FY 2016 – 2017 Microbiological Sampling Assignment

Summary Report: Hot Peppers

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 hot peppers in 2015 under the agency's 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 5), centers on the testing of a large number of samples of targeted foods over a relatively short period, about 18 months, to ensure that enough data are available to inform decisions. This approach may help the agency determine if there are common factors – such as origin, variety or season – associated with pathogen findings.

The FDA issued the hot peppers assignment in November 2015 under its new sampling model. The agency collected 1,615 samples to test to determine the prevalence of select pathogens in the commodity. The agency collected about 80 percent of its samples from imported hot peppers and the rest from domestically produced hot peppers, comparable to their respective market shares at the outset of the assignment. The agency designed its sampling plan such that if contamination of one percent or greater was present in the commodity, the agency would be likely to detect it. The agency monitored the assignment closely to gather lessons learned and to make changes to its sampling procedures if needed to address trends or food safety issues.

For purposes of this assignment, the FDA targeted the fruit of the genus *Capsicum* (e.g., habanero, jalapeño and serrano peppers) and not the fruit of the genus *Piper* (e.g., black or green peppercorns), following an agency prioritization of foods to be sampled based on potential microbial risk and associated data gaps.

The FDA tested the hot pepper samples for *Salmonella* and *Escherichia coli* (*E. coli*) O157:H7, as well as for other types of Shiga toxin-producing *E. coli* (STEC). Based on the test results, the FDA found the overall prevalence of *Salmonella* in the samples collected to be 2.85 percent. The agency did not detect *E. coli* O157:H7 in any samples. The FDA detected another STEC strain in one sample, but further testing determined that the strain was incapable of causing severe illness.

Among the FDA's other findings, the agency found the prevalence of *Salmonella* to be significantly higher in imported hot peppers (3.48%) than in domestically produced hot peppers (0.31%). Among the countries from which the FDA collected 50 or more import samples, the agency found hot peppers grown in Mexico to have a *Salmonella* prevalence of 2.61 percent (29 positive, out of 1,112 tested), and hot peppers grown in the Dominican Republic to have a *Salmonella* prevalence of 8.33 percent (7 positive, out of 84 tested).

The initial findings for hot peppers grown in the Dominican Republic warranted further investigation, and so the FDA conducted intensified screening (i.e., additional sampling of hot peppers from the island nation) as a supplement to the agency's main assignment. The results of the intensified screening were consistent with the initial findings. The FDA placed two firms in the Dominican Republic and their product on Import Alert 99-23, "Detention without Physical Examination of Produce Due to Contamination with Human Pathogens." Also, as the circumstances did not meet the agency's criteria for a countrywide import alert, the agency

worked directly with the country's Ministry of Agriculture and Fisheries. The ministry instituted training sessions for growers, packers and transportation intermediaries. It also conducted its own sampling of hot peppers in 2017 and did not detect *Salmonella*.

In response to the *Salmonella*-positive samples from Mexico, the National Agro-Alimentary Health, Safety and Quality Service (SENASICA), or state investigators acting on its behalf, conducted follow-up visits at the identified firms. The visits included sampling of water, food contact surfaces, non-food contact surfaces, hot peppers and other produce. The sampling did not detect *Salmonella* in the samples tested. Additionally, SENASICA carried out environmental investigations at nine firms having the highest incidence of positive findings by the FDA, where they identified practices to be corrected. The FDA placed seven firms in Mexico and their product on Import Alert 99-23.

The FDA addressed all the import samples that tested positive for *Salmonella* in the same manner. Specifically, the agency refused entries of hot peppers in lots associated with positive samples and, where the criteria was met, placed the responsible firms and product on Import Alert 99-23. In all, the agency placed 10 firms on the import alert (seven in Mexico, two in the Dominican Republic, and one in Haiti). The FDA also worked with importers to conduct five voluntary recalls. To address the domestic sample that tested positive for *Salmonella*, the FDA worked with the firm that owned the affected hot peppers to conduct a voluntary recall.

The FDA will continue to evaluate methods to reduce microbial contamination of hot peppers. Such contamination remains a concern to the FDA given this assignment's findings and the results of other research, even though the available consumption data indicates that hot peppers are frequently subjected to a 'kill step,' such as cooking or pickling, prior to consumption. The findings of this assignment underscore the need for importers of hot peppers to comply with the agency's Foreign Supplier Verification Programs Rule[1] as applicable. Though the findings indicate a significantly lower *Salmonella* prevalence in domestically produced hot peppers as compared to imported hot peppers, the agency advises growers, both domestic and foreign, to familiarize themselves with the agency's Produce Safety Rule[2], as applicable, since hot peppers are "covered produce" subject to the provisions of the Produce Safety Rule.

The agency will continue to sample hot peppers, including targeted surveillance sampling of imported product from countries of interest. As part of the targeted import sampling, the agency will use its PREDICT tool,[3] which assists entry reviewers in targeting higher-risk shipments to be examined. The FDA also may sample hot peppers using its longstanding approach to food sampling, which centers on (but is not limited to) the following criteria:

[1] The Foreign Supplier Verification Programs Rule requires that importers perform certain risk-based activities to verify that food imported into the United States has been produced in a manner that meets applicable U.S. safety standards.

[2] The Produce Safety Rule establishes, for the first time, science-based minimum standards for the safe growing, harvesting, packing, and holding of fruits and vegetables grown for human consumption.

[3] PREDICT improves import screening and targeting to prevent entry of adulterated, misbranded, or otherwise violative goods and expedites the entry of non-violative goods.

- 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.

Employing the approaches described above, the FDA will sample hot peppers as warranted and take other steps consistent with its mission to protect consumers.

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 must 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 developed a new 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 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 score commodities based on microbial risk. The group reviewed sampling data collected over a five-year period, systematically considering criteria such as linkage to foodborne illness, consumption of product without a mitigating kill step, and available research studies. 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. Following 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. In FY2016-2017, the FDA chose to sample cucumbers and hot peppers under the new model. This report details the rationale and findings for the sampling and testing of hot peppers.

Why Hot Peppers?

Fresh hot peppers were implicated in a nationwide outbreak that caused 1,500 illnesses, 308 hospitalizations and two deaths in 2008.[4] In addition, 11 product recalls involving *Salmonella* on fresh hot peppers occurred in the United States from 2010 to 2015, the latter year marking the start of this assignment. In 2013, the Centers for Disease Control and Prevention (CDC) released a study that found *Salmonella* contamination of salsa or guacamole had resulted in 26 outbreaks and 1,872 illnesses during the 35-year period examined.[5] A common ingredient in salsa, hot peppers may come into contact with contaminated water, soil, or equipment during growing, harvesting, and/or post-harvest activities. Hot peppers also can be a 'stealth component' in multi-ingredient dishes, meaning people may not know they are eating them. Prior to this assignment, the FDA had limited data on the prevalence of *Salmonella*, as well as *E. coli* O157:H7 and other STEC in hot peppers. Given the circumstances, the agency saw a need to better understand the prevalence of these pathogens in the commodity, and to identify common factors among contaminated samples, if possible, with the end goal of helping to protect consumers.

Hot Peppers Production

Hot peppers are cultivated mainly in tropical and subtropical climates. Grown in fields, they feature thousands of varieties and come in many shapes and sizes. Though eaten as vegetables, hot peppers are fruit by scientific classification.

Hot peppers production involves from two to four harvests from the same plants. Almost all hot peppers are picked by hand because harvesting machinery can damage the crop, rendering it unsuitable for market. The multiple harvests are a possible risk factor that can contribute to contamination because every time farm workers or farm equipment enter the growing field, the probability of contamination increases. Other possible risk factors include animal intrusion into growing fields, improper cleaning or sanitizing of food contact surfaces (such as harvesting totes), insanitary wash water, and – if a customer requirement – waxing that may trap bacteria on the product surface.

The United States produced approximately 470 million pounds of fresh chili peppers in 2016 while importing about one billion pounds of the commodity that same year, according to the U.S. Department of Agriculture (USDA).[6,7] Chili peppers are the fruit of plants from the genus *Capsicum*.

Worldwide the largest producers of hot peppers are China and Mexico.[8] Most of the hot peppers imported by the United States are grown in Mexico.

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[4] Behravesh, C.B., Mody, R.K., Jungk, J., Gaul, L., Redd, J.T., Chen, S., et al. (2011). 2008 Outbreak of Salmonella Saintpaul infections associated with raw produce. *The New England Journal of Medicine*, 364, 918-927.

[5] Kendall, M., Mody, R., Mahon, B., Doyle, M., Herman, K. & Tauxe, R. (2013). Emergence of salsa and guacamole as frequent vehicles of foodborne disease outbreaks in the United States, 1973–2008. *Foodborne Pathogens and Disease*.

[6] USDA National Agricultural Statistics Service. (2016). National Statistics for "Peppers, Chile – Production, Measured in CWT" [Data Query]. Retrieved from NASS website, www.nass.usda.gov, on February 21, 2018.

[7] USDA Economic Research Service. (2016). U.S. Import Sources by Volume: Fresh or Chilled Chili Peppers [Data Query]. Retrieved from ERS website, www.ers.usda.gov, on February 21, 2018.

[8] Food and Agriculture Organization of the United Nations. (2016). Crops: Chilies and Peppers, Green [Data Query]. Retrieved from FAO website, www.fao.org/faostat, on February 21, 2018.

OBJECTIVES

The objectives of the FDA's FY2016-2017 hot peppers sampling assignment were:

- To determine the prevalence of *Salmonella*, *E. coli* O157:H7, and other STEC in hot peppers.
- To determine if there are common factors associated with positive findings (such as by origin, variety or season).
- To take appropriate regulatory action in response to violations.

SAMPLE COLLECTION

The FDA collected 1,615 samples of hot peppers from November 2015 to July 2017 for this assignment. The samples were collected in proportions comparable to their respective market share based on origin (i.e., domestic vs. import).

Agency field staff collected samples one at a time from individual lots. In cases where the collection site(s) featured multiple lots, the field staff generally collected one sample from each lot. This approach, which avoided commingling samples from different lots, was designed to help the FDA identify the likely source of the contamination, if present, and to facilitate targeted removal of adulterated product from the food supply.

The field staff collected samples to ensure they were representative of the lot and to facilitate analyzing cross sections of operation types (e.g., growers, packinghouses, distributors and retailers) for the domestic samples and countries of origin for the import samples. The agency collected the samples over 18 months, enabling the capture of seasonal data as well.

The FDA did not collect hot peppers that it knew to be intended for a kill step (such as pickling, brining or drying), or other processed hot peppers (such as chopped, frozen or jarred).

The field staff collected all samples aseptically to prevent contamination during the collection process. The FDA's aseptic sampling methods, which entail the use of sterile implements and containers, and prescribed collection procedures, are published in the agency's Investigations Operations Manual (Section 4.3.6).

Domestic Sample Collection

As directed by the assignment, the field staff collected 322 domestic samples of hot peppers, with most of them collected at distribution facilities and packinghouses (Table 1). Samples were collected in 35 states, with the largest number collected in California (65), followed by Florida (32), and Colorado (23).

Table 1: Domestic Sample Collection Sites

Collection Site	Number of Samples Collected	Percentage of Domestic Samples	Percentage of All Samples*
Distributor/Warehouse	190	59%	12%
Packinghouse	64	20%	4%
Grower	42	13%	3%
Retail	26	8%	2%
Total	322	100%	20%

* Numbers do not add up to 20 percent due to rounding.

Import Sample Collection

As directed by the assignment, the field staff collected 1,293 import samples, most exported to the United States from Mexico. The FDA used two approaches to collect import samples: port-of-entry and domestic import (DI) collection. Of the total, 1,027 samples (79 percent) were collected at ports of entry or other locations where the product was being held prior to release into domestic commerce (Table 2).

Additionally, 266 samples (21 percent) were collected as DI samples and counted toward the import sample total. DI samples are samples collected after being released into domestic commerce. They often are collected near their port of entry, usually at a warehouse, but may also be collected from retail stock rooms, prior to consumer handling. DI sampling allows for imported products to be released and sold domestically or to undergo processing. For purposes of this report, DI samples are included in the import sample data (Table 2).

Table 2: Import Sample Collection Sites

Collection Site	Number of Samples Collected	Percentage of Import Samples	Percentage of All Samples
Port of Entry	1,027	79%	64%
Domestic Import	266	21%	16%
Total	1,293	100%	80%

The FDA collected import samples from 13 countries. The large majority were grown in Mexico (1,112), followed by the Dominican Republic (84), the Netherlands (19), Belgium (14), Haiti (14), Jamaica (14), Trinidad and Tobago (14), Honduras (8), Canada (7), Israel (3), Saint Lucia (2), Grenada (1), and Peru (1).

Sample Collection by Hot Pepper Variety

The FDA collected samples of 36 different varieties of hot peppers, as well as some that it categorized as "unspecified" and others that it categorized as a "mix," meaning they contained two or more varieties. The most frequently collected were jalapeño (36%) and serrano (18%), as well as habanero, pasilla, and poblano, each constituting 6 percent. A table with all the varieties and accompanying sample counts is provided in Appendix A: Sample Collection by Variety.

Sample Collection by Season

The FDA was able to collect samples year-round because the U.S. harvest season and the Mexican and Caribbean harvest seasons are complementary. The agency collected most of its samples in the summer of 2016 (445 samples). Other sizeable collections, in descending order by volume, were collected in the winter of 2015 (323 samples), the spring of 2016 (284 samples), and the fall of 2016 (280 samples).

Sample Composition

Each sample consisted of 20 subsamples, and each subsample consisted of one pound of hot peppers. The FDA divided the subsamples for testing purposes, with one half to be tested for *Salmonella* and the other for Shiga toxin-producing *E. coli*, including *E. coli* O157:H7.

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 present, given that microbial hazards may not be uniformly present. Accordingly, if one subsample tested positive for a target pathogen, the FDA regarded the sample to be positive for the organism.

PATHOGEN FINDINGS

This section provides the overall prevalence(s) of *Salmonella*, as well as *E. coli* O157:H7 and other STEC in hot peppers, based on the FDA's test results, and other noteworthy findings. The test methods the agency used are described in Appendix B: Test Methods.

Pathogen Findings: *Salmonella*

The agency detected *Salmonella* in 46 of the samples, a prevalence of 2.85 percent. Of the total number of *Salmonella*-positive samples, 45 were import samples, and one was domestic. The most common serotypes were *Salmonella* Newport and *Salmonella* Aberdeen. The complete list of the *Salmonella*-positive samples by serotype is provided in Appendix C.

Pathogen Findings: *E. coli* O157:H7

None of the samples tested positive for *E. coli* O157:H7.

Pathogen Findings: Other STEC

One sample tested positive for STEC (i.e., non-O157 STEC). Upon further characterization, the FDA determined that the bacteria were incapable of causing severe illness. Specifically, analysis showed the bacteria did not possess any of the known characteristics that would enable it to adhere to intestinal epithelium (i.e., the cells in the luminal portion of the intestines), which is essential for infection to begin.

Pathogen Findings: By Hot Pepper Variety

The FDA's *Salmonella*-positive findings by variety are provided below (Table 3). The agency cautions against making inferences based solely on the findings by variety, which are provided for informational purposes (Table 3).

Table 3: Pathogen (*Salmonella*) Findings by Hot Pepper Variety

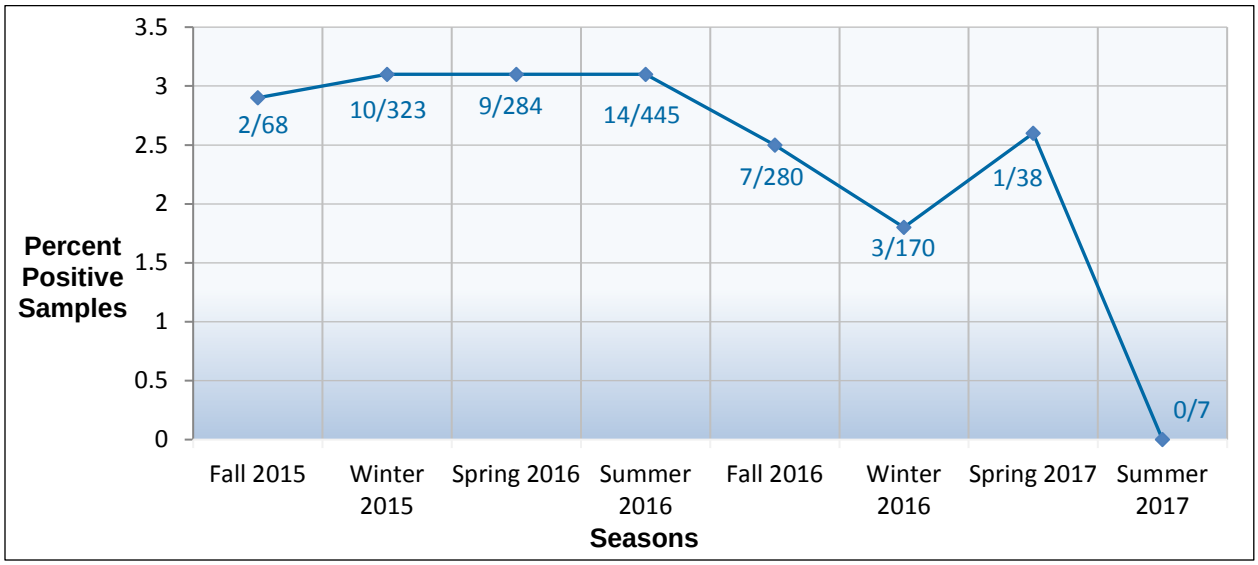
Variety	No. Samples Collected	No. Samples Positive
Ají	5	2
Anaheim	88	3
Caribe	23	1
Finger	6	1
Green Chili *	28	6
Habanero	102	3
Jalapeño	574	4
Pasilla	95	2
Pimiento	2	1
Poblano	91	1
Scotch Bonnet	10	1
Serrano	285	12
Thai Chili	46	6
Yellow Chili *	23	1
Unspecified	60	2

* Variety not further specified on labeling information, invoices or bills of lading.

Pathogen Findings: By Season

The FDA detected *Salmonella* in each season and observed modest seasonal fluctuation in the percentage of positive samples, except for the summer of 2017, when the agency did not detect any positive samples out of the seven samples collected (Figure 1). The FDA found the winter of 2015 and spring and summer of 2016 to be the seasons with the highest percentage of positive samples, approximately 3.1 percent.

Figure 1: Pathogen (*Salmonella*) Findings by Season

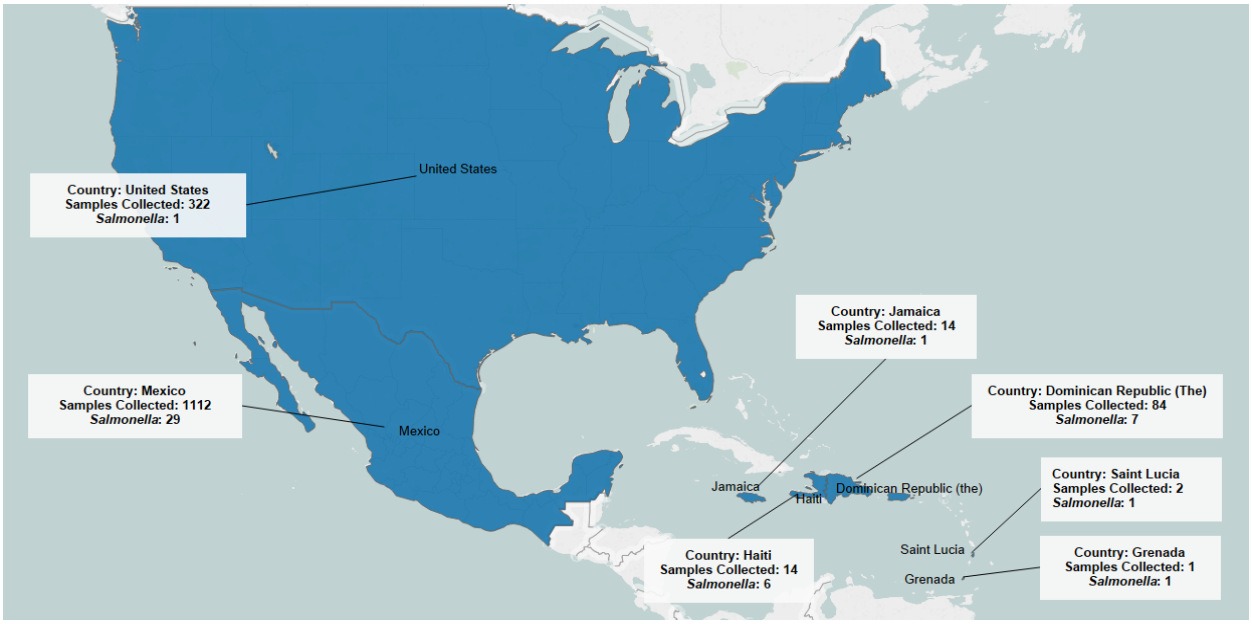


The fractions under the graph's data points provide the number(s) of positive samples out of each season's collection total. The date ranges defining the seasons are posted at the U.S. Naval Observatory site.

Pathogen Findings: By Country of Origin

The FDA obtained country-of-origin information for all samples collected. The 45 import samples that tested positive for *Salmonella* were collected from product exported to the United States from Mexico (29), the Dominican Republic (7), Haiti (6), Grenada (1), Jamaica (1), and Saint Lucia (1). Additionally, one domestic sample tested positive for *Salmonella* (Figure 2).

Figure 2: Pathogen Findings by Country of Origin



This map displays the pathogen findings by country of origin, with sources of contaminated samples in blue.

Pathogen Findings: By 'Repeat Offender' Firms (De-Identified), and Related Actions

For the purpose of this subsection, 'repeat offender' firms are defined as firms responsible for one or more positive samples during each of two or more sample collections. Thirty-seven of the 46 *Salmonella*-positive samples were not associated with 'repeat offenders.' Nine of the *Salmonella*-positive samples detected by the FDA were associated with four 'repeat offender' firms, which were placed on Import Alert 99-23 (Table 4).

Table 4: Pathogen Findings by 'Repeat Offender' Firms (De-Identified), and Related Actions

Firm ID	Firm Type	Firm Location	Collection Date *	Action
A	Grower	Dominican Republic	2/2016	Import Alert
			4/2016	Import Alert
B	Grower	Dominican Republic	2/2016	Import Alert
			3/2016	Import Alert
C	Grower	Haiti	1/2016	Import Alert
			2/2016	Import Alert
			2/2016	Import Alert
D	Grower	Mexico	11/2016	Import Alert
			5/2017	Import Alert

* Only months and years are listed to avoid identifying firms.

STATISTICAL EVALUATION

The FDA estimated the overall prevalence of *Salmonella* in hot peppers based on the data collected and, where possible, also estimated the prevalence of *Salmonella* in the commodity by origin, variety and season. The FDA found the overall prevalence of *Salmonella* in hot peppers to be 2.85 percent with a 95 percent confidence interval of 2.09 percent to 3.78 percent. The agency also observed the following:

Sample Origin: Domestic vs. Import

The prevalence of *Salmonella* in imported hot peppers was significantly higher ($p < 0.01$) than in domestically produced hot peppers based on a Fisher's Exact Test (Table 5). The FDA is not aware of other bacterial surveillance sampling studies of hot peppers that may be considered for comparison. The difference in the *Salmonella* prevalence between domestically produced and imported hot peppers may be attributable to one or more risk factors. To determine what factor(s) contribute to the difference would require further study.

Table 5: Pathogen (*Salmonella*) Findings: Domestic vs. Import

Origin	No. of Samples Collected	No. of Samples Positive	Estimated Prevalence	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Domestic	322	1	0.31%	0.01%	1.72%
Import	1,293	45	3.48%	2.55%	4.63%

Sample Origin: Country of Origin

The FDA did not design its sample collection to compare bacterial prevalence by country of origin and therefore cautions against making inferences based solely on the analytical results that follow, which are provided for informational purposes (Table 6). The table lists countries whose collection counts totaled at least 50 samples. The complete list of countries and data is provided in Appendix D: *Salmonella* Findings by Country of Origin.

Table 6: Pathogen (*Salmonella*) Findings by Country of Origin

Country	No. of Samples Collected	No. of Samples Positive	Estimated Prevalence	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Dominican Republic	84	7	8.33%	3.42%	16.42%
Mexico	1,112	29	2.61%	1.75%	3.72%
United States	322	1	0.31%	0.01%	1.72%

Hot Pepper Variety

The FDA did not design its sample collection to compare bacterial prevalence by hot pepper variety and therefore cautions against making inferences based solely on the analytical results

that follow, which are provided for informational purposes (Table 7). The table lists varieties whose collection counts totaled at least 50 samples. The complete list of varieties and data is provided in Appendix E: *Salmonella* Findings by Hot Pepper Variety.

Table 7: Pathogen (*Salmonella*) Findings by Hot Pepper Variety

Variety	No. of Samples Collected	No. of Samples Positive	Estimated Prevalence	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Anaheim	88	3	3.41%	0.71%	9.64%
Habanero	102	3	2.94%	0.61%	8.36%
Jalapeno	574	4	0.70%	0.19%	1.77%
Pasilla	95	2	2.11%	0.26%	7.40%
Poblano	91	1	1.10%	0.03%	5.97%
Serrano	285	12	4.21%	2.19%	7.24%

Season

The FDA did not design its sample collection to compare bacterial prevalence by season and therefore cautions against making inferences based solely on the analytical results that follow, which are provided for informational purposes (Table 8). Based on the data collected, there was no significant difference in the prevalence of *Salmonella* by season.

Table 8: Pathogen (*Salmonella*) Findings by Season

Season	No. of Samples Collected	No. of Samples Positive	Estimated Prevalence	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Fall	348	9	2.59%	1.19%	4.85%
Winter	493	13	2.64%	1.41%	4.47%
Spring	322	10	3.11%	1.50%	5.64%
Summer	452	14	3.10%	1.70%	5.14%

REGULATORY APPROACH

The Federal Food, Drug, and Cosmetic Act (FD&C Act) authorizes the FDA to take regulatory action regarding adulterated food. Regulatory tools at the agency's disposal include warning letters, import alerts, import refusals, administrative detentions, seizures, injunctions, suspension of registration, and mandatory recalls (if a firm does not conduct an adequate voluntary recall).

Hot peppers that test positive for *Salmonella*, *E. coli* O157:H7 or other pathogenic STEC are 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

Consistent with the FDA's mission to protect consumers, this assignment helped identify hot peppers as the likely vehicle in a multistate outbreak of salmonellosis that involved 32 people in 2016.

In April of that year, FDA field staff collected as part of this assignment a sample at a port of entry at the U.S.-Mexico border that tested positive for *Salmonella*. Whole genome sequencing of the sample determined that it was linked to the outbreak.

Upon detecting *Salmonella* in the sample, the agency refused entries of hot peppers in lots associated with the positive and placed the responsible firm and product on Import Alert 99-23. The import alert put additional controls in place for future entries. FDA field staff witnessed the destruction of half the lot that had contained the positive. Representatives of the firm reported that they discarded the other half of the lot upon learning of the contamination.

As part of the outbreak investigation, local and state health departments in seven states interviewed patients using standard foodborne illness questionnaires, which were followed by more thorough, open-ended interviews by the CDC. Fourteen of the people interviewed reported eating, or possibly eating fresh hot peppers, or reported eating an item that contained fresh hot peppers, most at restaurants. The illnesses occurred from May 6 to July 9, based on the available information. Eight people were hospitalized.[9]

FDA field staff conducted follow-up inspections at two facilities in Texas that the agency knew to be associated with the responsible firm. Upon completing the inspections, the agency cited the facilities for insanitary conditions and other violations.

The FDA also conducted a traceback investigation to trace the hot peppers eaten by the ill patients to the source of the contamination, if possible. Due to the complexities of the hot pepper supply chain, and the practice in Mexico of commingling product from multiple farms, the FDA was unable to identify a single source farm or point of contamination.

The CDC received no further reports of illness caused by the *Salmonella* strain in question (i.e., Anatum), and closed the investigation on August 19, 2016.

Other Findings

Apart from the salmonellosis outbreak, the FDA could not determine whether any of the other bacteria on the samples it collected and tested were the likely cause of human illness, due to the limited epidemiological data available.

A more detailed explanation of the FDA's genetic analysis of the 46 *Salmonella*-positive samples is provided in Appendix F: Genetic Evaluation. Of particular note in this analysis is the increasing importance of whole genome sequencing in identifying the scope and source of

[9] Hassan R, Rounds J, Sorenson A, et al. Multistate Outbreak of Salmonella Anatum Infections Linked to Imported Hot Peppers — United States, May–July 2016. Morbidity and Mortality Weekly Report 2017;66:663–667.

microbial contamination. For that reason, the FDA will continue to expand its efforts in whole genome sequencing, gradually moving away from lower resolution approaches.

In conducting this assignment, when the FDA detected *Salmonella* in samples it collected at ports of entry, it refused to admit the lots associated with those positive samples and, where the criteria was met, placed the responsible firms and product on Import Alert 99-23. The agency placed 10 firms on the import alert in all. The FDA also worked with importers to conduct five voluntary recalls. To address the domestic sample positive for *Salmonella*, the agency worked with the firm that owned the affected hot peppers to conduct a voluntary recall. Each recall removed potentially contaminated product from the marketplace, thus preventing consumption and potentially averting illnesses.

Additionally, with respect to the initial findings for hot peppers from the Dominican Republic, the FDA conducted intensified screening as a supplement to its main assignment. The results of the intensified screening were consistent with the initial findings. The FDA placed two firms in the Dominican Republic and their product on import alert. Also, given that the circumstances did not meet the FDA's criteria for a countrywide import alert, the agency worked directly with the country's Ministry of Agriculture and Fisheries, which instituted training sessions for growers, packers and transportation intermediaries. The ministry also conducted its own sampling of hot peppers in 2017 and did not detect *Salmonella*.

In response to the *Salmonella*-positive samples from Mexico, the National Agro-Alimentary Health, Safety and Quality Service (SENASICA), or state investigators acting on its behalf, conducted follow-up visits at the identified firms. The visits included sampling of water, food contact surfaces, non-food contact surfaces, hot peppers and other produce. The sampling did not detect *Salmonella* in the samples tested. SENASICA carried out environmental investigations at nine firms having the highest incidence of positive findings by the FDA, where they identified practices to be corrected. The FDA placed seven firms in Mexico and their product on Import Alert 99-23.

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 prevalence of *Salmonella*, *E. coli* O157:H7 and other STEC in hot peppers.

As detailed in the Statistical Evaluation section of this report (page 12), the assignment found the prevalence of *Salmonella* in hot peppers to be 2.85 percent with a 95 percent confidence interval of 2.09 percent to 3.78 percent. None of the samples tested positive for *E. coli* O157:H7. The FDA detected another STEC strain in one sample, but further testing determined that the strain was incapable of causing severe illness.

Due to resource constraints and anticipated challenges with sample availability, the FDA limited the primary objective of its study to determining the overall prevalence(s) of the target pathogens associated with hot peppers. Despite the limitations, the FDA also evaluated its analytical results preliminarily and throughout the assignment for signals (i.e., variations in prevalence by origin,

variety and season) to determine if more targeted sampling or further study may be warranted. The FDA did not detect any signals other than the *Salmonella* prevalence in hot peppers grown in the Dominican Republic, which the agency addressed and continues to monitor.

As to common factors among the FDA's findings, the agency performed a statistical test to determine whether the prevalence of *Salmonella* in the commodity differed based on origin (i.e., domestic vs. import). The FDA found that the prevalence of *Salmonella* in imported hot peppers (3.48%) was significantly higher than in domestically grown hot peppers (0.31%). To determine what risk factors contribute to the difference in prevalence would require further study.

Where possible the FDA also estimated the prevalence of *Salmonella* in the commodity by country of origin, variety and season. The FDA did not design its sample collection to compare prevalence by country of origin, variety or season and thus cautions against making inferences based solely on its observations by these breakdowns, which are provided for informational purposes:

Country of Origin: Among the countries from which the FDA collected 50 or more import samples, the agency found hot peppers grown in Mexico to have a *Salmonella* prevalence of 2.61 percent (29 positive, out of 1,112 tested), and hot peppers grown in the Dominican Republic to have a *Salmonella* prevalence of 8.33 percent (7 positive, out of 84 tested), based on the test data. With respect to the hot peppers from the Dominican Republic, the reliability of the estimated *Salmonella* prevalence is limited by the small number of samples.

Variety: Among the varieties of hot peppers whose collection counts totaled 50 or more samples, the estimated *Salmonella* prevalence ranged from 0.7 percent in jalapeño peppers to 4.21 percent in serrano peppers. For some varieties, the reliability of the estimated prevalence may be limited by the small sample size. Also, the differences in the estimated prevalence among the varieties may be confounded by agricultural practice, water supply, transportation intermediaries, or other elements in the production and distribution chain.

Season: There was no significant difference in the prevalence of *Salmonella* by season.

Upon detecting each positive sample, the FDA took appropriate action as warranted. The FDA refused entries of hot peppers in lots associated with each positive sample and, where the criteria was met, placed the responsible firm and product on import alert. The FDA also worked with importers to conduct five voluntary recalls. For the domestic sample that tested positive for *Salmonella*, the agency worked with the firm that owned the affected hot peppers to conduct a voluntary recall.

In addition, this assignment helped identify hot peppers as the likely vehicle in a multistate outbreak of salmonellosis that involved 32 people in 2016. Whole genome sequences from a sample collected as part of this assignment were found to be highly related to sequences of *Salmonella* strains from most of the ill patients. Upon detecting the positive sample, the FDA placed the responsible firm on import alert, preventing its future hot pepper shipments from entering the U.S. marketplace (unless subject to certain controls). The FDA also conducted a traceback investigation.

Hot peppers are a raw agricultural commodity, and the samples collected by the FDA were not packaged. As a result, when the FDA detected a pathogen in a sample, the available information did not definitively identify the point of origin of the contamination, as it could have occurred at any of several points in the supply chain. Similarly, in the 2016 outbreak, due to complexities in the hot pepper supply chain (mainly the practice of consolidators mixing product from different farms) the FDA was unable to identify the point of contamination.

The findings of this assignment underscore the need for importers of hot peppers to comply with the agency's Foreign Supplier Verification Programs Rule, as applicable. Additionally, as the FDA implements and growers and other operators comply with the agency's Produce Safety Rule, all points in the hot peppers distribution chain, including at the farm, should review the rule, and other information regarding adequately reducing pathogens in or on fresh produce, such as the FDA's "Guide to Minimize Microbial Food Safety Hazards for Fruits and Vegetables." The agency also is developing guidance to help industry comply with the Produce Safety Rule. The rule's compliance dates vary depending on farm size and type, and based on whether a farm may be eligible for a qualified exemption. The agency has posted updated information on the rule's compliance dates.

The FDA will continue to sample hot peppers, including targeted surveillance sampling of imported product from countries of interest. As part of the targeted import sampling, the FDA will use its PREDICT tool, which aids entry reviewers in targeting higher-risk shipments for examination.

Underpinning the objectives of this assignment, the FDA's intent was to strengthen its understanding of the public health issues associated with hot peppers 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 FDA will continue to evaluate methods to reduce microbial contamination of hot peppers. The presence of harmful bacteria in the commodity remains a concern to the FDA in view of this assignment's findings and the history of reported outbreaks associated with hot peppers. Hot peppers require appropriate protection from environmental pathogens during growing, harvesting, packing and holding, as this study confirms.

APPENDIX A: SAMPLE COLLECTION BY HOT PEPPER VARIETY

The FDA collected samples of 36 different varieties of hot peppers, as well as some that it categorized as "unspecified" and others that it categorized as a "mix." All are listed in the table that follows.

Variety	No. of Samples Collected	Percent of Total Collection
Ají	5	0.3%
Anaheim	88	5.4%
Árbol	7	0.4%
Banana	6	0.4%
Cachucha	4	0.2%
California	18	1.1%
Caribe	23	1.4%
Cayenne	1	0.1%
Cherry	5	0.3%
Chilaca	6	0.4%
Cubanelle	4	0.2%
Finger Hot Pepper	6	0.4%
Fresno	26	1.6%
Green Chili *	28	1.7%
Habanero	102	6.3%
Hatch Green Chili	10	0.6%
Hungarian	16	1.0%
Indian Chili *	1	0.1%
Jalapeño	574	35.5%
Jamaican	6	0.4%
Japanese *	7	0.4%
Korean *	5	0.3%
Long Chili *	9	0.6%
Manzano	12	0.7%
Mix (Green & Red) *	1	0.1%
Orange Chili *	1	0.1%
Padrón	1	0.1%
Pasilla	95	5.9%
Pimento	2	0.1%
Poblano	91	5.6%
Pueblo Chili	1	0.1%
Red Chili *	21	1.3%
Scotch Bonnet	10	0.6%
Serrano	285	17.6%
Shishito	9	0.6%
Thai Chili *	46	2.8%
Yellow Chili *	23	1.4%
Unspecified	60	3.7%
Total	1,615	100%

* Variety not further specified on labeling information, invoices or bills of lading.

APPENDIX B: TEST METHODS

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

Salmonella

A soak method was used to detect *Salmonella* contamination on the hot peppers. The analysts soaked the samples in a pre-enrichment broth of modified buffered peptone water (without blending) and incubated them for 24 hours at 35 degrees Celsius. The analysts then used VIDAS *Salmonella* SLM (OMA 2004.03) or VIDAS *Salmonella* Easy (2011.03) methods to detect *Salmonella*. The FDA's Bacteriological Analytical Manual (chapter 5) culture method for *Salmonella* was then used to confirm the VIDAS results. Sample enrichments positive for *Salmonella* were plated onto selective/differential agars. Isolates were confirmed, serotyped, and subtyped using SNP base whole genome sequence analysis.

E. coli O157:H7, STEC

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

The FDA's Bacteriological Analytical Manual (BAM) method for STEC and *E. coli* O157:H7 is a polymerase chain reaction (PCR) assay that tests specifically for the *stx* genes and for genes in the O157:H7 serotype. The sample preparation procedure used is described in Chapter 4A of the BAM. Briefly, 25-gram samples of product are mixed with 225 milliliters of enrichment medium containing antibiotic that selects for the growth of STEC. After enrichment overnight, DNA was extracted from an aliquot of the enrichment and tested by PCR. The sample found to be positive for *stx* or O157:H7-specific genes was plated onto agar media to isolate the bacteria and confirmed for STEC or O157:H7 using biochemical, serological and genetic assays.

APPENDIX C: *SALMONELLA*-POSITIVE SAMPLES BY SEROTYPE

Organized by sample ID, the table that follows provides the serotype of each strain of *Salmonella* detected. Some samples produced multiple isolates. Antigenic formulas are provided in cases where the FDA observed unnamed serotypes.

Sample ID	Isolate No. 1	Isolate No. 2	Isolate No. 3	Isolate No. 4	Isolate No. 5	Isolate No. 6	Isolate No. 7	Isolate No. 8
720675	Antigenic Formula IV 45: z36,z38:-							
792027	Muenster							
931293	Typhimurium							
936278	Michigan	Michigan						
938426	Tucson							
941569	Minnesota							
941573	Aberdeen	Aberdeen						
944804	Aberdeen	Muenster						
945548	Antigenic Formula IV 42:z36:-							
945549	Corvallis	Corvallis	Uganda					
947564	Weltevreden							
948105	Aberdeen							
948463	Uganda							
948887	Luciana	Oranienburg	Oranienburg					
950816	Pharr							
951331	Antigenic Formula IV 48:z4,z32:-							
955374	Aberdeen							
956658	Antigenic Formula IIIa 42:g,z51:-							
957831	Anatum	Anatum						
958119	Rubislaw							
960226	Aberdeen							
963008	Newport							

Sample ID	Isolate No. 1	Isolate No. 2	Isolate No. 3	Isolate No. 4	Isolate No. 5	Isolate No. 6	Isolate No. 7	Isolate No. 8
968399	Denver	Denver						
970184	Braenderup	Braenderup	Braenderup	Braenderup				
973803	Antigenic Formula IIIa 41:z4,z24:-	Antigenic Formula IIIa 41:z4,z24:-	Antigenic Formula IIIa 41:z4,z24:-	Antigenic Formula IIIa 41:z4,z24:-	Antigenic Formula IIIa 53:z4,z23:-			
973992	Newport	Newport	Newport					
974577	Antigenic Formula IIIa 21:z4,z23:-	Antigenic Formula IIIa 21:z4,z23:-						
975328	Newport	Newport						
975575	Antigenic Formula IIIa 63:z4,z23:-	Antigenic Formula IIIa 63:z4,z23:-	Antigenic Formula IIIa 63:z4,z23:-					
975618	Antigenic Formula IIIa 63:z4,z23:-	Antigenic Formula IIIa 63:z4,z23:-						
975808	Newport	Newport						
976150	Newport	Newport						
976361	Rubislaw							
977679	Bama	Bama	Bama	Bama	Bama			
977927	Anatum	Anatum						
982708	Pomona							
986113	Luciana	Luciana	Luciana					
986320	Weltevreden							
986363	Javiana							
986369	Michigan	Michigan	Michigan	Michigan	Michigan	Michigan	Michigan	Michigan
987431	Soerenga	Soerenga						
988195	Muenchen	Muenchen						
994956	Rubislaw	Rubislaw	Rubislaw					
996570	Infantis							
1001470	Antigenic Formula IIIb 60:r:e,n,x,z15							
1012070	Minnesota							

APPENDIX D: ESTIMATED *SALMONELLA* PREVALENCE BY COUNTRY OF ORIGIN

The table below lists estimated *Salmonella* prevalences by country or origin, based on this study's test results. Confidence interval upper and lower bounds also are provided. The FDA did not design its sample collection to be representative by country of origin and therefore cautions against making inferences based solely on the information in the table.

Country	No. of Samples Collected	No. of Samples Positive	Estimated Prevalence	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Belgium	14	0	0.00%	0.00%	23.16%
Canada	7	0	0.00%	0.00%	40.96%
Dominican Republic	84	7	8.33%	3.42%	16.42%
Grenada	1	1	100.00%	2.50%	100.00%
Haiti	14	6	42.86%	17.66%	71.14%
Honduras	8	0	0.00%	0.00%	36.94%
Israel	3	0	0.00%	0.00%	70.76%
Jamaica	14	1	7.14%	0.18%	33.87%
Mexico	1,112	29	2.61%	1.75%	3.72%
Netherlands	19	0	0.00%	0.00%	17.65%
Peru	1	0	0.00%	0.00%	97.50%
Saint Lucia	2	1	50.00%	1.26%	98.74%
Trinidad & Tobago	14	0	0.00%	0.00%	23.16%
United States	322	1	0.31%	0.01%	1.72%
Total	1,615	46	2.85%	2.09%	3.78%

APPENDIX E: *SALMONELLA* FINDINGS BY HOT PEPPER VARIETY

The table below lists estimated *Salmonella* prevalences by hot pepper variety, based on this study's test results. Confidence interval upper and lower bounds also are provided. The FDA did not design its sample collection to be representative by variety and therefore cautions against making inferences based solely on the information in the table.

Variety	No. of Samples Collected	No. of Samples Positive	Estimated Prevalence	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Ají	5	2	40.00%	5.27%	85.34%
Anaheim	88	3	3.41%	0.71%	9.64%
Árbol	7	0	0.00%	0.00%	40.96%
Banana	6	0	0.00%	0.00%	45.93%
Cachucha	4	0	0.00%	0.00%	60.24%
California	18	0	0.00%	0.00%	18.53%
Caribe	23	1	4.35%	0.11%	21.95%
Cayenne	1	0	0.00%	0.00%	97.50%
Cherry	5	0	0.00%	0.00%	52.18%
Chilaca	6	0	0.00%	0.00%	45.93%
Cubanelle	4	0	0.00%	0.00%	60.24%
Finger Hot Pepper	6	1	16.67%	0.42%	64.12%
Fresno	26	0	0.00%	0.00%	13.23%
Green Chili*	28	6	21.43%	8.30%	40.95%
Habanero	102	3	2.94%	0.61%	8.36%
Hatch Green Chili	10	0	0.00%	0.00%	30.85%
Hungarian	16	0	0.00%	0.00%	20.59%
Indian Chili*	1	0	0.00%	0.00%	97.50%
Jalapeño	574	4	0.70%	0.19%	1.77%
Jamaican	6	0	0.00%	0.00%	45.93%
Japanese*	7	0	0.00%	0.00%	40.96%
Korean*	5	0	0.00%	0.00%	52.18%
Long Chili*	9	0	0.00%	0.00%	33.63%
Manzano	12	0	0.00%	0.00%	26.46%
Mix (Green & Red)*	1	0	0.00%	0.00%	97.50%
Orange Chili*	1	0	0.00%	0.00%	97.50%
Padrón	1	0	0.00%	0.00%	97.50%
Pasilla	95	2	2.11%	0.26%	7.40%
Pimento	2	1	50.00%	1.26%	98.74%
Poblano	91	1	1.10%	0.03%	5.97%
Pueblo Chili	1	0	0.00%	0.00%	97.50%
Red Chili*	21	0	0.00%	0.00%	16.11%
Scotch Bonnet	10	1	10.00%	0.25%	44.50%
Serrano	285	12	4.21%	2.19%	7.24%
Shishito	9	0	0.00%	0.00%	33.63%
Thai Chili*	46	6	13.04%	4.94%	26.26%
Unspecified	60	2	3.33%	0.41%	11.53%
Yellow Chili*	23	1	4.35%	0.11%	21.95%

* Variety not further specified on labeling information, invoices or bills of lading.

APPENDIX F: GENETIC EVALUATION

This section describes the FDA's further analysis of the samples that tested positive for *Salmonella* – and their comparison to clinical isolates – to determine whether those bacteria, or microorganisms 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 *Salmonella* findings.

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.

Information on disease surveillance in the United States is available at 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.

Importantly, while the FDA uses indistinguishable PFGE patterns to cluster genetically similar bacterial strains and investigate potential foodborne illness outbreaks, other information, usually food histories from ill persons and isolates from the site where the food was grown or processed, are needed to determine that an adulterated food caused an illness, or multiple illnesses in the case of an outbreak.

The FDA's evaluation found considerable diversity among the PFGE patterns of the species of *Salmonella* detected. Specifically, the 46 samples that tested positive for *Salmonella* produced 47 PFGE patterns across 88 isolates. Upon querying the PulseNet USA database, the FDA found that 19 PFGE patterns in the food isolates were associated with clinical entries. The database query covered the period from January 13, 2016 (i.e., shortly after the agency detected the first *Salmonella*-positive hot pepper sample) to December 13, 2017. The agency's findings are as follows:

- Four isolates from four hot pepper samples, all with the inferred PFGE pattern[1] of ADNX01.0008, were indistinguishable by PFGE from three clinical isolates uploaded from two states. Upon detecting the positives, the FDA refused entries of hot peppers in

[1] An inferred PFGE pattern is a PFGE pattern that has not been officially named by the CDC but is identical to a PFGE pattern found in another sample that has been officially named by the CDC.

lots associated with the positives. The agency placed one firm, from which two of the samples had originated, on import alert. Additionally, the agency conducted intensified screening of shipments of hot peppers from the other two responsible firms.

- Three isolates from one hot pepper sample, all with the inferred PFGE pattern of ADNX01.0023, were indistinguishable by PFGE from one clinical isolate uploaded from one state. Upon detecting the positive, the FDA refused entries of hot peppers in lots associated with the positive and placed the responsible firm and product on import alert.

- One isolate from one hot pepper sample, with the PFGE pattern of JAX01.0188, was indistinguishable by PFGE from 30 clinical isolates uploaded from 10 states. Upon detecting the positive, the FDA refused entries of hot peppers in lots associated with the positive and placed the responsible firm and product on import alert.

- Four isolates from one hot pepper sample, all with the PFGE pattern of JBPX01.0104, were indistinguishable by PFGE from 21 clinical isolates uploaded from 11 states. Upon detecting the positive, the FDA refused entries of hot peppers in lots associated with the positive and conducted intensified screening of shipments of hot peppers from the responsible firm.

- Two isolates from one hot pepper sample, both with the inferred PFGE pattern of SCVX01.0010, were indistinguishable by PFGE from six clinical isolates uploaded from four states. Upon detecting the positive, the FDA refused entries of hot peppers in lots associated with the positive and placed the responsible firm and product on import alert.

- Two isolates from one hot pepper sample, both with an unnamed PFGE pattern for *Salmonella* Denver, were indistinguishable by PFGE from one clinical isolate uploaded from one state. Upon detecting the positive, the FDA refused entries of hot peppers in lots associated with the positive, worked with the importer to carry out a recall, and conducted intensified screening of shipments of hot peppers from the responsible firm.

- One isolate from one hot pepper sample, with an unnamed PFGE pattern for *Salmonella* IIIa 53:z4,z23:-, was indistinguishable by PFGE from one clinical isolate uploaded from one state. Upon detecting the positive, the FDA refused entries of hot peppers in lots associated with the positive and placed the responsible firm and product on import alert.

- One isolate from one hot pepper sample, with the PFGE pattern of JFXX01.0070, was indistinguishable by PFGE from 11 clinical isolates uploaded from eight states. Upon detecting the positive, the FDA refused entries of hot peppers in lots associated with the positive and conducted intensified screening of shipments of hot peppers from the responsible firm.

- One isolate from one hot pepper sample, with the PFGE pattern of JGGX01.0032, was indistinguishable by PFGE from 16 clinical isolates uploaded from 12 states. Upon detecting the positive, the FDA refused entries of hot peppers in lots associated with the positive and placed the responsible firm and product on import alert.

- Eight isolates from one hot pepper sample, all with the inferred PFGE pattern of MCHX01.0001, were indistinguishable by PFGE from 15 clinical isolates uploaded from 10 states. Upon detecting the positive, the FDA worked with the importer to carry out a recall and placed the responsible firm and product on import alert.

- Four isolates from one hot pepper sample, all with the PFGE pattern of JJPX01.0438, were indistinguishable by PFGE from 148 clinical isolates uploaded from 27 states. Upon detecting the positive, the FDA refused entries of hot peppers in lots associated with the positive and conducted intensified screening of shipments of hot peppers from the responsible firm.

- One isolate from one hot pepper sample, with the PFGE pattern of JJPX01.0613, was indistinguishable by PFGE from three clinical isolates uploaded from two states. Upon detecting the positive, the FDA refused entries of hot peppers in lots associated with the positive and conducted intensified screening of shipments of hot peppers from the responsible firm.

- Three isolates from one hot pepper sample, all with the PFGE pattern of JJPX01.3473, were indistinguishable by PFGE from 33 clinical isolates uploaded from 21 states. Upon detecting the positive, the FDA refused entries of hot peppers in lots associated with the positive and conducted intensified screening of shipments of hot peppers from the responsible firm.

- Two isolates from one hot pepper sample, both with the PFGE pattern of JJXX01.0134, were indistinguishable by PFGE from four clinical isolates uploaded from three states. Upon detecting the positive, the FDA refused entries of hot peppers in lots associated with the positive and placed the responsible firm and product on import alert.

- One isolate from one hot pepper sample, with the PFGE pattern of JLPX01.0059, was indistinguishable by PFGE from 228 clinical isolates uploaded from 20 states. Upon detecting the positive, the FDA worked with the importer to carry out a recall and placed the responsible firm and product on import alert.

- Two isolates from one hot pepper sample, both with the inferred PFGE pattern of SRNX01.0003, were indistinguishable by PFGE from eight clinical isolates uploaded from six states. Upon detecting the positive, the FDA refused entries of hot peppers in lots associated with the positive and placed the responsible firm and product on import alert.

- One isolate from one hot pepper sample, with the PFGE pattern of JPXX01.1996, was indistinguishable by PFGE from six clinical isolates uploaded from four states. Upon detecting the positive, the FDA worked with the responsible firm to carry out a recall.

- One isolate from one hot pepper sample, with the PFGE pattern of TDWX01.0117, was indistinguishable by PFGE from 11 clinical isolates uploaded from 10 states. Upon

detecting the positive, the FDA refused entries of hot peppers in lots associated with the positive and placed the responsible firm and product on import alert.

- One isolate from one hot pepper sample, with the unnamed PFGE pattern for *Salmonella* Weltevreden, was indistinguishable by PFGE from one clinical isolate uploaded from one state. Upon detecting the positive, the FDA refused entries of hot peppers in lots associated with the positive and conducted intensified screening of shipments of hot peppers from the responsible firm.

- One isolate from one hot pepper sample, with the PFGE pattern of JQPX01.0048, was indistinguishable by PFGE from 20 clinical isolates uploaded from seven states. Upon detecting the positive, the FDA refused entries of hot peppers in lots associated with the positive and placed the responsible firm and product on import alert.

Other than the information gathered during the 2016 salmonellosis outbreak (described in this report's Public Health Impact section, page 14), no epidemiological information was available to link the clinical entries to hot peppers. However, upon detecting each positive sample, the FDA took appropriate action to keep contaminated products from reaching consumers, as described above.

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.

The FDA compared the whole genome sequences of the 46 samples that tested positive for *Salmonella* with sequences from environmental and clinical isolates, and other food isolates, all housed in a database at the National Center for Biotechnology Information.

The FDA found sequences from nine of the 46 samples to be highly related to sequences from clinical isolates, suggesting that the clinical isolates and those obtained from the hot peppers may have originated from common sources of contamination. Of those nine samples, one was the sample associated with the salmonellosis outbreak. Of the other eight samples, the sequences of seven were highly related not only to sequences from clinical isolates but also to sequences from other isolation sources (both food and environmental), meaning the illnesses may have been caused by one of the other food vehicles, or by an environmental source, as shown in the table that follows. For the one remaining sample, hot peppers were the only isolation source, but attribution could not be assigned absent epidemiological information.

Sample ID	Sample Origin	Isolation Source(s)	No. of Matching Clinical Isolates
987431	Mexico	alfalfa sprouts/seeds, beef, hot peppers, pork	9
945549*	Dominican Republic	fish, hot peppers, pistachios, pork	92†
945549* 948463	Dominican Republic	beef, chicken, hot peppers, swine	58‡
973992	Mexico	almonds, beef, environmental swabs, hot peppers, pork	144
970184	Mexico	Beef, chicken, hot peppers, pork	118
957831**	Mexico	animal feed, cilantro, hot peppers, soil/water	33
960226	Jamaica	avocado, curry powder, hot peppers, tea	1
975808	Mexico	hot peppers	2
986363	Mexico	cucumbers, hot peppers	2

* This sample contained two *Salmonella* strains, each highly related to strains from separate clinical isolates.
** This sample was associated with the 2016 salmonellosis outbreak.
† Fifty-six of these illnesses occurred in England (i.e., the isolates were listed by Public Health England).
‡ Eight of these illnesses occurred in England (i.e., the isolates were listed by Public Health England).