



U.S. FOOD & DRUG
ADMINISTRATION

FY 2016 – 2017 Microbiological Sampling Assignment
Summary Report: Cucumbers

Office of Compliance
Center for Food Safety and Applied Nutrition

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EXECUTIVE SUMMARY

The U.S. Food and Drug Administration (FDA) set out to collect and test cucumbers 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 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 cucumbers assignment in November 2015 under its then new sampling model. The agency collected 1,601 samples to test to determine the prevalence of *Salmonella* spp. and *Escherichia coli* (*E. coli*) O157:H7 in the commodity. The agency collected about 76 percent of its samples from imported cucumbers and the rest from domestically produced cucumbers, comparable to their respective U.S. market shares at the start 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.

The FDA collected cucumbers grown in fields and greenhouses, with all varieties of the commodity eligible to be sampled. The agency did not collect frozen, chopped, sliced, pureed or pickled cucumbers, or those that it knew to be intended for a 'kill step' to eliminate pathogens. The agency found the prevalence of *Salmonella* in the samples collected to be 1.75 percent, based on the test results. The FDA did not detect *E. coli* O157:H7 (or other pathogenic *E. coli*) in any samples.

Consistent with the FDA's mission to protect consumers, this assignment helped identify cucumbers as the vehicle in an outbreak of salmonellosis that involved 10 people in three states in 2016. Three patients were hospitalized during the outbreak, which is summarized in the Public Health Impact section of this report (page 12).

When the FDA detected *Salmonella* in domestic samples, the agency worked with the firm(s) that owned or distributed the affected cucumbers to conduct a voluntary recall, although in some cases there was no product to recall, or low likelihood of product available to recall due to the commodity's relatively short shelf life. In instances where no recall was carried out, the agency provided the firm with guidance on minimizing microbial hazards in fruits and vegetables and shared its findings with state partners (as is done with samples that result in recalls).

When the FDA detected *Salmonella* in samples collected at ports of entry, the agency refused to admit the lot(s) associated with the positive samples and, where the criteria were met, placed the responsible firm(s) and product on [Import Alert 99-23](#), "Detention without Physical Examination of Produce Due to Contamination with Human Pathogens." In all, the agency placed seven firms on the import alert as a result of this sampling assignment. The agency also conducted intensified

screening (i.e., additional sampling) of cucumbers from 10 foreign firms whose products tested positive at entry. One voluntary recall was conducted by an importer.

The findings of this assignment affirm that *Salmonella* may be present on cucumbers and so underscore the need for growers and others in the distribution chain to comply with the FDA's Produce Safety Rule,¹ as applicable, and for importers to comply with the FDA's Foreign Supplier Verification Programs Rule,² as applicable. Cucumbers require appropriate protection from human pathogens during growing, harvesting, packing and holding. The FDA intends to engage cucumber growers and distributors to make them aware of the findings of this assignment and to provide additional information on resources on steps to mitigate for contamination of cucumbers.

Consumers can take simple steps to reduce any possible microbial risks related to the consumption of cucumbers. [Foodsafety.gov](https://www.foodsafety.gov) recommends that consumers "wash all produce thoroughly under running water before eating, cutting or cooking." The site also advises, "Even if you plan to cut the rind or peel off the produce before eating, it is still important to wash it first so dirt and bacteria aren't transferred from the knife onto the fruit." The site furthermore advises consumers to scrub firm produce (including cucumbers) with a clean produce brush, and then dry it with a clean cloth towel or paper towel to further reduce bacteria that may be present.

The FDA will continue to evaluate methods to prevent microbial contamination of cucumbers. Such contamination remains a concern to the agency given this assignment's findings and the history of outbreaks associated with cucumbers. *Salmonella* is able to survive on cucumbers past their recommended shelf life at refrigeration temperatures.³ Additionally, cucumbers are a ready-to-eat food, meaning consumers do not typically subject them to a kill step (such as cooking) prior to consumption.

The FDA will continue to sample cucumbers for pathogens. Cucumbers are covered produce under the Produce Safety Rule, and among its next steps, the agency and its state partners began inspections of large produce farms for compliance with the Produce Safety Rule in the spring of 2019. The agency also will continue to sample imported cucumbers, including targeted sampling of product from countries of interest. With respect to the sampling of imported product, the FDA will use its [PREDICT tool](#), which identifies shipments of interest based on certain risk criteria, to target future cucumber sampling efforts. In addition, the FDA will sample cucumbers using its longstanding approach to food sampling, which centers on (but is not limited to) the following criteria:

- A firm has a history of unmitigated microbial contamination in the environment (e.g., as evidenced by being a confirmed source of human illness, recalled or seized product, prior

¹ [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.

² [The Foreign Supplier Verification Programs Rule](#) requires that importers perform certain risk-based activities to verify that food imported into the U.S. has been produced in a manner that meets applicable U.S. safety standards.

³ Bardsley, C. A., Truitt, L. N., Pfunter, R. C., Danyluk, M. D., Rideout, S. L., & Strawn, L. K. (2019). [Growth and Survival of *Listeria monocytogenes* and *Salmonella* on Whole and Sliced Cucumbers](#). *Journal of Food Protection*, Vol. 82, No. 2, 301 to 319.

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 cucumbers 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 prevent contamination by disease-causing microorganisms.

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 out of 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 of cucumbers.

Why Cucumbers?

From 2009 to 2014, federal and state public health agencies linked three outbreaks of *Salmonella* infections and one outbreak of *E. coli* O157:H7 infections in the United States to the consumption of cucumbers contaminated with those pathogens. Cucumbers were confirmed to be the food vehicle

in one of the *Salmonella* outbreaks and suspected to be the food vehicle in the *E. coli* O157:H7 and two other *Salmonella* outbreaks. The four outbreaks resulted in 260 illnesses and 32 hospitalizations.⁴

Additionally, a nationwide outbreak of *Salmonella* Poona infections associated with cucumbers was underway in 2015 when the FDA selected cucumbers for surveillance sampling. That outbreak resulted in 907 reported illnesses, 204 hospitalizations, and six deaths. The U.S. Centers for Disease Control and Prevention (CDC) reported that [the outbreak](#) appeared to be over as of March 2016.

Cucumbers may be exposed to contaminated water, soil, animals or equipment during growing, harvesting, and/or post-harvest activities. Cucumbers also may be handled by workers who may transmit pathogens. Prior to this assignment, the FDA had limited data on the prevalence of *Salmonella* and *E. coli* O157:H7 in cucumbers. Given the circumstances, the FDA saw a need to better understand the prevalence of these pathogens in the commodity, and if possible, to identify common factors among contaminated samples with the goal of helping to protect consumers.

Cucumber Production

Cucumbers are grown in both tropical and temperate climates, and thus in much of the world. Mexico and the United States are the biggest commercial producers of cucumbers in the Western Hemisphere. Domestically grown cucumbers made up about 27 percent of the U.S. market and imported cucumbers made up about 73 percent of the U.S. market in 2015, the year leading up to this sampling assignment.^{5,6} Most of the cucumbers imported by the United States are harvested in Mexico.

Cucumbers are grown in fields and greenhouses. Production usually involves two or more harvests from the same plantings. The multiple harvests can be a possible risk factor that can contribute to contamination. As entry to growing operations by farm workers or farm equipment increases, the probability of contamination may increase. Other possible risk factors include animal intrusion into growing fields, improper cleaning or sanitizing of food contact surfaces (such as harvesting totes), use of agricultural water that is not safe and of adequate quality, and, if a customer requirement, waxing that may trap bacteria on the surface of the cucumbers.

Cucumber Types

There are three basic types of cucumbers: slicing, pickling and seedless. Slicing cucumbers are the type most commonly available in grocery stores in the United States. Grown to be eaten fresh, slicing cucumbers range from six to nine inches long, have soft, edible seeds, and may be waxed to preserve moisture and reduce abrasion. Pickling cucumbers are grown for flavor and have a longer shelf life. They tend to be shorter, thicker and less uniformly shaped. Pickling cucumbers are never waxed, as the wax coating can interfere with the pickling process. Seedless

⁴ FDA. *Outbreaks and Illnesses Associated with FDA-Regulated Produce, 1996 – 2014*. Reference Type: Query Return of CORE Outbreak Database.

⁵ USDA National Agricultural Statistics Service. (2015). National Statistics for “Cucumbers, Fresh Market – Production, Measured in CWT” [Data Query]. Retrieved from [NASS website](#) on August 24, 2018.

⁶ USDA Economic Research Service. (2015). U.S. Imports by Volume: Fresh or Chilled Cucumbers [Data Query]. Retrieved from [ERS website](#) on August 24, 2018.

cucumbers are commonly thin and smooth-skinned and grow to up to two feet in length. They tend to be sweeter than the other types and may be seedless or nearly so. Seedless cucumbers also are called “English cucumbers” because several varieties of that type originated in England, or elsewhere in Europe. Cucumbers are fruit by scientific classification.

OBJECTIVES

The objectives of the FDA’s FY2016-2017 cucumbers sampling assignment were:

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

SAMPLE COLLECTION

The FDA collected 1,601 cucumber samples from November 2015 to October 2017 for this assignment. The samples were collected in proportions comparable to their respective U.S. market share based on origin (i.e., domestic vs. import).

Agency field staff collected samples one at a time from individual lots and multiple lots. In instances where the collection site featured multiple lots, the field staff generally collected one sample from each lot. The FDA’s approach, which avoided commingling samples from different lots, was designed to help the agency 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 analysis of cross sections of establishment types (e.g., packinghouses, distribution centers, and retail stockrooms) for the domestic samples and countries of origin for the import samples. The FDA collected the samples over nearly two years, also enabling the capture of seasonal data.

The FDA did not collect cucumbers that were frozen, chopped, sliced, pureed or pickled, or that it knew to be intended for a ‘kill step’ to eliminate pathogens, such as blanching.

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 384 domestic samples of cucumbers, with most of them collected at distribution centers and warehouses (Table 1). Samples were collected

in 36 states, with the largest number collected in Michigan (42), followed by New York (38), and California (28).

Table 1: Domestic Sample Collection Sites

Collection Site	Domestic Samples Collected	Percentage of Domestic Samples	Percentage of All Samples
Distribution Center/Warehouse	204	53%	13%
Packinghouse/Repacker	51	13%	3%
Farm/Growing Operation*	80	21%	5%
Retail	49	13%	3%
Total	384	100%	24%

* Samples collected at farms or growing operations were obtained post-harvest from product holding facilities.

Import Sample Collection

As directed by the assignment, the field staff collected 1,217 import samples, most of which were grown in Mexico. The agency used two approaches to collect import samples: port-of-entry and domestic import (DI) collection. Of the total, 956 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, 261 samples (21 percent) were collected as DI samples and counted toward the import sample total. DI samples are samples of foreign origin 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 stockrooms, prior to consumer handling. Unlike samples collected at ports of entry, 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 as import sample data because they originated outside of the United States (Table 2).

Table 2: Import Sample Collection Sites

Collection Site	Import Samples Collected	Percentage of Import Samples	Percentage of All Samples
Port of Entry	956	79%	60%
Domestic Import	261	21%	16%
Total	1,217	100%	76%

The FDA collected import samples from eight countries. The large majority were grown in Mexico (1,049), followed by Canada (44), Honduras (33), the Netherlands (30), the Dominican Republic (28), Spain (25), Guatemala (6), and the Bahamas (2).

Sample Collection by Season

The FDA was able to collect samples year-round. Cucumbers are field-grown throughout much of the year in Central America and the Caribbean, and many operations in the United States and Canada grow them in greenhouses. The agency collected most of its samples in the summer of 2016 (443 samples). Other sizeable collections, in descending order by volume, were collected in

the fall of 2016 (303 samples), the spring of 2016 (293 samples), and the winter of 2015 (222 samples).

Sample Composition

Each sample consisted of 20 subsamples, and each subsample consisted of one pound of cucumbers. The FDA divided the subsamples evenly for testing purposes, testing half for *Salmonella* and half for *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 entire sample as positive for the organism.

PATHOGEN FINDINGS

This section provides the overall prevalence(s) of *Salmonella* and *E. coli* O157:H7, as well as other findings. The test methods the FDA employed are described in [Appendix A: Test Methods](#).

Salmonella

The FDA detected *Salmonella* in 28 samples, a prevalence of 1.75 percent. Of the total number of *Salmonella* positives, 17 were import samples, and 11 were domestic samples. The most common serotypes were *Salmonella* Newport and *Salmonella* Saintpaul, which the FDA detected in five and four samples, respectively. The complete list of the *Salmonella* positives by serotype is provided in [Appendix B: *Salmonella*-Positive Samples by Serotype](#).

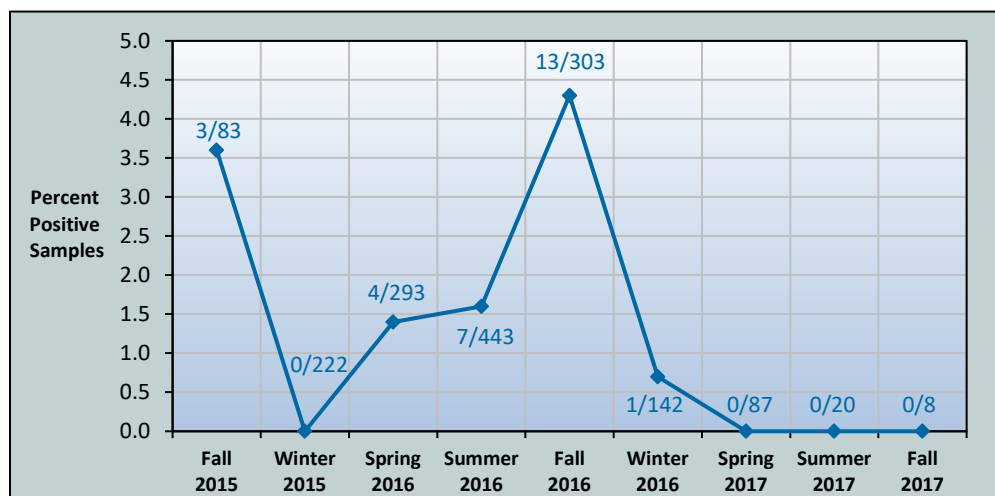
***E. coli* O157:H7**

None of the samples tested positive for *E. coli* O157:H7, or any other pathogenic *E. coli*.

By Season

The FDA detected *Salmonella* in each season, with most of the positives observed in the fall (Figure 1). The agency found the fall of 2016 to be the season with the highest percentage of positive samples, approximately 4.3 percent. Please see the “Statistical Evaluation” section on page 11 for further discussion of these findings.

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



The fractions in the graph's plot area report the number(s) of samples that tested positive for *Salmonella* out of the total number of samples collected for the indicated season. The date ranges defining the seasons may be obtained at the [U.S. Naval Observatory](#) site.

By Origin

The FDA obtained country-of-origin information for all samples collected. The 28 samples that tested positive for *Salmonella* were grown in Mexico (17) and the United States (11).

By Variety

The FDA is unable to report the pathogen findings by variety. While all varieties of the commodity were eligible for collection, it was not within the scope of this assignment to document the varieties.

By 'Repeat Violation' Firms (De-Identified), and Related Actions

For purposes of this subsection, 'repeat violation' firms are defined as physical locations where the agency detected one or more positive samples during each of two or more sample collections. Twenty-six of the 28 samples were not associated with 'repeat violation' firms.

A single firm in Mexico met the FDA's definition of 'repeat violation' under this assignment. Specifically, the FDA detected *Salmonella* in two imported cucumbers grown by the same firm, which the agency collected in June and November 2016, respectively, at a port of entry. The FDA placed the firm on [Import Alert 99-23](#) and worked with the importer to voluntarily recall the affected cucumbers.

STATISTICAL EVALUATION

The FDA estimated the overall prevalence of *Salmonella* in cucumbers based on the data collected under this assignment and, where possible, also estimated the prevalence of *Salmonella* in the commodity by origin and season.

Overall Prevalence

The FDA found the prevalence of *Salmonella* in cucumbers to be 1.75 percent with a 95 percent confidence interval of 1.17 percent to 2.52 percent (Table 3).

Table 3: *Salmonella* Findings

Samples Collected	Samples Positive	Estimated Prevalence	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
1,601	28	1.75%	1.17%	2.52%

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.

The prevalence of *Salmonella* in domestically grown cucumbers was 2.86 percent with a 95 percent confidence interval of 1.44 percent to 5.07 percent, and the prevalence of *Salmonella* in imported cucumbers was 1.4 percent with a 95 percent confidence interval of 0.82 percent to 2.23 percent (Table 4).

Table 4: *Salmonella* Findings: Domestic vs. Import

Origin	Samples Collected	Samples Positive	Estimated Prevalence	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Domestic	384	11	2.86%	1.44%	5.07%
Import	1,217	17	1.40%	0.82%	2.23%

The FDA also calculated the *Salmonella* prevalence in Mexican cucumbers given that all 17 of the import samples positive for the pathogen were grown in that country. The prevalence of *Salmonella* in cucumbers from Mexico was 1.62 percent with a 95 percent confidence interval of 0.95 percent to 2.58 percent (Table 5).

Table 5: *Salmonella* Findings: Mexico

Samples Collected	Samples Positive	Estimated Prevalence	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
1,049	17	1.62%	0.95%	2.58%

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 6). The prevalence of *Salmonella* in cucumbers collected in the fall was higher than the other seasons. Fisher’s Exact Test shows that the difference was significant in each case: fall vs. spring ($p = 0.01$); fall vs. winter ($p = 0.0003$); and fall vs. summer ($p = 0.03$). Additional study would be required to determine what factors contribute to the difference(s). The FDA will endeavor to identify any factors that may be contributing to the differences in seasonal findings through discussions with cucumber growers and distributors and through examining the findings of Produce Safety Rule inspections.

Table 6: *Salmonella* Findings by Season

Season	Samples Collected	Samples Positive	Estimated Prevalence	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Fall	394	16	4.06%	2.34%	6.51%
Winter	364	1	0.27%	0.01%	1.52%
Spring	380	4	1.05%	0.29%	2.67%
Summer	463	7	1.51%	0.61%	3.09%

The FDA is not aware of other published bacterial surveillance sampling studies of cucumbers that may be considered for comparison.

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).

Cucumbers that test positive for *Salmonella*, *E. coli* O157:H7 or other pathogenic Shiga toxin-producing *E. coli* 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, the agency analyzed the *Salmonellae* detected in the cucumber samples to identify their genetic patterns and determine whether those pathogens may be linked to human illness.

The FDA employed two technologies in conducting its genetic analyses, [pulsed-field gel electrophoresis](#) (PFGE) and [whole genome sequencing](#) (WGS), both commonly used to subtype disease-causing bacteria. Explanations of these technologies are provided in [Appendix C: Genetic Evaluation](#).

The FDA’s analyses – and more broadly, this assignment – helped identify cucumbers as the vehicle in an outbreak of *Salmonella* infections that involved 10 people in three states (Arizona, California and Washington) in the summer of 2016. Three patients were reported hospitalized during the outbreak. Of the seven patients interviewed, six reported consuming cucumbers in the week prior to illness onset. Additionally, the FDA determined based on testing by the CDC and state public health laboratories that clinical isolates from the 10 patients were highly related to *Salmonella* isolates from a cucumber sample collected by the FDA under this assignment in June 2016. The CDC closed its investigation on August 19 of that year.

Upon detecting *Salmonella* in the sample (collected in June 2016), the FDA notified the U.S. importer, which voluntarily recalled the affected lots. The FDA placed the responsible firm and product on Import Alert 99-23, thus requiring additional controls for future entries. Notified of the positive, the firm that was responsible for the product ceased cucumber shipments to the

United States, evaluated its packing line, and made food safety improvements before resuming operations. The FDA has since removed the firm and its product from the import alert.

The FDA's analyses also showed that eight other cucumber samples had yielded *Salmonella* isolates highly related to clinical isolates from one or more ill persons (and to 42 patients in total). However, in the case of these clinical isolates, the available epidemiological information did not indicate whether the consumption of cucumbers was implicated in the illnesses.

The FDA followed up as warranted in response to all the *Salmonella* detections to keep contaminated cucumbers from reaching consumers. When the agency detected *Salmonella* in samples collected at ports of entry, it refused to admit the lots associated with the positives and, where the criteria were met, placed the responsible firms and product on Import Alert 99-23. When the criteria to place a firm on import alert were not met, the agency carried out intensified sampling (i.e., additional sampling) of cucumbers from the responsible firms. The agency placed seven firms on import alert in all. To address the domestic samples positive for *Salmonella*, the FDA worked with four firms that owned or released the product to conduct voluntary recalls. The recalls removed potentially contaminated product from the marketplace, thus preventing consumption and potentially averting illnesses. In seven other domestic cases where the agency detected *Salmonella*, the firm did not conduct a recall because no cucumbers remained in stock, or were likely to be in stock, given their limited shelf life. The FDA provided these firms with guidance on minimizing microbial hazards in fruits and vegetables and shared its findings with state partners.

A more detailed explanation of the FDA's genetic analyses of the 28 *Salmonella*-positive samples is provided in Appendix C: Genetic Evaluation. Of particular note in this analysis is the increasing importance of WGS in identifying the scope and source of microbial contamination. For that reason, the FDA will continue to expand its efforts in WGS, gradually moving away from lower resolution approaches.

CONCLUSION AND NEXT STEPS

The FDA accomplished the objectives that it set for this assignment, the most fundamental being to estimate the prevalence of *Salmonella* and *E. coli* O157:H7 in cucumbers.

As detailed in this report's Statistical Evaluation section (page 10), the assignment found the prevalence of *Salmonella* in cucumbers consumed in the United States to be 1.75 percent with a 95 percent confidence interval of 1.17 percent to 2.52 percent, based on the test results. None of the samples tested positive for *E. coli* O157:H7 or other pathogenic *E. coli*.

While the study was designed primarily to determine the overall prevalence(s) of the target pathogens associated with cucumbers, the FDA also evaluated its analytical results preliminarily and throughout the assignment for signals (i.e., variations in prevalence by origin and season) to determine if more targeted sampling or further study may be warranted. The FDA did not detect any signals by origin or season that warranted more targeted sampling or additional study.

As to common factors among the FDA's findings, the agency estimated the prevalence of *Salmonella* in cucumbers by country of origin and season. The agency did not design its sample collection to compare prevalence by country of origin or season and thus cautions against making inferences based solely on its observations by these breakdowns, which are provided for informational purposes.

Origin: Domestic/Import: The prevalence of *Salmonella* in domestically grown cucumbers was 2.86 percent, and the prevalence of *Salmonella* in imported cucumbers was 1.4 percent.

Season: The prevalence of *Salmonella* in cucumbers collected in the fall was higher than the other seasons.

Additional study would be required to determine what factors contribute to the difference(s) noted in the findings provided above.

Consistent with the FDA's mission to protect consumers, this assignment helped identify cucumbers as the vehicle in an outbreak of salmonellosis that involved 10 people in three states in the summer of 2016. The FDA found whole genome sequences from a cucumber sample collected under this assignment to be highly related to sequences of a *Salmonella* strain from clinical isolates in the cluster. The outbreak appeared to be over by August 2016.

The FDA took action as warranted upon detecting each *Salmonella* positive. For domestic positives, the agency worked with the firm(s) that owned or distributed the affected cucumbers to conduct voluntary recalls in all cases in which product was in stock or likely to be in stock. With respect to the positives detected at ports of entry, the FDA refused to admit lots associated with the positive samples and/or, where the criteria were met, placed the responsible firms and product on import alert. The FDA placed seven firms on the import alert in all. The FDA also conducted additional sampling of cucumbers from 10 Mexico-based firms whose product tested positive at entry. One voluntary recall was conducted by an importer.

The findings of this assignment underscore the need for growers and others in the cucumber distribution chain to comply with the FDA's Produce Safety Rule, as applicable. The agency has published draft guidance documents to help industry comply with this rule, and FDA and state partners began Produce Safety Rule inspections in the spring of 2019. In addition, FDA is reaching out to cucumber growers and distributors to make them aware of the findings of this assignment and to provide additional information on resources on steps to mitigate contamination of cucumbers.

For importers of cucumbers, compliance with the FDA's Foreign Supplier Verification Programs (FSVP) Rule is an additional way to help ensure the safety of imported cucumbers. The FDA has developed draft guidance on the [FSVP Rule](#) and began FSVP inspections in 2017.

The FDA will continue to sample cucumbers for pathogens. Cucumbers are covered produce under the Produce Safety Rule, and among its next steps, the agency and its state partners began inspections of large produce farms for compliance with the Produce Safety Rule in the spring of

2019. The agency also will continue to sample imported cucumbers, including targeted sampling of product from countries of interest.

The FDA likewise will continue to evaluate methods to prevent microbial contamination of cucumbers. The presence of harmful bacteria in the commodity remains a concern to the FDA given this assignment's findings, the history of reported outbreaks associated with cucumbers, and the fact that cucumbers are a ready-to-eat food, meaning consumers typically eat them without cooking or otherwise subjecting them to a 'kill step' to reduce or eliminate pathogens. Cucumbers require appropriate protection from human pathogens during growing, harvesting, packing and holding, as this study confirms.

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 cucumbers. 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 based whole genome sequence analysis.

***E. coli* O157:H7, STEC**

The FDA's [Bacteriological Analytical Manual](#) (BAM) method for *E. coli* O157:H7 is a polymerase chain reaction (PCR) assay that tests specifically for genes in the O157:H7 serotype. The sample preparation procedure used is described in [Chapter 4A](#) of the BAM. Briefly, one-pound samples of product are mixed with enrichment medium equivalent to 1.5 times the sample weight containing antibiotic that selects for the growth of Shiga toxin-producing *E. coli*. After enrichment overnight, DNA was extracted from an aliquot of the enrichment and tested by PCR. The FDA did not detect any positives for *E. coli* O157:H7. If the pathogen had been detected, analysts would have plated the bacteria onto agar media to isolate it and confirm that it was O157:H7 using biochemical, serological and genetic assays.

APPENDIX B: SALMONELLA-POSITIVE SAMPLES BY SEROTYPE

Organized by sample ID, the table below provides the serotype of each *Salmonella* strain detected. Some samples produced more than one isolate. Antigenic formulas are provided in cases where the FDA observed unnamed serotypes.

Sample ID	Isolate No. 1	Isolate No. 2
907217	Cerro	
929379	Saintpaul	
936584	Cerro	
938513	Buzu	
938876	Newport	
943150	subsp. arizonae serovar IIIa 48:z4,z23,z32:-	
950473	Norwich	Newport
951368	Newport	Newport
952004	subsp. diarizonae serovar IIIb 16:z10:e,n,x,z15	
956966	Weltevreden	
962875	Saintpaul	Saintpaul
966665	Saintpaul	
968724	Muenchen	Aqua
970382	I 4,5,12:i:-	
970565	Infantis	
978498	Oranienburg	
979335	Mdandaka	Mdandaka
980839	Michigan	
981000	Poona	Saphra
982801	Newport	
983017	Saintpaul	Saintpaul
984313	Oranienburg	
984477	Luciana	
986574	Luciana	
986593	Michigan	Javiana
987958	Minnesota	
991957	Javiana	
1002342	Newport	Newport

APPENDIX C: 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 subtype 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.

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.

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 location where the food was grown, packed or processed are needed to determine that an adulterated food caused a given illness, or multiple illnesses in the case of an outbreak. PFGE enables less specificity than WGS subtyping and cannot be relied upon as the sole determinant of illness attribution.

The FDA's evaluation found considerable diversity among the PFGE patterns of the species of *Salmonella* detected. Specifically, the 28 samples that tested positive for *Salmonella* produced 32 PFGE patterns across 37 isolates. Upon querying the PulseNet USA database, the FDA found that 14 PFGE patterns in the food isolates were associated with one or more clinical entries. The database search covered 700 days, from October 17, 2016 (shortly after the agency detected the first *Salmonella*-positive cucumber) to November 16, 2018.

Other than the information gathered during the 2016 salmonellosis outbreak (described in this report's Public Health Impact section, page 12), the available epidemiological information was inconclusive as to the food or other vehicle involved in the illnesses, or no epidemiological information was available to link the clinical entries to cucumbers. The PFGE findings are as follows:

- One isolate from one cucumber sample, with an unnamed PFGE pattern for *Salmonella* Michigan, was indistinguishable by PFGE from three clinical isolates uploaded from three states (New Mexico, New York and Washington).
- One isolate from one cucumber sample, with an unnamed PFGE pattern for *Salmonella* Michigan, was indistinguishable by PFGE from three clinical isolates uploaded from two states (California and Michigan).
- One isolate from one cucumber sample, with the PFGE pattern of JFXX01.1103, was indistinguishable by PFGE from two clinical isolates uploaded from two states (South Carolina and Virginia).
- One isolate from one cucumber sample, with the PFGE pattern of JGGX01.0032, was indistinguishable by PFGE from 11 clinical isolates uploaded from nine states (California, Florida, Kansas, Minnesota, North Carolina, Ohio, Oklahoma, Texas, Wisconsin).
- One isolate from one cucumber sample, with the PFGE pattern of JJPX01.0025, was indistinguishable by PFGE from 300 clinical isolates uploaded from 38 states throughout the country.
- Two isolates from one cucumber sample, with the PFGE pattern of JJPX01.0238, were indistinguishable by PFGE from 164 clinical isolates uploaded from 31 states throughout the country.
- Two isolates from one cucumber sample, with the PFGE pattern of JJPX01.0289, were indistinguishable by PFGE from six clinical isolates uploaded from six states (California, Florida, Indiana, Oklahoma, North Dakota, and Nevada).
- One isolate from one cucumber sample, with the PFGE pattern of JJPX01.0709, was indistinguishable by PFGE from six clinical isolates uploaded from four states (Arkansas, California, Texas and Wisconsin).
- One isolate from one cucumber sample, with the PFGE pattern of JJPX01.0035, was indistinguishable by PFGE from 146 clinical isolates uploaded from 30 states throughout the country.
- One isolate from one cucumber sample, with the PFGE pattern of JN6X01.0030, was indistinguishable by PFGE from 91 clinical isolates uploaded from 17 states throughout the country.
- One isolate from one cucumber sample, with the PFGE pattern of JN6X01.0031, was indistinguishable by PFGE from 14 clinical isolates uploaded from nine states (Connecticut, Florida, Georgia, Mississippi, New Jersey, New York, South Carolina, Tennessee, and Texas).

- Two isolates from one cucumber sample, with the PFGE pattern of JN6X01.0137, were indistinguishable by PFGE from 55 clinical isolates uploaded from 14 states throughout the country.
- Two isolates from one cucumber sample, with the PFGE pattern of JN6X01.1234, were indistinguishable by PFGE from one clinical isolate uploaded from California.
- One isolate from one cucumber sample, with the PFGE pattern of TDTX01.0036, was indistinguishable by PFGE from 10 clinical isolates uploaded from eight states (Connecticut, Massachusetts, Maryland, North Carolina, New Jersey, New York, Pennsylvania, and Virginia).

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 28 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 agency's analysis showed that sequences from nine of the *Salmonella* positives were highly related to sequences from one or more clinical isolates, suggesting the possibility that the clinical isolates and those from the cucumbers may have originated from common source contamination. Of those nine positives, one was the sample associated with the salmonellosis outbreak described in the Public Health Impact section of this report. With respect to the other eight positives, the available epidemiological information was inconclusive as to the food or other vehicle involved in the illnesses.