Sprouts Unlimited on March 19, 2020 to verify all violations noted had been corrected.

c. Laboratory Results

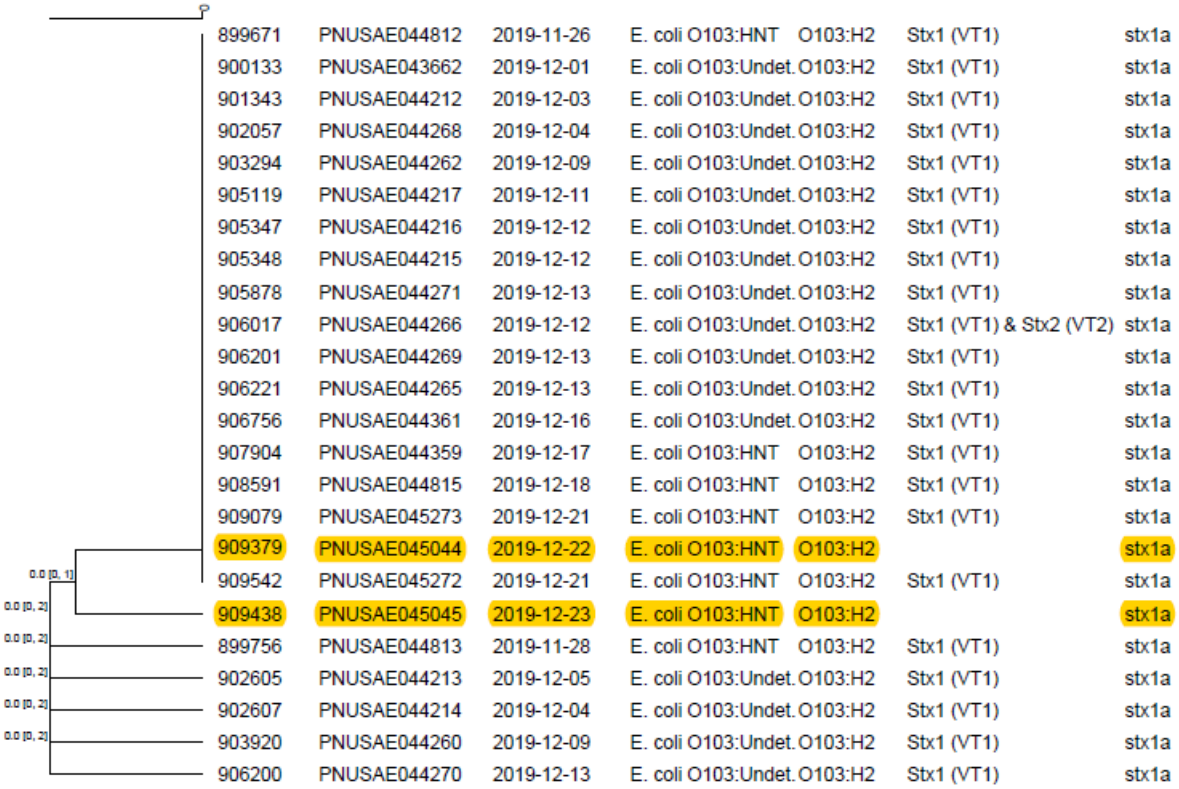
All clinical and environmental samples submitted to SHL were identified as carrying the *stx1a* toxin gene using real-time PCR, serotyped by conventional agglutination, and sequenced using PulseNet procedures. SHL identified *E.coli* O103 from 22 ill people, 15/16 clover sprouts packages and corresponding SIW (Table 6).

Table 5: Sprouts Unlimited environmental samples and results

Product Location	SHL Lab #	Result for E.coli O103
Bean tanks 1 & 2	909378	Negative
Sprout racks 1 & 2	909379	Positive
Clover sprouts (16 packages/ subsamples from rack 1)	909438	Positive

Both environmental (one clover sprout package and the SIW) isolates were highly related to the 22 human clinical isolates by whole genome sequencing by 0-2SNPs (Figure 3).

Figure 3: wgMLST (Core) tree



899671	PNUSAE044812	2019-11-26	E. coli O103:HNT	O103:H2	Stx1 (VT1)	stx1a
900133	PNUSAE043662	2019-12-01	E. coli O103:Undet.	O103:H2	Stx1 (VT1)	stx1a
901343	PNUSAE044212	2019-12-03	E. coli O103:Undet.	O103:H2	Stx1 (VT1)	stx1a
902057	PNUSAE044268	2019-12-04	E. coli O103:Undet.	O103:H2	Stx1 (VT1)	stx1a
903294	PNUSAE044262	2019-12-09	E. coli O103:Undet.	O103:H2	Stx1 (VT1)	stx1a
905119	PNUSAE044217	2019-12-11	E. coli O103:Undet.	O103:H2	Stx1 (VT1)	stx1a
905347	PNUSAE044216	2019-12-12	E. coli O103:Undet.	O103:H2	Stx1 (VT1)	stx1a
905348	PNUSAE044215	2019-12-12	E. coli O103:Undet.	O103:H2	Stx1 (VT1)	stx1a
905878	PNUSAE044271	2019-12-13	E. coli O103:Undet.	O103:H2	Stx1 (VT1)	stx1a
906017	PNUSAE044266	2019-12-12	E. coli O103:Undet.	O103:H2	Stx1 (VT1) & Stx2 (VT2)	stx1a
906201	PNUSAE044269	2019-12-13	E. coli O103:Undet.	O103:H2	Stx1 (VT1)	stx1a
906221	PNUSAE044265	2019-12-13	E. coli O103:Undet.	O103:H2	Stx1 (VT1)	stx1a
906756	PNUSAE044361	2019-12-16	E. coli O103:Undet.	O103:H2	Stx1 (VT1)	stx1a
907904	PNUSAE044359	2019-12-17	E. coli O103:HNT	O103:H2	Stx1 (VT1)	stx1a
908591	PNUSAE044815	2019-12-18	E. coli O103:HNT	O103:H2	Stx1 (VT1)	stx1a
909079	PNUSAE045273	2019-12-21	E. coli O103:HNT	O103:H2	Stx1 (VT1)	stx1a
909379	PNUSAE045044	2019-12-22	E. coli O103:HNT	O103:H2		stx1a
909542	PNUSAE045272	2019-12-21	E. coli O103:HNT	O103:H2	Stx1 (VT1)	stx1a
909438	PNUSAE045045	2019-12-23	E. coli O103:HNT	O103:H2		stx1a
899756	PNUSAE044813	2019-11-28	E. coli O103:HNT	O103:H2	Stx1 (VT1)	stx1a
902605	PNUSAE044213	2019-12-05	E. coli O103:Undet.	O103:H2	Stx1 (VT1)	stx1a
902607	PNUSAE044214	2019-12-04	E. coli O103:Undet.	O103:H2	Stx1 (VT1)	stx1a
903920	PNUSAE044260	2019-12-09	E. coli O103:Undet.	O103:H2	Stx1 (VT1)	stx1a
906200	PNUSAE044270	2019-12-13	E. coli O103:Undet.	O103:H2	Stx1 (VT1)	stx1a

VI. Conclusion

All 22 clinical specimens and two environmental samples isolated at SHL were highly genetically related by WGS. This further supported the epidemiological investigation that showed strong correlations between cases and exposure to clover sprouts produced by Sprouts Unlimited and sold at select Jimmy John's locations.

There were some limitations to this investigation. First, recall bias could have affected data results. This outbreak was identified seven days after the last exposure, which could have resulted in cases having difficulty remembering their food history and purchase dates. Cases that reported sprouts had a better recall because it is a menu item that had to be requested and did not routinely come on all Jimmy John's sandwiches.

VII. Recommendations

STEC testing of SIW can be used as best practice for improving sprout safety to prevent future outbreaks of toxigenic *E.coli*. In addition, method validations should be performed on SIW for STEC testing. SHL was able to successfully detect the STEC using the same enrichment procedures recommended and used for *E.coli* O157 testing in this complex matrix.

VIII. Prepared By

Iowa Department of Public Health
Iowa Department of Inspections and Appeals – Bureau of Food Protection
State Hygienic Laboratory

IX. Supporting Documentations

a. IDPH routine *E.coli* questionnaire
b. Outbreak-specific supplemental questionnaire
c. FDA warning letter to Jimmy John's
d. FDA warning letter to Sprouts Unlimited
e. Sprout Unlimited Recall