FY 2014 – 2016 Microbiological Sampling Assignment

Summary Report: Sprouts

Office of Compliance
Center for Food Safety and Applied Nutrition

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CONTENTS

EXECUTIVE SUMMARY

The U.S. Food and Drug Administration (FDA) set out to collect and test sprouts in 2014 as part of a new proactive and preventive approach to deploying its sampling resources with the ultimate goal of keeping contaminated food from reaching consumers.

The new approach, detailed in the Background section of this report (page 4), centers on the testing of a statistically determined number of samples of targeted foods over a relatively short period of time, 12 to18 months, to ensure a statistically valid amount of data is available for decision making. This approach helps the agency determine if there are common factors – such as origin, season, or variety – associated with pathogen findings.

The FDA issued the sprouts assignment in January 2014 under its new sampling model. The assignment targeted sprouts at three points in the production process (seeds, finished product and spent irrigation water), with the aim of collecting and testing 1,600 samples to determine the prevalence of select pathogens in the commodity. As background, the FDA designed its sampling plan such that if contamination of one percent or greater was present in the commodity, the agency would detect it. The FDA monitored the assignment closely to gather lessons learned and make changes to its sampling procedures if needed to address trends or food safety issues. About one year into the assignment, the FDA decided to stop its collection and testing at 825 samples because it had already collected samples on more than one occasion from many of the sprouting operations known to the agency and its state partners. The sample set acquired was sufficient for the FDA to estimate the bacterial prevalences in the commodity with a 95 percent confidence interval of 0% to 2% for a one percent contamination rate.

The FDA tested only domestically grown sprouts for this assignment because virtually all sprouts eaten in the United States are grown domestically due to the commodity's delicate nature and relatively short shelf-life. Of note, the industry features a preponderance of relatively small operations.

The FDA tested the sprout samples for three pathogens: *Salmonella*, *Listeria monocytogenes* and *Escherichia coli* (*E. coli*) O157:H7. Based on the test results, the FDA found the prevalence of *Salmonella* in the finished product sprouts to be 0.21 percent. The agency also found that the prevalence of *Salmonella* in seeds (2.35%) was significantly higher than in finished product (0.21%) and in spent irrigation water (0.54%). Based on the test results, the FDA found the prevalence of *Listeria monocytogenes* in the finished product to be 1.28 percent. There was no significant difference in the prevalence of *Listeria monocytogenes* based on point in the production process. None of the samples tested positive for *E. coli* O157:H7. The agency did not test seed for *E. coli* O157:H7 due to limitations associated with the test method.

Among the FDA's other findings, the agency found most of the positive samples at a small number of sprouting operations. Specifically, the FDA found violative samples at eight (8.5%) of the 94 sprouting operations visited for purposes of this assignment. The fact that the agency found multiple positive samples at some of these operations underscores the need for sprouting operations to comply with the agency's Produce Safety Regulation (published November 2015), which seeks to prevent outbreaks of foodborne illness and improve sprout safety.

To address the positive samples, the FDA worked with the firms that owned or released the affected product to conduct voluntary recalls or to have their consignees destroy it, and then followed up with inspections. Of particular note, this sampling assignment helped detect and stop an outbreak of listeriosis while it still entailed a small number of cases, as described in the Public Health Impact section of this report (page 14). This assignment also prompted six product recalls.

The FDA will continue to consider microbial contamination of sprouts and how best to reduce it. Such contamination remains a concern to the FDA given the aforementioned outbreak and the recalls initiated. Going forward, the FDA intends to inspect sprouting operations to ensure they are complying, as applicable, with the Produce Safety Rule, which includes new requirements for sprouts growers. The agency has no plans to conduct additional large-scale sampling of sprouts at this time but may sample the commodity in accordance with its longstanding approach to food sampling, which centers on (but is not limited to) the following criteria:

- A firm has a previous history of unmitigated microbial contamination in the environment (e.g., human illness, recalled or seized product, previous inspectional history, or environmental pathogens without proper corrective actions by the facility), or

- Inspectional observations that warrant collection of samples for microbiological analyses.

BACKGROUND

The FDA Food Safety Modernization Act (FSMA) amended the Federal Food, Drug, and Cosmetic Act (FD&C Act) to provide the FDA with additional authority to better prevent problems before they occur. To develop better prevention-based systems, the FDA needs data and other information to help identify hazards that should be addressed and minimized. That is why sampling is an important part of the agency's preventive approach to food safety and why the FDA has developed a new microbiological sampling model designed to identify patterns that may help predict and prevent contamination by disease-causing bacteria.

The new model complements the FDA's longstanding approach to sampling, which has employed for-cause and targeted strategies to monitor known hazards. The FDA will continue its longstanding approach to sampling while also undertaking larger, in-depth surveys of products and commodities to help evaluate risks. These large sample collections enable the FDA to determine the prevalence of contamination (i.e., the number of samples that tested positive for a pathogen in proportion to the total number of samples tested for the given commodity) in instances where it does not otherwise have enough data to do so. Such studies also may shed light on areas of needed focus or issues of food safety that must be addressed, or conversely may help identify effective industry practices to control or minimize food safety hazards.

As a starting point for the new model – and because it is not feasible to sample every product and/or commodity extensively – an FDA work group developed a system to rank commodities based on microbial risk. The work group reviewed sampling data collected over a five-year period, systematically considering criteria such as association with foodborne illness,

consumption of product without a mitigating "kill" step, and prevalence data. Foods that ranked comparatively high were evaluated by subject matter experts to determine their feasibility as candidates for a large-scale survey and the remaining data needs for the commodity. After the work group review, the FDA chose to sample avocados (whole pit fruit), raw milk cheese (aged 60 days), and sprouts (seeds, finished product and spent irrigation water) in FY2014-2016, as the first commodities under the new model. This report details the rationale and findings for the sampling and testing of sprouts.

Why Sprouts?

From 1996 to July 2016, there were 46 reported outbreaks of foodborne illness in the United States associated with sprouts. These outbreaks accounted for 2,474 illnesses, 187 hospitalizations, and three deaths.[1] Prior to this assignment, the FDA had limited data on the prevalence of foodborne pathogens in sprout seed and the extent to which it correlated with their prevalence in spent sprout irrigation water and finished product. The agency also had planned to develop guidance to help sprouting operations comply with the Produce Safety Rule, and these data on the prevalence of foodborne pathogens would help inform the guidance. Ultimately, the FDA saw a need to better understand the estimated prevalence of pathogens at key points in the production process, and to identify common factors among contaminated samples, if possible, with the end goal of helping to protect consumers. Sprouts are especially vulnerable to pathogen contamination given the warm, moist and nutrient-rich conditions needed to grow them.

Sprouts Production

Sprouts are grown from seeds or beans. Example varieties include alfalfa, buckwheat, clover, mung bean, and soybean. Sprouts take their name from the germination process, or "sprouting," that describes their development.

Sprouts production features a wide array of practices and depends on the type of operation and amount of sprouts produced. Most firms are relatively small operations, although some produce sprouts industrially. Sprouts production is generally conducted indoors (e.g., in warehouses or greenhouses), and thus occurs year round. It commonly features the following steps:

- **Seed receipt and storage**. Seeds or beans are delivered to the sprouting operation and held for sprout production.
- **Seed treatment and rinsing**. Seeds or beans are treated to reduce pathogens.
- **Pre-germination soak**. Seeds or beans are pre-soaked in water to initiate sprouting.
- **Germination and growth**. Seeds or beans are transferred to growing containers such as bins, trays or rotary drums for germination. (Typically partitioned into four sections, rotary drums separate the sprouts to keep them from clumping. Water is sprayed intermittently on the growing sprouts as the drums rotate to facilitate germination.)

[1] Food and Drug Administration. (2017). *Compliance with and Recommendations for Implementation of the Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption for Sprout Operations: Guidance for Industry – Draft Guidance.* p. 7. Docket No. 2017-D-0175 in the Federal Register.

- **Microbial testing**. About 48 hours into germination, sampling and testing for pathogens occurs. The spent water passing through the batch of sprouts, or the sprouts themselves, may be sampled.
- **Harvest**. Sprouts are removed from growing containers and washed with cool water to lower their temperature and remove excess hulls. Some operations spin dry the product in a centrifuge to prepare it for packaging.
- **Packaging**. Once cool and dry, sprouts are manually packaged, usually in plastic clamshell containers, and refrigerated.

There are few estimates of the number of sprouts producers in the United States as well as the size of the sprouts market. That is largely due to the preponderance of relatively small operations throughout the industry and the related difficulty in tracking their production. In 2013, the FDA estimated the average annual domestic production volume of sprouts to be about 55 million pounds,[2] based on data obtained in 1998 by the U.S. Department of Agriculture (USDA), the International Sprouts Growers Association, and International Specialty Supply, a provider of sprout seeds and equipment.

OBJECTIVES

The objectives of the FDA's FY2014-2016 sprouts sampling assignment were:

- To determine the prevalences of *Salmonella*, *Listeria monocytogenes* and *E. coli* O157:H7 in sprouts (through testing of seeds, finished product and spent irrigation water).
- To determine if there are common factors associated with positive findings (such as by sprout variety, or point in the production process).
- To inform the FDA's guidance to help sprouting operations comply with the Produce Safety Rule.
- To take appropriate regulatory action in response to violations.

SAMPLE COLLECTION

The FDA collected 825 samples from February 2014 through September 2015 for this assignment. All of the samples were collected domestically because the vast majority of sprouts eaten in the United States are grown in this country.

The FDA originally planned to collect 1,600 samples, but after about one year of sampling decided to stop its collection and testing because it had already collected samples on more than one occasion from many of the sprouting operations known to the agency and its state partners.

[2] FSMA Proposed Rule on Produce Safety (pp. 240 to 242), available at: http://www.fda.gov/downloads/Food/GuidanceRegulation/FSMA/UCM334116.pdf.

As directed by the assignment, FDA field staff collected the samples from three points in the production process (seeds, finished product and spent irrigation water), seeking in each case to obtain complete sample sets, where possible. A complete set comprised at least one sample from each point in the production process, with the samples of finished product and spent irrigation water related to the same seed lot from which the seed was collected.[3] This approach was designed to allow the agency to evaluate the likely source of the contamination, if present, and to facilitate the targeted removal of adulterated product from the food supply chain.

Depending on the size of the sprouting operation, FDA field staff typically collected multiple samples during a collection visit, with the greatest emphasis on finished product. The assignment specifically directed agency field staff to collect about twice as many finished product samples as seed or spent irrigation water samples. This emphasis on finished product was intended to help the FDA achieve its target number of samples and assess the microbial safety of the commodity in the form that reaches consumers. Additionally, the FDA also collected samples from other participants in the food supply chain besides sprout growing operations, such as distributors and retail food establishments. Collection of finished product at retail enables the FDA to consider a dataset more representative of foodborne pathogen prevalence(s) across the industry.

In all cases, collections were carried out to ensure samples were representative of the lot and to allow the FDA to obtain cross sections of operation types (i.e., growers, distributors and retailers).

Collection Sites

FDA field staff collected samples from growers, distributors and retailers, with most of the collection occurring at grower sites (Table 1). Retail samples were collected from intact plastic clamshell containers, or other intact packaging, to minimize the likelihood that any identified contamination could have occurred after the product left the sprouting operation.

Table 1: Sample Collection Sites

Collection Site	Number of Samples Collected	Percentage of Samples Collected
Grower	608	74%
Distributor	101	12%
Retailer	116	14%
Total	825	100%

Sample Collection by Sprout Variety

The FDA collected samples of 14 different varieties of sprouts in total. The most frequently collected were mung bean (36%), alfalfa (21%), clover (10.4%), and soybean (10.4%). Other sprout varieties collected under this assignment include adzuki bean, broccoli, kale, mustard

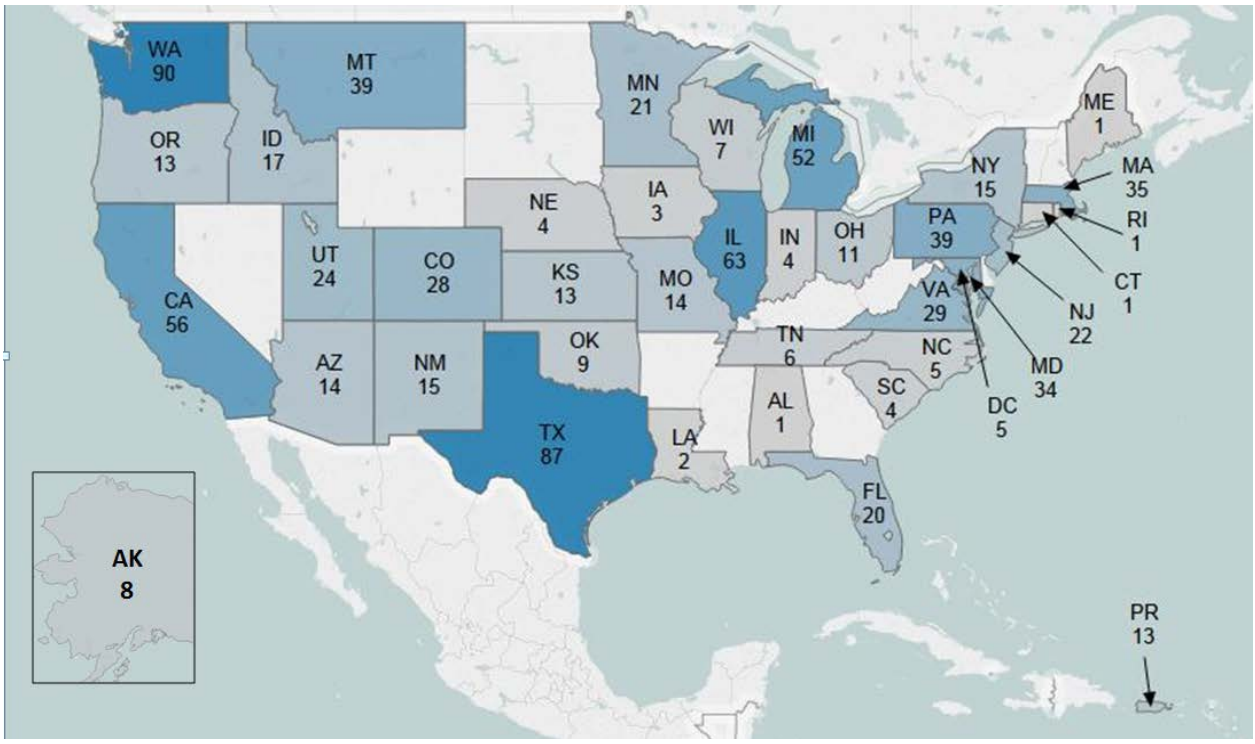
[3] FDA field staff collected the samples that made up each complete set at the same time and place; they did not return to the sprouting operations to collect finished product and spent irrigation water samples matching the production cycle of the seed.

seed, onion, pea, radish, snow pea, sunflower, and wheat. In addition, 5 percent of the samples collected were described as “mixes,” meaning they contained two or more varieties.

Sample Collection by U.S. States

The FDA collected samples from 37 U.S. states, Puerto Rico and the District of Columbia. In designing its surveillance sampling assignments, the agency ordinarily takes production statistics into account to inform its sample allocation but was unable to do so in this case because data on sprouts production is limited. That being the case, the FDA sought to collect samples from every region of the country and as many states as was feasible (Figure 1).

Figure 1: Sample Collection by State



This map shows the numbers of samples collected by state. The darker the color, the greater the number of samples collected.

Though all of the samples were collected in the United States, 72 of the 170 seed samples were of international origin (from Australia, Canada, China and Italy), based on labeling information, invoices and bills of lading.

Sample Collection by Sample Type (i.e., Point in the Production Process)

A breakdown of the sample collection by sample type (seed, finished product and spent irrigation water) is provided (Table 2).

Table 2: Collection by Point in Production Process

Sample Type	Number of Samples Collected	Percentage of Samples Collected *
Seed	170	20.6%
Finished Product	469	56.8%
Spent Irrigation Water	186	22.5%
Total	825	100%

* Numbers do not add up to 100 percent due to rounding.

To the extent possible, the FDA sought to collect complete sample sets, and managed to obtain 62 complete sets in all.

Sample Composition

Each sample consisted of a designated number of subsamples, or in the case of spent irrigation water, of a designated volume, as follows:

Seed. Each seed sample consisted of 30 subsamples from the seed lot of a given sprout type. Each seed subsample consisted of at least 100 grams of seed collected from at least two areas within a seed bag.

Finished Product. Each finished product sample consisted of a minimum of 34 subsamples from the same lot. Each finished product subsample consisted of approximately 50 grams of sprouts, also from the same lot.

Spent Irrigation Water. Each spent irrigation water sample consisted of 2 liters of water, with one sample collected for each type of sprout being produced at the time of collection.

The FDA apportioned the subsamples for testing purposes. This approach – the collection and testing of samples composed of multiple subsamples – is more reflective of actual conditions, and it increases the odds of finding pathogens if they are present, given that microbial hazards may not be uniformly present.

All samples were collected aseptically to prevent contamination, per the FDA's Investigation Operations Manual (Section 4.3.6).

PATHOGEN FINDINGS

This section provides the overall prevalence(s) of *Salmonella*, *Listeria monocytogenes* and *E. coli* O157:H7 in sprouts as well as the prevalence of each target pathogen by sample type, based on the FDA's test results, and other noteworthy findings. The test methods the agency used are described in Appendix A: Test Methods.

Pathogen Findings: *Salmonella*

The FDA detected *Salmonella* in six of the 825 samples tested. Four of those six samples were seed samples; one was finished product; and one was spent irrigation water (Table 3).

Table 3: *Salmonella* Findings

Sample Type	Samples Collected	Number of Positive Samples	Percentage of Positive Samples (by Sample Type)
Seed	170	4	2.35%
Finished Product	469	1	0.21%
Spent Irrigation Water	186	1	0.54%

Serotyping classified three of the six aforementioned positive samples as *Salmonella* Soerenga (all collected at the same facility), one as *Salmonella* Oranienburg, one as *Salmonella* Tennessee, and one as an unnamed serotype (antigenic formula 47:a:1,5).

Pathogen Findings: *Listeria monocytogenes*

The FDA detected *Listeria monocytogenes* in eight of the 825 samples tested. Six of those eight samples were finished product; one was seed; and one was spent irrigation water (Table 4).

Table 4: *Listeria monocytogenes* Findings

Sample Type	Samples Collected	Number of Positive Samples	Percentage of Positive Samples (by Sample Type)
Seed	170	1	0.59%
Finished Product	469	6	1.28%
Spent Irrigation Water	186	1	0.54%

Pathogen Findings: *E. coli* O157:H7

None of the samples tested positive for *E. coli* O157:H7. The FDA tested the finished product and spent irrigation water samples for *E. coli* O157:H7 but not the seed samples due to limitations associated with the test method.

Pathogen Findings: By Complete Sets

The FDA collected 62 complete sets of samples. As described in the Sample Collection section of this report, a complete set comprised at least one sample from each point in the production process, with the samples of finished product and spent irrigation water related to the same seed lot from which the seed was collected. Of the 62 sets, four were found to contain *Salmonella* or *Listeria monocytogenes* at one or more points in the production process.

- **Set No. 1**, collected at a grower site in the Southwest, contained *Salmonella* at all three points in the production process.
- **Set No. 2**, collected at a grower site in the Midwest, contained *Listeria monocytogenes* in the finished product and spent irrigation water.
- **Set No. 3**, collected at a grower site in the West, contained *Listeria monocytogenes* in the finished product.
- **Set No. 4**, collected at a grower site in the Southwest, contained *Listeria monocytogenes* in the seed.

Pathogen Findings: By Sprout Variety

The FDA did not design its sample collection to be representative by sprout variety and thus cautions against making inferences based solely on the findings by variety, which are provided below.

- **Alfalfa**. Of the 100 samples of alfalfa sprouts, the FDA detected *Salmonella* in one sample. Of the 36 samples of alfalfa seed, the FDA detected *Salmonella* in four of the samples, which had been collected from three growers. None of the other varieties tested positive for *Salmonella*. The FDA did not detect other pathogens in its alfalfa samples.

- **Mung Bean**. Of the 163 samples of mung bean sprouts, three were contaminated with *Listeria monocytogenes*. The FDA did not detect other pathogens in its mung bean samples.

- **Soy Bean**. Of the 45 samples of soy bean sprouts, three were contaminated with *Listeria monocytogenes*. The FDA did not detect other pathogens in its soy bean samples.

Further results by variety are provided in Appendix B: Positive Findings by Bacterial Type.

Pathogen Findings: By Collection Site

Of the 14 samples that tested positive for a pathogen, all of them were collected by the FDA at grower sites. With that said, the agency collected the large majority of this assignment's samples (74%) at grower sites, and comparatively few at distributor and retail sites.

Pathogen Findings: By Firms and Related Actions

The FDA found positive samples at eight (8.5%) of the 94 sprouting operations visited for purposes of this assignment. Additionally, of the 14 positive samples detected by the FDA in all, 10 were collected at just four establishments, as shown in rows A-D of the table below (Table 5). The four remaining positive samples were found at four other sprouting operations.

Table 5: Pathogen Findings by Firms and Related Actions

Firm ID	Firm Type	Firm Location	Collection Date *	Pathogen	Point in Production	Action
A	Grower	Southwest	7/2014	*Salmonella*	Finished Product	Product Destroyed
			7/2014	*Salmonella*	Water	Product Destroyed
			7/2014	*Salmonella*	Seed	Product Destroyed
B	Grower	Southeast	9/2014	*Salmonella*	Seed	Class I Recall[4]
			9/2014	*Salmonella*	Seed	Class I Recall
C	Grower	Midwest	8/2014	*Listeria monocytogenes*	Finished Product	Class I Recall, Injunction
			8/2014	*Listeria monocytogenes*	Water	Class I Recall, Injunction
			8/2014	*Listeria monocytogenes*	Finished Product	Class I Recall, Injunction
D	Grower	West	11/2014	*Listeria monocytogenes*	Finished Product	Class I Recall, Regulatory Mtg.,[5] Follow-up Inspection
			1/2015	*Listeria monocytogenes*	Finished Product	Class I Recall, Regulatory Mtg.
E	Grower	Mid Atlantic	5/2015	*Salmonella*	Seed	Product Destroyed
F	Grower	Northwest	12/2014	*Listeria monocytogenes*	Finished Product	Class I Recall, Referred to State
G	Grower	Southwest	1/2015	*Listeria monocytogenes*	Seed	Class I Recall
H	Grower	West	4/2015	*Listeria monocytogenes*	Finished Product	Referred to State, Follow-up Inspection by State

STATISTICAL EVALUATION

The FDA evaluated the pathogen findings using Fisher's Exact Test, a statistical significance test, to determine whether the contamination rates differed statistically by sample type, sprout variety or collection site. The FDA observed the following:

Sample Type

Based on this assignment's findings, the prevalence of *Salmonella* in seeds (2.35%) was significantly higher ($p = 0.02$) than in finished product (0.21%) and in spent irrigation water (0.54%). This finding aligns with other studies that have identified contaminated seed as the most likely source for most reported sprout-associated *Salmonella* outbreaks.[6]

In contrast, there was no significant difference in the prevalence of *Listeria monocytogenes* by sample type. This finding aligns with other studies indicating that this pathogen is most often transmitted to produce via the production environment. Additionally, when *Listeria monocytogenes* becomes established in a sprouting operation, it can be a source of repeated

[4] A Class 1 recall is a situation in which there is a reasonable probability that the use of or exposure to a violative product will cause serious adverse health consequences or death.

[5] The FDA holds a Regulatory Meeting to inform responsible individuals or firms about how one or more products, practices, processes, or other activities are considered to be in violation of the law. Such a meeting can be effective in obtaining prompt voluntary compliance.

[6] National Advisory Committee on Microbiological Criteria for Foods. (1999). Microbiological safety evaluations and recommendations on sprouted seeds. International Journal of Food Microbiology, Vol. 52, pp. 123-153.

contamination of sprouts, food contact surfaces and the production environment.[7, 8] As a consequence, the FDA expects that all sample types (seeds, sprouts, spent irrigation water) collected at an operation in which *Listeria monocytogenes* has become established in the production environment would have similar likelihood of contamination.

Sprout Variety

The FDA did not design its sample collection to be representative by sprout variety and thus cautions against making inferences based solely on the following findings. One sample of finished product alfalfa sprouts tested positive for *Salmonella* out of 100 samples of this variety of finished product sprouts, for an estimated prevalence of one percent. None of the other sprout varieties tested positive for *Salmonella*. Additionally, based on this assignment's findings, the estimated prevalence of *Listeria monocytogenes* is 6.67 percent in finished product soybean sprouts; 1.84 percent in finished product mung bean sprouts; and zero percent in all other varieties combined. While these estimates of prevalence of *Listeria monocytogenes* vary based on sprout variety, other studies indicate that this pathogen is most often transmitted to produce by means of the production environment.[9]

Collection Site

The rate of total microbial contamination (*Salmonella* plus *Listeria monocytogenes*) at grower sites is significantly higher than at distributor and retail sites ($p = 0.02$). Specifically, 7 positive finished product samples out of 256 were collected at grower sites, while zero positive finished product samples out of 213 were collected at distributor and retail sites. This information alone does not definitively identify the point of origin of the contamination, as the contamination may be occurring at any of various points in production and contaminated seed could have been received by the sprouting operations.[10]

REGULATORY APPROACH

The Food, Drug, and Cosmetic Act (FD&C Act) empowers the FDA to take regulatory action regarding adulterated food. Regulatory tools at the agency's disposal include warning letters, import alerts, import refusals, administrative detention and seizures, injunctions, and voluntary and mandatory recalls.

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[7] Tompkin, R.B. (2002). Control of *Listeria monocytogenes* in the Food-Processing Environment. *Journal of Food Protection*, Vol. 65, No. 4, pp. 709–725.

[8] Garner, D., & Kathariou, S. (2016). Fresh Produce–Associated Listeriosis Outbreaks, Sources of Concern, Teachable Moments, and Insights. *Journal of Food Protection*, Vol. 79, No. 2, pp. 337-344.

[9] Ibid

[10] The *Listeria monocytogenes* contaminated samples associated with the 2014 outbreak detailed in the Public Health Impact section of this report make for an exception as additional sampling by the FDA as part of the outbreak investigation indicated the presence of the pathogen in the firm's sprout production area. Also, among the complete sets, the occurrence of contamination in some or all of the sample types sheds light on the possible source(s) of the pathogen in those particular cases.

The FD&C Act applies to finished sprout products, seeds and beans used to grow them, and the water used to irrigate them. Seeds and beans used to grow sprouts meet the definition of food in section 201(f) of the FD&C Act. The Produce Safety Regulation applies to sprouts. If water used to irrigate sprouts tests positive for a pathogen, the FDA considers the production batch of sprouts grown in the contaminated water to be adulterated, and thus the sprouts may be subject to regulatory action.

Salmonella

Sprouts and/or seeds for sprouting that test positive for *Salmonella* are considered to be adulterated under Section 402(a)(1) of the FD&C Act in that they bear or contain a poisonous or deleterious substance which may render them injurious to health. Such foods may be subject to regulatory action.

Listeria monocytogenes

Sprouts and/or seeds for sprouting that test positive for *Listeria monocytogenes* are considered to be adulterated under Section 402(a)(1) of the FD&C Act in that they bear or contain a poisonous or deleterious substance which may render them injurious to health. Such foods may be subject to regulatory action.

PUBLIC HEALTH IMPACT

Of greatest significance to the public health, this assignment helped to detect and stop an outbreak of listeriosis linked to sprouts while it still entailed a small number of cases, consistent with the FDA mission to protect consumers.

In August 2014, agency field staff collected samples under this assignment from a sprouting operation in the Midwest, three of which (two finished product, and one spent irrigation water) tested positive for *Listeria monocytogenes*. Whole genome sequences of the bacteria detected in those samples and environmental samples collected at the operation two weeks later were found to be highly related to sequences of *Listeria* strains from five people who became ill from June through August 2014.

Of the five people who became ill, all were hospitalized and two of them, both elderly, died. All five cases were in the Midwest, according to the U.S. Centers for Disease Control and Prevention (CDC). As part of the outbreak investigation, state public health officials interviewed two of the patients and both reported eating bean sprouts.

The sprouting operation management, having been informed of the positive sample findings, agreed in late August to perform a voluntary recall of their bean sprouts (only to determine that no product remained on the market) and subsequently took corrective action at their operation. FDA field staff returned to the operation in early October and collected additional product and environmental samples. The testing that followed detected *Listeria monocytogenes* in three of the environmental samples.

After finding the additional contamination, the FDA concluded that sprouts were not being safely produced by the firm at its operation. Representatives of the firm agreed to close the operation in early November and cease production and distribution of bean sprouts. A few days later, the firm issued a letter to its customers and employees, notifying them that the firm had permanently ceased operations. The CDC received no further reports of illness caused by the *Listeria* strains in question and closed the outbreak investigation in late January 2015.

The FDA and CDC jointly concluded that bean sprouts were the vehicle involved in the outbreak based on the whole genome sequencing findings, the sprout consumption history of the two patients interviewed, and the inspectional findings at the sprouting operation.

To help ensure the protection of consumers going forward, the FDA and U.S. Department of Justice pursued an injunction against the firm. In April 2015, the United States District Court for the Northern District of Illinois entered into its record a consent decree of permanent injunction against the firm. It cannot resume operations until it meets specific requirements in the consent decree.

The FDA has continued to monitor the building in question as it was rented to another sprouts grower in 2015. FDA field staff inspected the building anew in March 2016, including collecting environmental samples, one of which tested positive for *Listeria monocytogenes*. Notified about the positive sample finding, representatives of the firm removed and replaced the equipment from which the positive sample had been collected. They also took corrective action to address other inspectional findings, including writing a plan to implement preventive controls. Agency field staff returned to the building in July 2016 and collected four finished product samples that each consisted of 50 subsamples, none of which tested positive for pathogens.

The FDA plans to continue to monitor the building. Additionally, as part of its ongoing surveillance, the agency routinely checks to see whether the genomic sequences of isolates it obtained match those of clinical isolates in a National Center for Biotechnology Information database that includes cases of human illness. The FDA has not identified any further matches as of the publication date of this report.

Other Samples, Collection Sites

Apart from the aforementioned outbreak of listeriosis, no illnesses are known to have been associated with the samples collected at other sites and subsequently determined to be contaminated, based on the FDA's evaluation of the samples and the limited epidemiological and clinical data currently available. A detailed explanation of the FDA's evaluation is provided in Appendix C: Genetic Evaluation. Of particular note in this analysis is the increasing importance of whole genome sequencing in identifying the scope and source of microbial contamination. For these reasons, the FDA will continue to expand its efforts in whole genome sequencing, gradually moving away from lower resolution approaches.

In conducting this assignment, the FDA detected pathogens in sprout, seed and spent irrigation water samples from certain firms and worked with firm representatives to take voluntary corrective actions to remove contaminated products from the marketplace, thus preventing consumption and potentially averting illnesses.

As sprouts growers comply with the Produce Safety Rule, they should be aware of the FDA's "Draft Guidance for Industry: Compliance with and Recommendations for Implementation of the Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption for Sprout Operations," which the agency released for comment January 19, 2017. The largest sprouting operations covered by the Produce Safety Rule must be in compliance with all applicable provisions at present, while covered sprouting operations that are small and very small businesses have compliance dates of January 26, 2018 and January 28, 2019, respectively.

Voluntary Industry Actions, Regulatory Activities in Response to Pathogen Findings

In all, the FDA's investigation of the positive samples resulted in six Class I recalls, two follow-up inspections, two referrals to state authorities, two instances of product destruction, one regulatory meeting, and one case of a sprouts grower discontinuing its use of seed from a particular supplier, as well as the previously noted consent decree of permanent injunction. The recalls occurred as follows:

- In August 2014, after the FDA detected *Listeria monocytogenes* in three samples (one spent irrigation water, and two finished product), a firm in the Midwest voluntarily recalled sprouts that had been distributed in the state where they were produced.

- In October 2014, after the FDA detected *Salmonella* in one seed sample, a firm in the Southeast voluntarily recalled seeds that had been distributed throughout the United States and to Bermuda and Canada.

- In November 2014, after the FDA detected *Salmonella* in one seed sample, a firm in the Southeast voluntarily recalled seeds that had been distributed throughout the United States and to Canada.

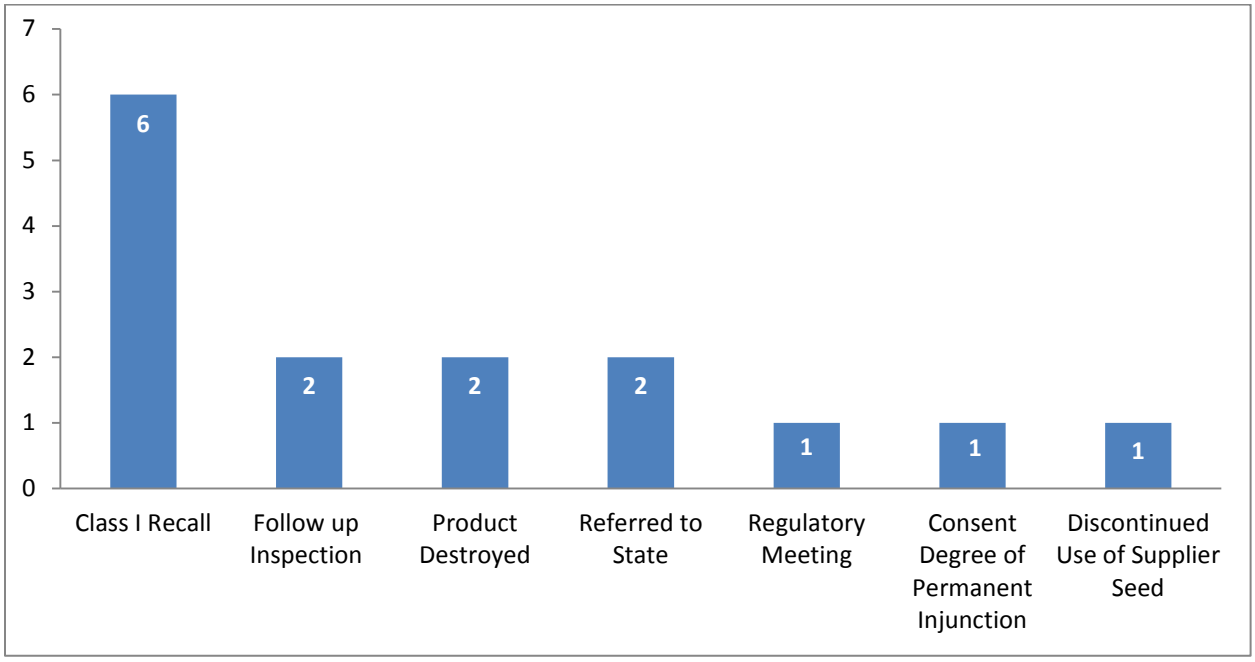
- In December 2014, after the FDA detected *Listeria monocytogenes* in one finished product sample, a firm in the Northwest voluntarily recalled sprouts that had been distributed to two states in the region.

- In February 2015, after the FDA detected *Listeria monocytogenes* in two finished product samples, a firm in the West voluntarily recalled sprouts that had been distributed in the state where they were produced.

- In February 2015, after the FDA detected *Listeria monocytogenes* in one seed sample, a firm in the Southwest voluntarily recalled sprouts that had been distributed in the state where they were produced.

The chart that follows shows the regulatory activities and voluntary industry actions for all positive samples (Figure 2).

Figure 2: Regulatory and Voluntary Industry Actions for All Positive Samples



CONCLUSION AND NEXT STEPS

The FDA accomplished the objectives that it set at the outset of this assignment, the most fundamental being to determine the *Salmonella*, *Listeria monocytogenes* and *E. coli* O157:H7 contamination rates associated with sprouts.

As detailed in the Pathogen Findings section of this report (page 9), the assignment found the prevalence of *Salmonella* in the finished product sprouts to be 0.21 percent and the prevalence of *Listeria monocytogenes* in the finished product to be 1.28 percent. None of the samples tested positive for *E. coli* O157:H7. The agency did not test seed for *E. coli* O157:H7 due to limitations associated with the test method. The FDA also obtained baseline estimates of the prevalence of the pathogens by sample type, which are provided in Appendix B: Positive Findings by Bacterial Type.

As to common factors among the FDA's findings, the agency performed Fisher's Exact Test to determine whether the contamination rates differed statistically by sample type, sprout variety or collection site. The test findings are examined in the Statistical Evaluation section of this report (page 12). Briefly stated, the agency observed the following:

> **Sample Type:** The prevalence of *Salmonella* in seeds (2.35%) was significantly higher ($p = 0.02$) than in finished product (0.21%) and in spent irrigation water (0.54%). There was no significant difference in the prevalence of *Listeria monocytogenes* by sample type. Both findings align with other studies.
>
> **Variety:** This assignment was not designed to be representative by sprout variety, and thus the FDA cautions against making inferences based solely on its variety-specific findings,

which are as follows: 1) The estimated prevalence of *Salmonella* is one percent in alfalfa sprouts; and 2) The estimated prevalence of *Listeria monocytogenes* is 6.67 percent in soybean sprouts, and 1.84 percent in mung bean sprouts. These findings may be confounded by sprout grower, seed supplier, seed lot, water supply, or other elements in the production and supply chain.

Collection Site: The rate of total microbial contamination (*Salmonella* plus *Listeria monocytogenes*) in finished sprout products collected at grower sites is significantly higher than at distributor and retail sites ($p = 0.02$). Of the 7 positive samples of finished sprout products detected in the course of this assignment, all were collected at grower sites.

Upon detecting each positive sample, the FDA took regulatory action as warranted. In each case, the FDA worked with the firm that owned or released the product to conduct a voluntary recall or to have consignees destroy it, except in two instances in which it provided referrals to state authorities. The FDA also followed up with inspections as appropriate to assess whether the production environment was the source of the contamination and to evaluate the firms' production practices.

Of greatest significance to the public health, this assignment helped to detect and stop an outbreak of listeriosis linked to sprouts in 2014 while it was still small in case count, as described in the Public Health Impact section of this report (page 14). The firm whose product was implicated in the outbreak ceased operations shortly after the FDA concluded that the firm was not safely producing sprouts in its operation, and the United States District Court for the Northern District of Illinois subsequently entered into its record a consent decree of permanent injunction against the firm, preventing it from resuming operations until it meets certain requirements.

Underpinning the assignment objectives, it is important to understand that the FDA's ultimate goal in conducting this assignment was to strengthen its understanding of the public health issues associated with sprouts and how they may compare to those of other foods so that the agency can make the best use of its resources as it protects consumers. The agency found most of the positive samples at a small number of sprouting operations. The finding of multiple positive samples in some operations underscores the need for sprouts growers to comply with the FDA's produce safety regulation. Additionally, the data affirm that seeds for sprouting can be an important source of *Salmonella* contamination and that the production environment can be an important source of *Listeria monocytogenes* contamination.

The FDA at this time does not anticipate additional large-scale sampling of sprouts, given the findings of this assignment and the possibility that the contamination rates may well decrease in coming years as sprouts growers comply with the Produce Safety Rule, which includes new requirements for sprouting operations. The FDA has released for comment draft guidance to sprouts growers to help them comply with the Produce Safety Rule, and draft guidance to help industry control *Listeria monocytogenes* in ready-to-eat foods, which provide the agency's current thinking on these topics. In addition, the agency in coming months intends to conduct a study at establishments that grow, harvest, condition, pack/re-pack, and/or supply seeds and beans for sprouting to gain insights on production practices, sanitation methods and

manufacturing processes and to help the establishments achieve compliance in the event that insanitary conditions are observed. Apart from the aforementioned study, the FDA also intends to inspect sprouting operations to ensure they are complying with the Produce Safety Rule and may continue to sample sprouts and the production environment in accordance with the agency's longstanding approach to food sampling, which centers on (but is not limited to) the following criteria:

- A firm has a previous history of unmitigated microbial contamination in the environment (e.g., human illness, recalled or seized product, previous inspectional history, or environmental pathogens without proper corrective actions by the facility), or

- Inspectional observations that warrant collection of samples for microbiological analyses.

The FDA will continue to consider microbial contamination of sprouts and how best to reduce it. The presence of harmful bacteria in the commodity remains a concern to the agency in view of the history of reported outbreaks associated with sprouts, including five in 2016,[11, 12] and the fact that this assignment resulted in six product recalls. As to other efforts by the agency to assess the microbiological safety of sprouts, the FDA commissioned a study of ready-to-eat foods sampled at retail from 2010 to 2014 and found foodborne pathogen prevalences in sprouts similar to those reported in this study. One scientific manuscript based on the commissioned study was published in April[13] and another is anticipated to be published later this year.

Employing the approaches described above, the FDA will sample sprouts as warranted and take other steps consistent with its vigilance in protecting consumers.

[11] FDA. Memorandum of Record. 2015-2016 Sprout and Sprout-Derived Product Related Outbreak Data. 2016. Ref Type: Data Summary.

[12] FDA. Memorandum of Record. July-December 2016 Sprout Related Outbreak Data. 2017 Ref Type: Data Summary.

[13] Luchansky, J., et al. (2017). Survey for Listeria monocytogenes in and on Ready-to-Eat Foods from Retail Establishments in the United States (2010 through 2013): Assessing Potential Changes of Pathogen Prevalence and Levels in a Decade. *Journal of Food Protection*, Vol. 80, No. 6, pp. 903–921.

APPENDIX A: TEST METHODS

Analysts tested the samples using aseptic methods specific to each pathogen, as follows:

Salmonella

Seeds: The FDA used a soak method to detect *Salmonella* contamination in the seeds. Analysts removed 25 grams of seed from each of the 30 subsamples (that made up a sample) to form two composites, each comprising 375 grams of sprout seed. The analysts then soaked each seed composite in 3,375 milliliters of a pre-enrichment lactose broth without blending and incubated the seeds for 24 hours at approximately 35 degrees Celsius. The analysts then used VIDAS Easy (2011.03) or VIDAS SLM (OMA 996.08 or 2004.03) immunoassays to detect *Salmonella.* The FDA's *Bacteriological Analytical Manual* culture method for *Salmonella* was then used to confirm the VIDAS results.

Finished Product: A soak method was used to detect *Salmonella* contamination in the finished sprouts. Analysts removed 25 grams of sprouts from each of 30 subsamples to form two 375 gram sprout composites. Analysts then soaked each composite in 3,375 milliliters of a pre-enrichment lactose broth without blending and incubated them for 24 hours at approximately 35 degrees Celsius. The analysts then used VIDAS Easy (2011.03) or VIDAS SLM (OMA 996.08 or 2004.03) immunoassays to detect *Salmonella.* The FDA's *Bacteriological Analytical Manual* culture method for *Salmonella* was then used to confirm the VIDAS results.

Spent Irrigation Water: Two 375-milliliter composites were analyzed. Analysts added 375 milliliters of spent irrigation water to 3,375 milliliters of a pre-enrichment lactose broth and incubated them for 24 hours at approximately 35 degrees Celsius. The analysts then used VIDAS Easy (2001.03) or VIDAS SLM (OMA 996.08 or 2004.03) immunoassays to detect *Salmonella.* The FDA's *Bacteriological Analytical Manual* culture method for *Salmonella* was then used to confirm the VIDAS results.

Listeria monocytogenes

Seeds: Each sample to be tested consisted of ten 50-gram subsamples. Analysts made two composites from each sample, with each composite consisting of five 50-gram subsamples. The analysts transferred 5 grams from each of the first 5 subsamples into a sterile stomach bag, added 225 milliliters of Buffered *Listeria* Enrichment Broth (BLEB) and mixed thoroughly. The analysts then repeated the procedure for the other 5 subsamples. Following sample preparation, the analysts analyzed the samples using the methods described in the *Listeria* chapter of the FDA's *Bacteriological Analytical Manual*. Briefly, analysts incubated the enrichment mixtures for four hours at 30 degrees Celsius and then added selective agents. The incubation was continued at 30 degrees Celsius for a total of 48 hours. Alternatively, AOAC OMA 999.06 (VIDAS LIS *Listeria*), OMA AOAC 2004.06 (VIDAS LMO2 *Listeria monocytogenes*) or 2004.02 (Modified VIDAS LIS *Listeria*) were used for rapid screening.

Finished Product: Two 50-gram subsamples were analyzed. Analysts aseptically transferred 25 grams of sprouts from each subsample into a sterile stomach bag, added 225 milliliters of

BLEB and mixed them in a stomacher machine at medium speed for two minutes. Analysts then repeated the procedure with the second subsample. Following sample preparation, they analyzed the samples using the methods described in the chapter on *Listeria* in the FDA's *Bacteriological Analytical Manual*. Briefly, analysts incubated the enrichment mixtures for four hours at 30 degrees Celsius and then added selective agents. The incubation was continued at 30 degrees Celsius for a total of 48 hours. Alternatively, AOAC OMA 999.06 (VIDAS LIS *Listeria*), OMA AOAC 2004.06 (VIDAS LMO2 *Listeria monocytogenes*) or 2004.02 (Modified VIDAS LIS *Listeria*) were used for rapid screening.

Spent Irrigation Water: Two 100 milliliter subsamples were analyzed. Analysts transferred 100 milliliter subsamples into a 1 liter flask, added 900 milliliters of BLEB and mixed thoroughly. Analysts repeated the procedure with a second 100 milliliter aliquot. Following sample preparation, they analyzed the samples using the methods described in the chapter on *Listeria* in the FDA's *Bacteriological Analytical Manual*. Briefly, analysts incubated the enrichment mixtures for four hours at 30 degrees Celsius and then added selective agents. The incubation was continued at 30 degrees Celsius for a total of 48 hours. Alternatively, AOAC OMA 999.06 (VIDAS LIS *Listeria*), OMA AOAC 2004.06 (VIDAS LMO2 *Listeria monocytogenes*) or 2004.02 (Modified VIDAS LIS *Listeria*) were used for rapid screening.

E. coli O157:H7

E. coli O157:H7 is the best known serotype of the Shiga toxin-producing *E. coli* (STEC) group, and it causes most foodborne STEC infections. STEC are classified based on the production of shiga toxins (Stx), which are encoded by the *stx* genes.

Finished Product: The FDA's *Bacteriological Analytical Manual* method (Chap. 4A) used to test for *E. coli* O157:H7 is a polymerase chain reaction (PCR) assay that is specific for the *stx* genes and for genes in the O157:H7 serotype. The sample preparation is a modification of that in Chap. 4A, where 25-gram samples of sprouts were mixed with 225 milliliters of enrichment medium containing antibiotic that selects for the growth of STEC. The procedure was repeated for a duplicate sample. After incubation at 42 degrees Celsius overnight, DNA was extracted from an aliquot and tested by PCR. Though none were detected as part of this assignment's testing, samples found to contain *stx* and O157-specific genes would have been plated onto agar media to isolate the bacteria and confirmed for STEC or for O157:H7 using biochemical, serological and genetic assays.

Spent Irrigation Water: The testing method is the same as that described above. The sample preparation was a modification of that in Chap. 4A, where 100 milliliters of spent sprout irrigation water was mixed with 100 milliliters of 2X of enrichment medium containing antibiotic that selects for the growth of STEC. The procedure was repeated for a duplicate sample. After incubation at 42 degrees Celsius overnight, DNA was extracted from an aliquot and tested by PCR. Though none were detected as part of this assignment's testing, samples found to contain *stx* and O157-specific genes would have been plated onto agar media to isolate the bacteria and confirmed for STEC or for O157:H7 using biochemical, serological and genetic assays.

APPENDIX B: POSITIVE FINDINGS BY BACTERIAL TYPE

The tables that follow provide breakdowns, based on this assignment's test results, of the prevalence for each bacterial type by sample type, sprout variety and collection site. The data in the latter two tables pertains to finished sprouts.

Sample Type

Bacteria	Sample Type	Positive	Collected	Prevalence	Conf. Interval Lower Bound*	Conf. Interval Upper Bound**
Salmonella	Seeds	4	170	2.35%	0.64%	5.91%
	Finished Product	1	469	0.21%	0.01%	1.18%
	Spent Irrigation Water	1	186	0.54%	0.01%	2.96%
Listeria Monocytogenes	Seeds	1	170	0.59%	0.01%	3.23%
	Finished Product	6	469	1.28%	0.47%	2.76%
	Spent Irrigation Water	1	186	0.54%	0.01%	2.96%

Sprout Variety

Bacteria	Sprout Variety	Positive	Collected	Prevalence	Conf. Interval Lower Bound*	Conf. Interval Upper Bound**
Salmonella	Mung	0	163	0.00%	0.00%	2.23%
	Alfalfa	1	100	1.00%	0.18%	5.45%
	Clover	0	43	0.00%	0.00%	8.22%
	Soybean	0	45	0.00%	0.00%	7.87%
	All Others Combined	0	118	0.00%	0.00%	3.08%
Listeria Monocytogenes	Mung	3	163	1.84%	0.48%	6.74%
	Alfalfa	0	100	0.00%	0.00%	3.62%
	Clover	0	43	0.00%	0.00%	8.22%
	Soybean	3	45	6.67%	1.84%	21.37%
	All Others Combined	0	118	0.00%	0.00%	3.08%

Collection Site

Bacteria	Collection Site	Positive	Collected	Prevalence	Conf. Interval Lower Bound*	Conf. Interval Upper Bound**
Salmonella	Grower	1	256	0.39%	0.07%	2.19%
	Distributor	0	98	0.00%	0.00%	3.69%
	Retail	0	115	0.00%	0.00%	3.16%
Listeria Monocytogenes	Grower	6	256	2.34%	0.87%	6.12%
	Distributor	0	98	0.00%	0.00%	3.69%
	Retail	0	115	0.00%	0.00%	3.16%

*The lower confidence bound of a 95% confidence interval calculated using the binomial "exact" calculation.
**The upper confidence bound of a 95% confidence interval calculated using the binomial "exact" calculation.

APPENDIX C: GENETIC EVALUATION

This section describes the FDA's further analysis of the samples that tested positive for pathogens – and their comparison to clinical isolates – to determine whether those pathogens, or pathogens of the same species, may have caused foodborne illness.

In carrying out its further analysis, the FDA employed two technologies, pulsed-field gel electrophoresis (PFGE) and whole genome sequencing (WGS), which are commonly used to identify microorganisms. Subsections on each technology are provided below, along with the specific findings for each pathogen.

It is important to note that not all consumers exposed to contaminated foods become ill. Additionally, not all persons who become ill seek care in the health care system, and among those who obtain care, not all receive microbial testing. Regardless of whether or not a link to reported human illness can be demonstrated, removal of contaminated foods from the marketplace serves to prevent potential human illnesses.

For information on disease surveillance in the United States, please visit www.cdc.gov.

Pulsed-Field Gel Electrophoresis (PFGE) Evaluation

PFGE is a laboratory technique used to separate DNA fragments for purposes of bacterial subtyping. After conducting PFGE analysis, the FDA queried the PulseNet USA database, the nation's established repository of PFGE test results, to see whether any of the PFGE patterns associated with the samples that tested positive for a pathogen under this assignment matched any of the PFGE patterns reported previously in association with ill individuals.

The FDA's comparisons found that most of the bacterial strains from the sprout samples were indistinguishable by PFGE from clinical isolates in the PulseNet USA database, although no epidemiological information was available to link the clinical entries to sprouts, apart from the data obtained during the investigation into the 2014 listeriosis outbreak, as noted in the Public Health Impact Section of this report (page 14). While the agency uses indistinguishable PFGE patterns to cluster genetically similar bacterial strains and investigate potential outbreaks of foodborne illness, further information, usually food histories from ill persons and isolates from the site where the food was produced or processed, are needed to ascertain that an adulterated food caused a particular illness, or multiple illnesses in the case of an outbreak.

Salmonella. The six samples that tested positive for *Salmonella* yielded four PFGE patterns across nine isolates. The FDA queried the PulseNet USA database to compare the four patterns to those of reported human biological (i.e., clinical) isolates uploaded between February 24, 2014 and January 31, 2017. The FDA found two PFGE patterns that were associated with human clinical entries. More specifically:

- Six isolates from three sprout samples, all with the same PFGE pattern, were indistinguishable by PFGE from 13 clinical isolates uploaded from nine different states. Upon detecting the pathogen in the samples, the FDA worked with the responsible firm

to have its consignees return or destroy the product. The firm also discontinued its use of seed from its supplier.

- One PFGE pattern found in an isolate from one sprout sample was indistinguishable by PFGE from two human clinical entries from two different states. Upon detecting the pathogen in the sample, the FDA worked with the responsible firm to carry out a recall.

- The other two PFGE patterns found in the sprouts samples did not match isolates uploaded to PulseNet in the above specified date range. Upon detecting the pathogen in the samples, the FDA worked with the responsible firms to carry out a recall in one case, and to oversee the destruction of product in the other.

***Listeria monocytogenes*.** The eight sprout samples that tested positive for *Listeria monocytogenes* yielded five PFGE pattern combinations across fifteen isolates. The FDA queried the PulseNet USA database to compare the fifteen isolates to clinical isolates uploaded to PulseNet by January 31, 2017. The FDA found six sprout isolates to be indistinguishable from clinical isolates, more specifically:

- One sprout isolate was indistinguishable by PFGE from 76 clinical isolates from 21 states. Upon detecting the pathogen in the sample, the FDA worked with the responsible firm to carry out a recall.

- Three isolates from two samples were found to be indistinguishable by PFGE from each other and from two clinical isolates from two different states. Upon detecting the pathogen in the samples, the FDA worked with the firm to carry out a recall. The FDA also conducted a follow-up inspection of the firm, which found two environmental samples to be positive for *Listeria monocytogenes*. The firm's product ultimately was implicated in an outbreak of listeriosis following a multi-agency investigation. The firm ceased operations in late 2014, and the United States District Court for the Northern District of Illinois entered a consent decree of permanent injunction against the firm in April 2015, as described in the Public Health Impact section of this report (page 14).

- Two isolates from two sprout samples were found to be indistinguishable by PFGE from each other and from 133 clinical isolates from 28 states. These findings pertain to the same firm noted in the bullet immediately above, and therefore the FDA's follow-up actions were one and the same. For additional information, please refer to the Public Health Impact section of this report (page 14).

- Two PFGE patterns including nine isolates found in four sprout samples did not match clinical isolates. Upon detecting the pathogen in the samples, the FDA in three instances worked with the firms to carry out recalls and referred the fourth to the state, based on jurisdiction. The FDA also held two regulatory meetings in two of the cases and in one instance carried out a follow-up inspection.

Whole Genome Sequencing (WGS) Evaluation

Whole genome sequencing reveals the complete DNA make-up of an organism, enabling the FDA to better understand variations both within and between species. This in turn helps the FDA to differentiate between organisms with a precision that other technologies do not allow.

***Salmonella*.** WGS of clinical *Salmonella* isolates is not routinely performed by the CDC and state public health laboratories at this time, and therefore the FDA was not able to compare the WGS profile(s) of isolates from the sprout samples to isolates from clinical samples. However, as noted above, the FDA is increasingly using WGS to compare profiles of isolates from product samples to those of environmental samples.

***Listeria monocytogenes*.** Beginning in the fall of 2013, WGS technology has been used for analysis of clinical *Listeria monocytogenes* isolates in the United States. The FDA compared whole genome sequences from the five *Listeria monocytogenes* strains isolated from the sprout samples collected under this assignment to clinical case sequences housed in the National Center for Biotechnology Information (NCBI) database. Four of the isolates from a single sprouting operation were highly related to each other, and to seven clinical isolates in the NCBI database. The FDA actions associated with these four isolates are described in the subsection above on PFGE evaluation, as well as in the Public Health Impact section (page 14). The remaining isolate did not match any clinical isolates in the database as of the publication date of this report.

APPENDIX D: INSPECTIONAL CHECKLIST RESULTS

As part of this assignment, FDA field staff conducted general inspections of the sprouting operations and in doing so completed an inspectional checklist that included questions on food safety practices based on the agency's 1999 Sprout Guidance and Subpart M (Sprouts) of what at the time was the proposed Produce Safety Rule. Agency field staff completed the checklist based on observations, discussion with firm representatives and records review during the inspections. The checklist questions pertained to topics such as seed treatment, sprout production, irrigation, sampling and testing of spent irrigation water, finished product and production environment.

The FDA's purpose in using the inspectional checklist was to gather information to inform the development of the final Produce Safety Rule and the agency's draft guidance to help sprouting operations comply with the Produce Safety Rule, which the agency released January 19, 2017.

The FDA conducted 69 inspections of sprouting operations under this assignment, completing a checklist for each. The tabulated checklist results include the following:

General Information

- **Production Volume:** On average, the growers inspected produced 20,225 pounds of sprouts per week (min., 25 pounds; max. 280,158 pounds).

- **Building Type (Fully vs. Partially Enclosed):** Almost all of the sprouting operations inspected (96 percent) were within a fully enclosed building.

- **Other Products:** More than half of the firms (56 percent) grew, packed/repacked, manufactured/processed or held products other than sprouts.

- **Soil-Grown Sprouts:** The FDA estimated that 10 of the 69 firms inspected grew sprouts (including wheatgrass) in soil.

Seeds for Sprouting

- **Good Agricultural Practices:** Of the sprouting operations inspected as part of this assignment, the FDA found that 32 of them (46 percent) purchased seeds from a supplier who obtained seed from growers who followed good agricultural practices (GAPs), and of those 32 operations, almost all (94 percent) estimated that the entirety of their seeds for sprouting were grown under GAPs.

- **Lot Identity:** Almost all of the sprouting operations inspected (91 percent) maintained lot identity for the seeds they receive and use, such as by ensuring that seed lot numbers were clearly marked and maintained on bags or other containers.

- **Visual Inspection:** The FDA found that most of the sprouting operations (84 percent) visually examined seeds/beans and their packaging for signs of potential contamination, such as evidence of rodent intrusion, prior to use.

- **Seed Treatment:** Nearly half of the sprouting operations inspected (48 percent) had a written seed treatment program to reduce microorganisms of public health significance prior to sprouting. Most of the operations used a seed treatment, either chemical (80 percent) or physical (3 percent), the FDA found. About half of the sprouting operations (56 percent) used seed treatments cited as examples by the FDA in the 1999 Sprout Guidance or other international sprout guidelines, or used seed treatments shown to be comparable with respect to microbial reduction in the scientific literature. Additionally, 27 percent of the spouting operations that used a chemical seed treatment verified the treatment solution's concentration either daily or weekly.

Growing Process

- **Water Source:** The agency found that all of the sprouting operations inspected as part of this assignment used either municipal water (59 percent) or ground water (41 percent) to irrigate sprouts. Less than half of the sprouting operations (45 percent) conducted microbiological testing of water sources used for sprout irrigation. None of the sprouting operations capture and reuse spent irrigation water, the FDA found.

- **Sprouting Duration:** The average germination period for the various sprout types was 141 hours or almost six days (min., 8 hours; max., 720 hours).

- **Growing Containers:** Examples of growing containers used by sprouting operations included trays, bins, tanks, roofing sheets, rotary drums, and metal tubes.

Spent Irrigation Water Testing

- **Microorganisms Targeted:** The FDA found that more than half of the firms inspected as part of this assignment (59 percent) conducted microbiological testing of spent irrigation water. These firms tested for a variety of microorganisms, including generic *E. coli*, *E. coli* O157:H7, *Salmonella* species, and *Listeria* species. Of the firms inspected, 55 percent tested spent irrigation water for both *E. coli* O157:H7 and *Salmonella*, as recommended by the 1999 Sprout Guidance.

- **Sample Collection:** The FDA found that only 27 percent of firms collected a unique spent irrigation water sample from every production batch of sprouts (i.e., from each set of sprouts started at the same time in a single growing unit), as now required under the Produce Safety Rule. Many sprouting operations pooled samples (i.e., collected subsamples from many different growing containers and combined them as one sample for analysis), sampled only a subset of all growing containers or collected samples at a lower frequency (e.g., once a week).

- **Written Sampling Plans:** Of the firms inspected, 38 percent had a written sampling plan for spent irrigation water.

- **Laboratory Testing:** The FDA found that 19 percent of the firms inspected conducted initial microbial testing of spent irrigation water through an in-house laboratory, and 46 percent used a contract laboratory.

- **Corrective Action Plans:** About half of the firms (51 percent) told investigators that they had a corrective action plan for what to do were a pathogen to be detected in a spent irrigation water sample; however, these plans were not always documented in writing or available to be reviewed by an investigator.

- **Testing of In-Process Sprouts:** In lieu of spent irrigation water testing, one firm (1.4 percent) collected and tested in-process sprouts.

Other Testing

- **Environmental Monitoring:** The FDA found that 38 percent of the firms inspected under this assignment conducted some environmental monitoring. Twenty-two percent had a written environmental monitoring plan, and 17 percent tested either for *Listeria* species or *Listeria monocytogenes*, as now required under the Produce Safety Rule. The FDA also found that 20 percent of the firms inspected conducted environmental testing for other microorganisms, including *Salmonella*, *E. coli* O157:H7, and generic *E. coli*. Fifty-four percent of the firms that conduct some environmental monitoring collected environmental samples during production, as now required under the Produce Safety Rule, whereas 38 percent of the firms conducting environmental monitoring instead collected environmental samples immediately after cleaning and sanitizing but before beginning production.