U.S. FOOD & DRUG ADMINISTRATION

Summary Report: Frozen Berries (Strawberries, Raspberries and Blackberries)

FY 2019 – 2023
Microbiological Sampling Assignment

Office of Microbiological Food Safety
Human Foods Program

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At-A-Glance

- The FDA under this assignment collected and tested 1,558 samples of frozen berries for hepatitis A virus (HAV) and norovirus from November 2018 to September 2023. Of the total, 585 were frozen strawberries, 528 were frozen raspberries, and 445 were frozen blackberries.

- The FDA detected HAV in eight samples (1 strawberry, 5 raspberry, and 2 blackberry) and norovirus in 10 samples (3 strawberry, 3 raspberry, and 4 blackberry). These results made for HAV and norovirus contamination rates of less than 1% for each of the three berry types.

- The FDA did not find a statistically significant difference in the HAV or norovirus detection rates by origin (i.e., domestic versus import), for any of the three berry types.

- The assignment did not find evidence linking any violative products to foodborne illness outbreaks.

- Working with industry experts and other interest holders, the FDA has developed a prevention strategy to help limit or prevent enteric virus contamination and outbreaks linked to fresh or frozen berries. It outlines actions for the FDA, industry, and other interest holders to ensure accurate and timely communication and consistent application of effective prevention measures across the global berry industry. A summary of the strategy is available on FDA.gov.

Executive Summary

Seeking to better understand the occurrence of hepatitis A virus (HAV) and norovirus in frozen strawberries, raspberries and blackberries, the U.S. Food and Drug Administration collected and tested more than 1,500 samples of the three commodities from November 2018 to September 2023 as part of its prevention-based work and mission to keep contaminated food from reaching consumers.

Fresh and frozen berries have been linked to outbreaks of HAV and norovirus infections in several countries, including in the United States. The outbreaks prompted the FDA to conduct this assignment to gain insights into the risks of HAV and norovirus associated with the three commodities.

Assignment Overview

In all, the FDA collected and tested 1,558 samples of the three frozen commodities (585 strawberries, 528 raspberries, and 445 blackberries) for this assignment. The total is less than the number of samples that the FDA set out to collect and test because the agency encountered circumstances that necessitated adjustments to the collection scheme, including to the collection target, as explained in the Sample Collection section of this report.

As to the design of the assignment, the FDA sought to estimate the HAV and norovirus contamination rates in both domestic and import samples for each type of berry, and secondarily to compare them by origin (i.e., domestic versus import). The agency collected samples in finished packaging and from bulk containers. The agency did not collect berry pulp or frozen berries with added ingredients.

Ten months into the assignment, the agency ceased its collection of samples at retail in favor of sampling at earlier stages of the distribution chain so that, per industry common practice,[1] greater volumes of product impacted by testing would be held prior to entering commerce or not enter commerce in the event of a pathogen detection. In making this update, the agency's intent was to better protect consumers while minimizing market disruption.

Additionally, after engaging in dialogue with external interest holders, the FDA in the first year of the assignment strengthened its testing protocol by adding amplification and subsequent Sanger sequencing of a second region of the viral genome. This approach was put in place to help address some of the challenges that exist when detecting and characterizing non-cultivable enteric pathogens. Details on this update to the assignment may be found in "Appendix A: Analytical Methods and Procedures."

1. Firms whose product is being tested for pathogens by the FDA typically place the associated lots on a hold status, pending receipt of test results. The circumstances surrounding the agency's decision to stop collecting samples at retail are detailed in the Sample Collection section of this report.

Findings and Follow-up Actions

The FDA detected HAV in eight samples (1 strawberry, 5 raspberry, and 2 blackberry) and norovirus in 10 samples (3 strawberry, 3 raspberry, and 4 blackberry). These results made for HAV and norovirus prevalences of less than 1% for each of the three berry types.

The FDA compared the HAV and norovirus detection rates by origin (i.e., by their domestic-versus-import collection status) for each of the commodities. The agency did not find any statistically significant difference in contamination rates based on origin.

Whenever the agency detected HAV or norovirus under this assignment, the FDA oversaw the removal of all affected product from the U.S. distribution chain. All the domestic samples found to be violative resulted in a voluntary recall; all the import samples found to be violative were either refused entry into the United States, or they resulted in a voluntary recall of the affected lot. Additionally, in four cases, the FDA either flagged, for 180 days, all future shipments of the commodity by the responsible firm, such that all were subject to screening upon entry, or the FDA placed the firm and its product on Import Alert 99-35, which requires a firm to overcome the appearance of adulteration and be removed from the import alert before any entries can be released into domestic commerce.

The findings of this assignment, combined with the history of foodborne illness outbreaks linked to fresh and frozen berries, underscore the need for berry growers to comply with the FDA's Produce Safety Rule, for frozen berry manufacturers and others in their distribution chain to comply with the FDA's Preventive Controls for Human Food Rule, and for importers of these commodities to comply with the FDA's Foreign Supplier Verification Programs Rule.

Strategy to Prevent Enteric Virus Outbreaks Associated with Fresh and Frozen Berries

In tandem with this assignment, the FDA has worked with industry experts and other interest holders to develop a prevention strategy for enteric viruses in fresh and frozen berries to help limit or prevent HAV and norovirus contamination and outbreaks associated with fresh or frozen berries. The prevention strategy was initiated in response to a history of HAV and norovirus outbreaks linked to the consumption of both fresh and frozen berries. It was not initiated as a result of the findings of this sampling assignment. The prevention strategy summary features goals and activities for the FDA, industry and other interest holders to prevent contamination of fresh and frozen berries with enteric viruses. Hygienic practices and challenges for the control of enteric viruses in berries and other hand-harvested produce apply globally. An approach that identifies, leverages, and shares effective practices can benefit domestic and global operators that grow, process, and source berries and other produce with similar risk factors.

Background

In 2011, the FDA Food Safety Modernization Act (FSMA) amended the Federal Food, Drug, and Cosmetic Act (FD&C Act) to provide the agency with additional authority to better prevent food safety problems before they occur. To develop better prevention-based systems, the FDA needs data and

other information to help identify hazards that should be addressed and minimized. That is why sampling is an important part of the FDA's preventive approach to food safety. To help achieve its objectives, the FDA revised its approach to sampling in 2013 to selectively acquire large sample sets (such as this assignment's) to estimate the prevalence and to identify patterns of occurrence of targeted pathogens, with the end goal of preventing microbial contamination.

Why Frozen Berries?

Frozen berries are used as ingredients in many foods and like other produce can be an important part of a healthy diet. Frozen berries are used in pies, cakes and other baked goods. When baked, the berries are subject to a 'kill step' that reduces or eliminates the risk of any present enteric viruses to humans. However, frozen berries are also consumed ready-to-eat in fruit salads, yogurt and smoothies, as well as other foods that do not typically include a kill step. The vast majority of frozen berries eaten in the United States are consumed safely. There were, however, at least three HAV outbreaks (one attributed to frozen pomegranate arils consumed as part of a frozen organic antioxidant blend) and one norovirus outbreak linked to the consumption of frozen berries in the United States from 1997 to 2016. These were the data that the FDA reviewed prior to initiating its frozen berries sampling assignment in FY19.[2] Since then, the FDA and the CDC also investigated a 2022 outbreak of HAV infections linked to fresh organic strawberries and a 2023 HAV outbreak linked to frozen organic strawberries. Both outbreaks were linked to a single grower in Baja California, in Mexico, and to strawberries harvested during the same time period.

Humans are the primary reservoir of HAV and norovirus that can be introduced into foods by human contact.[3,4] Strawberries, raspberries and blackberries may become contaminated with HAV or norovirus at any point from farm to frozen packaging if handled by an infected worker who does not use appropriate hand hygiene or if exposed to contaminated agricultural water or a contaminated surface, like a harvesting tote or processing equipment. While freezing preserves berries and otherwise extends their shelf life, viruses can survive at freezing temperatures.

After reviewing the history of viral outbreaks linked to frozen berries, the FDA determined that a sampling assignment may help the agency to better understand the prevalence[5] of HAV and norovirus in frozen strawberries, raspberries, and blackberries and potentially aid in identifying common factors among contaminated samples, with the end goal of protecting consumers. While HAV and norovirus in frozen berries could have originated during growing, harvesting or transport of berries, fresh berries were not included in the sampling assignment.

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2. The outbreaks of HAV infections linked to frozen berries occurred in 1997, 2013 and 2016. The outbreak of norovirus infections linked to frozen berries occurred in 2016.
3. Hepatitis A. World Health Organization online resource, retrieved September 12, 2024.
4. Hall, A. J., Vinjé, J., Lopman, B., Park, G. W., Yen, C., Gregoricus, N., et al. (2011). Updated Norovirus Outbreak Management and Disease Prevention Guidelines. Morbidity and Mortality Weekly Report. 60(RR03); 1-15.
5. Viral or bacterial prevalence is the number of samples that test positive for a pathogen in proportion to the total number of samples tested for the analyte.

Production and Consumption

Frozen strawberries, raspberries, and blackberries are prepared from the plants' ripened fresh fruit. Workers perform steps such as removing the stems and cleaning the fruit, as applicable. The berries are then packed with or without packing media, flash frozen and stored at temperatures necessary to preserve them.[6] Because the freezing process typically occurs shortly after the fruit is picked, when the berries' nutrients are at or near their peak, the fruit largely retains its nutritional value.[7]

The United States produced about 440 million pounds of frozen strawberries, raspberries and blackberries and imported about 455 million pounds of the three commodities on average each year from 2019 to 2023.[8] The federal government likewise collects data on the consumption of frozen berries via the National Health and Nutrition Examination Survey (NHANES) and estimates that it has increased substantially in recent decades, in part due to the growth in frozen smoothies consumption.[9]

Objectives

The objectives of the FDA's FY 2019-2023 frozen berries assignment were:

- To estimate the prevalence of HAV and norovirus in frozen strawberries, raspberries, and blackberries;
- To determine if there were common factors associated with pathogen findings (such as by origin), where possible; and
- To take follow-up action in response to pathogen findings, as warranted, with the goal of protecting consumers.

Sample Collection

The FDA originally planned to collect and test 2,000 samples of frozen berries for this assignment. Of the total, one half were to be domestic samples; the other, import samples. The design of the assignment and robust sample size were intended to support statistical evaluation, including comparison of contamination rates by whether the frozen berries were domestically produced versus imported into the United States.

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6. Frozen Berries Grades and Standards, USDA Agricultural Marketing Service. Web resource retrieved September 12, 2024.
7. Berries subject to Individual Quick Freeze (IQF) largely retain their nutritional value provided that they are properly packaged, not subject to temperature fluctuations (i.e., multiple freeze-thaw cycles) and do not exceed their shelf life. Memo to the file prepared by the Office of Nutrition and Food Labeling, FDA Human Foods Program.
8. Breakdowns of these five-year averages are provided in "Appendix B: Domestic Production and Import Data."
9. "Berries led growth in frozen fruit consumption over the last three decades." USDA Economic Research Service, Food Availability (Per Capita) Data System. Page retrieved: September 12, 2024.

Methodology

The agency initially directed its field staff to collect only frozen berries in finished packaging. Each sample consisted of three subsamples. Each subsample was made up of a finished package of frozen strawberries, raspberries or blackberries, weighing at least one pound. The FDA routinely collects and tests samples composed of multiple subsamples. This approach is more reflective of actual conditions, and it increases the probability of detecting pathogens, if present, given that microbial hazards may not be uniformly present. The agency did not collect units of mixed frozen berries, berry pulp or frozen berry products with added ingredients, like sugar, or frozen berries coated with chocolate or yogurt, for example.

The agency encountered unforeseen circumstances during the assignment that necessitated adjustments. Accordingly, the FDA made updates to the assignment to aid in its progress while also ensuring that it meet its public health goals. The adjustments are detailed below.

Adjustments to the Collection Scheme

Sampling from bulk containers permitted. Beginning in November 2018, when the FDA initiated the assignment, there was no collection of samples from bulk containers (only collection of samples in finished packaging). Agency field staff experienced difficulty meeting the monthly collection targets[10] based on the initial assignment directives. That being the case, the agency updated the assignment collection scheme in May 2019 to allow for samples to be collected from bulk containers. If product in finished packaging was not available at a collection site, the field staff would collect one sample from any given bulk container. The samples of bulk product were made up of three portions of frozen berries from the same container (i.e., three subsamples), each one weighing at least one pound. The FDA collected the bulk samples aseptically to prevent contamination during collection. The agency's aseptic sampling methods, which involve the use of sterile implements and containers, and prescribed collection procedures, may be found in the agency's Investigations Operations Manual (Section 4.3.6).

Sampling at retail stopped. From the outset of the assignment, the FDA collected samples at processors, distribution facilities, ports of entry and retail establishments. This approach was intended, in part, to help the FDA achieve its collection target, as the agency had also committed to not collect samples from any one establishment more than three times in a six-month period to avoid overburdening entities in the distribution chain. However, firms whose product is being tested for pathogens typically place the associated lots on a hold status, pending receipt of test results. Bearing in mind this practice, and following a mid-year review of the assignment, the agency ceased its collection of samples at retail in August 2019 in favor of collection at earlier stages of the distribution chain. This adjustment to the collection scheme strengthened the assignment's public health benefit in that greater volumes of product impacted by testing were held before entering commerce or, in the event of a pathogen finding, did not enter commerce. Stopping the collection of samples at retail also forestalled the possibility of contamination being attributable to handling at retail, irrespective of the source. The adjusted approach to sample collection was less burdensome to industry, too, in that sampled product

10. The difficulty in meeting the monthly collection targets was largely due to the twofold challenge of limited stock in grocery store freezers and the fact that the FDA was seeking to collect product from a diversity of manufacturers to achieve a substantive representation of what the industry produces.

in some instances had not yet entered retail commerce, leading to less extensive recalls when positive samples were identified. In stopping the collection of samples at retail, the FDA's aims were to better protect consumers, minimize market disruption and respond to stakeholder feedback.

Collection target lowered. In 2022 the FDA reassessed the assignment's planned sample size and in January 2023 made a final adjustment to its collection scheme, namely, to lower the collection target to help bring the assignment to completion. This change was necessitated by the fact that the assignment had already surpassed its planned duration by more than 18 months, due to external factors. The agency initially was compelled to pause its sampling work because of a 35-day lapse in federal appropriations that began on December 22, 2018. Also, in responding to the onset of the coronavirus (COVID-19) pandemic, the FDA placed all field activities on hiatus in March 2020 and then gradually resumed them, beginning with its mission-critical work. Resumption of the frozen berries assignment was further complicated by the fact that, for a time, it was not clear whether cold chain transmission of COVID-19 was possible.[11] The agency resumed its collection and testing in August 2022. The FDA ultimately lowered the assignment's collection target to 1,547 samples, following its reassessment to ensure that the revised target would be adequate to meet the agency's public health goals.[12]

Overall Collection, Including by Berry Type

In all, the FDA collected 1,558 samples for this assignment. Of the total, 585 were frozen strawberries, 528 were frozen raspberries, and 445 were frozen blackberries.

Domestic Sample Collection

Of the 1,558 samples collected, 538 were domestic samples (i.e., berries produced in the United States). Table 1 provides a breakdown of the domestic sample collection by frozen berry type.

Table 1: Domestic Sample Collection by Frozen Berry Type

Frozen Berry Type	Samples Collected
Strawberry	222
Raspberry	145
Blackberry	171
Total	538

Import Sample Collection

The FDA collected 1,020 samples of imported frozen berries. The agency used two approaches to collect samples of imported product: collection in "import status" and domestic import (DI) sampling.

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11. A study published in the Food and Environmental Virology journal concluded in 2022 that the risk of cold-chain food being contaminated by SARS-CoV-2 was slight and the likelihood of transmitting COVID-19 via contaminated food or food packaging was low. Prior to the publication of that study, the FDA and USDA said in a joint statement in 2021 that there was no scientific information indicating that COVID-19 could be transmitted through food or food packaging.
12. The reduced number of samples compared to the original target of 2,000 samples did not negate the FDA's ability to perform statistically valid analyses.

"Import status" refers to samples collected at ports of entry or other sites where the product was being held prior to its release into domestic commerce. The field staff collected 473 samples in import-status locations, representing 46% of the total collection of imported product sampled. In addition, 547 samples (54% of the total collection of imported product sampled) were collected as DI samples and counted toward the import sample total. DI samples are samples of international origin collected after being released into domestic commerce. Typically, they are collected near the port of entry, at a storage facility or distribution hub. Unlike samples collected in import status, DI sampling allows for imported products to be released and sold domestically or to undergo processing. For purposes of this report, the DI samples are included in the import sample data because they originated outside of the United States (Table 2).

Table 2: Import Sample Collection by Frozen Berry Type

Frozen Berry Type	Import Status Collection	Domestic Import Collection	Total
Strawberry	188	175	363
Raspberry	163	220	383
Blackberry	122	152	274
Total	473	547	1,020

Collection by Country of Origin

The FDA collected frozen berries, including the 538 domestic samples, originating from 22 countries in all. Of the import samples, the largest number originated in Chile (378), followed by Mexico (286), and Serbia (130), as shown below (Table 3).

Table 3: Sample Collection by Country of Origin

Origin	Strawberries	Raspberries	Blackberries	Total
Argentina	13	0	0	13
Canada	3	29	17	49
Chile	93	151	134	378
China	19	0	3	22
Mexico	137	79	70	286
Morocco	17	0	0	17
Peru	42	0	1	43
Serbia	0	104	26	130
Turkey	22	3	2	27
U.S.	222	145	171	538
Other*	15	10	15	40
Unknown**	2	7	6	15
Total	585	528	445	1,558

* In addition to the countries listed in this table, the FDA collected samples from 12 other countries: Brazil, Colombia, Costa Rica, Ecuador, Egypt, Guatemala, Kosovo, Lithuania, Poland, Russia, Spain and Yugoslavia. The agency collected less than 10 samples in all from each of these 12 countries. That being the case, for analytical purposes, the report groups them in the table above under the heading "Other."

** The FDA collected 15 samples at import whose country of origin could not be determined or was not documented upon collection.

Collection by Season

The FDA collected samples year-round. Frozen berries are available throughout the year given their status as a frozen commodity. The availability also is due to the number of countries that produce and export frozen berries coupled with the fact that their harvest seasons vary. However, this report does not provide seasonal breakdowns because, these being frozen foods, the agency's sample collection dates may not reliably indicate the seasonal timing of detected viruses. In addition, not all frozen berry products feature a 'manufacture' date on their packaging, nor can a manufacture date be reliably calculated from a 'best by' date due to varying company practices and shelf life.

Collection by Finished Packaging vs. from Bulk Containers

In all, the agency collected 1,478 samples (95%) in finished packaging and 80 samples (5%) from bulk containers. The table that follows provides a breakdown of this variable by frozen berry type (Table 4).[13]

Table 4: Collection by Finished Packaging vs. from Bulk Container, and Frozen Berry Type

Frozen Berry Type	Finished Packaging	From Bulk Container	Total
Strawberry	544	41	585
Raspberry	508	20	528
Blackberry	426	19	445
Total	1,478	80	1,558

Test Methods

At the outset of the assignment and for its duration, the FDA used the agency's Bacteriological Analytical Manual (BAM) method for viral extraction (Chapter 26 B), which had undergone a matrix extension study to validate the method for use with soft fruit.[14, 15] The extraction method was followed by RT-qPCR to detect HAV and norovirus, if present, with each analytical method having undergone multi-laboratory validation prior to implementation of the assignment. These methods are described in "Appendix A: Analytical Methods and Procedures."

In the first year of the assignment, the FDA received feedback from frozen food industry stakeholders, including with respect to the analytical methods employed. The FDA considered the concerns raised and addressed them, making the case that its use of RT-qPCR was scientifically defensible but also agreeing to strengthen its protocol by adding amplification of a second region of the viral genome followed by Sanger sequence characterization of that region. Ten months into the assignment (as of August 2019), the FDA used Sanger sequencing to characterize the viral genome of RT-qPCR

13. To determine the collection status of the samples (i.e., in finished packaging versus from a bulk container), the FDA used a natural language processing tool to classify collection report data and then conducted two instances of human review of the tool's result.
14. Strawberries, raspberries and blackberries.
15. The FDA used RT-qPCR testing in seeking to detect HAV and norovirus in the samples as opposed to viral culture because no effective viral culture method has been developed for use in food.

positives, from that point forward. This approach was put in place to help address some of the challenges that exist when detecting and characterizing non-cultivable enteric pathogens.

The FDA has received feedback from external stakeholders that the test methods used in the assignment do not differentiate between the presence and infectivity of the virus. The agency agrees that enhanced testing methods that characterize viral infectivity would benefit the further characterization of food safety risks associated with the presence of viruses in foods. The FDA looks forward to continuing to collaborate with the scientific community in the furtherance of this research as part of the prevention strategy.

Pathogen Findings

This section provides the prevalence of HAV and norovirus in frozen strawberries, raspberries and blackberries, as well as select breakdowns, based on the assignment test results. The agency describes its approach to its statistical evaluation in “Appendix C: Statistical Analysis.”

HAV Findings

Of the 1,558 samples of frozen berries tested, the agency detected HAV in eight of them (1 strawberry, 5 raspberry, and 2 blackberry). Of those eight samples in which HAV was detected via RT-qPCR, seven were subjected to secondary RT-PCR and amplicon Sanger sequenced. The remaining sample (a frozen raspberry sample) was tested via RT-qPCR, prior to the agency incorporating secondary amplification and Sanger sequencing into the assignment. The table that follows provides the HAV prevalence for each type of frozen berry (Table 5).

Table 5: HAV Findings by Frozen Berry Type

Frozen Berry Type	Samples Collected	Samples Positive	Prevalence	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Strawberry	585	1	0.17%	0.00%	0.95%
Raspberry	528	5	0.95%	0.31%	2.20%
Blackberry	445	2	0.45%	0.05%	1.61%

The three tables below compare the HAV prevalence for each type of frozen berry based on their domestic-versus-import origin (Tables 6, 7 and 8).

Table 6: HAV Findings in Strawberries - Domestic vs. Import

Strawberry	Samples Collected	Samples Positive	Prevalence	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Domestic	222	1	0.45%	0.01%	2.48%
Import	363	0	0.00%	0.00%	1.01%

Table 7: HAV Findings in Raspberries - Domestic vs. Import

Raspberry	Samples Collected	Samples Positive	Prevalence	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Domestic	145	1*	0.69%	0.02%	3.78%
Import	383	4	1.04%	0.29%	2.65%

* The FDA detected HAV in this sample via RT-qPCR testing, prior to incorporating secondary amplification and Sanger sequencing into the assignment.

Table 8: HAV Findings in Blackberries - Domestic vs. Import

Blackberry	Samples Collected	Samples Positive	Prevalence	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Domestic	171	1	0.58%	0.01%	3.22%
Import	274	1	0.36%	0.01%	2.02%

With respect to each of the three types of frozen berries, the FDA did not detect a statistical difference in the prevalence of HAV by domestic-versus-import origin (*P*-value > .05, in all cases).

The FDA also calculated the prevalence of HAV in the samples by country of origin. That data is provided in “Appendix D: Viral Prevalence by Country of Origin.” It is included in this report for informational purposes only as the agency did not design its assignment to compare the viral prevalence by country of origin.

Norovirus Findings

Of the 1,558 samples of frozen berries tested, the agency detected norovirus in 10 of them (3 strawberry, 3 raspberry, and 4 blackberry). Of those 10 samples in which norovirus was detected via RT-qPCR, seven (2 strawberry, 1 raspberry, and 4 blackberry) were subjected to secondary RT-PCR and amplicon Sanger sequenced. The other three samples (1 strawberry, and 2 raspberry) tested positive for norovirus via RT-qPCR testing, prior to the FDA incorporating secondary amplification and Sanger sequencing into the assignment. The prevalence of norovirus for each type of frozen berry is provided below (Table 9).

Table 9: Norovirus Findings by Frozen Berry Type

Frozen Berry Type	Samples Collected	Samples Positive	Prevalence	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Strawberry	585	3	0.51%	0.11%	1.49%
Raspberry	528	3	0.57%	0.12%	1.65%
Blackberry	445	4	0.90%	0.25%	2.29%

The three tables below compare the prevalence of norovirus for each type of frozen berry based on their domestic-versus-import origin (Tables 10, 11 and 12).

Table 10: Norovirus Findings in Strawberries - Domestic vs. Import

Strawberry	Samples Collected	Samples Positive	Prevalence	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Domestic	222	1	0.45%	0.01%	2.48%
Import	363	2*	0.55%	0.07%	1.98%

* The FDA detected norovirus in one sample via RT-qPCR testing, prior to incorporating secondary amplification and Sanger sequencing into the assignment. The other detection of norovirus in the second of the two import samples resulted from the use of all three methods.

Table 11: Norovirus Findings in Raspberries - Domestic vs. Import

Raspberry	Samples Collected	Samples Positive	Prevalence	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Domestic	145	2*	1.38%	0.17%	4.89%
Import	383	1*	0.26%	0.01%	1.45%

* The FDA detected norovirus in one domestic sample and one import sample via RT-qPCR testing, prior to incorporating secondary amplification and Sanger sequencing into the assignment. The other detection of norovirus in the second domestic sample resulted from the use of all three methods.

Table 12: Norovirus Findings in Blackberries - Domestic vs. Import

Blackberry	Samples Collected	Samples Positive	Prevalence	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Domestic	171	1	0.58%	0.01%	3.22%
Import	274	3	1.09%	0.23%	3.17%

With respect to each of the three types of frozen berries, the FDA did not detect a statistical difference in the prevalence of norovirus by domestic-versus-import origin (*P*-value > .05, in all cases).

The FDA also calculated the prevalence of norovirus in the samples by country of origin. That data may be found in “Appendix D: Viral Prevalence by Country of Origin.” It is included in this report for informational purposes only as the FDA did not design its assignment to compare the viral prevalence by country of origin.

Findings by Finished Packaging vs. Bulk Container

All the positives for each virus were detected in the samples collected in finished packaging. However, the FDA collected 95% of the samples in finished packaging, and only 5% from bulk containers. Full analysis of this breakdown is available in “Appendix E: Viral Prevalence by Finished Packaging vs. Bulk Container.”

Findings By ‘Repeat-Violation’ Brand (De-Identified)

For purposes of this subsection, repeat-violation brands are defined as brands for which the agency detected two or more positive samples during the assignment. Of the 18 positive samples, four were associated with two brands, as shown below (Table 13).

Table 13: Findings by 'Repeat Violation' Brand (De-Identified)

Brand	Sample Collection Date *	Frozen Berry Type	Analyte
A	2019	Strawberry	HAV
A	2019	Raspberry	HAV
B	2019	Blackberry	HAV
B	2020	Blackberry	Norovirus

* Only the years are listed to avoid identifying firms.

Product Commingling

Domestically produced and imported frozen berries of the same type are not commonly commingled in finished packaging; the exception would be when a need arises due to a shortfall, a surplus, or contractual limitations. In light of these circumstances, the FDA did not make any adjustments on this basis to its statistical evaluation.

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, depending on the circumstances, include mandatory recalls, enhanced screening of imported products at ports of entry, import alerts, and import refusals, among other mechanisms.

Although test methods cannot determine viability, the FDA views a frozen strawberry, raspberry or blackberry that tests positive for HAV or norovirus as adulterated under the FD&C Act. For example, under section 402(a)(4) of the FD&C Act, a food is deemed adulterated if it was “prepared, packed, or held under insanitary conditions whereby it may have become contaminated with filth, or whereby it may have been rendered injurious to health.” Such foods are subject to regulatory action by the FDA.

Public Health Impact and Follow-up Actions

In analyzing the genetic patterns of the HAV and norovirus detected under this assignment, the FDA determined that none of the strains of either virus matched or was closely related to any known active clinical illnesses. Additionally, during the four-year period of the assignment, the FDA did not respond to any outbreaks of HAV or norovirus infections attributed to frozen strawberries, raspberries or blackberries in the United States during active sampling of the respective berry types.[16]

Whenever the agency detected a pathogen under this assignment, the FDA pursued the removal of all affected product from the U.S. distribution chain. Removal of contaminated products from the distribution chain prevents consumption and so avoids potential illnesses, consistent with the agency's prevention efforts.

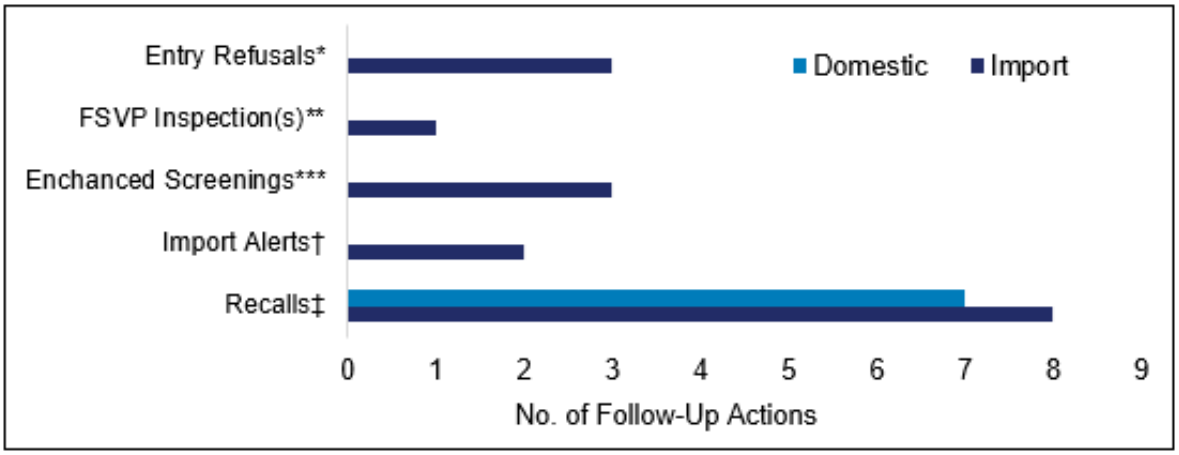
In determining the appropriate follow-up action(s) in response to each positive finding, the FDA evaluated the facts and circumstances specific to each case.

With respect to the domestic samples that tested positive for HAV or norovirus, during the first 10 months of the assignment when the agency's RT-qPCR testing detected either of the target pathogens, the FDA would work with the firm that owned or distributed the affected product to conduct a voluntary recall. Once Sanger sequencing was incorporated into the assignment testing scheme in August 2019, the agency would work with firms to conduct a voluntary recall if and when successful characterization affirmed the likelihood of an intact virus. The agency detected a target pathogen in seven domestic samples, and all resulted in a recall (Figure 1).

With respect to the import samples that tested positive for HAV or norovirus, in all cases the FDA either refused to admit the shipments associated with the positive findings into the United States or worked with the firm that owned or distributed the product to conduct a voluntary recall of the affected lot. All the lots associated with the samples collected in import status that tested positive for a target pathogen were refused entry, and all the lots associated with the domestic import samples found to be positive for either HAV or norovirus resulted in a voluntary recall. Additionally, in four cases, the FDA either flagged, for 180 days, all future shipments of the commodity by the responsible firm, such that all were subject to screening upon entry, or placed the firm and its product on Import Alert 99-35. The agency also conducted one Foreign Supplier Verification Program (FSVP) inspection in response to an import sample that tested positive for a target virus. Once the agency incorporated Sanger sequencing into its testing scheme beginning in August 2019, the FDA would take action as described if and when successful characterization affirmed the likelihood of an intact virus. The chart below provides a breakdown of all the follow-up actions (Figure 1).

16. An outbreak of HAV infections that involved frozen organic strawberries occurred in the U.S. in 2023. While the FDA collected frozen strawberry samples in connection with the outbreak, collection of frozen strawberries under this assignment had ceased because the agency had obtained all the samples of that berry type that it needed for its statistical evaluation. Of the three berry types collected for purposes of this assignment, the greatest number were frozen strawberries.

Figure 1: Follow-Up Actions



The follow-up actions involved, respectively:

* 2 raspberry samples, and 1 strawberry sample;
** 1 raspberry sample;
*** 2 raspberry samples, and 1 strawberry sample;
† 2 raspberry samples; and
‡ Domestic (■) samples: 2 strawberry, 3 raspberry, and 2 blackberry; Import (■) samples: 1 strawberry, 3 raspberry, and 4 blackberry.

Discussion

Consumption of frozen berries (including strawberries, raspberries and blackberries) has been increasing in the United States. These berries are used as ingredients in many foods and, given their nutrient content, they can be an important part of a healthy diet.[17] As with other produce, the FDA is committed to ensuring the microbiological safety of these commodities.

This assignment did not find evidence linking any violative products to foodborne illness outbreaks.

The assignment found rates of HAV and norovirus contamination of less than 1% in frozen strawberries, raspberries and blackberries. The FDA did not detect a statistical difference in the prevalence of HAV or norovirus by origin (i.e., domestic vs. import) in the case of each type of frozen berry.

In light of this assignment's findings and the history of enteric virus illness outbreaks linked to fresh and frozen berries, including one in the United States as recently as 2023,[18] the FDA remains focused on the importance of reducing the microbial risks associated with these commodities.

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17. All three types of berries contain vitamins, fiber and antioxidants. USDA MyPlate Fruit Group – One of the Five Food Groups
18. In 2023, an outbreak of HAV infections linked to frozen organic strawberries resulted in 10 illnesses and four hospitalizations in the United States.

An approach that identifies, leverages and shares effective practices can benefit domestic and global operators that grow, process and source berries and other produce with similar risk factors.

The FDA, international regulatory partners, state departments of food and agriculture, and industry all have a role to play in helping to ensure the safety of frozen berries, as follows:

FDA. Working with industry experts and other interest holders, the FDA has developed a prevention strategy to help limit or prevent enteric virus contamination and outbreaks linked to fresh or frozen berries. It outlines actions for the FDA, industry, and other interest holders to ensure accurate and timely communication and consistent application of effective prevention measures across the global berry industry. A summary of the prevention strategy, which features goals and activities for the FDA, industry, regulatory partners and other interest holders to prevent contamination of fresh and frozen berries, is available on FDA.gov. The FDA intends to work with industry, public health authorities and other interest holders to conduct and facilitate research to further the understanding of enteric virus ecology and detection. The FDA at this time does not anticipate additional large-scale sampling of frozen berries. However, going forward the agency may conduct inspections, investigations, or for-cause sampling at growers or manufacturers in response to signals, such as product recalls or outbreaks of foodborne illness, should any occur.

International Regulatory Partners. The FDA's international counterparts can encourage and support growers and manufacturers in their respective countries to implement or reinforce basic food safety best practices, as warranted, to help ensure that their products comply with the FDA's food safety standards and U.S. import requirements.

State Departments of Food and Agriculture. States that participate in the Cooperative Agreement Program (CAP) with the FDA can work through that program to enhance the safety of berries and achieve high rates of compliance with the FDA's Produce Safety Rule by way of regulatory programs focused on quality and national consistency, education and technical assistance, and local/state/federal coordination. State officials in the U.S. also can work with frozen berry manufacturers to help ensure consistent adherence to basic food safety best practices.

Industry. The findings of this assignment, combined with the history of foodborne illness outbreaks linked to frozen berries, underscore the need for berry growers to comply with the FDA's Produce Safety Rule, for frozen berry manufacturers and others in their distribution chain to comply with the FDA's Preventive Controls for Human Food Rule, and for importers of these commodities to comply with the FDA's Foreign Supplier Verification Programs Rule. These FDA regulations provide standards for the effective design, consistent application and robust verification of hygienic and sanitary controls for fresh and frozen berry operations. Industry food safety experts should identify and communicate effective practices and ensure application globally by growers and processors of fresh and frozen berries and produce operations with similar risk factors.

Consumers. The vast majority of fresh and frozen berries eaten in the U.S. are consumed safely. However, produce such as fresh and frozen berries may be contaminated by foodborne pathogens due to failures of food safety systems in place to prevent contamination. Safe handling practices can decrease risk of illness.

This sampling assignment featured three strategic objectives: 1) to estimate the prevalence of HAV and norovirus in frozen strawberries, raspberries, and blackberries; 2) to determine if there were common factors associated with pathogen findings (such as by origin), where possible; and 3) to take follow-up action in response to pathogen findings, as warranted, with the goal of protecting consumers. The agency achieved all three strategic objectives.

Frozen strawberries, raspberries and blackberries—like their fresh counterparts—require appropriate protection from microbial hazards during growing, harvesting, packing, processing, and holding, as this study affirms.

Appendix A: Analytical Methods and Procedures

Analytical Methods

All berries for this sampling assignment were tested for human hepatitis A virus (HAV) and norovirus using multi-laboratory validated methods described in the FDA Bacteriological Analytical Manual (BAM) Chapter 26 and Appendices: Concentration, Extraction and Detection of Enteric Viruses from Food, 2022. The agency employed a matrix extension of a multi-laboratory validated protocol to concentrate and extract the viruses from produce. HAV and norovirus were detected using Reverse Transcription quantitative Polymerase Chain Reaction (RT-qPCR) protocols that had likewise undergone multi-laboratory validation prior to implementation of the agency's frozen berries sampling assignment. The method validation process followed the agency's Guidelines for the Validation of Analytical Methods for the Detection of Microbial Pathogens in Foods and Feeds.

In the first year of this assignment, the FDA received feedback from frozen food industry stakeholders regarding the agency's analytical methods. The agency considered the comments raised and addressed them, making the case that its use of RT-qPCR was scientifically defensible. Additionally, the agency agreed that its RT-qPCR testing protocol could be strengthened by adding a second confirmatory step that included sequencing. This additional step was added to the protocol by amplifying a second, larger region of the viral genome and subjecting it to Sanger sequencing. Because this second and larger region of the viral genome is different from the region detected by RT-qPCR, the method ensures a very high confidence level in positively identifying viruses. This further characterization began ten months into the assignment (August 2019) and continued from that point forward.

Analytical Procedures

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

Enteric Virus Concentration and Extraction from Berries

Viruses (HAV or norovirus) were extracted and concentrated from three separate 50-gram portions of frozen berries using the following procedure. A solution of glycine/beef extraction buffer containing pectinase was added to each sample portion and shaken. Each portion was centrifuged (12,000 x *g*; 15 min) and the resulting supernatant was subjected to ultracentrifugation (170,000 x *g*; 45 min). The resulting pellet was suspended in 600 µl of phosphate buffered saline (PBS) and subjected to chloroform extraction. The aqueous phase of extraction solvent was processed through commercial column kits to extract and purify RNA from the sample concentrates.

Detection via RT-qPCR

Purified RNA from berry concentrates was analyzed with RT-qPCR assays for the presence of HAV, murine norovirus (extraction control), and norovirus genogroups I and II (GI and GII). Each RT-qPCR contained an internal amplification control to avoid false negative reporting. Description of RT-qPCR components, thermal cycling conditions for the AB 7500 platform, and data interpretation for HAV,

murine norovirus, and norovirus GI and GII are in sections B1, B2, and B4 of the BAM Chapter 26, respectively.

Sequence Characterization

RNA from samples in which HAV or norovirus were detected were then subjected to a separate RT-PCR for strain characterization.[19] This second round of amplification targeted a larger portion of the viral genome, non-contiguous to the initial RT-qPCR detection assays. Specifically, for HAV, the VP1-2A region of the genome was targeted, and regions B or C were targeted for norovirus. Once amplified, secondary amplification of one of these diagnostic regions was conducted using specific M13-tailed primers. Amplicons from this procedure were gel purified and sequenced in forward and reverse directions following the sequencing instrument manufacturer recommendations. Protocols for Sanger sequencing were consistent with manufacturer and published guidelines for base calling quality scores and sequence trimming. Sequence similarities from berry samples were identified by both NCBI BLAST and CDC's HAV and CaliciNet databases.

19. The FDA began characterizing the viral genome of RT-qPCR positives ten months into the assignment (as of August 2019). That being the case, 4 of the 18 RT-qPCR positives were not subject to sequence characterization.

Appendix B: Domestic Production and Import Data

Table B1: Approximate Domestic Production of Frozen Berries (Million Pounds)

Year	Strawberries[1]	Raspberries[2]	Blackberries[3]
2019	369.00	67.65	36.95
2020	359.70	65.45	30.22
2021	353.90	45.42	20.61
2022	349.80	49.92	31.10
2023	331.90	59.68	30.14
5-year avg.	352.86	57.62	29.80

1. Processing Strawberry Advisory Board of California
2. Washington Red Raspberry Commission
3. Oregon Raspberry & Blackberry Commission

Table B2: Approximate Imports of Frozen Berries (Million Pounds)

Year	Strawberries	Raspberries	Blackberries
2019	278.95	45.92	31.74
2020	351.34	54.02	37.76
2021	423.82	64.25	36.93
2022	390.47	73.48	41.30
2023	355.12	57.87	30.49
5-year avg.	359.94	59.11	35.64

Source: USDA Foreign Agricultural Service Global Agricultural Trade System (FAS GATS).

Harmonized Tariff Schedule (HTS) codes:

- 0811100020, frozen strawberries
- 0811100050, frozen strawberries <25% sugar
- 0811100060, frozen strawberries >25% sugar
- 0811100070, frozen strawberry (other)
- 0811202025, frozen red raspberries
- 0811202035, frozen raspberries (other than red)
- 0811204030, frozen blackberries

Appendix C: Statistical Analysis

All statistical analyses were carried out using SAS software (SAS Institute Inc., Version 9.4 TS1M8). The SURVEYFREQ procedure was used to compute viral prevalence and its 95% confidence interval using the modified Clopper Pearson exact method. Viral prevalence estimation for each frozen berry type was stratified by product origin (domestic vs. imported). For each frozen berry type, viral prevalence and its 95% confidence interval were also computed when sufficient numbers of samples were collected from a country of origin. The SURVEYLOGISTIC procedure was used to compare prevalence estimates between domestic and imported products. Fisher's Exact method of the FREQ procedure was used to explore differences in the viral prevalence of each type of frozen berry by collection status (i.e., finished packaging vs. bulk container).

Appendix D: Viral Prevalence by Country of Origin

The agency calculated the prevalence of HAV and norovirus by country of origin in the case of all countries that were the source of at least 200 samples. These findings are provided for informational purposes only as the assignment was not designed to compare the viral prevalence by country of origin.

Table D1: HAV Prevalence by Country of Origin and Berry Type

Frozen Berry Type	Origin	Samples Collected	Number Positive	Prevalence	95% C.I. Lower Bound	95% C.I. Upper Bound
Strawberry	Chile	93	0	0	0	3.80
Strawberry	Mexico	137	0	0	0	2.66
Strawberry	United States	222	1	0.45	0.01	2.48
Strawberry	Other*	133	0	0	0	2.74
Raspberry	Chile	151	2	1.32	0.16	4.70
Raspberry	Mexico	79	0	0	0	4.56
Raspberry	United States	145	1	0.69	0.02	3.78
Raspberry	Other*	153	2	1.31	0.16	4.64
Blackberry	Chile	134	0	0	0	2.72
Blackberry	Mexico	70	1	1.43	0.04	7.70
Blackberry	United States	171	1	0.58	0.01	3.22
Blackberry	Other*	70	0	0	0	5.13

* Argentina, Brazil, Canada, China, Colombia, Costa Rica, Ecuador, Egypt, Guatemala, Kosovo, Lithuania, Morocco, Peru, Poland, Russia, Serbia, Spain, Turkey and Yugoslavia.

See next page for “Table D2: Norovirus Prevalence by Country of Origin and Berry Type.”

Appendix D: Viral Prevalence by Country of Origin (Continued)

Table D2: Norovirus Prevalence by Country of Origin and Berry Type

Frozen Berry Type	Origin	Samples Collected	Number Positive	Prevalence	95% C.I. Lower Bound	95% C.I. Upper Bound
Strawberry	Chile	93	0	0	0	3.89
Strawberry	Mexico	137	0	0	0	2.66
Strawberry	United States	222	1	0.45	0.01	2.48
Strawberry	Other*	133	2	1.50	0.18	5.33
Raspberry	Chile	151	1	0.66	0.02	3.63
Raspberry	Mexico	79	0	0	0	4.56
Raspberry	United States	145	2	1.38	0.17	4.89
Raspberry	Other*	153	0	0	0	2.38
Blackberry	Chile	134	2	1.49	0.18	5.29
Blackberry	Mexico	70	1	1.43	0.04	7.70
Blackberry	United States	171	1	0.59	0.01	3.22
Blackberry	Other*	70	0	0	0	5.13

* Argentina, Brazil, Canada, China, Colombia, Costa Rica, Ecuador, Egypt, Guatemala, Kosovo, Lithuania, Morocco, Peru, Poland, Russia, Serbia, Spain, Turkey and Yugoslavia.

Appendix E: Viral Prevalence by Finished Packaging vs. Bulk Container

The FDA compared the HAV and norovirus contamination rates by collection status (i.e., in finished packaging vs. bulk container) for frozen strawberries, raspberries and blackberries. The FDA did not detect a statistically significant difference in the rate of contamination for either pathogen in the case of all three types of berries (*P*-value = 1). Below are the prevalence and confidence intervals for HAV and norovirus, respectively (Tables E1 and E2).

Table E1: HAV Prevalence by Finished Packaging vs. from Bulk Container

Berry Type	Finished Packaging Samples Collected	Finished Packaging Number Positive	Finished Packaging Prevalence	Finished Packaging 95% C.I.	Bulk Samples Collected	Bulk Positive	Bulk Prevalence	Bulk 95% C.I.
Strawberry	544	1	0.18	(0, 1.02)	41	0	0	(0, 8.60)
Raspberry	508	5	0.98	(0.32, 2.28)	20	0	0	(0, 16.84)
Blackberry	426	2	0.47	(0.06, 1.69)	19	0	0	(0, 17.65)

Table E2: Norovirus Prevalence by Finished Packaging vs. from Bulk Container

Berry Type	Finished Packaging Samples Collected	Finished Packaging Number Positive	Finished Packaging Prevalence	Finished Packaging 95% C.I.	Bulk Samples Collected	Bulk Positive	Bulk Prevalence	Bulk 95% C.I.
Strawberry	544	3	0.5515	(0.11, 1.60)	41	0	0	(0, 8.60)
Raspberry	508	3	0.5906	(0.12, 1.72)	20	0	0	(0, 16.84)
Blackberry	426	4	0.9390	(0.26, 2.39)	19	0	0	(0, 17.65)