FY 2014 – 2016 Microbiological Sampling Assignment
Summary Report: Raw Milk Cheese Aged 60 Days

Office of Compliance
Center for Food Safety and Applied Nutrition

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CONTENTS

EXECUTIVE SUMMARY

In 2014, the U.S. Food and Drug Administration (FDA) set out to collect and test cheese made from unpasteurized milk, also referred to as "raw milk cheese," aged 60 days as part of a new proactive and preventive approach to sampling with the ultimate goal of keeping contaminated food from reaching consumers.

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

The FDA issued the raw milk cheese assignment in January 2014 along with two others (for sprouts and avocados) as the initial commodities under its new sampling model. As planned, the FDA collected 1,606 raw milk cheese samples (exceeding its target by 6 samples). The FDA designed its sampling plan such that if contamination of one percent or greater was present in the commodity, the agency would detect it. The agency closely monitored the assignment to gather lessons learned and make changes to the sampling if needed to address trends or food safety issues.

Of the 1,606 raw milk cheese samples collected and tested, 473 samples (29 percent) were domestic samples, and 1,133 samples (71 percent) were of international origin. The FDA sought to design its sampling plan to approximate the ratio of domestically made versus imported product on the U.S. market but was unable to do so in this case because the federal government does not track production volume of raw milk cheese.[1] Details on the assignment design are provided in the Sample Collection section of this report (page 6).

The FDA tested samples for the presence of the pathogens *Salmonella*, *Listeria monocytogenes*, *E. coli* O157:H7 and Shiga toxin-producing *E. coli*, as well as for generic *E. coli*. The overall contamination rate for each of the pathogens was less than one percent, and the overall contamination rate for generic *E. coli* was 5.4 percent. While the prevalence for generic *E. coli* was comparatively high, it bears mention that it rarely causes illness even as it may signal insanitary processing conditions.

Because the contamination frequencies among the pathogens were below one percent, the FDA was limited in its ability to detect differences in contamination rates based on the type of cheese or its origins (i.e., domestic vs. import), even with the large number of samples.

In addressing the violative samples of domestic raw milk cheese, the FDA worked with the responsible firms to carry out recalls as appropriate and followed up with facility inspections. In addressing the violative samples of imported raw milk cheese, the FDA refused entries of raw

[1] The USDA Economic Research Service tracks the supply and commercial use of cheese in the United States but has no figures specific to raw milk cheese, which differs mainly with respect to the lack of pasteurization even as it comes in many varieties.

milk cheese and placed the responsible firms/product on Import Alert 12-10. The FDA also worked with a regulatory partner in the European Union to address further follow-up of manufacturing locations abroad.

Listeria monocytogenes in cheeses, particularly semi-soft varieties, remains a concern, as demonstrated by the nearly one percent contamination rate in semi-soft cheeses (see Appendix B: Positive Findings by Bacterial Type). The FDA believes this contamination rate may be related to product handling practices or procedures. Given the serious public health implications of *Listeria monocytogenes* contamination associated with ready-to-eat foods, the FDA plans to continue to work with the cheese industry to identify and correct practices that lead to *Listeria monocytogenes* contamination in cheese.

BACKGROUND

The FDA Food Safety Modernization Act is an amendment to the Federal Food, Drug, and Cosmetic Act (FD&C Act) that is intended to provide the FDA with additional authority to better prevent problems before they occur. However, in order to develop 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 has developed a new microbiological sampling model designed to identify patterns that may help predict and prevent contamination by disease-causing bacteria.

The new model complements the FDA's longstanding approach to sampling, which has employed for-cause and targeted strategies to monitor known hazards. The FDA will continue its longstanding approach to sampling, but the prevention mandate outlined in FSMA gives cause for larger, in-depth surveys of products and commodities to better evaluate risks. These large sample collections enable the FDA to determine the prevalence of contamination 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. However, if a contamination rate is substantially lower than one percent, then even extensive sampling may not provide actionable insight into the source of the contamination.

As a starting point for the new model – and because it is not feasible to sample every product and/or commodity extensively – an FDA work group developed a system to rank commodities based on microbial risk. The work group reviewed sampling data collected over a five-year period, systematically considering criteria such as association with foodborne illness, consumption of product without a mitigating "kill" step, and prevalence data (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). Foods that ranked comparatively high were evaluated by subject matter experts to determine their feasibility as candidates for a large-scale survey and the remaining data needs for the commodity. After the work group review, the FDA chose to sample raw milk cheese (aged 60 days), sprouts (seeds, spent irrigation water, and finished product), and avocados (whole pit fruit) in FY2014-2016, as the first commodities under the new model. This report details the rationale and findings for the sampling and testing of raw milk cheese.

Why Raw Milk Cheese?

Evidence indicates that aging raw milk cheese for 60 days may not eliminate or adequately reduce *E. coli* O157:H7 and *Salmonella* in raw milk cheese, thus posing a potential hazard to consumers.[2, 3] Additionally, *Listeria monocytogenes* may grow in certain types of cheese during aging.[4] The Centers for Disease Control and Prevention reported in a 2012 study that cheese made from raw milk was involved in 27 outbreaks of foodborne illness from 1993 to 2006.[5]

Raw Milk Cheese Production

The USDA Economic Research Service (ERS) tracks the supply and commercial use of cheese in the United States but has no figures specific to cheese made from unpasteurized milk. In 2014 the United States produced more than 11 billion pounds of cheese and imported 363 million pounds of the product.[6,7] The same year, the most recent for which statistics are available, USDA ERS also reported that Americans ate an average of 33.9 pounds of cheese per person. That figure has more than doubled since 1975 when U.S. cheese consumption was just 14.2 pounds per person.[8] Based on anecdotal information, the FDA believes that the volume of unpasteurized cheese on the U.S. market may also have increased over this time period, in part because of the apparent growth in specialty cheese manufacturing. Despite the lack of quantitative data on raw milk cheese, the increase in cheese consumption in the United States suggests that consumption of raw milk cheese may, too, be on the rise.

Making raw milk cheese entails the same basic steps as are required for any cheese, whether made by an artisan or a comparatively large manufacturer. Typically, the cheesemaker receives raw milk for processing, heats it and adds starter culture, followed by rennet, then allows it to set (or coagulate). Once set, the curd is cut to release the whey. The mixture is cooked and stirred until the desired temperature and firmness are reached, and the whey is drained. The

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[2] Reitsma, C.J., Henning, D.R. (1996). Survival of Enterohemorrhagic *Escherichia coli* O157:H7 during the Manufacture and Curing of Cheddar Cheese. *Journal of Food Protection*, Vol. 59, No. 5, Pages 460-464.

[3] Schlesser, J.E., Gerdes, R., Ravishankar, S., Madsen, K., Mowbray, J. & TEO, A.Y. (2006). Survival of a Five-Strain Cocktail of *Escherichia coli* O157:H7 During the 60-Day Aging Period of Cheddar Cheese Made from Unpasteurized Milk. *Journal of Food Protection.* Vol. 69, No. 5, Pages 990-998.

[4] Joint FDA / Health Canada Quantitative Assessment of the Risk of Listeriosis from Soft-Ripened Cheese Consumption in the United States and Canada.
(http://www.fda.gov/Food/FoodScienceResearch/RiskSafetyAssessment/ucm429410.htm)

[5] Langer, A.J., Ayers, T., Grass, J., Lynch, M., Angulo, F., & Mahon, B.E. (2012). Non-pasteurized Dairy Products, Disease Outbreaks, and State Laws—United States, 1993–2006. *Emerging Infectious Diseases*. Vol. 18, No. 3, Pages 385-391.

[6] USDA Dairy Products 2015 Summary. (http://usda.mannlib.cornell.edu/usda/current/DairProdSu/DairProdSu-04-28-2016.pdf) See page No. 30, "Total Cheese (Excluding Cottage Cheese) Production by Month – States, United States, and Regions: 2015 and Total 2014-2015."

[7] USDA Data Set: Value of U.S. Dairy Products.
(http://www.ers.usda.gov/datafiles/US_Food_Imports/__Value_of_US_food_imports_detailed_tables_by_food_group/dairy2_1_.xlsx)

[8] Dairy products: Per capita consumption, United States, 1975-2014.
(http://www.ers.usda.gov/datafiles/Dairy_Data/pcconsp_1_.xlsx)

cheesemaker typically salts and presses the curds to remove more whey, then molds the curds together. Finally, cheese made from unpasteurized milk must be aged at not less than 35 degrees Fahrenheit for at least 60 days, in accordance with 21 C.F.R. part 133.

Worldwide there are more than 2,000 cheese varieties. Virtually all of them fall within the basic categories of "soft (fresh)," "soft-ripened," "semi-soft," "hard," or "hard grating." But, cheese is a broad category of food that features many variables. For example, it may be made from the milk of cows, goats, or sheep, or a combination thereof, or even other animals. It may be mold-ripened, have a washed rind, or a natural rind. It may have a closed texture, like Cheddar, or have an open texture, like Swiss. And, it may be made from pasteurized, thermized or raw milk.

OBJECTIVES

The objectives of the FY2014-2016 raw milk cheese sampling assignment were:

- To determine the prevalence of *Salmonella*, *Listeria monocytogenes* and Shiga toxin-producing *E. coli* (primarily *E. coli* O157:H7) in raw milk cheese aged 60 days.
- To determine if there are common factors associated with positive findings (such as origin, variety or manufacturing practice).
- To take appropriate regulatory action when positive findings are observed.
- To explore new processes and parameters to strengthen the FDA's current approach to sample collection and analysis.

SAMPLE COLLECTION

The FDA collected 1,606 samples of raw milk cheese from February 19, 2014 to November 3, 2015 under this assignment. Of the total, 473 samples (29 percent) were taken from domestically produced raw milk cheese, and 1,133 samples (71 percent) were taken from raw milk cheese of international origin.

The FDA sought to design its sample collection to approximate the ratio of domestically made versus imported product on the U.S. market but was unable to do so in this case given the absence of market-share data on raw milk cheese. Thus, the agency based its sample collection ratio largely on the fact that many popular raw milk cheeses, such as Gouda, Provolone and Roquefort are traditionally of international (e.g., European) origin. The FDA collected samples to ensure that they were representative of the lot and to help enable the agency to obtain cross sections of manufacturer types for the domestic samples and countries of origin for the import samples.

Domestic Collection

As directed by the assignment, FDA field staff collected 473 domestic samples of raw milk cheese from three types of establishments: manufacturers, distribution centers or warehouses,

and retail stores (Table 1). Samples were collected in 38 states and Puerto Rico. Among the state totals, the FDA collected the largest number of samples in Wisconsin (78), followed by California (35), and Illinois (33).

Import Collection

As directed by the assignment, FDA field staff collected nearly all of the 1,133 import samples from raw milk cheese in import status, which is the status of articles in the admissibility process prior to their release into commerce. These samples were collected at the port of entry or a location where the product was being held prior to being released into domestic commerce. In addition, the agency collected a small number of import samples at retail (Table 1). The samples collected at import originated from 22 countries, with the largest number sent from France (531), followed by Spain (145), and Italy (137). The FDA also sampled raw milk cheese from Austria, Belgium, Bulgaria, Canada, Cyprus, Denmark, Germany, Greece, Hungary, Ireland, Lithuania, Mexico, the Netherlands, Nicaragua, Poland, Portugal, Switzerland, Turkey and the United Kingdom. The two biggest exporters of cheese to the United States are France and Italy.

Table 1: Sample Collection Sites

Origin	Site Type	Number of Samples Collected	Percentage of Samples Collected
Domestic	**Distributor/Warehouse**	68	4.23%
	Manufacturer	251	15.63%
	Retail	154	9.59%
Import	**Ports of Entry**	1,121	69.80%
	Domestic Import (DI)	12	0.75%
Total		1,606	100%

Sample Collection by Cheese Type (i.e., Texture)

The FDA collected samples of soft (fresh),[9] semi-soft,[10] soft-ripened,[11] and hard[12] raw milk cheese (Table 2), with priority placed on the softer types because their relatively high moisture content makes them susceptible to the growth of *Listeria monocytogenes*, if present. The FDA collected samples in solid, shredded, grated, curd and extruded forms.

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[9] **Soft (Fresh)** cheese: There is no standard of identity for soft (fresh) cheese under U.S. federal law. However, internationally, Codex Standard 283-1978, General Standard for Cheese, indicates that a soft cheese will have greater than 67 percent moisture on a fat-free basis. Examples of soft (fresh) cheese include Feta, fresh Mozzarella and Ricotta.

[10] **Semi-Soft** cheese contains more than 39 percent, but no more than 50 percent, of moisture, and its solids contain no less than 50 percent of milkfat. For the complete standard of identity for semi-soft cheese, please see 21 C.F.R. part 133.187. Examples of semi-soft cheese include Fontina, Gouda and Provolone.

[11] **Soft-Ripened** cheese contains no less than 50 percent of milkfat in solid form. For the complete standard of identity for soft-ripened cheese, please see 21 C.F.R. part 133.182. Examples of soft-ripened cheese include Brie, Camembert and Milano.

[12] **Hard** cheese contains no more than 39 percent of moisture, and its solids contain no less than 50 percent of milkfat. For the complete standard of identity for hard cheese, please see 21 C.F.R. part 133.150. Examples of hard cheese include Appenzeller, Cheddar and Romano.

Table 2: Sample Collection by Cheese Type /Texture

Type/Texture	Number of Samples Collected	Percentage of Samples Collected
Soft (Fresh)	24	1.5%
Semi-Soft	1,013	63%
Soft-Ripened	48	3%
Hard	521	32.5%
Total	1,606	100%

Sample Collection by Milk Source (i.e., Type of Animal)

Provided they were samples of raw milk cheese, the assignment allowed for the collection of product samples made from the milk of cows, goats or sheep, or a combination thereof. The FDA did not require its field staff to document milk source. That being the case, the FDA obtained the milk source of 443 samples from labeling information and field staff voluntary reporting. The milk source for the other 1,163 samples is listed as "unspecified," for reporting purposes.

Of the samples for which the FDA knows the milk source, most of the samples of domestically produced raw milk cheese were made from the milk of cows, reflecting the predominance of bovine animals among U.S. dairy herds. By comparison, significant numbers of import samples were made from the milk of cows, goats and sheep (Table 3).

Table 3: Sample Collection by Milk Source

Milk Source	Origin	Number of Samples Collected	Percentage of Samples Collected
Cow	Domestic	102	6.4%
	Import	44	2.7%
Goat	Domestic	47	2.9%
	Import	39	2.4%
Sheep	Domestic	18	1.1%
	Import	189	11.8%
Mixture	Domestic	2	0.1%
	Import	2	0.1%
Unspecified	Domestic	304	18.9%
	Import	859	53.5%
Total		1,606	99.9% *

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

Size and Weight as Sample Collection Factors

Because cheese comes in different sizes, the FDA designated three categories of subsamples for purposes of its collection scheme. Each sample consisted of a fixed number of subsamples based on weight, as follows:

- **Retail units of less than 1 pound**: Samples in this category consisted of 10 subsamples, each weighing at least 1 pound. (That being the case, if for example the product came in 8 oz. packages, two retail packages would be needed to constitute one subsample.)

- **Retail units of 1 to 5 pounds**: Samples in this category consisted of 10 subsamples (i.e., individual packages from the same lot).

- **Cheese wheels, loaves or bricks greater than 5 pounds**: Samples in this category consisted of one or two intact units from the same lot. (The FDA initially required that two wheels, loaves or bricks be collected at the assignment's outset, but the agency amended its guidance to its field staff on May 7, 2014 to permit the collection of a single unit. The FDA made the change based on sample availability and cost, and after determining that it would not adversely affect test results.)

This approach – the collection and testing of samples that comprise multiple subsamples – is more reflective of actual conditions as it increases the odds of finding pathogens in cheese from a common establishment, given that microbial hazards may not be uniformly present.

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

PATHOGEN FINDINGS

This section provides the prevalence of *Salmonella*, *Listeria monocytogenes*, *E. coli* O157:H7 and other Shiga toxin-producing *E. coli* in raw milk cheese, based on the results of the 1,606 samples tested, along with other noteworthy findings. The test methods are described in Appendix A: Test Methods. In addition, a complete breakdown of the prevalence of each pathogen by origin (i.e., domestic vs. import) and cheese type/texture is included in Appendix B: Positive Findings by Bacterial Type.

Pathogen Findings: *Salmonella*

The FDA detected *Salmonella* in three of the 1,606 samples tested, for a *Salmonella* contamination rate of 0.19 percent. The FDA found each instance of *Salmonella* contamination in an import sample (two having been exported to the United States from France, and one from Italy). The samples from France were semi-soft cheeses. The sample from Italy was a hard cheese.

Pathogen Findings: *Listeria monocytogenes*

The FDA detected *Listeria monocytogenes* in 10 samples of the 1,606 tested, for a *Listeria monocytogenes* contamination rate of 0.62 percent. The FDA found the pathogen in five samples of domestically produced raw milk cheese, with three of those five collected at a single firm. The agency likewise found the pathogen in five import samples (Table 4).

Nine of the 10 samples that tested positive for *Listeria monocytogenes* were of the semi-soft type/texture. That being the case, the FDA found a *Listeria monocytogenes* contamination rate of 0.89 percent in semi-soft raw milk cheese.

Table 4: *Listeria monocytogenes* Positive Findings by Product Origin and Type/Texture

Country	Type/Texture
Italy	Semi-Soft
France	Semi-Soft
	Semi-Soft
	Semi-Soft
	Semi-Soft
United States	Semi-Soft
	Semi-Soft
	Semi-Soft
	Semi-Soft
	Hard

Pathogen Findings: *E. coli* O157:H7

The FDA did not detect *E. coli* O157:H7 in any of the 1,606 samples it tested, irrespective of origin or type/texture.

Pathogen Findings: Shiga toxin-producing *E. coli*

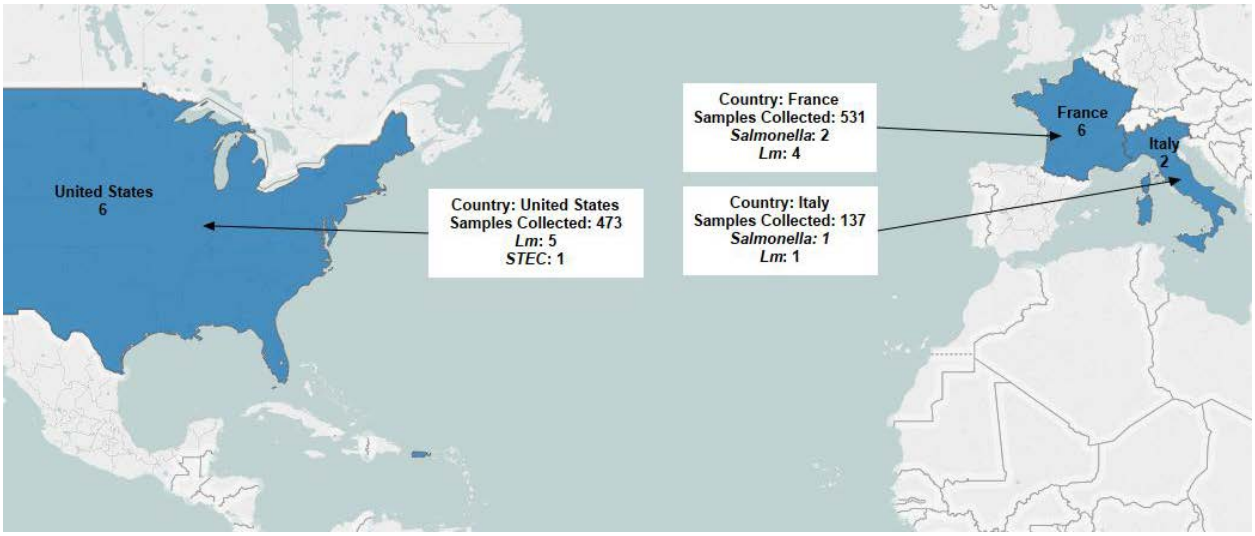
The FDA detected Shiga toxin-producing *E. coli* in 11 of the 1,606 samples tested, for a contamination rate of 0.68 percent. After further characterization of the 11 samples, the agency determined one of them to be pathogenic (i.e., potentially injurious to human health). The pathogenic sample, *E. coli* O111:H8 serotype, was found in a hard, raw goat milk cheese, collected in the Midwest. This one pathogenic sample of Shiga toxin-producing *E. coli* made for a contamination rate of 0.06 percent.

Pathogen Findings: By Country of Origin

Of the 1,606 samples tested, the FDA detected pathogens in 14 samples in all (Figure 1). The three samples that tested positive for *Salmonella* were found in raw milk cheese made in France (2) and Italy (1). The 10 samples that tested positive for *Listeria monocytogenes* were found in raw milk cheese made in Italy (1), France (4), and the United States (5). Significantly, three of the five positive samples of domestically produced raw milk cheese were collected at a single firm in the West. Additionally, one sample that tested positive for pathogenic Shiga toxin-producing *E. coli* was made from raw milk cheese collected in the Midwest (Figure 1).

Given the low prevalence of samples that tested positive for *Listeria monocytogenes*, no meaningful conclusions can be made about country-specific comparisons in relation to risk for contamination of raw milk cheeses from this assignment.

Figure 1: All Pathogen Findings by Country of Origin



Pathogen Findings: By Repeat Firm with Adulterated Samples

One domestic firm was responsible for multiple samples (three in total) that tested positive for *Listeria monocytogenes*. No other domestic or international firms had more than one violative sample.

OTHER FINDINGS: GENERIC *E. COLI*

This section reports the prevalence of violative levels of generic *E. coli* in raw milk cheese, based on the results of the 1,606 samples tested, and other noteworthy findings. In determining samples to be violative due to the presence of generic *E. coli*, the FDA employed the criteria described in Appendix C (in short, that levels of the bacteria exceeding 10 MPN/g and less than 100 MPN/g in three or more subsamples of the five tested constitute a violation).

Generic *E. coli* is a type of *E. coli*, a diverse group of bacteria that ordinarily lives in the intestines of people and animals. Some *E. coli* are pathogenic, but generic *E. coli* rarely causes illness. Many countries, including the United States, have historically used the presence of *E. coli* as an indicator of insanitary processing conditions.

Prevalence

The FDA detected violative levels of generic *E. coli* in 87 of the 1,606 samples tested, which makes for an overall contamination rate of 5.4 percent.

The agency found the generic *E. coli* contamination rate in domestically produced raw milk cheese samples to be 3.8 percent, as 18 of the 473 domestic samples contained violative levels of the bacteria.

The agency found the generic *E. coli* contamination rate in imported raw milk cheese samples to be 6.1 percent, as 69 of the 1,133 samples of international origin contained violative levels of the bacteria.

A complete breakdown of the prevalence of generic *E. coli* by origin (i.e., domestic vs. import) and cheese type/texture is included in Appendix B: Positive Findings by Bacterial Type.

As Appendix B shows, most of the samples that contained violative levels of generic *E. coli* were of semi-soft raw milk cheese (Table 5).

Table 5: Generic E. coli Positive Samples by Domestic vs. Import, and Type/Texture

Domestic vs. Import	Type/Texture	Number of Samples Positive
Domestic	Soft (Fresh)	0
	Semi-Soft	10
	Soft-Ripened	0
	Hard	8
Import	Soft (Fresh)	0
	Semi-Soft	54
	Soft-Ripened	3
	Hard	12
Total		87

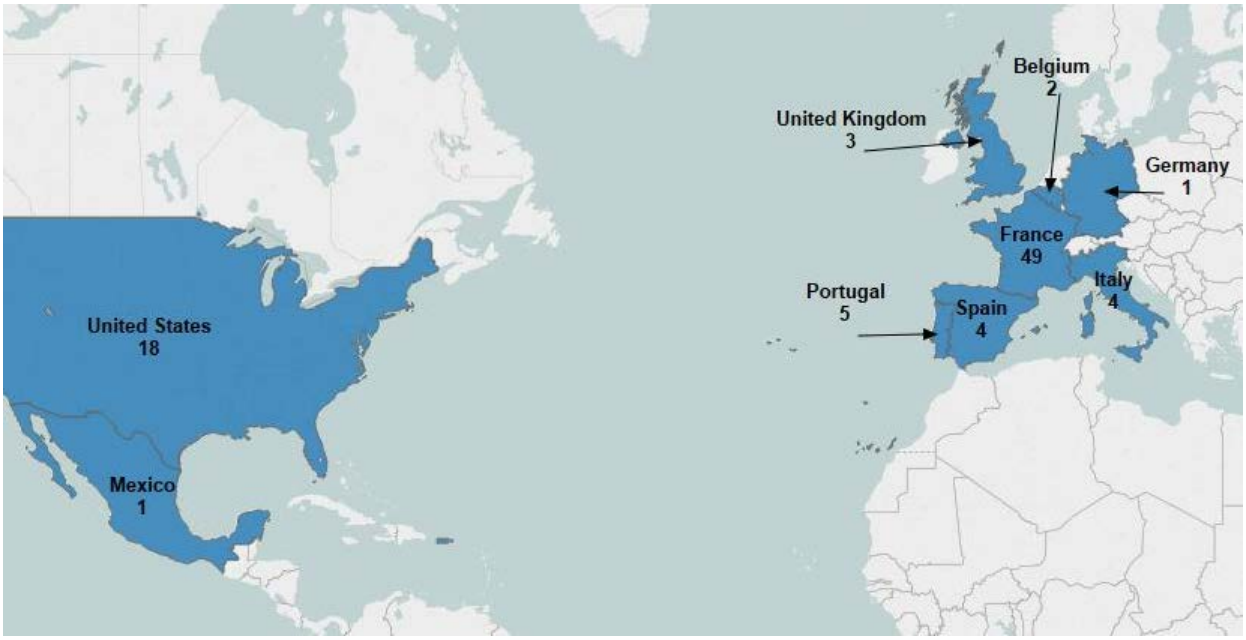
See Appendix B: Positive Findings by Bacterial Type, section on generic *E. coli.*

Generic *E. coli* Findings: By Country of Origin

In addition to the 18 samples of domestically produced raw milk cheese that the FDA found to be violative for generic *E. coli*, the agency detected violative levels of the bacteria in samples of raw milk cheese exported to the United States from seven European countries and Mexico, with the largest number of violative samples shipped from France (49), followed by Portugal (5), and Italy and Spain (4, each).

The map that follows shows the countries of origin and country-specific totals for the violative samples of generic *E. coli*. The caption below the map provides each country's sample collection total (Figure 3).

Figure 2: Generic *E. coli* Violative Samples by Country of Origin



Shown are the numbers of violative samples and their countries of origin. The corresponding sample collection totals for each country are: France (531), the United States (473), Spain (145), Italy (137), the United Kingdom (125), Portugal (42), Germany (21), Belgium (10), and Mexico (9).

Occurrence with Pathogens

Of the 1,606 samples tested, the FDA found one sample contaminated with both violative levels of generic *E. coli* and a pathogen (namely, *Listeria monocytogenes*).

The FDA evaluated the relationship between the bacteria and the pathogens detected under this assignment, using Pearson's chi-squared test (χ^2). The test provided no evidence of an association between the presence of generic E. *coli* and *Salmonella*, *Listeria monocytogenes*, *E. coli* O157:H7 or Shiga toxin-producing *E. coli* (p-value = 1). The table is provided below (Table 6).

Table 6: Chi Squared Table for Pathogens in Relation to Violative Samples of *E. coli*

	Negative for Pathogen(s)	Positive for Pathogen(s)
Not Violative for Generic *E. coli*	1506	13
Violative for Generic *E. coli*	86	1

It is established in scientific literature that the presence of index or indicator organisms, such as *E. coli*, coliforms, fecal coliforms and Enterobacteriaceae, generally does not correlate well with the presence of foodborne pathogens and is not useful for determining contamination of individual lots of food by pathogens. Likewise, the absence of such index/indicator organisms

from a lot of food generally does not correlate with the absence of foodborne pathogens.[13] Written assessments by the United Nations' Food and Agriculture Organization and the National Research Council's Subcommittee on Microbiological Criteria concluded that levels of *E. coli*, coliforms, fecal coliforms and Enterobacteriaceae should not be used to predict the safety of food products.[14, 15] On the other hand, detection of index/indicator organisms, such as *E. coli*, are useful in assessment of facility hygiene and the potential loss of process control.[16]

REGULATORY APPROACH

The Food, Drug, and Cosmetic Act (FD&C Act) empowers the FDA to take regulatory action when it finds adulterated food. Regulatory tools at the agency's disposal include warning letters, import alerts, import refusals, administrative detention and seizures, injunctions, food facility registration suspension, and voluntary and mandatory recalls. The FDA's regulatory approach for each listed bacteria is as follows:

Salmonella

Because *Salmonella* infections are often serious and can occur in a healthy individual from consumption of even a very small amount of the bacteria, foods that test positive for *Salmonella* are considered to be adulterated under Section 402(a)(1) of the FD&C Act in that they bear or contain a poisonous or deleterious substance which may render them injurious to health and subject to regulatory action.

Listeria monocytogenes

Because *Listeria monocytogenes* infections can lead to fetal loss in pregnant women and to serious illness or death in certain high-risk groups (such as the elderly or people with weakened immune systems), ready-to-eat foods that test positive for *Listeria monocytogenes* are considered to be adulterated under Section 402(a)(1) of the FD&C Act in that they bear or contain a poisonous or deleterious substance which may render them injurious to health. Therefore, the presence of the pathogen in raw milk cheese is considered a violation of the act and subject to regulatory action.

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[13] Enterobacteriaceae, Coliforms, and Escherichia coli as Quality and Safety Indicators. 2015. Kornacki, J., Gurtler, J. and Stawick, B. In: Compendium of Methods for the Microbiological Examination of Foods, 5th edition. Eds. Y. Salfinger and M.L. Tortorello. Ch. 9. American Public Health Association, Washington DC.

[14] FAO/WHO. 1979. Report of a joint FAO/WHO Working Group on Microbiological Criteria for Foods, Geneva, February 20-26, WHO Geneva. Document WG/Microbiol. 79/1.

[15] NRC. National Research Council, Food and Nutrition Board, Committee on Food Protection, Subcommittee on Microbiological Criteria. 1985. An evaluation of the role of microbiological criteria for foods and food ingredients. P. 436. National Academy Press, Washington, DC.

[16] Enterobacteriaceae, Coliforms, and Escherichia coli as Quality and Safety Indicators. 2015. Kornacki, J., Gurtler, J. and Stawick, B. In: Compendium of Methods for the Microbiological Examination of Foods, 5th edition. Eds. Y. Salfinger and M.L. Tortorello. Ch. 9. American Public Health Association, Washington DC.

Shiga toxin-producing *E. coli* (STEC)

E. coli O157:H7, the most commonly identified STEC in North America, and selected other STEC can cause severe intestinal distress as well as a life-threatening complication known as hemolytic uremic syndrome in 5 to 10 percent of those diagnosed with a STEC infection. Therefore, under Section 402(a)(1) of the FD&C Act, the presence of *E. coli* O157:H7 and other pathogenic STEC in a ready-to-eat food (including raw milk cheese) renders the food adulterated in that it bears or contains a poisonous or deleterious substance which may render it injurious to health, and thus in violation of the act and subject to regulatory action.

Generic *E. coli*

E. coli has traditionally been used as a microbiological indicator of insanitation during processing. Raw milk cheese may be considered adulterated under Section 402(a)(4) of the FD&C Act in that it has been prepared, packed, or held under insanitary conditions whereby it may have become contaminated with filth when *E. coli* is found at violative levels. Please see Appendix C: Generic *E. coli* Testing Scheme and Regulatory Strategy for an explanation of how the FDA determines whether or not a sample is violative for generic *E. coli*.

As of February 9, 2016, the FDA has paused its testing for generic *E. coli* in raw milk cheese as it considers implementation of the FSMA rules and what role generic *E. coli* should have in identifying and preventing insanitary conditions and food safety hazards for both domestic and foreign cheese producers. However, the samples obtained under this assignment were collected and tested prior to the pause and the FDA intends to consider the data yielded as part of its deliberation on the role of generic *E. coli* testing going forward.

PUBLIC HEALTH IMPACT

Based on the FDA's evaluation of the raw milk cheese samples, and the limited clinical data currently available, no illnesses are known to have been caused by the raw milk cheese that the FDA found contaminated. A detailed explanation of the FDA's evaluation is available in Appendix D: Genetic Evaluation. Of particular note in this analysis is the increasing importance of whole genome sequencing in identifying the scope and source of microbial contamination. For these reasons, the agency will continue to expand its efforts in whole genome sequencing, gradually moving away from lower resolution approaches.

In conducting this assignment, the agency found several instances of pathogens in select products produced from certain facilities and subsequently worked with domestic industry to take voluntary corrective actions to remove contaminated cheese from the marketplace, thus preventing consumption and potentially averting illnesses. When the FDA found contaminated cheeses at ports of entry, the agency refused those entries and placed the responsible firms and product on Import Alert 12-10, thereby requiring additional controls for future entries, and likewise potentially averting illnesses.

Given that the FDA collected previously unopened packaged cheese under this assignment, when the agency found pathogens in that cheese it demonstrated that the contamination occurred where the cheese was made. Protection of cheese, a ready-to-eat food, from environmental pathogens,

during processing and prior to packaging, requires vigilance. To control *Listeria monocytogenes*, in refrigerated ready-to-eat food, the FDA published draft guidance for industry.

As the FDA implements and industry complies with the FSMA Final Rule for Preventive Controls for Human Food, ready-to-eat food facilities will need to conduct verification activities that may include product testing and environmental monitoring as possible verification activities. Verification activities will only be required as appropriate to the food, facility, nature of the preventive control, and the role of that control in the facility's food safety system. Food facilities will be required to conduct environmental monitoring if contamination of a ready-to-eat food with an environmental pathogen is a hazard requiring a preventive control. Businesses that have more than 500 employees must comply with the FSMA Final Rule for Preventive Controls for Human Food by September 19, 2016. Smaller businesses have an additional one or two years to comply, depending on their size.

Voluntary Industry Actions, Regulatory Activities for Domestic Sample Pathogen Findings

FDA investigation of the violative domestic samples resulted in five Class I recalls,[17] one regulatory meeting,[18] and one untitled letter.[19] The recalls of domestically produced raw milk cheese occurred as follows:

- In June 2014, after the FDA found a sample positive for *Listeria monocytogenes*, a company in the Northeast voluntarily recalled 21 wheels of waxed raw milk semi-soft cheese that had been distributed to retail stores in the Northeast.

- In July 2014, after the FDA found a sample positive for pathogenic Shiga toxin-producing *E. coli*, a company in the West voluntarily recalled about 750 pounds of a raw milk hard cheese that had been distributed in the Midwest and Southwest.

- In December 2014, after the FDA found samples positive for *Listeria monocytogenes*, a company in the West voluntarily recalled about 2,000 pounds of raw milk cheeses that had been distributed in six states. Approximately two weeks later, the company voluntarily expanded its recall to include all cheese made at the facility over a seven-month period that occurred earlier the same year. The FDA, CDC and state officials conducted an outbreak investigation involving the company and its products in 2015. Using whole genome sequencing, the investigation found the bacteria in the cheeses to be closely related to a small number of clinical isolates. However, a leafy vegetable sample obtained within a 30-mile radius of the cheese manufacturer exhibited the same close association to the clinical

[17] A Class 1 recall is a situation in which there is a reasonable probability that the use of or exposure to a violative product will cause serious adverse health consequences or death.
[18] The FDA holds a regulatory meeting to inform responsible individuals or firms about how one or more products, practices, processes, or other activities are considered to be in violation of the law. Such a meeting can be effective in obtaining prompt voluntary compliance.
[19] An untitled letter cites violations that do not meet the threshold for regulatory action but warrant corrective action on the part of the company.

isolates, precluding specific attribution. That being the case, the FDA took no further regulatory action.

- In December 2014, after the FDA found a sample positive for *Listeria monocytogenes*, a company in the Midwest voluntarily recalled about 1,100 pounds of hard raw milk cheese that had been distributed through retail stores in the Midwest.

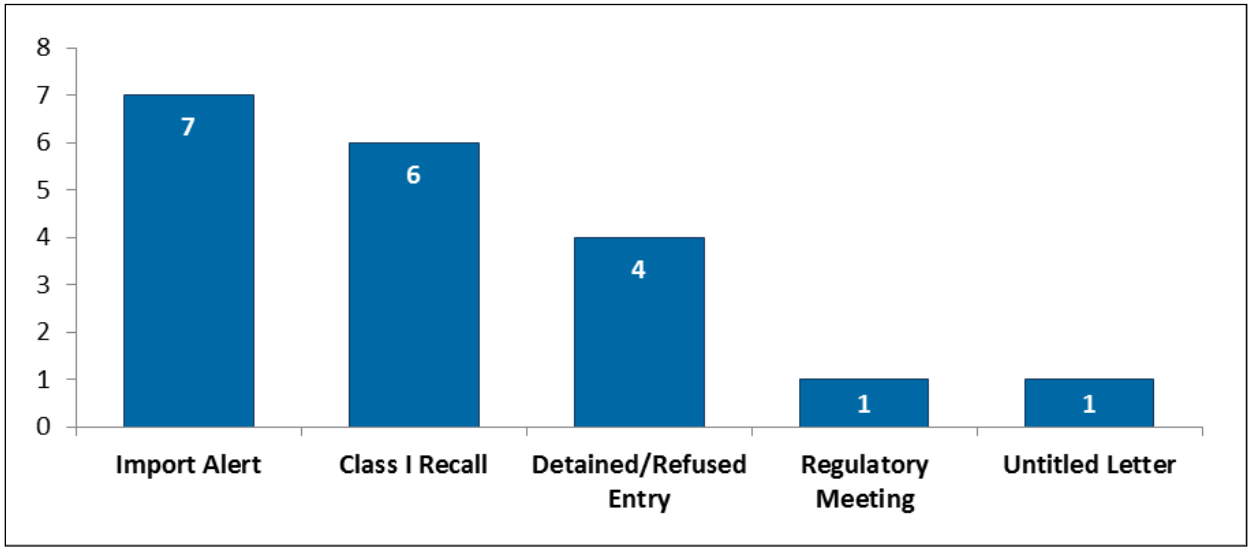
Voluntary Industry Actions, Regulatory Activities for Imported Sample Pathogen Findings

FDA investigation of the violative import samples resulted in seven companies being placed on Import Alert 12-10, "Detention without Physical Examination of Cheese Due to Microbiological Contamination." Three of the seven companies subsequently were removed from the import alert, with four remaining at the time of this report's publication.

One grocery chain voluntarily recalled all cut and wrapped raw milk organic semi-soft cheese of a certain variety exported to the United States from a company in France. The retailer recalled the cheese from its stores nationwide after the FDA found *Listeria monocytogenes* contamination in a sample of cut cheese wedges. In response to this recall, the FDA conducted further sampling of imported product from the company in France, and no further *Listeria monocytogenes* was found.

The chart that follows shows the regulatory activities and voluntary industry actions for all violative samples, both domestic and imported (Figure 2). The FDA took more than one regulatory action in response to several of the samples that it found to be violative.

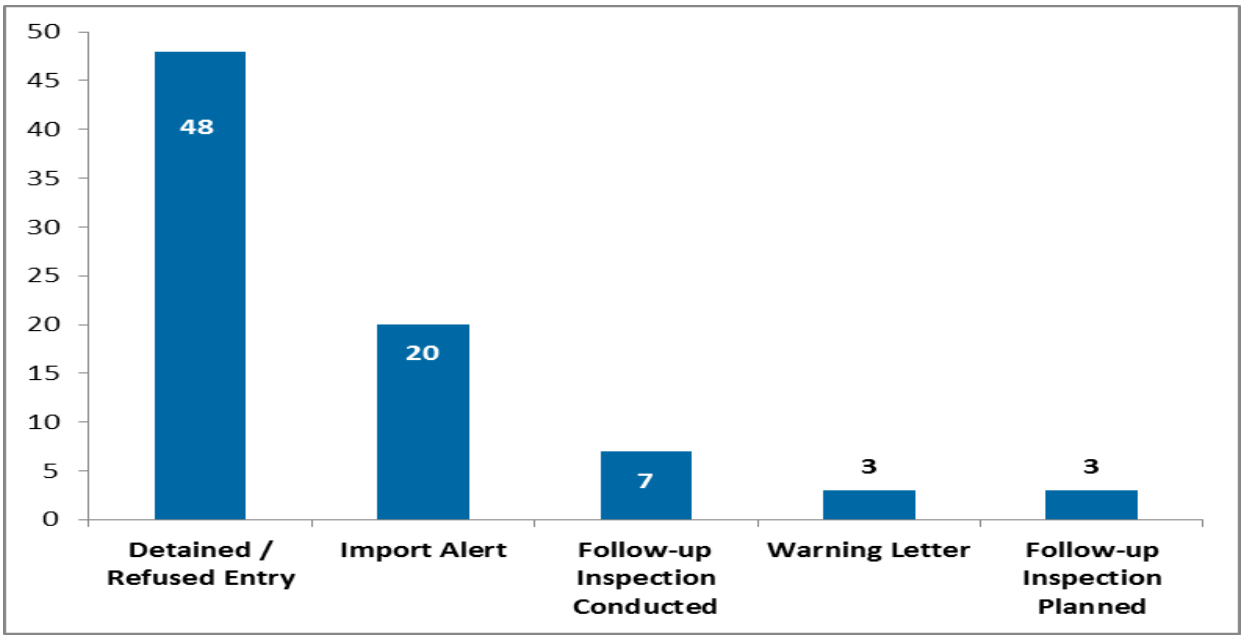
Figure 3: Regulatory Activities and Voluntary Industry Actions for All Violative Samples



Voluntary Industry Actions, Regulatory Activities for Generic *E. coli* Findings

The chart that follows reports voluntary industry actions and the regulatory activities that the FDA carried out in response to all samples determined to be violative based on the presence of generic *E. coli*. Activities numbering fewer than three are listed in the caption (Figure 4).

Figure 4: Voluntary Industry Actions and Regulatory Activities for All Samples Violative Due to Generic *E. coli*



In addition to the regulatory activities shown in the chart, the FDA issued two notices of non-compliance with a consent decree of permanent injunction, and worked with three firms to carry out, respectively, a Class I, Class II, and Class III recall. (The Class I recall centered on a sample positive for both *Listeria monocytogenes* and generic *E. coli*.) Additionally, the FDA determined no enforcement action was warranted in response to a single violative sample after reviewing the firm's compliance history and determining that the product was no longer in the marketplace.

CONCLUSION AND NEXT STEPS

The FDA accomplished the objectives that it set at the outset of the assignment, the most fundamental being to determine the contamination rates of *Salmonella*, *Listeria monocytogenes* and Shiga toxin-producing *E. coli* (primarily, *E. coli* O157:H7) in raw milk cheese.

As detailed in Appendix B, the FDA found the overall contamination rates for each of the pathogens to be less than one percent. Further, the agency found the overall contamination rate of violative levels of generic *E. coli* to be 5.4 percent. While the prevalence for generic *E. coli* was comparatively high, it should be noted that it rarely causes illness even as it may signal insanitary processing conditions.

With respect to common factors among the FDA's findings, the less than one percent contamination frequencies limited the agency's ability to detect differences in prevalence, such as based on the type of cheese or its origin. Thus, the agency concluded that it could not reliably make inferences with respect to possible common factors.

The FDA took regulatory action as warranted in response to each violative sample. To address the violative domestic samples, the agency worked with the responsible firms to carry out recalls and followed up with inspections of manufacturing facilities to ascertain their adherence to good manufacturing practices. To address the violative samples of imported raw milk cheese, the FDA placed the responsible firms/products on Import Alert 12-10. Details on the regulatory actions that the FDA took are available in this report's section on Public Health Impact (pages 15-17).

Underpinning the assignment objectives, it is important to understand that the FDA's ultimate goal in carrying out the sampling assignment was to strengthen its understanding of the public health risks associated with raw milk cheese 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 data collected by the FDA indicate that the prevalences of *Salmonella* and pathogenic Shiga toxin-producing *E. coli* are relatively low and similar to the contamination rates in many other foods. *Listeria monocytogenes* prevalence, especially in semi-soft cheese, remains a concern and the agency will be actively working with industry to address strategies to significantly minimize or prevent contamination. In light of the findings, the FDA does not anticipate additional large-scale sampling of raw milk cheese but plans to continue to utilize its Domestic and Imported Cheese Compliance Program for routine sampling of cheeses. In addition, the agency intends to continue to sample raw milk cheese using its longstanding approach to food sampling, which centers on the following criteria:

- A firm has a previous history of unmitigated microbiological contamination in the environment and/or in finished product (e.g., illness complaints, recalled or seized product, previous inspectional history, or environmental pathogens without proper corrective actions by the facility), or
- For cause (i.e., when inspectional observations warrant collection of samples for microbiological analyses).

In employing the approach described immediately above, the FDA will sample raw milk cheese as warranted and consistent with its vigilance in protecting the U.S. food supply.

APPENDIX A: TEST METHODS

FDA analysts tested the samples using established and widely recognized methods specific to each pathogen. In testing for *Salmonella*, *Listeria monocytogenes* and Shiga toxin-producing *E. coli* (STEC), the analysts did not conduct enumeration (i.e., the determination of the number of viable pathogens in a sample). The test methods the FDA employed are as follows:

Salmonella

A blend method was used to detect *Salmonella* contamination in the cheese as described in the FDA's *Bacteriological Analytical Manual* (BAM) culture method for *Salmonella* (Chap.5). Analysts blended cheese samples with a pre-enrichment lactose broth in sterile jars and incubated them for 24 hours at 35 degrees Celsius. The analysts then used AOAC[20] official methods: VIDAS Easy (OMA 2011.03) or VIDAS SLM (OMA 996.08 or 2004.03) enzyme-linked immunofluorescent assay(s) to detect *Salmonella.* The BAM culture method was then used to confirm the VIDAS results.

Listeria monocytogenes

The method described in the BAM (Chap. 10) or equivalent AOAC methods were used to analyze samples. For the BAM method, analysts blended cheese samples with a buffered *Listeria* enrichment broth in sterile jars and incubated them for four hours at 30 degrees Celsius. They next added selective reagents and continued the incubation until it totaled 48 hours in all. After incubation, enrichment mixes were streaked onto selective agars and further characterized to confirm *Listeria monocytogenes*. Alternatively, analysts used VIDAS LIS (OMA 999.06) or VIDAS LMO2 (OMA 2004.06) immunoassay(s) to screen for *Listeria* species or *Listeria monocytogenes.* The FDA's BAM culture method for *Listeria monocytogenes* was then used to confirm the VIDAS results.

Shiga toxin-producing *E. coli* (STEC)

STEC are classified based on the production of shiga toxins (Stx), which are encoded by the *stx* genes. The FDA's BAM method (Chap. 4A) used to test for STEC in cheese is a polymerase chain reaction (PCR) assay that is specific for the *stx* genes and for O157:H7 serotype. Briefly, 10 subsamples of cheese, 25 grams each, were blended and enriched in 225 milliliters of broth medium containing antibiotic that selects for the growth of STEC. After incubation at 42 degrees Celsius overnight, DNA was extracted from an aliquot and tested by PCR. Samples that were O157- negative, but positive for *stx* are suspected to contain STEC. Since not all STEC appear to be pathogenic to humans, STEC were isolated from agar media and further characterized to differentiate low health risk strains from those that pose high health risks and have the potential to cause severe disease. The further characterization is based on serotype, the toxin subtypes

[20] At times referred to as the Association of Analytical Communities, AOAC International is a globally recognized, independent, not-for-profit association that develops analytical methods for foods and beverages, among other products.

present, and adherence factors, which determine to what extent the bacteria may attach to cell surfaces in the gastrointestinal tract.

Generic *E. coli*

Generic *E. coli* has historically been used worldwide as an indicator of insanitary processing conditions. Unlike pathogens, which are tested with qualitative methods (presence or absence), *E. coli* are tested with quantitative methods to determine whether the level present has exceeded the *E. coli* limit set by a given country. The method used to enumerate *E. coli* levels in cheese is the statistical Most Probable Number (MPN) method,[21] described in the FDA's BAM (Chap. 4). The MPN method has been used worldwide for decades for the enumeration of *E. coli* in foods. Briefly, five subsamples of cheese, 50 grams each, were blended with 450 milliliters of buffer. A series of ten-fold dilutions were made from this preparation and inoculated into sets of lactose-based broth medium to detect acid and gas production from the fermentation of lactose. Gas-positive tubes were sub-cultured to a broth that is more selective for *E. coli,* incubated and again, checked for gas production. Aliquots from gas positive tubes were plated onto agar medium and isolated colonies were identified biochemically as *E. coli*. The MPN is estimated from the combination of *E. coli*-positive tubes.

[21] The Most Probable Number method is a statistical, multi-step assay employed to determine the viable bacterial population in a sample.

APPENDIX B: POSTIVE FINDINGS BY BACTERIAL TYPE

The table that follows provides a complete breakdown of the prevalence for each bacterial type by origin (i.e., domestic vs. import) and cheese type/texture. Readers will note that when added together the positive findings for the subcategories listed under the "Cheese" heading exceed the total provided in each row labeled "All." This occurs because of overlap among subcategories.

Bacteria	Cheese	Positive	No. Collected	Prevalence	Conf. Interval Lower Bound	Conf. Interval Upper Bound
Salmonella	All	3	1,606	0.19%	0.04	0.54
	Semi-Soft	2	1,013	0.2%	0.02	0.71
	Semi-Soft (Domestic)	0	229	0%	0	1.6
	Semi-Soft (Import)	2	784	0.26%	0.03	0.92
	Soft, Fresh	0	24	0%	0	14.25
	Soft- Ripened	0	48	0%	0	7.4
	Hard	1	521	0.19%	0	1.06
	Hard (Domestic)	0	220	0%	0	1.66
	Hard (Import)	1	301	0.33%	0.01	1.84
	Domestic	0	473	0%	0	0.78
	Import	3	1,133	0.26%	0.05	0.77
L. mono.	All	10	1,606	0.62%	0.3	1.14
	Semi-Soft	9	1,013	0.89%	0.41	1.68
	Semi-Soft (Domestic)	4	229	1.75%	0.48	4.41
	Semi-Soft (Import)	5	784	0.64%	0.21	1.48
	Soft, Fresh	0	24	0%	0	14.25
	Soft-Ripened	0	48	0%	0	7.4
	Hard	1	521	0.19%	0	1.06
	Hard (Domestic)	1	220	0.45%	0.01	2.51
	Hard (Import)	0	301	0%	0	1.22
	Domestic	5	473	1.06%	0.34	2.45
	Import	5	1,133	0.44%	0.14	1.03
***E. coli* O157**	All	0	1,606	0%	0	0.23
	Semi-Soft	0	1,013	0%	0	0.36
	Soft, Fresh	0	24	0%	0	14.25
	Soft-Ripened	0	48	0%	0	7.4
	Hard	0	521	0%	0	0.71
	Domestic	0	473	0%	0	0.78
	Import	0	1,133	0%	0	0.33

The table is continued on the next page, and includes data on Shiga toxin-producing *E. coli*, and generic *E. coli*.

Bacteria	Cheese	Positive	No. Collected	Prevalence	Conf. Interval Lower Bound	Conf. Interval Upper Bound
STEC	All	10	1,606	0.62%	0.3	1.14
	Semi-Soft	7	1,013	0.69%	0.28	1.42
	Semi-Soft (Domestic)	1	229	0.44%	0.01	2.41
	Semi-Soft (Import)	6	784	0.77%	0.28	1.66
	Soft, Fresh	0	24	0%	0	14.25
	Soft-Ripened	1	48	2.08%	0.05	11.07
	Hard	2	521	0.38%	0.05	1.38
	Hard (Domestic)	1	220	0.45%	0.01	2.51
	Hard (Import)	1	301	0.33%	0.01	1.84
	Domestic	2	473	0.42%	0.05	1.52
	Import	8	1,133	0.71%	0.31	1.39
Pathogenic STEC *	All	1	1,606	0.06%	0	0.35
	Hard	1	521	0.19%	0	1.06
	Hard (Domestic)	1	220	0.45%	0.01	2.51
	Hard (Import)	0	301	0%	0	1.22
	Domestic	1	473	0.21%	0.01	1.17
	Import	0	1,133	0%	0	0.33
Generic *E. coli*	All	87	1,606	5.42%	4.36	6.64
	Semi-Soft	64	1,013	6.32%	4.9	8
	Semi-Soft (Domestic)	10	229	4.37%	2.11	7.88
	Semi-Soft (Import)	54	784	6.89%	5.22	8.89
	Soft, Fresh	0	24	0%	0	14.25
	Soft-Ripened	3	48	6.25%	1.31	17.2
	Soft-Ripened (Domestic)	0	12	0%	0	26.46
	Soft-Ripened (Import)	3	36	8.33%	1.75	22.47
	Hard	20	521	3.84%	2.36	5.87
	Hard (Domestic)	8	220	3.64%	1.58	7.04
	Hard (Import)	12	301	3.99%	2.08	6.86
	Domestic	18	473	3.81%	2.27	5.95
	Import	69	1,133	6.09%	4.77	7.64

* ***E. coli* O111:H8**

APPENDIX C: GENERIC *E. COLI* TESTING SCHEME AND REGULATORY STRATEGY

The FDA established a three-class attribute sampling plan to support its monitoring for generic *E. coli* in cheese, consistent with recommendations from the International Commission on Microbiological Specifications for Foods (ICMSF), a leading source for impartial, science-based guidance on controlling the microbiological safety of foods.

The three-class attribute plan, implemented for purposes of the raw milk cheese assignment, is defined as *n=5*, *c=2*, and *m=10 MPN/g* to *M=100 MPN/g*, wherein:

- *n=5* refers to the number of subsamples that make up one sample;
- *c=2* refers to the number of allowable defects (i.e., the number of subsamples allowed to test positive) per sample; and
- *m=10 MPN/g* to *M=100 MPN/g* provides the range for the unacceptable levels in any one subsample.

In short, the three-class attribute plan specifies that levels of generic *E. coli* exceeding 10 MPN/g and less than 100 MPN/g in three or more subsamples of the five tested are unacceptable, and thus render the sample violative. Additionally, a level of generic *E. coli* exceeding 100 MPN/g in any single subsample renders the sample violative.

The FDA considers that its plan, in which *n* is relatively low, is appropriate because generic *E. coli* is not a pathogen. The FDA thus determined that *n* equal to five would suffice for the number of individual units or subsamples of cheese to be tested.

Further, in deciding upon a final level for *m*, the FDA considered ICMSF guidance that *m* reflect a level that is acceptable and attainable in the food as well as implementation of good hygienic practices. The FDA concluded that *m* at 10 cfu/g is consistently attainable. In deciding upon a final level for *M*, the FDA considered ICMSF guidance that, as a general hygiene indicator, *M* should represent clearly unacceptable conditions of hygiene. The scientific literature, international standards in use, and the FDA's own analytical results for generic *E. coli* in cheese, led the agency to conclude that *M* at 100 cfu/g is consistently attainable and that exceeding this level in cheese is indicative of conditions meeting the adulteration standard of section 402(a)(4) of the FD&C Act.

The FDA implemented the three-class attribute sampling plan throughout the assignment, with the exception of the first several months, when the agency applied a level of scrutiny greater than *n=5* and *c=2*. The FDA took steps to ensure the intended level of scrutiny going forward, along with other corrective action, namely the removal of four firms from an import alert. All findings of generic *E. coli* presented in this report are consistent with the agency's three-class attribute sampling plan.

As of February 9, 2016, the FDA has paused its testing for generic *E. coli* in raw milk cheese as it considers implementation of the FSMA rules and what role generic *E. coli* should have in identifying and preventing insanitary conditions and food safety hazards for both domestic and international cheese producers.

APPENDIX D: GENETIC EVALUATION

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

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

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

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

Pulsed-Field Gel Electrophoresis

PFGE 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 to determine whether any of the PFGE patterns associated with the raw milk cheese that tested positive for a pathogen under this sampling assignment matched any of the PFGE patterns reported previously in association with ill individuals.

The FDA's comparisons found that most of the bacterial strains from the raw milk cheese samples were indistinguishable from clinical isolates in the PulseNet USA database. While the FDA uses indistinguishable PFGE patterns to cluster genetically similar bacterial strains and investigate potential outbreaks of foodborne illness, further data, usually food histories from ill persons and isolates from the site(s) where the food was produced or processed, are needed to ascertain that an adulterated food caused a particular illness, or multiple illnesses in the case of an outbreak.

Salmonella. Of the three samples of raw milk cheese that tested positive for *Salmonella*, one produced two isolates (yielding four *Salmonella* isolates in all). Three distinct PFGE patterns were found from the four *Salmonella* isolates. The FDA queried the PulseNet USA database to compare the four *Salmonella* isolates taken from raw milk cheese samples to reported human biological (clinical) isolates from June 12, 2014 to June 01, 2016. In this survey the FDA did not find any instances in which a *Salmonella* isolate from raw milk cheese was determined to be indistinguishable from a clinical isolate.

***Listeria monocytogenes*.** Of the 10 samples of raw milk cheese that tested positive for *Listeria monocytogenes*, several produced more than one isolate (yielding 15 *Listeria monocytogenes* isolates in all). The FDA queried the complete PulseNet USA database to compare the 15 *Listeria monocytogenes* isolates taken from raw milk cheese samples to

reported human biological (clinical) isolates, entered from 1998 to June 1, 2016. The FDA found 11 bacterial isolates from raw milk cheese to be indistinguishable from clinical isolates when compared by subtype. The bulleted information that follows provides details on the 11 isolates and related regulatory activities that the FDA undertook.

- Six raw milk cheese isolates were found to be indistinguishable by PFGE from each other and were found to be indistinguishable by PFGE from seven clinical isolates from three states. Five of the seven clinical isolates were from the West. (Upon detecting the pathogen in the cheese samples, the FDA worked with the responsible firm to carry out a Class I recall in 2014, which was expanded approximately two weeks after its issuance to include all cheese produced at the facility over a seven month period. In addition, the FDA, CDC and state officials conducted a foodborne outbreak investigation in 2015 involving the six raw milk cheese isolates and matching clinical cases. The investigation was inconclusive and therefore did not lead to further regulatory action.

The remaining five isolates indicated possible illness causality but absent other evidence were inconclusive. As stated earlier, further data, usually food histories from ill persons and isolates from the site(s) where the food was produced or processed, are needed to ascertain that an adulterated food caused a particular illness, or multiple illnesses. Ultimately, the whole genome sequence information (see Whole Genome Sequencing subsection, page 27) revealed that the bacteria involved in the findings below did not match any clinical illnesses.

- Two of the 15 raw milk cheese isolates were found to be indistinguishable by PFGE from each other and those two isolates were found to be indistinguishable by PFGE pattern from 15 clinical isolates from eight states. (Upon detecting the pathogen in the cheese sample, the FDA refused the shipment entry into the United States and placed the responsible firm/product on Import Alert 12-10.)
- One raw milk cheese isolate was found to be indistinguishable by PFGE from three clinical isolates from two states. (Upon detecting the pathogen in the cheese sample, the FDA worked with the U.S. retailer to carry out a Class I recall, which instructed all consignees to destroy the product.)
- One raw milk cheese isolate was found to be indistinguishable by PFGE from 11 clinical isolates from seven states. (Upon detecting the pathogen in the cheese sample, the FDA refused the shipment entry into the United States and placed the responsible firm/product on Import Alert 12-10.)
- One raw milk cheese isolate was found to be indistinguishable by PFGE from 250 clinical isolates from 33 states and territories. However, the isolate found in the cheese ultimately did not match the human clinical isolates when further tested by WGS. (Upon detecting the pathogen in the cheese sample, the FDA worked with the responsible firm to carry out a Class I recall.)

Shiga toxin-producing *E. coli* (STEC). The one sample of raw milk cheese that tested positive for pathogenic STEC (specifically, *E. coli* O111:H8 serotype) produced two isolates. The FDA queried the PulseNet USA database to compare the two STEC isolates taken from raw milk cheese samples to reported human biological (clinical) isolates from

1998 to June 1, 2016. The FDA found both raw milk cheese isolates to be indistinguishable by PFGE from each other as well as to two clinical isolates.

Whole Genome Sequencing

Whole genome sequencing (WGS) 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.

***Listeria monocytogenes*.** Beginning in the fall of 2013, WGS technology has been used for analysis of clinical *Listeria monocytogenes* isolates in the United States. The FDA compared its raw milk cheese isolates to those in a National Center for Biotechnology Information database of cases of human illness. Six of the isolates from raw milk cheese collected in 2014 were identical to a single case of illness from 2013. The remaining five raw milk cheese isolates did not match any clinical isolates currently in the database.

***Salmonella*, Shiga toxin-producing *E. coli*.** WGS of clinical *Salmonella* and Shiga toxin-producing *E. coli* isolates are not routinely performed by the CDC and state public health laboratories at this time, and therefore the FDA was not able to compare the WGS profile of isolates from raw milk cheese to isolates from human clinical samples. No WGS matches for Salmonella or STEC were found in the limited clinical data currently available. But, this is not significant as relates to *Salmonella* given that there were no identified, indistinguishable PFGE matches between the food and clinical isolates reported in PulseNet USA.