Conference for Food Protection
2020 Issue Form

Issue: 2020 III-025

Council Recommendation:	Accepted as Submitted	_____	Accepted as Amended	_____	No Action	_____
Delegate Action:	Accepted	_____	Rejected	_____		

All information above the line is for conference use only.

Issue History:

This is a brand new Issue.

Title:

Amend definition of TCS to include caramel apples with an inserted stick

Issue you would like the Conference to consider:

Historically, uncooked fruits have been considered non-TCS food unless they were epidemiologically implicated in foodborne illness outbreaks and are capable of supporting the growth of pathogenic bacteria in the absence of temperature control. In light of a 2014 multi-state outbreak of listeriosis associated with consumption of caramel apples contaminated with *Listeria monocytogenes*, and subsequent scientific investigations into the factors that could have led to the outbreak, we recommend the Conference to consider modifying the definition of Time/Temperature Control for Safety Food to include "caramel apples with an inserted stick" in Chapter 1, Section 1-201.10.

Public Health Significance:

In 2014, there was a multi-state foodborne illness outbreak of listeriosis associated with consumption of caramel apples; these caramel apples had an inserted stick (FDA Report 2014; CDC Report 2015). At the end of its outbreak investigation, the Centers for Disease Control and Prevention of the U.S. Department of Health and Human Services had reported that a total of 35 people in 12 states were infected with the outbreak strain of *L. monocytogenes* (CDC Report). Of those 35 people:

- Thirty-four people were hospitalized;
- Listeriosis contributed to at least three of the seven deaths reported;
- Eleven illnesses were pregnancy-related (occurred in a pregnant woman or her newborn infant), with one illness resulting in a fetal loss;
- Three invasive illnesses (meningitis) were reported among otherwise healthy children aged 5-15 years; and
- Twenty-eight (90%) of the 31 ill people interviewed reported eating commercially produced, prepackaged caramel apples before becoming ill.

More information about FDA's investigation of this outbreak is available in the outbreak investigation report (FDA Report 2014). Three manufacturers of caramel apples issued voluntary recalls of caramel apples because they had the potential to be contaminated with *L. monocytogenes*. In addition, the apple supplier that provided apples to each of these manufacturers recalled apples implicated in the outbreak.

L. monocytogenes is a bacterium that can contaminate foods and cause a mild illness (called listerial gastroenteritis) or a severe, sometimes life-threatening, illness (called invasive listeriosis (Codex, 2007). Invasive listeriosis has a relatively high mortality rate compared to most other foodborne illness (approximately 20 percent compared to less than 1 percent for *Salmonella* or *Escherichia coli* O157) (Scallan et al, 2011). Persons who have the greatest risk of experiencing listeriosis after consuming foods contaminated with *L. monocytogenes* are pregnant women and their fetuses, the elderly, and persons with weakened immune systems (Pouillot et al, 2015,). It is well established that foods that pose the greatest risk of foodborne listeriosis are those ready-to-eat (RTE) foods that have intrinsic characteristics (such as pH and water activity) that support the growth (i.e., multiplication to increase in number) of *L. monocytogenes*, whereas the RTE foods that pose the least risk of foodborne listeriosis are foods that have intrinsic characteristics that prevent the growth of *L. monocytogenes* (Codex, 2007). For example, *L. monocytogenes* does not multiply in a food that has a pH of 4.4 or below or in a food that has a water activity of the food that is less than or equal to 0.92 (Codex, 2007). Although *L. monocytogenes* can grow slowly during refrigerated storage and, thus, refrigeration is less effective as a control measure for *L. monocytogenes* than for other foodborne pathogens (such as *Salmonella*), *L. monocytogenes* grows more slowly under refrigeration than at room temperature.

Outbreaks of listeriosis from caramel apples were surprising because apples have a pH less than 4.0 and the caramel coating has a water activity less than 0.80, which are below the limits that allow growth of *L. monocytogenes* (Glass et al., 2015). However, research on the survival and growth of *L. monocytogenes* in caramel apples in which a stick was inserted at the stem end suggests that inserting the stick may release juices from the apple that leads to a microenvironment at the interface of the caramel and the apple in which significant growth of *L. monocytogenes* can occur at room temperature (Glass et al. 2015; Salazar et al., 2016). *L. monocytogenes* inoculation of the apple followed by stick insertion at the stem end and caramel coating resulted in significantly more growth in caramel-coated apples with sticks than in caramel-coated apples without sticks (Glass et al., 2015). *L. monocytogenes* did not grow on fresh apples (uncoated) stored at 25°C (77°F) for 49 days (Salazar et al., 2016) and showed limited growth on caramel-coated apples without sticks when stored at 25°C for 28 days (Glass et al., 2015). In contrast, *L. monocytogenes* increased by several logs in caramel apples with an inserted stick (Glass et al. 2015; Salazar et al., 2016). *L. monocytogenes* growth was significantly reduced when caramel apples on a stick were stored at refrigeration temperatures (5-7°C; 41-45°F) (Glass et al., 2015; Salazar et al., 2016). The interface between the stem end of the apple and the caramel layer may have a microenvironment with sufficiently high water activity and pH when the stick penetrates the apple. Thus, caramel-coated apples on a stick present a lower risk for illness when stored refrigerated storage compared to storage at room temperature.

Recommended Solution: The Conference recommends...:

The Conference recommends a letter be sent to FDA to request amending the definition of "Time/Temperature Control for Safety Food" by adding "caramel apples with an inserted stick" in part 2 of the definition of "Time/Temperature Control for Safety Food" in Chapter 1, Section 1-201.10.

Submitter Information:

Name:	Brett Podoski
Organization:	U.S. Food and Drug Administration
Address:	5001 Campus Drive
City/State/Zip:	College Park, MD 20740
Telephone:	240-402-3022
E-mail:	brett.podoski@fda.hhs.gov

Supporting Attachments:

- "Outbreaks of Foodborne Illness"
- "Multistate Outbreak of Listeriosis Linked to Commercially Produced..."
- "Guidelines on the application of the General Principles of Food Hygiene"
- "Foodborne illness acquired in the US - major pathogens"
- "Listeria monocytogenes dose response revisted"
- "Fate of Listeria monocytogenes in Fresh Apples and Caramel Apples"
- "Growth of Listeria monocytogenes within a caramel-coated apple microenv..."

It is the policy of the Conference for Food Protection to not accept Issues that would endorse a brand name or a commercial proprietary process.

FDA Investigated Listeria monocytogenes Illnesses Linked to Caramel Apples

Update
August 21, 2015
After a U.S. Food and Drug Administration review, the agency considers the **January 9, 2015** recall of Gala and Granny Smith apples supplied by Bidart Bros. to be complete.

On this page:

The U.S. Food and Drug Administration (FDA) along with the Centers for Disease Control and Prevention (CDC) and state and local authorities investigated a listeriosis outbreak linked to commercially-produced, prepackaged

whole caramel apples. Listeriosis is caused by the bacterium Listeria monocytogenes. According to the CDC, the outbreak appeared to be over as of February 12, 2015.

What was the Problem and What was Done?

The FDA, CDC and state and local officials investigated an outbreak of listeriosis linked to commercially-produced, prepackaged whole caramel apples.

According to the CDC, 35 people from 12 states were infected with the outbreak strains of *Listeria monocytogenes.* The CDC reports that 34 ill people were hospitalized. Listeriosis contributed to at least three of the seven deaths that were reported. Eleven illnesses were pregnancy-related, with one illness resulting in a fetal loss. Illness onset dates ranged from October 17, 2014 to January 6, 2015.

The CDC reports that 28 of the 31 ill people interviewed reported eating commercially-produced, prepackaged whole caramel apples. To date, caramel apple brands named in interviews include Happy Apple, Carnival and Merb's Candies.

On December 18, 2014, the **Minnesota Department of Health** reported four illnesses. The Minnesota cases purchased caramel apples from Cub Foods, Kwik Trip, and Mike's Discount Foods, which carried Carnival brand and Kitchen Cravings brand caramel apples. These two brands are no longer available for purchase at retail locations.

The Public Health Agency of Canada (PHAC) has identified **two cases of listeriosis in Canada** with the same DNA fingerprints, or pulsed-field gel electrophoresis (PFGE) patterns, as seen in the US outbreak. PHAC is working with its provincial and territorial partners to determine the source of these illnesses. Since the investigation began, more detailed testing on the two Canadian cases has been completed, concluding that only a single case in Manitoba is genetically related to the U.S. outbreak of listeriosis.

Three companies have issued voluntary recalls of caramel apples because they have the potential to be contaminated with *Listeria monocytogenes*. These companies are:

- Happy Apple Company of Washington, Missouri
- California Snack Foods, of South El Monte, California
- Merb's Candies of St. Louis, Missouri

Each company reported receiving notice from Bidart Bros., an apple supplier headquartered in Bakersfield, California, that there may be a connection between the listeriosis outbreak and the apples supplied to them by Bidart Bros.

Investigating agencies worked to trace the origin of the caramel apples eaten by 11 ill

people involved in the outbreak. Although the manufacturers of the brands reported by these cases (including Happy Apple Company and Merb's Candies) received apples from other growers, the traceback investigation confirmed that Bidart Bros. is the only apple grower that supplied apples to each company.

On December 22, 2014, the FDA and the California Department of Public Health (CDPH) briefed Bidart Bros. on the status of the investigation.

On December 22, 2014, Bidart Bros. issued a recall of Granny Smith apples it sold in 2014 to those customers known to produce caramel apples. Then, on December 24, 2014, Bidart Bros. notified all customers receiving Granny Smith apples in 2014 to recall those apples if they had been used to make caramel apples.

On December 23, 2014, FDA and CDPH activated the California Food Emergency Response Team (CalFERT), a team comprised of CDPH and FDA specialists who rapidly respond to food emergencies in California. CalFERT conducted a joint investigation of the firm. The team took environmental samples, swabbing surfaces likely to come into contact with apples. Analyses of the samples revealed that several of these samples contained Listeria monocytogenes. CalFERT shared these laboratory results with Bidart Bros. on January 5, 2015.
On January 6, 2015, Bidart Bros. sent letters to its distributors, expanding its voluntary recall. Bidart Bros. is recalling all Granny Smith and Gala apples shipped from the company's Shafter, California packing facility in 2014.

On January 8, 2015, pulsed-field gel electrophoresis (PFGE) analysis of the *Listeria monocytogenes* isolated from environmental samples collected at Bidart Bros. confirmed that the PFGE patterns, or DNA fingerprints, of the pathogen matched the outbreak strains of *Listeria monocytogenes* isolated from people affected by the outbreak. *Listeria monocytogenes* matching the outbreak strains, by PFGE type, also was isolated from samples of Bidart Bros. whole apples collected along the distribution chain by FDA and state investigators in December 2014.

On January 9, 2015, Bidart Bros. issued a **news release announcing the recall** and reporting that December 2, 2014, was the last shipment date for the company's apples.

Other varieties of apples and apples from other growers are not affected by the recall.

On January 18, 2015, whole genome sequence (WGS) analysis of the *Listeria monocytogenes* isolated from environmental samples collected at Bidart Bros. confirmed that the genomes of the pathogens were highly related to the outbreak strains of *Listeria monocytogenes* isolated from people affected by the outbreak. Highly related *Listeria monocytogenes* strains were also isolated from samples of Bidart Bros. whole apples collected along the distribution chain by FDA and state investigators in December 2014.

According to the CDC, the outbreak appears to be over as of February 12, 2015.

back to top

What are the Symptoms of Listeriosis?

Listeriosis is a rare but serious illness caused by eating food contaminated with the bacterium called *Listeria monocytogenes*. Anyone who experiences fever and muscle aches, sometimes preceded by diarrhea or other gastrointestinal symptoms, or develops fever and chills after eating commercially-produced, prepackaged caramel apples should seek medical care and tell the health care provider about any history of eating those caramel apples. Symptoms can appear from a few days up to a few weeks after consumption of the contaminated food.

Who is at Risk?

Listeriosis can be fatal, especially in certain high-risk groups. These groups include the elderly, and people with weakened immune systems and certain chronic medical conditions (such as cancer). In pregnant women, listeriosis can cause miscarriage, stillbirth, premature labor, and serious illness or death in newborn babies.

back to top

What Specific Products were recalled?

On December 24, 2014, the Happy Apple Company of Washington, Missouri, issued a voluntary recall of Happy Apple Brand caramel apples with a best use by date between August 25th and November 23rd 2014, because they have the potential to be contaminated with *Listeria monocytogenes*.

Happy Apple caramel apples are sold in single pack, three packs, four packs and eight packs and each package will have a best use by date on the front of the label. They were available for retail sale through grocery, discount and club stores, generally in the produce section and were distributed to retailers in the following states: Alabama, Arizona, Arkansas, California, Colorado, Florida, Georgia, Hawaii, Illinois, Indiana, Iowa, Kansas, Louisiana, Massachusetts, Minnesota, Mississippi, Missouri, Montana, Nebraska, Nevada, New Mexico, North Carolina, Ohio, Oklahoma, Oregon, Pennsylvania, Tennessee, Texas, Utah, Washington, Wisconsin.

Also, on December 24, 2014, the Canadian Food Inspection Agency (CFIA) announced the recall in Canada of Happy Apple brand caramel apples due to possible Listeria monocytogenes contamination.

On December 31, 2014, the Happy Apple Company expanded this recall to include Kroger Brand caramel apples produced by the Happy Apple Company with a best use by date between September 15th and November 18th 2014 because they have the

potential to be contaminated with *Listeria monocytogenes*.

Kroger brand caramel apples produced by Happy Apple are sold in single packs and three packs. Each package will have a best use by date on the front of the label. Some caramel apples sold under the Kroger brand are labeled as candy apples and some are labeled as caramel apples. The apples were distributed to retailers in the following states: Arizona, Alaska, Kansas, Idaho, Louisiana, Montana, Missouri, Nebraska, Nevada, Oregon, Texas, Utah, Washington and Wyoming.

On December 27, 2014, California Snack Foods, of El Monte, California, issued a voluntary recall of California Snack Foods Karm'l Dapple brand caramel apples with a best use by date between August 15th and November 28th, 2014, because they have the potential to be contaminated with *Listeria monocytogenes*.

California Snack Foods caramel apples are sold in single packs and three packs and each package will have a best use by date on the front of the label. They were available for retail sale through grocery, discount and club stores, generally in the produce section and were distributed to retailers in the following states: Arizona, California, Nevada, Texas and Utah.

On December 29, 2014, Merb's Candies of St. Louis, Missouri, issued a voluntary recall of the Merb's Candies brand Bionic Apples and Double Dipped Apples because they have the potential to be contaminated with *Listeria monocytogenes.*

Bionic Apples and Double Dipped Apples were available for retail sales at St. Louis area locations, through local supermarkets (located in the produce section) and through mail orders nationwide. The product is individually packaged in a clear, burgundy and gold cellophane bag and would have been available from September 8th through November 25th 2014 – no identifying lot codes were used.

The recalling companies report that the recalled caramel apples should no longer be available for purchase in stores.

On January 6, 2015, Bidart Bros. of Bakersfield, California issued a voluntary recall of all Gala and Granny Smith apples shipped from its Shafter, California packing facility in 2014.

On January 7, 2015, the CFIA announced the recall in Canada of Granny Smith apples and Gala apples from Bidart Bros due to possible *Listeria monocytogenes* contamination. According to CFIA, Bidart Apples are sold under the brand names "Big B" and "Granny's Best."

What Do Consumers Need To Do?

After a U.S. Food and Drug Administration review the agency considers that the January 9, 2015 recall of Gala and Granny Smith apples supplied by Bidart Bros. to

be complete.

Recommendations for preventing listeriosis are available at the CDC *Listeria* website: http://www.cdc.gov/listeria/prevention.html.

Listeria monocytogenes can grow at refrigerator temperatures, as low as 40 degrees Fahrenheit (4 degrees Celsius). The longer ready-to-eat refrigerated foods are stored in the refrigerator, the more opportunity *Listeria* has to grow.

For refrigerators and other food preparation surfaces and food cutting utensils that may have come in contact with commercially-produced, prepackaged caramel apples, including those containing nuts, sprinkles, chocolate, or other toppings, it is very important that the consumers thoroughly clean the following areas:

- Wash the inside walls and shelves of the refrigerator, cutting boards and countertops; then sanitize them with a solution of one tablespoon of chlorine bleach to one gallon of hot water; dry with a clean cloth or paper towel that has not been previously used.

In addition, consumers can follow these simple steps for food safety:

- Wash hands with warm water and soap for at least 20 seconds before and after handling food.
- Wipe up spills in the refrigerator immediately and clean the refrigerator regularly.
- Always wash hands with warm water and soap following the cleaning and sanitization process.

back to top

What Do Retailers and Restaurants Need To Do?

Retailers and restaurants should work with their suppliers to ensure that they are not selling the Granny Smith and Gala apples being recalled by Bidart Bros., or caramel apples made using the recalled Bidart Bros. apples. This includes caramel apples containing nuts, sprinkles, chocolate, or other toppings.

Restaurants and retailers should also:

- Wash and sanitize display cases and refrigerators where potentially contaminated products were stored.
- Wash and sanitize cutting boards, surfaces, and utensils used to cut, serve, or store potentially contaminated products.
- Wash hands with warm water and soap following the cleaning and sanitation process.
- Retailers, restaurants, and other food service operators who have processed and

packaged any potentially contaminated products need to be concerned about cross contamination of cutting surfaces and utensils through contact with the potentially contaminated products.

- Regular frequent cleaning and sanitizing of cutting boards and utensils used in processing may help to minimize the likelihood of cross-contamination.

Listeria can grow at refrigeration temperatures. *Listeria* can also cross contaminate other food cut and served on the same cutting board or stored in the same area. Retailers, restaurants, and other food service operators may wish to consider whether other foods available for sale could have been cross-contaminated from the potentially contaminated products, and should be discarded.

Who Should be Contacted?

Consumers with questions about the Bidart Bros. recall may contact the company at 661-399-0978.

Consumers with questions about the California Snack Foods recall may contact the company at 800-966-5501 Monday through Friday during normal business hours or via email at info@californiasnackfoods.com.

Consumers with questions about the Happy Apple recall may contact the company at 800-527-7532 Monday through Friday during normal business hours or via email at customercare@happyapples.com.

Consumers with questions about the Merb's Candies recall may contact the firm at customercare.merbscandies@gmail.com or during normal business hours Monday through Friday 9 a.m. to 5 p.m. CST at (314) 832-7206.

The FDA encourages consumers with questions about food safety to call 1-888-SAFEFOOD Monday through Friday between 10 a.m. and 4 p.m. Eastern time, or to consult http://www.fda.gov.

back to top

The information in this posting reflects the FDA's best efforts to communicate what it has learned from the manufacturer, the CDC, and the state and local public health and food regulatory agencies involved in the investigation. The agency will update this page as more information becomes available.

For more information:

- CDC Vital Signs Listeria
- FoodSafety.gov on Listeria
- CDC Web Page

- Minnesota Press Release

More in Outbreaks

Outbreak Investigations

Environmental Assessments

About the CORE Network

Resources & Related Links

Page Last Updated: 11/03/2016
Note: If you need help accessing information in different file formats, see Instructions for Downloading Viewers and Players.
Language Assistance Available: Español | 繁體中文 | Tiếng Việt | 한국어 | Tagalog | Русский | العربية | Kreyòl Ayisyen | Français | Polski | Português | Italiano | Deutsch | 日本語 | فارسی | English

U.S. Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993
1-888-INFO-FDA (1-888-463-6332)

Contact FDA

Listeria (Listeriosis)

Commercially Produced, Prepackaged Caramel Apples

Posted February 12, 2015 4:30 PM ET

This investigation is closed, and the shelf life of recalled products has passed. Read the Advice to Consumers to learn about products that were recalled.

- **Read the Advice to Consumers and Retailers>>**
- This outbreak appears to be over. However, recalled products may still be in people's homes. Consumers unaware of the recalls could continue to eat the products and get sick.
- On January 6, 2015, Bidart Bros. of Bakersfield, California voluntarily recalled Granny Smith and Gala apples because environmental testing revealed contamination with *Listeria monocytogenes* at the firm's apple-packing facility.
    - On January 18, 2015, FDA laboratory analyses using whole genome sequencing (WGS) showed that these *Listeria* isolates were highly related to the outbreak strains.

A total of 35 people infected with the outbreak strains of *Listeria monocytogenes* were reported from 12 states.

- Of these, 34 people were hospitalized. Listeriosis contributed to at least three of the seven deaths reported.
- Eleven illnesses were pregnancy-related (occurred in a pregnant woman or her newborn infant), with one illness resulting in a fetal loss.
- Three invasive illnesses (meningitis) were among otherwise healthy children aged 5–15 years.
- Twenty-eight (90%) of the 31 ill people interviewed reported eating commercially produced, prepackaged caramel apples before becoming ill.

- The Public Health Agency of Canada (PHAC) identified one case of listeriosis in Canada that is genetically related to the U.S. outbreak.

Previous Updates

Outbreak Summary

Introduction

CDC collaborated with public health officials in several states and with the U.S. Food and Drug Administration (FDA) to investigate an outbreak of *Listeria monocytogenes* infections (listeriosis). Joint investigation efforts indicated that commercially produced, prepackaged caramel apples made from Bidart Bros. apples were the likely source of this outbreak. *Listeria* can cause a serious, life-threatening illness. People at higher risk for listeriosis include adults 65 years or older, people with weakened immune systems, and pregnant

women.

Public health investigators used the PulseNet system to identify illnesses that were part of this outbreak. PulseNet is the national subtyping network of public health and food regulatory agency laboratories coordinated by CDC. DNA "fingerprinting" is performed on *Listeria* bacteria isolated from ill people using techniques called pulsed-field gel electrophoresis (PFGE) and whole genome sequencing (WGS). WGS gives a more detailed DNA fingerprint than PFGE. PulseNet manages a national database of these DNA fingerprints to identify possible outbreaks of enteric illness. Two outbreak clusters were identified by the PFGE technique. When WGS was used, two *Listeria* isolates (one within each cluster) were found to be highly related but distinct between the two clusters. CDC investigated the two clusters together because one person was infected with both *Listeria* strains simultaneously and also because illnesses in the two clusters occurred during a similar time period and in similar regions of the country.

The 35 ill people included in this outbreak investigation were reported from 12 states: Arizona (5), California (3), Colorado (1), Minnesota (4), Missouri (5), Nevada (1), New Mexico (6), North Carolina (1), Texas (4), Utah (1), Washington (1), and Wisconsin (3). Illness onset dates ranged from October 17, 2014, to January 6, 2015. Eleven illnesses were associated with a pregnancy (occurred in a pregnant woman or her newborn infant). One fetal loss was reported. Among people whose illnesses were not associated with a pregnancy, ages ranged from 7 to 92 years, with a median age of 62 years, and 33% were female. Three invasive illnesses (meningitis) occurred among otherwise healthy children aged 5–15 years. Thirty-four people were hospitalized, and listeriosis contributed to at least three of the seven deaths reported.

The outbreak can be visually described with a chart showing the number of people who were diagnosed each day. This chart is called an epidemic curve or epi curve.

The Public Health Agency of Canada (PHAC) identified two cases of listeriosis in Canada with the same PFGE patterns as those seen in the U.S. outbreak. More detailed testing using WGS showed that the isolate from only one of the two cases was genetically related to the U.S. outbreak. That person reported eating a caramel apple.

Investigation of the Outbreak

In interviews, ill people answered questions about foods consumed and other exposures in the month before becoming ill. Twenty-eight (90%) of the 31 ill people interviewed reported eating commercially produced, prepackaged caramel apples before becoming ill. Caramel apple brands named in interviews included Happy Apples, Carnival, and Merb's Candies. However, other brands may also have been consumed. The three ill people interviewed who did not report eating caramel apples did report eating whole or sliced green apples not covered in caramel. However, most (about 60%) of the general US population report eating apples [PDF – 29 pages] during a given week. The source of the reported whole or sliced green apples is unknown, and it is unknown whether these apples were linked to the patients' illnesses.

On January 6, 2015, Bidart Bros. of Bakersfield, California, voluntarily recalled Granny Smith and Gala apples because environmental testing revealed contamination with *Listeria monocytogenes* at the firm's apple-packing facility. The recall included all Granny Smith and Gala apples shipped from its Shafter, California, packing facility in 2014. On January 8, 2015, FDA laboratory analyses using PFGE showed that

environmental *Listeria* isolates from the Bidart Bros. facility were indistinguishable from the outbreak strains. On January 18, 2015, WGS found that these isolates were highly related to the outbreak strains. In addition, WGS showed that *Listeria* isolates from whole apples produced by Bidart Bros., collected along the distribution chain, also were highly related to the outbreak strains. CDC recommends that consumers not eat any of the recalled Granny Smith and Gala apples produced by Bidart Bros. and retailers not sell or serve them.

Three firms that produce caramel apples issued voluntary recalls after receiving notice from Bidart Bros. that there may be a connection between Bidart Bros. apples and this listeriosis outbreak. On December 24, 2014, Happy Apple Company of Washington, Missouri, voluntarily recalled Happy Apples brand caramel apples with a best use by date between August 25 and November 23, 2014. On December 31, 2014, Happy Apple Company expanded the recall to include Kroger brand caramel apples produced by Happy Apple Company with a best use by date between September 15 and November 18, 2014. On December 27, 2014, California Snack Foods voluntarily recalled Karm'l Dapple brand caramel apples with a best use by date between August 15 and November 28, 2014. On December 29, 2014, Merb's Candies of St. Louis, Missouri issued a voluntary recall of Merb's Candies Bionic Apples and Double Dipped Apples that would have been available from September 8 through November 25, 2014.

This outbreak appears to be over. However, recalled products may still be in people's homes. Consumers unaware of the recalls could continue to eat the products and get sick.

January 8, 2015

December 31, 2014

December 22, 2014

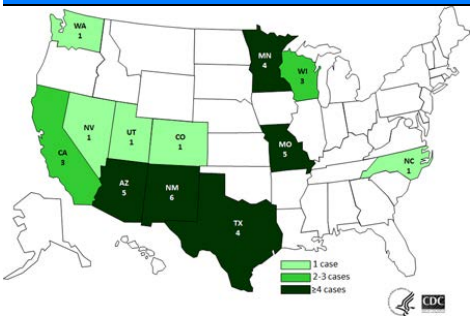
Initial Announcement

- **Case Count: 35**
- **States: 12**
- **Deaths: 7**
- **Hospitalizations: 34**
- **Recall: Yes**

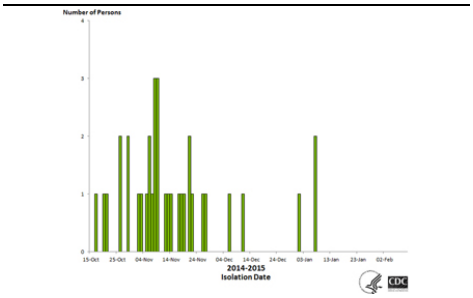
More Information:

- Recall & Advice to Consumers
- Signs & Symptoms
- Key Resources

CLICK TO VIEW CASE COUNT MAP.



CLICK TO VIEW EPI CURVE GRAPHS.



Caramel Apples

CDC recommends that U.S. consumers do not eat any commercially produced, prepackaged caramel apples that were made with Bidart Bros. apples produced in 2014.

Page last reviewed: February 12, 2015

- Wholesome Soy Products, Inc. Sprouts
- Roos Foods Dairy Products
- Crave Brothers Farmstead Cheeses
- Imported Frescolina Marte Brand Ricotta Salata Cheese
- Jensen Farms Cantaloupes

For Health Professionals

Surveillance

Publications

Related Links

- Food Safety
- Foodborne Illness Estimates
- Foodborne Illness Trends
- Foodborne Illness Attribution
- Foodborne Illness Outbreaks

Subscribe

RSS

HAVE QUESTIONS?

PDF file_external

GUIDELINES ON THE APPLICATION OF GENERAL PRINCIPLES OF FOOD HYGIENE TO THE CONTROL OF *LISTERIA MONOCYTOGENES* IN FOODS

CAC/GL 61 - 2007

Table of Content

Adopted in 2007; Annexes II and III adopted in 2009.

INTRODUCTION

Listeria (L.) monocytogenes is a Gram-positive bacterium that occurs widely in both agricultural (soil, vegetation, silage, faecal material, sewage, water), aquacultural, and food processing environments. *L. monocytogenes* is a transitory resident of the intestinal tract in humans, with 2 to 10% of the general population being carriers of the microorganism without any apparent health consequences.[1] In comparison to other non-spore forming, foodborne pathogenic bacteria (e.g., *Salmonella* spp., enterohemorrhagic *Escherichia coli*), *L. monocytogenes* is resistant to various environmental conditions such as high salt or acidity. *L. monocytogenes* grows at low oxygen conditions and refrigeration temperatures, and survives for long periods in the environment, on foods, in the processing plant, and in the household refrigerator. Although frequently present in raw foods of both plant and animal origin, sporadic cases or outbreaks of listeriosis are generally associated with ready-to-eat, refrigerated foods, and often involves the post-processing recontamination of cooked foods.

L. monocytogenes has been isolated from foods such as raw vegetables, raw and pasteurised fluid milk, cheeses (particularly soft-ripened varieties), ice cream, butter, fermented raw-meat sausages, raw and cooked poultry, raw and processed meats (all types) and raw, preserved and smoked fish. Even when *L. monocytogenes* is initially present at a low level in a contaminated food, the microorganism may multiply during storage in foods that support growth, even at refrigeration temperatures.

L. monocytogenes causes invasive listeriosis wherein the microorganism penetrates the lining of the gastrointestinal tract and then establishes infections in normally sterile sites within the body. The likelihood that *L. monocytogenes* can establish a systemic infection is dependent on a number of factors, including the number of microorganisms consumed, host susceptibility, and virulence of the specific isolate ingested. Almost all strains of *L. monocytogenes* appear to be pathogenic though their virulence, as defined in animal studies, varies substantially. Listeriosis is an infection that most often affects individuals experiencing immunosuppression including individuals with chronic disease (e.g., cancer, diabetes, malnutrition, AIDS), foetuses or neonates (assumed to be infected *in utero*), the elderly and individuals being treated with immunosuppressive drugs (e.g., transplant patients). The bacterium most often affects the pregnant uterus, the central nervous system or the bloodstream. Manifestations of listeriosis include but are not limited to bacteremia, septicaemia, meningitis, encephalitis, miscarriage, neonatal disease, premature birth, and stillbirth. Incubation periods prior to individuals becoming symptomatic can be from a few days up to three months. *L. monocytogenes* can also cause mild febrile gastro-enteritis in otherwise healthy individuals. The public health significance of this type of listeriosis appears to be much lower than that of invasive listeriosis.

Available epidemiological data show invasive listeriosis occurs both as sporadic cases and outbreaks, with the former accounting for the majority of cases. Invasive listeriosis is a relatively rare, but often severe disease with incidences typically of 3 to 8 cases per 1,000,000 individuals and fatality rates of 20 to 30% among hospitalised patients.[2] During recent years, the incidence of listeriosis in most countries has remained constant, with a number of countries reporting declines in the incidence of disease. These reductions likely reflect the efforts in those countries by industry and governments (a) to implement Good Hygienic Practice (GHP) and apply HACCP to reduce the frequency and extent of *L. monocytogenes* in ready-to-eat foods, (b) to improve the integrity of the cold chain through processing, distribution, retail and the home to reduce the incidence of temperature abuse conditions that foster the growth of *L. monocytogenes*, and (c) to enhance risk communication, particularly for consumers at increased risk of listeriosis. However, further actions are needed to achieve continuous improvement of public health by lowering the incidence of human foodborne listeriosis worldwide. Periodically transitory increases in incidence have been noted in several countries. These have been associated typically with foodborne outbreaks attributable to specific foods, often from specific manufacturers. In such cases, the incidence of listeriosis returned to prior baseline values after the causative food was removed from the market, and consumers received effective public health information pertaining to appropriate food choices and handling practices.

---

1 FAO (2000): Joint FAO/WHO Expert Consultation on Risk Assessment of Microbiological Hazards in Foods. FAO, Food and Nutrition Paper No. 71.

2 FAO and WHO (2001): Joint FAO/WHO Expert Consultation on Risk Assessment of Microbiological Hazards in Foods: Risk characterisation of Salmonella spp. in eggs and broiler chickens and *L. monocytogenes* in ready-to-eat foods. FAO, Food and Nutrition Paper No.72.

Listeriosis has been recognised as a human disease since the 1930's, however, it was not until the 1980's, when there were several large outbreaks in North America and Europe, that the role that foods play in the transmission of the disease was fully recognised. Foods are now considered to be the major vehicle for *L. monocytogenes*. A variety of specific foods have been implicated in outbreaks and sporadic cases of listeriosis (e.g., processed meats, soft cheeses, smoked fish, butter, milk, coleslaw). The foods associated with listeriosis have been overwhelmingly ready-to-eat products that are typically held for extended periods at refrigeration or chill temperatures.

The large number of ready-to-eat foods in which *L. monocytogenes* is at least occasionally isolated has made it difficult to effectively focus food control programs on those specific foods that contribute the greatest risk to foodborne listeriosis. As a means of addressing this and a number of related questions, several formal quantitative risk assessments have been undertaken to address issues related to the relative risks among different ready-to-eat foods and the factors that contribute to those risks. Available governmental risk assessments currently include (1) a comparative risk assessment of 23 categories of ready-to-eat foods conducted by the U.S. Food and Drug Administration and the Food Safety and Inspection Service (FDA/FSIS, 2003)[3], (2) a comparative risk assessment of four ready-to-eat foods conducted by FAO/WHO JEMRA at the request of the Codex Committee on Food Hygiene[4], and (3) a product/process pathway analysis conducted by the U.S. Food Safety and Inspection Service for processed meats[5], which examined the risk of product contamination from food contact surfaces.

Each of these assessments articulates concepts that countries can use to identify and categorise those ready-to-eat products that represent a significant risk of foodborne listeriosis. Five key factors were identified as contributing strongly to the risk of listeriosis associated with ready-to-eat foods:

- Amount and frequency of consumption of a food
- Frequency and extent of contamination of a food with *L. monocytogenes*
- Ability of the food to support the growth of *L. monocytogenes*
- Temperature of refrigerated/chilled food storage
- Duration of refrigerated/chilled storage

A combination of interventions is generally more effective in controlling the risk rather than any single intervention (FDA/FSIS, 2003)[3].

In addition to the factors above, which influence the number of *L. monocytogenes* present in the food at the time of consumption, the susceptibility of an individual is important in determining the likelihood of listeriosis.

The risk assessments that have been conducted have consistently identified the impact that the ability of a food to support the growth of *L. monocytogenes* has on the risk of listeriosis. Those foods that are able to support growth during the normal shelf life of a product increase substantially the risk that the food will contribute to foodborne listeriosis. Control of growth can be achieved by several different approaches, including reformulation of the product such that one or more of the parameters influencing the growth of the bacterium (e.g., pH, water activity, presence of inhibitory compounds) is altered so the food no longer supports growth. Alternatively, strict control of temperature so that ready-to-eat foods never exceed 6°C (preferably 2°C- 4°C) and/or shortening the duration of the product refrigerated/chilled shelf life are other means for assuring that growth to any significant degree does not occur before the product is consumed.

---

3 FDA/FSIS, 2003. Quantitative assessment of the relative risk to public health from foodborne *Listeria monocytogenes* among selected categories of ready-to-eat foods at www.cfsan.fda.gov

4 FAO/WHO, 2004. Risk assessment of *Listeria monocytogenes* in ready-to-eat foods. Technical Report. Microbiological Risk Assessment Series, No. 5.

5 FSIS Rule Designed to Reduce Listeria monocytogenes in Ready-to-Eat Meat & Poultry at http://www.fsis.usda.gov/factsheets/fsis_rule_designed_to_reduce_listeria/index.asp

Many of the ready-to-eat products that are associated with foodborne listeriosis include a step in their production that is listericidal. Thus, the frequency and level of contamination of these products with *L. monocytogenes* is typically associated with the recontamination of the product prior to final packaging or from subsequent handling during marketing or home use. Thus, another strategy to control foodborne listeriosis is to reduce recontamination of the product and/or to introduce an additional mitigation treatment after final packaging. Control of the frequency and level of contamination is likely to be influenced strongly by factors such as attention to the design and maintenance of equipment and the integrity of the cold chain, the latter clearly being identified as a risk factor (i.e., the temperature of refrigerated/chilled storage).

Some ready-to-eat foods do not include a listericidal treatment. Product safety in those instances is dependent on steps taken during primary production, processing, and subsequent distribution and use to minimise or reduce contamination/recontamination and to limit growth through maintaining the cold chain and limiting the duration of refrigerated storage.

The FAO/WHO risk assessment also clearly indicated that in order for food control programmes to be effective, they must be capable of consistently achieving the degree of control required; the risk of listeriosis is largely associated with failures to meet current standards for *L. monocytogenes*, be they at 0.04 or 100 CFU/g. The analyses conducted within that risk assessment clearly indicate that the greatest risk associated with ready-to-eat products is the small portion of the products with high contamination levels of *L. monocytogenes.* Thus, a key component of a successful risk management program is assurance that control measures (e.g., preventing contamination and growth of the pathogen) can be achieved consistently.

SECTION I - OBJECTIVES

These guidelines provide advice to governments on a framework for the control of *L. monocytogenes* in ready-to-eat foods, with a view towards protecting the health of consumers and ensuring fair practices in food trade. Their primary purpose of these guidelines is to minimise the likelihood of illness arising from the presence of *L. monocytogenes* in ready-to-eat foods. The guidelines also provide information that will be of interest to the food industry, consumers, and other interested parties.

SECTION II - SCOPE

2.1 SCOPE

These guidelines are intended for ready-to-eat foods and are applicable throughout the food chain, from primary production through consumption. However, based on the results of the FAO/WHO risk assessment, other available risk assessments and epidemiological evaluations, these guidelines will focus on control measures that can be used, where appropriate, to minimize and/or prevent the contamination and/or the growth of *L. monocytogenes* in ready-to-eat foods. These guidelines highlight key control measures that affect key factors that influence the frequency and extent of contamination of ready-to-eat foods with *L. monocytogenes* and thus the risk of listeriosis. In many instances, these control measures are articulated in a general manner in the Recommended International Code of Practice - General Principles of Food Hygiene (CAC/RCP 1-1969) as part of the general strategy for control of foodborne pathogens in all foods. In providing these guidelines, it is assumed that these General Principles of Food Hygiene are being implemented. Those principles that are restated reflect the need for special attention for the control of *L. monocytogenes.*

Good Hygienic Practices (GHPs) as specified in the Recommended International Code of Practice - General Principles of Food Hygiene (CAC/RCP 1-1969) and other applicable codes of hygienic practice should be suitable to control *L. monocytogenes* in non ready-to-eat foods. However, the additional measures described in the following guidelines should be consulted and implemented, as necessary to control *L. monocytogenes* in ready-to-eat foods.

2.2 DEFINITIONS

For the purpose of these Guidelines, the following definitions apply:

Definitions of the "Principles and Guidelines for the Conduct of Microbiological Risk Management" apply.

Ready-to-eat food – Any food which is normally eaten in its raw state or any food handled, processed, mixed, cooked, or otherwise prepared into a form which is normally eaten without further listericidal steps.

SECTION III - PRIMARY PRODUCTION

Many ready-to-eat foods receive one or more treatments during processing or preparation that inactivate or inhibit the growth of *L. monocytogenes*. For these foods animal health and general application of good agricultural practices, including animal husbandry, should be sufficient to minimise the prevalence of *L. monocytogenes* at primary production.

In those ready-to-eat foods that are manufactured without a listericidal treatment, extra attention at primary production is needed to assure specific control of the pathogen (e.g., control of *L. monocytogenes* mastitis in dairy cattle and sheep where the milk will be used to make raw milk cheeses, frequency of *L. monocytogenes* in raw milk as related to the feeding of inadequately fermented silage, high levels of *L. monocytogenes* in pork for fermented sausages resulting from wet feeding systems, faecal contamination of fresh produce), including increased focus on personal hygiene and water management programs at the primary production sites.

Analysis of raw material for *L. monocytogenes* can be, where appropriate, an important tool for validating and verifying that the control measures at the primary production level are adequately limiting the frequency and level of contamination to that needed to achieve the required level of control during subsequent manufacturing.

3.1 ENVIRONMENTAL HYGIENE

Refer to the Recommended International Code of Practice - General Principles of Food Hygiene (CAC/RCP 1-1969).

3.2 HYGIENIC PRODUCTION OF FOOD SOURCES

Refer to the Recommended International Code of Practice - General Principles of Food Hygiene (CAC/RCP 1-1969).

3.3 HANDLING, STORAGE AND TRANSPORT

Refer to the Recommended International Code of Practice - General Principles of Food Hygiene (CAC/RCP 1-1969).

3.4. CLEANING, MAINTENANCE AND PERSONNEL HYGIENE AT PRIMARY PRODUCTION

Refer to the Recommended International Code of Practice - General Principles of Food Hygiene (CAC/RCP 1-1969).

SECTION IV - ESTABLISHMENT: DESIGN AND FACILITIES

Objectives:

Equipment and facilities should be designed, constructed and laid out to ensure cleanability and to minimise the potential for *L. monocytogenes* harbourage sites, cross-contamination and recontamination.

Rationale:

- The introduction of *L. monocytogenes* into the ready-to-eat processing environment has resulted from inadequate separation of raw and finished product areas and from poor control of employees or equipment traffic.
- Inability to properly clean and disinfect equipment and premises due to poor layout or design and areas inaccessible to cleaning has resulted in biofilms containing *L. monocytogenes* and harbourage sites that have been a source of product contamination
- The use of spray cleaning procedures that aerosolize the microorganism has been linked to the spread of the *L. monocytogenes* in the processing environment.
- Inability to properly control ventilation to minimise condensate formation on surfaces in food processing plants may result in the occurrence of *L. monocytogenes* in droplets and aerosols which can lead to product contamination.

4.1 LOCATION

4.1.1 Establishments

Refer to the Recommended International Code of Practice - General Principles of Food Hygiene (CAC/RCP 1-1969).

4.1.2 Equipment

Whenever possible, equipment should be designed and placed in a manner that facilitates access for efficient cleaning and disinfection, and thus avoid the formation of biofilms containing *L. monocytogenes* and harbourage sites.

4.2 PREMISES AND ROOMS

4.2.1 Design and Layout

Whenever feasible, premises and rooms should be designed to separate raw and finished ready-to-eat product areas. This can be accomplished in a number of ways, including linear product flow (raw to finished) with filtered airflow in the opposite direction (finished to raw) or physical partitions. Positive air pressure should be maintained on the finished side of the operation relative to the "raw" side (e.g., maintain lower air pressures in raw areas and higher pressures in finished areas).

Where feasible, the washing areas for food equipment involved in the manufacture of the finished product should be located in a separate room from the finished product processing area. This latter area should be separate from the raw ingredient handling area and the cleaning area for equipment used in the handling of raw ingredients in order to prevent recontamination of equipment and utensils used for finished products. Rooms where ready-to-eat products are exposed to the environment should be designed so that they can be maintained as dry as possible; wet operations often enhance the growth and spread of *L. monocytogenes*.

4.2.2 New construction/renovations

Due to the ability of *L. monocytogenes* to survive in the plant environment for long periods of time, disturbances caused by construction or modification of layouts can cause reintroduction of *L. monocytogenes* from harbourage sites to the environment. Where appropriate, care should be taken to isolate the construction area, to enhance hygienic operations and to increase environmental monitoring to detect *Listeria* spp. during construction/renovation (see Section 6.5).

4.2.3 Temporary/mobile premises and vending machines

Refer to the Recommended International Code of Practice - General Principles of Food Hygiene (CAC/RCP 1-1969).

4.3 EQUIPMENT

4.3.1 General

Due to the ability of *L. monocytogenes* to exist in biofilms and persist in harbourage sites for extended periods, processing equipment should be designed, constructed and maintained to avoid, for example, cracks, crevices, rough welds, hollow tubes and supports, close fitting metal-to-metal or metal-to-plastic surfaces, worn seals and gaskets or other areas that cannot be reached during normal cleaning and disinfection of food contact surfaces and adjacent areas.

Racks or other equipment used for transporting exposed product should have easily cleaned cover guards over the wheels to prevent contamination of the food from wheel spray.

Cold surfaces (e.g., refrigeration units) can be sources for psychrotrophic bacteria, especially *L. monocytogenes*. Condensate from refrigeration unit pans should be directed to a drain via a hose or drip pans should be emptied, cleaned and disinfected on a regular basis.

Insulation should be designed and installed in a manner that it does not become a harbourage site for *L. monocytogenes* .

4.3.2 Food control and monitoring equipment

Refer to the Recommended International Code of Practice - General Principles of Food Hygiene (CAC/RCP 1-1969).

4.3.3 Containers for waste and inedible substances

Refer to the Recommended International Code of Practice - General Principles of Food Hygiene (CAC/RCP 1-1969).

4.4 FACILITIES

4.4.1 Water supply

Refer to the Recommended International Code of Practice - General Principles of Food Hygiene (CAC/RCP 1-1969).

4.4.2 Drainage and waste disposal

Refer to the Recommended International Code of Practice - General Principles of Food Hygiene (CAC/RCP 1-1969).

4.4.3 Cleaning

Refer to the Recommended International Code of Practice - General Principles of Food Hygiene (CAC/RCP 1-1969).

4.4.4 Personnel hygiene facilities and toilets

Refer to the Recommended International Code of Practice - General Principles of Food Hygiene (CAC/RCP 1-1969).

4.4.5 Temperature control

Refer to the Recommended International Code of Practice - General Principles of Food Hygiene (CAC/RCP 1-1969).

4.4.6 Air quality and ventilation

Control of ventilation to minimise condensate formation is of particular importance in *L. monocytogenes* control, since the organism has been isolated from a wide variety of surfaces in food processing plants. Wherever feasible, facilities should be designed so that droplets and aerosols from condensates do not directly or indirectly contaminate food and food contact surfaces.

4.4.7 Lighting

Refer to the Recommended International Code of Practice - General Principles of Food Hygiene (CAC/RCP 1-1969).

4.4.8 Storage

Where feasible and appropriate for the food product, and where food ingredients and products support growth of *L. monocytogenes,* storage rooms should be designed so that a product temperature should not exceed 6°C, (preferably 2°C - 4°C). Raw materials should be stored separately from finished, processed products.

SECTION V - CONTROL OF OPERATION

Objectives:

Processing operations should be controlled to reduce the frequency and level of contamination in the finished product, to minimise the growth of *L. monocytogenes* in the finished product and to reduce the likelihood that the product will be recontaminated and/or will support the growth of *L. monocytogenes* during subsequent distribution, marketing and home use.

Rationale:

For many ready-to-eat products listericidal processes[6] can ensure appropriate reduction in risk. However, not all ready-to-eat products receive such a treatment and other ready-to-eat products may be exposed to the environment and thus may be subject to potential recontamination. Prevention of cross-contamination, strict control of time and temperature for products in which *L. monocytogenes* can grow and formulation of products with hurdles to *L. monocytogenes* growth can minimise the risk of listeriosis.

5.1 CONTROL OF THE FOOD HAZARD

Control of *L. monocytogenes* for many ready-to-eat products will typically require a stringent application of Good Hygienic Practice and other supportive programs. These prerequisite programs, together with HACCP provide a successful framework for the control of *L. monocytogenes*.

The factors and attributes described below are components of Good Hygienic Practice programs that will typically require elevated attention to control *L. monocytogenes* and may be identified as critical control points in HACCP programs where *L. monocytogenes* is identified as a hazard.

5.2 KEY ASPECTS OF HYGIENE CONTROL SYSTEMS

5.2.1 Time and temperature control

The risk assessments done by the U.S. FDA/FSIS and FAO/WHO on *L. monocytogenes* in ready-to-eat foods demonstrated the tremendous influence of storage temperature on the risk of listeriosis associated with ready-to-eat foods that support *L. monocytogenes* growth. It is therefore necessary to control the time/temperature combination used for storage.

Monitoring and controlling refrigerated storage temperatures are key control measures. The product temperature should not exceed 6°C (preferably 2°C - 4°C). Temperature abuse that may occur supporting the growth of *L. monocytogenes* could result in a reduction of product shelf life.

The length of the shelf-life is another important factor contributing to the risk associated with foods that support *L. monocytogenes* growth. The shelf-life of such foods should be consistent with the need to control the growth of *L. monocytogenes.* Since *L. monocytogenes* is able to grow under refrigeration temperatures, the length of the shelf-life should be based on appropriate studies that assess the growth of *L. monocytogenes* in the food. Shelf-life studies and other information are important tools facilitating the selection of the length of shelf-life. If they are conducted, they should account for the fact that appropriate low temperatures may not be maintained throughout the entire food chain until the point of consumption. Temperature abuses may allow the growth of *L. monocytogenes*, if present, unless appropriate intrinsic factors are applied to prevent such growth. This should be taken into account when establishing shelf life.

5.2.2 Specific process steps

Listericidal processes should be validated to ensure that the treatments are effective and can be applied consistently (see Section V of the Recommended International Code of Practice - General Principles of Food Hygiene (CAC/RCP 1-1969).

In some products single parameters, such as a pH less than 4.4, a water activity less than 0.92 or freezing, may be relied upon to prevent *L. monocytogenes* growth. In other products a combination of parameters is used. Validation should be undertaken to ensure the effectiveness of these parameters in situations where combinations of parameters or bacteriostatic conditions are relied upon.

Products supporting the growth of *L. monocytogenes* that have undergone a listericidal treatment may be contaminated/recontaminated before final packaging. In these cases, additional control measures may be applied if necessary, (e.g., freezing the product, shortening the shelf life, reformulation of the product) to limit the extent of or prevent *L. monocytogenes* growth. Alternatively, a post-packaging listericidal treatment may be necessary (e.g. heating, high pressure treatment, irradiation, where accepted).

[6] Any appropriate treatment that kills listeria.

In raw, ready-to-eat food (e.g. lettuce), that support the growth of *L. monocytogenes*, that may be contaminated, specific control measures may be applied if necessary to limit the extent of or prevent the growth of *L. monocytogenes* (e.g. acid wash).

5.2.3 Microbiological and other specifications

Refer to the *RecommendedInternational Code of Practice-General Principles of Food Hygiene(CAC/RCP 1-1969) and Principles for the Establishment and Application of Microbiological Criteria for Foods (CAC/GL 21-1979).*

5.2.4 Microbiological cross-contamination

Microbiological cross-contamination is a major issue with respect to *L. monocytogenes.* It can occur through direct contact with raw materials, personnel, aerosols and contaminated utensils, equipment, etc.. Cross-contamination can occur at any step where the product is exposed to the environment, including processing, transportation, retail, catering, and in the home.

Traffic flow patterns for employees, food products, and equipment should be controlled between raw processing, storage area(s) and finished area(s) to minimise the transfer of *L. monocytogenes*. For example, a change of footwear or automated foam sprayers can be an effective alternative to footbaths where people, carts, forklifts and other portable equipment must enter an area where ready-to-eat foods are exposed. Another example is to use a colour coding system to identify personnel assigned to specific areas of the plant.

Utensils, pallets, carts, forklifts and mobile racks should be dedicated for use in either the raw area or the finished product area to minimise cross-contamination. Where this is not practical, they should be cleaned and disinfected before entry into the finished product area.

Reused brines and recycled process water used in direct contact with finished product should be discarded or decontaminated (e.g. chlorination for recycled water, heat treatment, or some other effective treatment) with sufficient frequency to ensure control of *L. monocytogenes*.

Ready-to eat foods that do not support the growth of *L. monocytogenes* but may have low levels of this pathogen should not be a source of contamination to other ready-to-eat foods that may support the growth of this pathogen. Consideration should be given to the fact that some ready-to-eat foods with special handling requirements (for example ice cream), that are handled after opening may present a lower risk for being a vector for cross contaminating other ready-to-eat foods, because such specially handled product is rapidly consumed. Other ready-to-eat products, however, with special formulation (for example dry fermented sausage), that are handled after opening may present a higher risk of being a vector for cross contaminating other ready-to-eat products if neither ready-to-eat product is rapidly consumed.

5.2.5 Physical and chemical contamination

Refer to the Recommended International Code of Practice - General Principles of Food Hygiene (CAC/RCP 1-1969).

5.3 INCOMING MATERIAL REQUIREMENTS

Refer to the Recommended International Code of Practice - General Principles of Food Hygiene (CAC/RCP 1-1969).

5.4 PACKAGING

Refer to the Recommended International Code of Practice - General Principles of Food Hygiene (CAC/RCP 1-1969).

5.5 WATER

Refer to the Recommended International Code of Practice - General Principles of Food Hygiene (CAC/RCP 1-1969).

5.5.1 In contact with food

Refer to the Recommended International Code of Practice - General Principles of Food Hygiene (CAC/RCP 1-1969).

5.5.2 As an ingredient

Refer to the Recommended International Code of Practice - General Principles of Food Hygiene (CAC/RCP 1-1969).

5.5.3 Ice and steam

Refer to the Recommended International Code of Practice - General Principles of Food Hygiene (CAC/RCP 1-1969).

5.6 MANAGEMENT AND SUPERVISION

Refer to the Recommended International Code of Practice - General Principles of Food Hygiene (CAC/RCP 1-1969).

5.7 DOCUMENTATION AND RECORDS

Refer to the Recommended International Code of Practice - General Principles of Food Hygiene (CAC/RCP 1-1969).

5.8 RECALL PROCEDURES

Based on the determined level of risk associated with the presence of *L. monocytogenes* in a given food product, a decision may be taken to recall the contaminated product from the market. In some instances, the need for public warnings should be considered.

5.9 MONITORING OF EFFECTIVENESS OF CONTROL MEASURES FOR *L. MONOCYTOGENES*

An effective environmental monitoring program is an essential component of a *Listeria* control program, particularly in establishments that produce ready-to-eat foods that support growth and may contain *L. monocytogenes*. Testing of food products can be another component of verification that control measures for *L. monocytogenes* are effective (see Section 5.2.3).

Recommendations for the design of an environmental monitoring program for *L. monocytogenes* in processing areas are given in Annex 1.

SECTION VI - ESTABLISHMENT: MAINTENANCE AND SANITATION

Objectives:

To provide specific guidance on how preventive maintenance and sanitation procedures, along with an effective environmental monitoring program can reduce contamination of food with *L. monocytogenes*, particularly when the foods support growth of *L. monocytogenes*:

Well structured cleaning and disinfection procedures should be targeted against *L. monocytogenes* in food processing areas where ready-to-eat foods are exposed to reduce

- the likelihood that the product will be contaminated after processing,
- the level of contamination in the finished product.

Rationale:

Basic cleaning and disinfection programs are critical to assuring control of *L. monocytogenes.* An environmental monitoring program for *Listeria* in processing areas where ready-to-eat foods are exposed is necessary to assess the effectiveness of control measures and, therefore, the likelihood of contamination of the food.

6.1 MAINTENANCE AND CLEANING

6.1.1 General

Establishments should implement an effective, scheduled preventive maintenance program to prevent equipment failures during operation and the development of harbourage sites. Equipment failures during production increase the risk of *L. monocytogenes* contamination as equipment is being repaired. The preventive maintenance program should be written and include a defined maintenance schedule.

The preventive maintenance program should include scheduled replacement or repair of equipment before it becomes a source of contamination. Equipment should be inspected periodically for parts that are cracked, worn or have developed spaces where food and moisture accumulate (i.e., harbourage sites). Preventive maintenance should include periodic examination and maintenance of conveyors, filters, gaskets, pumps, slicers, filling equipment, and packaging machines and support structures for equipment. Air filters for bringing outside air into the plant should be examined and changed based on manufacturer's specification or more frequently based on pressure differential or microbiological monitoring.

Wherever possible, tools used for maintenance of equipment to which ready-to-eat foods are exposed should be dedicated to the finished product area. Such tools should be washed and disinfected prior to use. Maintenance personnel in the finished product area should comply with the same hygiene requirements as the finished product production employees. Food contact surfaces on equipment should be cleaned and disinfected after maintenance work, prior to production use. Equipment that could have become contaminated during maintenance work on facility utilities, e.g. air system, water system, etc., or remodelling, should be cleaned and disinfected prior to use.

6.1.2 Cleaning procedures and methods

Experience indicates that over-reliance on the chemicals alone for cleaning can lead to increased levels of microbial contamination. The chemicals must be applied at the recommended use-concentration, for sufficient time, at the recommended temperature and with sufficient force (i.e., turbulence, scrubbing) to remove soil and biofilm. Instances of *L. monocytogenes* contamination have been linked, in particular, to insufficient manual scrubbing during the cleaning process.

Research and experience further indicates that *L. monocytogenes* does not possess an unusual ability to resist disinfectants or attach to surfaces. However, it is noted that *L. monocytogenes* has the ability to form biofilms on a variety of surfaces.

Solid forms of disinfectants (e.g., blocks of quarternary ammonium compounds (QAC)) can be placed in the drip pan of refrigeration units and solid rings containing disinfectants can be placed in drains to help control *L. monocytogenes* in drains. Granulated forms of disinfectants such as QAC, hydrogen peroxide and peroxyacetic acid can be applied to floors after routine cleaning and disinfecting. The development of antimicrobial resistance should be considered in the application and use of disinfectants.

The equipment used for cleaning, e.g. brushes, bottle brushes, mops, floor scrubbers, and vacuum cleaners should be maintained and cleaned so they do not become a source of contamination. The cleaning equipment should be dedicated either for raw areas or finished areas, and easily distinguishable (e.g., colour-coded cleaning tools).

To prevent aerosols from contacting ready-to-eat foods, food contact surfaces and food packaging materials, high-pressure water hoses should not be used during production or after equipment has been cleaned and disinfected.

It has been shown that *L. monocytogenes* can become established and persist in floor drains. Therefore, drains should be cleaned and disinfected in a manner that prevents contamination of other surfaces in the room. Utensils for cleaning drains should be easily distinguishable and be dedicated to that purpose to minimise the potential for contamination.

Floor drains should not be cleaned during production. High-pressure hoses should not be used to clear or clean a drain, as aerosols will be created that spread contamination throughout the room. If a drain backup occurs in finished product areas, production should stop until the water has been removed and the areas have been cleaned and disinfected. Employees who have been cleaning drains should not contact or clean food contact surfaces without changing clothes, and washing and disinfecting hands.

6.2 CLEANING PROGRAMS

The effectiveness of sanitation programs should be periodically verified and the programs modified as necessary to assure the consistent achievement of the level of control needed for a food operation to prevent *L. monocytogenes* contamination of ready-to-eat food and ready-to-eat food contact surfaces.

6.3 PEST CONTROL SYSTEMS

Refer to the Recommended International Code of Practice - General Principles of Food Hygiene (CAC/RCP 1-1969).

6.3.1 General

Refer to the Recommended International Code of Practice - General Principles of Food Hygiene (CAC/RCP 1-1969).

6.3.2 Preventing access

Refer to the Recommended International Code of Practice - General Principles of Food Hygiene (CAC/RCP 1-1969).

6.3.3 Harbourage and infestation

Refer to the Recommended International Code of Practice - General Principles of Food Hygiene (CAC/RCP 1-1969).

6.3.4 Monitoring and detection

Refer to the Recommended International Code of Practice - General Principles of Food Hygiene (CAC/RCP 1-1969).

6.3.5 Eradication

Refer to the Recommended International Code of Practice - General Principles of Food Hygiene (CAC/RCP 1-1969).

6.4 WASTE MANAGEMENT

Refer to the Recommended International Code of Practice - General Principles of Food Hygiene (CAC/RCP 1-1969).

6.5 MONITORING EFFECTIVENESS

Environmental monitoring (see 5.9) can also be used to verify the effectiveness of sanitation programs such that sources of contamination of *L. monocytogenes* are identified and corrected in a timely manner. Recommendations for the design of an environmental monitoring program in processing areas are given in Annex 1.

SECTION VII - ESTABLISHMENT: PERSONAL HYGIENE

Objectives:

To prevent workers from transferring *L. monocytogenes* from contaminated surfaces to food or food contact surfaces.

Rationale:

Workers can serve as a vehicle for cross-contamination and should be aware of the steps that need to be taken to manage this risk.

7.1 HEALTH STATUS

Refer to the Recommended International Code of Practice - General Principles of Food Hygiene (CAC/RCP 1-1969).

7.2 ILLNESS AND INJURIES

Refer to the Recommended International Code of Practice - General Principles of Food Hygiene (CAC/RCP 1-1969).

7.3 PERSONAL CLEANLINESS

Refer to the Recommended International Code of Practice - General Principles of Food Hygiene (CAC/RCP 1-1969).

7.4 PERSONAL BEHAVIOUR

Employee hygienic practices play an important role in preventing contamination of exposed ready-to-eat foods with *L. monocytogenes*. For example, employees who handle trash, floor sweepings, drains, packaging waste or scrap product, should not touch the food, touch food contact surfaces or food packaging material, unless they change their smock or outer clothing, wash and disinfect hands, and wear clean new gloves for tasks requiring gloves. Adequate training and supervision should be provided to assure hygienic practices are accomplished.

7.5 VISITORS

Refer to the Recommended International Code of Practice - General Principles of Food Hygiene (CAC/RCP 1-1969).

SECTION VIII – TRANSPORTATION

Objectives:

Measures should be taken where necessary to:

- protect food from potential sources of contamination including harbourage sites for *L. monocytogenes* in transportation equipment and to prevent the co-mingling of raw and ready-to-eat product;
- provide an adequately refrigerated environment (so that product temperature should not exceed 6°C, preferably 2°C - 4°C).

Rationale:

Food may become contaminated during transportation if not properly protected**.**

If refrigeration is inadequate, food may support the growth of *L. monocytogenes* to higher levels..

8.1 GENERAL

Transportation is an integral step in the food chain and should be controlled, particularly the product temperature which should not exceed 6°C (preferably2°C - 4°C).

Transportation vehicles should be regularly inspected for structural integrity, cleanliness, and overall suitability when unloading ingredients and prior to loading finished products. In particular, the structural integrity of transportation vehicles (e.g., tanker trucks) should be monitored for stress cracks that act as harbourage sites for *L. monocytogenes*. Tankers should be dedicated to transport either ingredients or finished products.

8.2 REQUIREMENTS

Refer to the Recommended International Code of Practice - General Principles of Food Hygiene (CAC/RCP 1-1969).

8.3 USE AND MAINTENANCE

Food transportation units, accessories, and connections should be cleaned, disinfected (where appropriate) and maintained to avoid or at least reduce the risk of contamination. It should be noted that different commodities may require different cleaning procedures. Where necessary, disinfection should be followed by rinsing unless manufacturer's instruction indicates on a scientific basis that rinsing is not required.[7] A record should be available that indicates when cleaning occurred.

SECTION IX - PRODUCT INFORMATION AND CONSUMER AWARENESS

Objectives:

Consumers should have enough knowledge of *L. monocytogenes* and food hygiene such that they:

- understand the importance of shelf-life, sell-by or use-by dates written on food label;
- can make informed choices appropriate to the individual's health status and concomitant risk of acquiring foodborne listeriosis;
- prevent contamination and growth or survival of *L. monocytogenes* by adequately storing and preparing ready-to-eat foods.

Health care providers should have appropriate information on *L. monocytogenes* in foods and listeriosis to give advice to consumers and in particular susceptible populations

Rationale:

Consumers (in particular, the susceptible populations), health care providers, need to be informed about ready-to-eat foods supporting growth of *L. monocytogenes,* food handling, preparation practices and avoidance of certain foods by susceptible populations.

9.1 LOT IDENTIFICATION

Refer to the Recommended International Code of Practice - General Principles of Food Hygiene (CAC/RCP 1-1969).

9.2 PRODUCT INFORMATION

Refer to the Recommended International Code of Practice - General Principles of Food Hygiene (CAC/RCP 1-1969).

9.3 LABELLING

Countries should give consideration to labelling of certain ready-to-eat foods so that consumers can make an informed choice with regard to these products. Where appropriate, product labels should include information on safe handling practices and/or advice on the time frames in which the product should be eaten.

9.4 CONSUMER EDUCATION

Since each country has specific consumption habits, communication programs pertaining to *L. monocytogenes* are most effective when established by individual governments.

Programs for consumer information should be directed:

- at consumers with increased susceptibility to contracting listeriosis, such as pregnant women, the elderly and immunocompromised persons;
  to help consumers make informed choices about purchase, storage, shelf-life labelling and appropriate consumption of certain ready-to-eat foods that have been identified in relevant risk assessment and other studies, taking into consideration the specific regional conditions and consumption habits;

[7] Code of Hygienic Practice for the Transport of Food in Bulk and Semi-packed Food (CAC/RCP 47-2001).

- to consumers to educate them on household practices and behaviours that would specifically keep the numbers of *L. monocytogenes* that may be present in foods, to as low a level as possible by
    - setting refrigerator temperatures so that product temperatures should not exceed 6°C (preferably 2°C - 4°C) since the growth of *L monocytogenes* is considerably reduced at temperatures below 6°C;
    - frequently washing and disinfecting the household refrigerator since *L. monocytogenes* can be present in many foods and grow at refrigerator temperatures, and thus contribute to cross-contamination;
    - respecting the shelf-life dates written on ready-to-eat foods;
    - using of thermometers inside home refrigerators.

Programs for health care providers should, in addition to information provided to consumers, be designed to provide them with guidance that

- facilitates rapid diagnosis of foodborne listeriosis;
- provides means to rapidly communicate information on preventing listeriosis to their patients, particularly those with increased susceptibility.

SECTION X - TRAINING

Objective:

Those engaged in food operation who come directly or indirectly in contact with ready-to-eat foods should be trained and/or instructed in the control of *L. monocytogenes* to a level appropriate to the operations they are to perform..

Rationale:

Controls specific to *L. monocytogenes* are generally more stringent than routine Good Hygiene Practices.

10.1 AWARENESS AND RESPONSIBILITIES

Industry (primary producers, manufacturers, distributors, retailers and food service/institutional establishments) and trade associations have an important role in providing specific instruction and training for control of *L. monocytogenes*.

10.2 TRAINING PROGRAMS

Personnel involved with the production and handling of ready-to-eat food should have appropriate training in:

- the nature of *L. monocytogenes*, its harbourage sites, and its resistance to various environmental conditions to be able to conduct a suitable hazard analysis for their products;
- control measures for reducing the risk of *L. monocytogenes* associated with ready-to-eat foods during processing, distribution, marketing, use and storage;
- the means for verifying effectiveness of control programs, including sampling and analytical techniques;

10.3 INSTRUCTION AND SUPERVISION

Refer to the Recommended International Code of Practice - General Principles of Food Hygiene (CAC/RCP 1-1969).

10.4 REFRESHER TRAINING

Refer to the Recommended International Code of Practice - General Principles of Food Hygiene (CAC/RCP 1-1969).

ANNEX I: RECOMMENDATIONS FOR AN ENVIRONMENTAL MONITORING[8] PROGRAM FOR *LISTERIA MONOCYTOGENES* IN PROCESSING AREAS

Manufacturers of ready-to-eat foods should consider the potential risk to consumers in the event their products contain *L. monocytogenes* when they are released for distribution. The necessity for an environmental monitoring program is highest for ready-to-eat foods that support *L. monocytogenes* growth and that are not given a post-packaging listericidal treatment. Recontamination has led to many of the recognised outbreaks of listeriosis. One effective element of managing this risk is to implement a monitoring program to assess control of the environment in which ready-to-eat foods are exposed prior to final packaging.

A number of factors (a – i) should be considered when developing the sampling program to ensure the program's effectiveness:

a) Type of product and process/operation

The need[9] for and extent of the sampling program should be defined according to the characteristics of the ready-to-eat foods (supporting or not supporting growth), the type of processing (listericidal or not) and the likelihood of contamination or recontamination (exposed to the environment or not). In addition, consideration also needs to be given to elements such as the general hygiene status of the plant or the existing history of *L. monocytogenes* in the environment.

b) Type of samples

Environmental samples consist of both food contact and non food contact surface samples. Food contact surfaces, in particular those after the listericidal step and prior to packaging, have a higher probability of directly contaminating the product, while for non food contact surfaces the likelihood will depend on the location and practices.

Raw materials may serve as a source of environmental contamination and may therefore be included in the monitoring program.

c) Target organisms

While this document addresses *L. monocytogenes*, effective monitoring programs may also involve testing for *Listeria* spp; their presence is a good indicator of conditions supporting the potential presence of *Listeria monocytogenes*. Where appropriate and shown to be valid, other indicator organisms may be used[10].

d) Sampling locations and number of samples

The number of samples will vary with the complexity of the process and the food being produced.

Information on appropriate locations can be found in published literature, can be based on process experience or expertise or in plant surveys. Sampling locations should be reviewed on a regular basis. Additional locations may need to be sampled depending on special situations such as major maintenance or construction or when new or modified equipment has been installed.

e) Frequency of sampling

The frequency of environmental sampling would be based primarily on the factors outlined under sub-heading "Type of product and process/operation". It should be defined according to existing data on the presence of *Listeria* spp. and/or *L. monocytogenes* in the environment of the operation under consideration.

---

8 Environmental monitoring is not to be confused with monitoring as defined in the HACCP.

9 Products such as in pack pasteurised foods which are not further exposed to environment may not necessarily require a monitoring.

10 Attributes contributing to the scientific support of the use of an indicator organism in view of a specific pathogen include: similar survival and growth characteristics; a shared common source for both organisms; direct relationship between the state or condition that contributes to the presence of the pathogen and the indicator organism; and practical, isolation, detection or enumeration methods for the potential indicator organism.

In the absence of such information sufficient suitable data should be generated to correctly define the appropriate frequency. These data should be collected over a sufficiently long period as to provide reliable information on the prevalence of *Listeria* spp. and/or *L. monocytogenes* and the variations over time.

The frequency of environmental sampling may need to be increased as a result of finding *Listeria* spp. and/or *L. monocytogenes* in environmental samples. This will depend on the significance of the findings (e.g. *L. monocytogenes* and a risk of direct contamination of the product).

f) Sampling tools and techniques

It is important to adapt the type of sampling tools and techniques to the type of surfaces and sampling locations. For example sponges may be used for large flat surfaces, swabs may be more appropriate for cracks and crevices or scrapers for hard residues.

g) Analytical methods

The analytical methods used to analyse environmental samples should be suitable for the detection of *L. monocytogenes* and of other defined target organisms. Considering the characteristics of environmental samples it is important to demonstrate that the methods are able to detect, with acceptable sensitivity, the target organisms. This should be documented appropriately.

Under certain circumstances it may be possible to composite (pool) certain samples without loosing the required sensitivity. However, in the case of positive findings additional testing will be necessary to determine the location of the positive sample.

Fingerprinting isolates by one or more of the available genetic techniques (e.g., pulsed field gel electrophoresis, ribotyping) can provide very useful information about the source(s) of *L. monocytogenes* and pathway(s) that lead to contamination of the food.

h) Data management

The monitoring program should include a system to record the data and their evaluation, e.g. performing trend analyses. A long-term review of the data is important to revise and adjust monitoring programs. It can also reveal low level, intermittent contamination that may otherwise go unnoticed.

i) Actions in case of positive results

The purpose of the monitoring program is to find *L. monocytogenes* or other target organisms if present in the environment. Generally manufacturers should expect to find them occasionally in the processing environment. Therefore an appropriate action plan should be designed and established to adequately respond to positive findings. A review of hygiene procedures and controls should be considered.

The manufacturer should react to each positive result; the nature of the reaction will depend upon the likelihood of contaminating the product and the expected use of the products.

The plan should define the specific action to be taken and the rationale. This could range from no action (no risk of recontamination), to intensified cleaning, to source tracing (increased environmental testing), to review of hygienic practices up to holding and testing of product.

ANNEX II: MICROBIOLOGICAL CRITERIA FOR *LISTERIA MONOCYTOGENES* IN READY-TO-EAT FOODS

1. INTRODUCTION

The microbiological criteria presented in this Annex are intended as advice to governments within a framework for control of *L. monocytogenes* in ready-to-eat foods with a view towards protecting the health of consumers and ensuring fair practices in food trade. They also provide information that may be of interest to industry.

This Annex references and takes into account the *Principles for the Establishment and Application of Microbiological Criteria for Foods* (CAC/GL 21 – 1997) and uses definitions, e.g. for microbiological criterion, as included in these principles. The provisions of this Annex should be used in conjunction with *Annex II: Guidance on Microbiological Risk Management Metrics of the Principles and Guidelines for the Conduct of Microbiological Risk Management* **(**CAC/GL 63-2007).

The risk assessments referenced in the introduction to the *Guidelines on the Application of General Principles of Food Hygiene to the Control of Listeria monocytogenes in Ready-to-Eat Food* (CAC/GL 61-2007) have indicated that food can be categorized according to the likelihood of *Listeria monocytogenes* being present and its ability to grow in the food. Available risk assessments have been taken into account in the development of the microbiological criteria in this Annex. In addition, factors that might impact upon the ability of governments to implement these microbiological criteria such as methodological limitations, costs associated with different types of quantitative testing, and statistics-based sampling needs were taken into account.

2. SCOPE

These microbiological criteria apply to specific categories of ready-to-eat foods, as described herein. The competent authority should consider the intended use and how specific ready-to-eat foods are likely to be handled during marketing, catering, or by consumers to determine the appropriateness of applying the microbiological criteria. Governments may apply these criteria, where appropriate, to assess the acceptability of ready-to-eat foods in international trade for imported products, at end of manufacture (finished product) for domestic products, and at point of sale for at least the expected shelf life[11] under reasonably foreseeable conditions of distribution, storage and use.

The microbiological criteria may be used as the basis for the development of additional criteria (e.g. process criteria, product criteria) within a food safety control system[12] to ensure compliance with these guidelines.

Different criteria or other limits may be applied when the competent authority determines that the use of such an approach provides an acceptable level of public health or when the competent authority determines a more stringent criterion is necessary to protect public health.

3. USE OF MICROBIOLOGICAL CRITERIA FOR *L. MONOCYTOGENES* IN READY-TO-EAT FOODS

There are various applications for microbiological criteria. As described, microbiological testing by lot can be used as a direct control measure, i.e., sorting of acceptable and unacceptable lots[13]. In this instance, microbiological criteria are implemented for those products and/or points of the food chain when other more

[11] See definition in the Code of Hygienic Practice For Milk and Milk Products (CAC/RCP 57–2004).
[12] See: Guidelines for the Validation of Food Safety Control Measures (CAC/GL 69-2008).
[13] See: Principles for the Establishment and Application of Microbiological Criteria for Foods (CAC/GL 21-1997).

effective tools are not available and where the microbiological criteria would be expected to improve the degree of protection offered to the consumer.

A microbiological criterion defines the acceptability of a product or food lot based on the absence or presence or number of microorganisms in the product. Testing for compliance with a microbiological criterion may be conducted on a lot by lot basis when there is little information about the conditions under which the product has been produced. Where there is information about the conditions of production, testing of lots for verification purposes may be conducted less frequently.

In addition, the application of the Hazard Analysis and Critical Control Point (HACCP) System describes how microbiological testing against a criterion can be used as a means of verifying the continuing effectiveness of a food safety control system[14]. Typically, such applications involve testing on less than a lot by lot basis and may be formalized into a system of process control verification testing (see Annex III).

Where possible and practicable, the risk-based approach to development of microbiological criteria as described in the Principles and Guidelines for the Conduct of Microbiological Risk Management (CAC/GL-63-2007) can be used to assure or contribute to the assurance, that a food control system will achieve the required level of consumer protection.

The competent authority should use a risk-based approach to sampling for *L. monocytogenes* such as that found in the Codex General Guidelines on Sampling (CAC/GL 50 – 2004). It may consider modifying the frequency of testing for process control verification based on additional consideration of the likelihood of contamination, characteristics of the food, product history, conditions of production and other relevant information. For example, testing against microbiological criteria may have limited utility immediately following certain processing steps or if the level of *L. monocytogenes* in a ready-to-eat food is consistently well below the limit of detection taking into account practical limits for sample sizes.

In particular, testing against microbiological criteria for *L. monocytogenes* may not be useful for:

(a) products that receive a listericidal treatment after being sealed in final packaging that ensures prevention of recontamination until opened by the consumer or otherwise compromised,

(b) foods that are aseptically processed and packaged[15] , and

(c) products that contain a listericidal component that ensures rapid inactivation of the pathogen if recontaminated (e.g., products that contain > 5 % ethanol)

Competent authorities may define other categories of products for which testing against microbiological criteria are not useful.

Different types of food present different risks from *L. monocytogenes*, hence different microbiological criteria could apply for the following categories of foods:

(a) ready-to-eat foods in which growth of *L. monocytogenes* will not occur, and

(b) ready-to-eat foods in which growth of *L. monocytogenes* can occur.

3.1 Ready-To-Eat foods in which growth of *L. monocytogenes* will not occur

Ready-to-eat foods in which growth of *L. monocytogenes* will not occur would be determined based on scientific justification[16], including the inherent variability of factors controlling *L. monocytogenes* in the

[14] See: Recommended International Code of Practice - General Principles of Food Hygiene (CAC/RCP 1-1969).
[15] See: Code of Hygienic Practice For Aseptically Processed And Packaged Low-Acid Foods (CAC/RCP 40-1993).

product. Factors such as pH, a_w, are useful in preventing growth. For example, *L. monocytogenes* growth can be controlled in foods that have:

- a pH below 4.4,
- an $a_w < 0.92$,
- a combination of factors (pH, a_w ,), e.g. the combination of pH < 5.0 with $a_w < 0.94$.

Such growth can also be controlled by freezing (during that period when the product remains frozen).

In addition, inhibitors can control the growth of *L. monocytogenes* and synergy may be obtained with other extrinsic and intrinsic factors that would result in no growth.

Demonstration that *L. monocytogenes* will not grow in a ready-to-eat food can be based upon, for example, food characteristics, the study of naturally contaminated food, challenge tests, predictive modelling, information from the scientific literature and risk assessments, historic records or combinations of these. Such studies would generally be conducted by food business operators (or by the appropriate product board, sector organizations or contract laboratories) and must be appropriately designed to validate that *L. monocytogenes* will not grow in a food[17].

The demonstration that *L. monocytogenes* will not grow in a ready-to-eat food should take into account the measurement error of the quantification method. Therefore, for example, for practical purposes, a food in which growth of *L. monocytogenes* will not occur will not have an observable increase in *L. monocytogenes* levels greater than (on average) 0.5 log CFU/g[18] for at least the expected shelf life as labelled by the manufacturer under reasonably foreseeable conditions of distribution, storage and use, including a safety margin.

For foods intended to be refrigerated, studies to assess whether or not growth of *L. monocytogenes* will occur should be conducted under reasonably foreseeable conditions of distribution, storage and use.

National governments should provide guidance on the specific protocols that should be employed to validate the studies demonstrating that growth of *L. monocytogenes* will not occur in a food during the expected shelf life.

If information is lacking to demonstrate that *L. monocytogenes* will not grow in a ready-to-eat food during its expected shelf life, the food should be treated as a ready-to-eat food in which growth of *L. monocytogenes* can occur.

3.2 Ready-to-eat foods in which growth of *L. monocytogenes* can occur

A ready-to-eat food in which there is greater than an average of 0.5 log CFU/g[118] increase in *L. monocytogenes* levels for at least the expected shelf life under reasonably foreseeable conditions of distribution, storage and use is considered a food in which growth of *L. monocytogenes* can occur.

---

[16] References that have been addressed for identifying properties of ready-to-eat foods which will categorize them as foods in which growth of *L. monocytogenes* will not occur, or as foods in which growth of the pathogen can occur, include *Microorganisms in Foods 5 – Characteristics of Microbial Pathogens* (ICMSF, 1996) and *Microbiological Risk Assessment Series 4 and 5: Risk assessment of Listeria monocytogenes in ready to eat foods: Interpretative Summary and Technical Report* (FAO/WHO, 2004).

[17] See: Guidelines for the Validation of Food Safety Control Measures (CAC/GL 69-2008).

[18] 0.5 log is two times the estimated standard deviation (i.e. 0.25 log) associated with the experimental enumeration using viable counting/plate counts.

4. MICROBIOLOGICAL CRITERIA FOR *L. MONOCYTOGENES* IN READY-TO-EAT FOODS

Microbiological criteria for *L. monocytogenes* in ready-to-eat foods are described.

Another procedure for establishing microbiological criteria for *L. monocytogenes* other than the criteria at specified points in the food chain that are described below, would be through the application of risk-based metrics (e.g., Food Safety Objective (FSO), Performance Objective (PO)) according to the general principles established in the *Annex II: Guidance on Microbiological Risk Management Metrics of the Principles and Guidelines for the Conduct of Microbiological Risk Management* (CAC/GL 63-2007).

4.1 Microbiological criteria for ready-to-eat foods in which growth of *L. monocytogenes* will not occur

The criterion in Table 1 is intended for foods in which *L. monocytogenes* growth will not occur under the conditions of storage and use that have been established for the product (see Section 3.1).

This criterion is based on the product being produced under application of the provisions of the general principles of food hygiene to the control of *L. monocytogenes* in ready-to-eat foods with appropriate evaluation of the production environment and process control and validation that the product meets the requirements of a food in which growth of *L. monocytogenes* will not occur (see Section 3.1).

If the factors that prevent growth cannot be demonstrated, the product should be evaluated based on criteria for ready-to-eat foods in which growth of *L. monocytogenes* can occur (see Section 4.2).

Another approach can also be used (see Section 4.3).

Table 1:

Microbiological criterion for ready-to-eat foods in which growth of *L. monocytogenes* will not occur

Point of application	Microorganism	n	c	m	Class Plan
Ready-to-eat foods from the end of manufacture or port of entry (for imported products), to the point of sale	*Listeria monocytogenes*	5 [a]	0	100 cfu/g [b]	2 [c]

Where n = number of samples that must conform to the criterion; c = the maximum allowable number of defective sample units in a 2-class plan;. m=a microbiological limit which, in a 2-class plan, separates acceptable lots from unacceptable lots.

[a] National governments should provide or support the provision of guidance on how samples should be collected and handled, and the degree to which compositing of samples can be employed.

[b] This criterion is based on the use of the ISO 11290-2 method.

Other methods that provide equivalent sensitivity, reproducibility, and reliability can be employed if they have been appropriately validated (e.g., based on ISO 16140).

[c] Assuming a log normal distribution, this sampling plan would provide 95% confidence that a lot of food containing a geometric mean concentration of 93.3 cfu/g and an analytical standard deviation of 0.25 log cfu/g would be detected and rejected based on any of the five samples exceeding 100 cfu/g *L. monocytogenes*. Such a lot may consist of 55% of the samples being below 100 cfu/g and up to 45% of the samples being above 100 cfu/g, whereas 0.002% of all the samples from this lot could be above 1000 cfu/g. The typical actions to be taken where there is a failure to meet the above criterion would be to (1) prevent the affected lot from being released for human consumption, (2) recall the

product if it has been released for human consumption, and/or (3) determine and correct the root cause of the failure.

4.2 Microbiological criteria for ready-to-eat foods in which growth of *L. monocytogenes* can occur

The criterion in Table 2 is intended for foods in which *L. monocytogenes* growth can occur under the conditions of storage and use that have been established for the product (see Section 3.2).

This criterion is based on the product being produced under application of general principles of food hygiene to the control of *L. monocytogenes* in ready-to-eat foods with appropriate evaluation of the production environment and process control (see Annex III).

The purpose of this criterion is to provide a specified degree of confidence that *L. monocytogenes* will not be present in foods at levels that represent a risk to consumers.

Another approach can also be used (see Section 4.3).

Table 2:

Microbiological criteria for ready-to-eat foods in which growth of *L. monocytogenes* can occur

Point of application	Microorganism	n	c	m	Class Plan
Ready-to-eat foods from the end of manufacture or port of entry (for imported products), to the point of sale	*Listeria monocytogenes*	5 [a]	0	Absence in 25 g (< 0.04 cfu/g) [b]	2 [c]

[a] National governments should provide or support the provision of guidance on how samples should be collected and handled, and the degree to which compositing of samples can be employed.

[b] Absence in a 25-g analytical unit. This criterion is based on the use of ISO 11290-1 method. Other methods that provide equivalent sensitivity, reproducibility, and reliability can be employed if they have been appropriately validated (e.g., based on ISO 16140).

[c] Assuming a log normal distribution, this sampling plan would provide 95% confidence that a lot of food containing a geometric mean concentration of 0.023 cfu/g and an analytical standard deviation of 0.25 log cfu/g would be detected and rejected if any of the five samples are positive for *L. monocytogenes.* Such a lot may consist of 55% of the 25g samples being negative and up to 45% of the 25 g samples being positive. 0.5 % of this lot could harbour concentrations above 0.1 cfu/g.

The typical actions to be taken where there is a failure to meet the above criterion would be to (1) prevent the affected lot from being released for human consumption, (2) recall the product if it has been released for human consumption, and/or (3) determine and correct the root cause of the failure.

4.3 Alternative approach

Further to the approaches described in sections 4.1 and 4.2 competent authorities may choose to establish and implement other validated limits for the *L. monocytogenes* concentration at the point of consumption or at other points that provide an acceptable level of consumer protection for foods in which *L. monocytogenes* will not grow as well as foods in which *L. monocytogenes* growth can occur.

Due to the large diversity among ready-to-eat food products in which growth of *L. monocytogenes* can occur, this approach would primarily be applied for specific categories or subcategories of ready-to-eat foods being

produced under application of the provisions of the general principles of food hygiene to the control of *L. monocytogenes* in ready-to-eat foods and that have a limited potential of growth over a specified shelf life.

In establishing such limits for *L. monocytogenes*, the competent authority needs to clearly articulate the types of information required of food business operators to ensure that the hazard is controlled and to verify that these limits are achieved in practice. Information needed by competent authorities should be obtained through validation studies or other sources, and may include

- specification for physicochemical characteristics of the products, such as pH, a_w, salt content, concentration of preservatives and the type of packaging system, taking into account the storage and processing conditions, the possibilities for contamination and the foreseen shelf life[19] including a safety margin, and

- consultations of available scientific literature and research data regarding the growth and survival characteristics of *L. monocytogenes*.

When appropriate on the basis of the above mentioned studies, additional studies should be conducted, which may include:

- predictive mathematical modelling established for the food in question, using critical growth or survival factors for *L. monocytogenes* in the product,

- challenge tests and durability studies to evaluate the growth or survival of *L. monocytogenes* that may be present in the product during the shelf life under reasonably foreseeable conditions of distribution, storage and use including seasonal and regional variations.

[19] See footnote 2 : Code of Hygienic Practice for Milk and Milk Products (CAC/RCP 57–2004).

ANNEX III: RECOMMENDATIONS FOR THE USE OF MICROBIOLOGICAL TESTING FOR ENVIRONMENTAL MONITORING AND PROCESS CONTROL VERIFICATION BY COMPETENT AUTHORITIES AS A MEANS OF VERIFYING THE EFFECTIVENESS OF HACCP AND PREREQUISITE PROGRAMS FOR CONTROL OF *LISTERIA MONOCYTOGENES* IN READY-TO-EAT FOODS

Introduction

These recommendations are for use by competent authorities if they intend to include environmental monitoring and/or process control testing as part of their regulatory activities. It is also anticipated that the annex will provide guidance that the competent authority can provide to industry. The recommendations provide an elaboration of the concepts in Sections 5 and 6 of the main text of this Code.

Guidance within Codex regarding microbiological testing is often restricted to the testing of end products using traditional lot-by-lot testing. However, the guidance provided in the main text of this Code emphasizes the criticality of enhanced control of sanitation, including the appropriate use of environmental monitoring. This is further elaborated in Annex I: *Recommendations for an Environmental Monitoring Program for Listeria monocytogenes in Processing Areas,* which provides recommendations to industry on implementation of environmental monitoring programs. The *Recommended International Code of Practice General Principles of Food Hygiene* (CAC/RCP 1-1969) emphasizes the need to apply control measures in a systematic manner using HACCP or other food safety control systems, including the testing of in-line or finished product samples for process control verification. This annex provides general recommendations on how competent authorities can use microbiological testing to verify the effectiveness of (a) general hygiene programs in the food operation environment and (b) control measures in facilities employing HACCP or other food safety control systems.

The two types of microbiological testing programs described below can be an important part of the ability of competent authorities to verify the effectiveness of *L. monocytogenes* control programs over time (see Section 5.9). In developing these recommendations, no attempt is made to establish specific decision criteria for the two types of microbiological testing or the specific actions that should be taken to re-establish control. Establishment of such specific criteria and actions is more appropriately the responsibility of competent authorities due to the diversity in products and manufacturing technologies.

a) Environmental Monitoring

In certain instances, competent authorities may incorporate the testing of the environment (food contact and/or non-food contact surfaces) for *L. monocytogenes* (or an appropriate surrogate microorganism (e.g., *Listeria* spp.)), as part of their regulatory requirements or activities. This can include sampling by a competent authority as part of its inspection activities or sampling performed by the individual food business operator that the competent authority can review as part of its verification of the business operator's controls (see Section 5.9). The aim of conducting and/or reviewing environmental testing programs by a competent authority is to verify, for example, that a manufacturer has successfully identified and controlled niches and harbourage sites for *L. monocytogenes* in the food plant and to verify that sanitation programs have been appropriately designed and implemented to control contamination by *L. monocytogenes*.

In developing environmental testing programs and the decision criteria for actions to be taken based on the results obtained, competent authorities should clearly distinguish between sampling of food contact surfaces and non-food contact surfaces. For example, sampling locations for competent authorities may be similar to those used by food business operators (See Annex I). In evaluating facilities that produce multiple products where at least one can support growth of *L. monocytogenes*, competent authorities should consider the importance of environmental sampling as a means of verifying that there is no cross contamination between the products (see Section 5.2.4). In the design of an environmental verification program, the competent authority should articulate the testing and sampling techniques that would be employed, including size, method and frequency of sampling, analytical method to be employed, locations where samples should be

taken, decision criteria, and actions to be taken if a decision criterion is exceeded (similar to recommendations in Annex I).

The competent authority should establish decision criteria that include specific conditions (e.g., specific number of positive samples) that will initiate follow-up actions (including additional testing) when an environmental sample is positive for *L. monocytogenes* or *Listeria* spp. The competent authority should also establish actions that the food business operator should anticipate if the criteria are exceeded. Detection of positive environmental samples by the competent authority exceeding the decision criteria should lead to an investigation by the food business operator and/or the competent authority to identify the source of contamination and action that should be taken by the food business operator to correct the problem. In reporting results of their analyses to food business operators, competent authorities should provide advice on the possible inferences the data provide in order to assist the food business operator in finding and correcting the source of contamination. For example, the competent authority could point out that the repetitive isolation of a specific subtype of *L. monocytogenes* is indicative of a harbourage site that current sanitation activities are insufficient to control.

Overall, sampling techniques and testing methods should be sufficiently sensitive for the decision criteria established and appropriate for the surface or equipment being evaluated. Methods used should be appropriately validated for the recovery of *L. monocytogenes* from environmental samples.

b) Process Control Verification

Business operators ensure the effectiveness of HACCP and other programs for the control of *L. monocytogenes* in their operating facilities. Further, business operators validate the food safety control systems they have in place. Competent authorities verify that the controls are validated and being implemented as designed, through activities such as monitoring of records and activities of production personnel.

For a well-designed food safety control system, a competent authority may consider establishing microbiological process control testing and decision criteria for products to identify trends that can be corrected before decision criteria are exceeded. When undesirable trends occur or decision criteria are exceeded, the food business operator will investigate the food safety control system to determine the cause and take corrective action(s). The competent authority verifies that appropriate actions are taken when criteria are exceeded. For example, the decision criteria for process control testing could be the frequency of contamination that would be indicative of a process no longer in control and likely to produce ready-to-eat foods that do not meet the microbiological criteria established in Annex II.

In addition to verifying that the process controls within the food safety control system are validated and operating as designed, process control testing of finished product (sometimes referred to as cross-lot or between-lot testing) has been used by business operators and/or competent authorities to detect changing patterns of contamination, which allows distinction between occasional 'in control' positive samples and an emerging loss of control. Process control testing of finished product contributes to the assessment of the continuing performance of a food safety control system and helps to ensure that corrective actions are implemented before microbiological criteria are exceeded. The competent authority verifies that the food safety control system remains 'in control' or ensures that the food business operator has taken corrective actions to prevent loss of control, which could include immediate corrections or changes to the food safety control system itself. The presence of *L. monocytogenes* in finished product can also indicate the lack of control of *L. monocytogenes* in the processing environment.

In certain instances, competent authorities may find it useful to establish an industry-wide process control-based criterion for *L. monocytogenes* for the purpose of ensuring that specific ready-to-eat foods undergo a consistent approach for verification of HACCP or other food safety control systems. This can include sampling by competent authorities as part of their inspection activities or sampling performed by the business operator that the competent authority can review as part of its verification of the food business operator's records.

As with other forms of verification via microbiological testing, the use of process control testing involves the establishment of decision criteria, specification of analytical methods, specification of a sampling plan, and actions to be taken in case of a loss of control. Details of process control testing principles and guidelines are beyond the scope of this annex, but are available through standard references.

Foodborne Illness Acquired in the United States—Major Pathogens

Elaine Scallan,[1] Robert M. Hoekstra, Frederick J. Angulo, Robert V. Tauxe, Marc-Alain Widdowson, Sharon L. Roy, Jeffery L. Jones, and Patricia M. Griffin

Estimates of foodborne illness can be used to direct food safety policy and interventions. We used data from active and passive surveillance and other sources to estimate that each year 31 major pathogens acquired in the United States caused 9.4 million episodes of foodborne illness (90% credible interval [CrI] 6.6–12.7 million), 55,961 hospitalizations (90% CrI 39,534–75,741), and 1,351 deaths (90% CrI 712–2,268). Most (58%) illnesses were caused by norovirus, followed by nontyphoidal *Salmonella* spp. (11%), *Clostridium perfringens* (10%), and *Campylobacter* spp. (9%). Leading causes of hospitalization were nontyphoidal *Salmonella* spp. (35%), norovirus (26%), *Campylobacter* spp. (15%), and *Toxoplasma gondii* (8%). Leading causes of death were nontyphoidal *Salmonella* spp. (28%), *T. gondii* (24%), *Listeria monocytogenes* (19%), and norovirus (11%). These estimates cannot be compared with prior (1999) estimates to assess trends because different methods were used. Additional data and more refined methods can improve future estimates.

Estimates of the overall number of episodes of foodborne illness are helpful for allocating resources and prioritizing interventions. However, arriving at these estimates is challenging because food may become contaminated by many agents (e.g., a variety of bacteria, viruses, parasites, and chemicals), transmission can occur by nonfood mechanisms (e.g., contact with animals or consumption of contaminated water), the proportion of disease transmitted by food differs by pathogen and by host factors (e.g. age and immunity), and only a small proportion of illnesses are confirmed by laboratory testing and reported to public health agencies.

Laboratory-based surveillance provides crucial information for assessing foodborne disease trends. However, because only a small proportion of illnesses are diagnosed and reported, periodic assessments of total episodes of illness are also needed. (Hereafter, episodes of illness are referred to as illnesses.) Several countries have conducted prospective population-based or cross-sectional studies to supplement surveillance and estimate the overall number of foodborne illnesses (*1*). In 2007, the World Health Organization launched an initiative to estimate the global burden of foodborne diseases (*2*).

In 1999, the Centers for Disease Control and Prevention provided comprehensive estimates of foodborne illnesses, hospitalizations, and deaths in the United States caused by known and unknown agents (*3*). This effort identified many data gaps and methodologic limitations. Since then, new data and methods have become available. This article is 1 of 2 reporting new estimates of foodborne diseases acquired in the United States (hereafter referred to as domestically acquired). This article provides estimates of major known pathogens; the other provides estimates for agents of acute gastroenteritis not specified in this article (*4*).

Methods

Adequate data for preparing national estimates were available for 31 pathogens. We estimated the number of foodborne illnesses, hospitalizations, and deaths caused by these 31 domestically acquired pathogens by using data shown in the online Appendix Table (www.cdc.gov/EID/content/17/1/7-appT.htm) and online Technical Appendix 1 (www.cdc.gov/EID/content/17/1/7-Techapp1.pdf).

Data were mostly from 2000–2008, and all estimates were based on the US population in 2006 (299 million persons). Estimates were derived from statistical models with many inputs, each with some measure of uncertainty (*5*). To reflect this uncertainty, we used probability distributions to describe a range of plausible values for all model

Author affiliation: Centers for Disease Control and Prevention, Atlanta, Georgia, USA

DOI: 10.3201/eid1701.P11101

[1]Current affiliation: Colorado School of Public Health, Aurora, Colorado, USA.

inputs. We expressed model outputs as probability distributions summarized by a mean point estimate with 90% credible intervals (CrIs). We used 2 types of modeling approaches for different types of data: 1) models that began with counts of laboratory-confirmed illnesses and were adjusted for undercounts (because of underreporting and underdiagnosis) and thus scaled up to the estimated number of illnesses and 2) models that began with a US population and used incidence data to scale down to the estimated number of illnesses (Table 1). The modeling approaches used and parameters of these probability distributions are detailed in online Technical Appendixes 2 and 3 (www.cdc.gov/EID/content/17/1/7-Techapp2.pdf and www.cdc.gov/EID/content/17/1/7-Techapp3.pdf, respectively); the proportions cited are modal values.

Illnesses

Laboratory-based surveillance data were available for 25 pathogens (online Appendix Table). The following events must occur for an illness to be ascertained and included in laboratory-based surveillance: the ill person must seek medical care, a specimen must be submitted for laboratory testing, the laboratory must test for and identify the causative agent, and the illness must be reported to public health authorities. If a break occurs in any of the first 3 steps of this surveillance chain, the causative agent will not be laboratory confirmed (underdiagnosis). Furthermore, although all laboratory-confirmed illnesses are reported by active surveillance, some will not be reported by passive surveillance (underreporting). Therefore, to estimate the number of illnesses caused by pathogens under public health surveillance, we determined the number of laboratory-confirmed illnesses and adjusted for underdiagnosis and, if necessary, for underreporting by using a series of component multipliers.

Laboratory-confirmed illnesses for these 25 pathogens were reported through 5 surveillance programs: the Foodborne Diseases Active Surveillance Network (FoodNet) for *Campylobacter* spp., *Cryptosporidium* spp., *Cyclospora cayetanensis*, Shiga toxin–producing *Escherichia coli* (STEC) O157, STEC non-O157, *Listeria monocytogenes*, nontyphoidal *Salmonella* spp., *Salmonella enterica* serotype Typhi, *Shigella* spp., and *Yersinia enterocolitica*; the National Notifiable Diseases Surveillance System (NNDSS) for *Brucella* spp., *Clostridium botulinum*, *Trichinella* spp., hepatitis A virus, and *Giardia intestinalis*; the Cholera and Other *Vibrio* Illness Surveillance (COVIS) system for toxigenic *Vibrio cholerae*, *V. vulnificus*, *V. parahemolyticus*, and other *Vibrio* spp.; the National Tuberculosis Surveillance System (NTSS) for *Mycobacterium bovis*; and the Foodborne Disease Outbreak Surveillance System (FDOSS) for *Bacillus cereus, Clostridium perfringens,* enterotoxigenic *E. coli* (ETEC), *Staphylococcus aureu*s, and *Streptococcus* spp. group A (online Appendix Table; online Technical Appendix 1). When data were available from >1 surveillance system, we used active surveillance data from FoodNet, except for *Vibrio* spp., for which we used COVIS because of geographic clustering of *Vibrio* spp. infections outside FoodNet sites. We used data on outbreak-associated illnesses from FDOSS only for pathogens for which no data were available from other systems.

Because FoodNet conducts surveillance at 10 sites (*6*), we estimated the number of laboratory-confirmed illnesses in the United States by applying incidence from FoodNet to the estimated US population for 2006 (*7*). We constructed a probability distribution based on extrapolation of rates by year (2005–2008) in each FoodNet site (online Technical Appendix 3). We used data from 2005–2008 because the FoodNet surveillance area was constant during that period and because FoodNet began collecting information on foreign travel in 2004. We used data from 2000–2007 for NNDSS, COVIS, and FDOSS and annual counts of reported illnesses for our probability distributions. Some evidence of trend was found for illness caused by hepatitis A virus, *S. aureus,* and *Vibrio* spp.; therefore, recent years were weighted more heavily (online Technical Appendixes

Table 1. Modeling approaches used to estimate the total number of illnesses for different types of data, United States*

Pathogens for which laboratory-confirmed illnesses were scaled up			Pathogens for which US population was scaled down
Active surveillance data	Passive surveillance data	Outbreak surveillance data	
Campylobacter spp.	*Brucella* spp.	*Bacillus cereus*	Astrovirus
Cryptosporidium spp.	*Clostridium botulinum*	*Clostridium perfringens*	Norovirus
Cyclospora cayetanensis	*Giardia intestinalis*	ETEC†	Rotavirus
STEC O157	Hepatitis A virus	*Staphylococcus aureus*	Sapovirus
STEC non-O157	*Mycobacterium bovis*	*Streptococcus* spp. group A	*Toxoplasma gondii*
Listeria monocytogenes	*Trichinella* spp.		
Salmonella spp., nontyphoidal‡	*Vibrio cholera*, toxigenic		
S. enterica serotype Typhi	*Vibrio parahaemolyticus*		
Shigella spp.	*Vibrio vulnificus*		
Yersinia enterocolitica	*Vibrio* spp., other		

*ETEC, enterotoxigenic *Escherichi coli*; STEC, Shiga toxin–producing *E. coli.*
†Numbers of *E. coli* other than STEC or ETEC assumed to be same as for ETEC.
‡Includes all serotypes other than Typhi.

2, 3). NTSS was used to determine the number of reported illnesses caused by *M. bovis* during 2004–2007.

We assumed that all laboratory-confirmed illnesses were reported to FoodNet active surveillance in the relevant catchment areas. Because COVIS and NNDSS conduct passive surveillance, we applied an underreporting multiplier (1.1 for bacteria and 1.3 for parasites) derived by comparing incidence of all nationally notifiable illnesses ascertained through FoodNet with that reported to NNDSS (online Technical Appendix 4, www.cdc.gov/EID/content/17/1/7-Techapp4.pdf). For the 5 bacteria for which only outbreak data were available, we estimated the number of laboratory-confirmed illnesses by creating an underreporting multiplier as follows. We determined the proportion of illnesses ascertained through FoodNet that were caused by *Campylobacter* spp., *Cryptosporidium* spp., *C. cayatanensis*, *L. monocytogenes*, *Salmonella* spp., *Shigella* spp., STEC, *Vibrio* spp., and *Y. enterocolitica* that were also reported to FDOSS as outbreak associated and applied the inverse of this proportion, 25.5, to those pathogens (online Technical Appendix 4). We assumed that all illnesses caused by *M. bovis* were reported to NTSS.

To adjust for underdiagnosis resulting from variations in medical care seeking, specimen submission, laboratory testing, and test sensitivity, we created pathogen-specific multipliers. To adjust for medical care seeking and specimen submission, we pooled data from FoodNet Population Surveys in 2000–2001, 2002–2003 (*8*), and 2006–2007 (Centers for Disease Control and Prevention, unpub. data) from which we estimated the proportion of persons who in the past month reported an acute diarrheal illness (≥3 loose stools in 24 hours lasting >1 day or resulting in restricted daily activities) and sought medical care and submitted a stool sample for that illness. Because persons with more severe illness are more likely to seek care (*9*), we estimated pathogen-specific proportions of persons with laboratory-confirmed infections who had severe illness (e.g., bloody diarrhea) and used medical care seeking and stool sample submission rates for bloody (35% and 36%, respectively) and nonbloody (18% and 19%, respectively) diarrhea as surrogates for severe and mild cases of most illnesses (online Technical Appendix 3). However, for infections with *L. monocytogenes*, *M. bovis*, and *V. vulnificus* and severe infections with hepatitis A virus, we assumed high rates of medical care seeking (i.e., we assumed that 100% of persons with *M. bovis* infection and 90% with *L. monocytogenes*, *V. vulnificus*, or severe hepatitis A virus infections sought care) and specimen submission (100% for hepatitis A virus and *M. bovis*, 80% for others). We accounted for percentage of laboratories that routinely tested for specific pathogens (25%–100%) and test sensitivity (28%–100%) by using data from FoodNet (*10,11*) and other surveys of clinical diagnostic laboratory practices (online Technical Appendix 3). For the 5 pathogens for which data were from outbreaks only, we used the nontyphoidal *Salmonella* spp. underdiagnosis multiplier.

Alternative approaches were used for infections not routinely reported by any surveillance system (i.e., diarrheagenic *E. coli* other than STEC and ETEC, *T. gondii*, astrovirus, rotavirus, sapovirus, and norovirus) (online Technical Appendixes 1–3). We assumed diarrheagenic *E. coli* other than STEC and ETEC to be as common as ETEC. Illnesses caused by *T. gondii* were estimated by using nationally representative serologic data from the 1999–2004 National Health and Nutrition Examination Survey (*12*) and an estimate that clinical illness develops in 15% of persons who seroconvert (*13*). We assumed that 75% of children experience an episode of clinical rotavirus illness by 5 years of age, consistent with findings from other studies (*14*), and used this estimate for astrovirus and sapovirus. We estimated norovirus illnesses by applying mean proportion of all acute gastroenteritis caused by norovirus (11%) according to studies in other industrialized countries (*15–18*) to estimates of acute gastroenteritis from FoodNet Population Surveys (online Appendix Table; online Technical Appendixes 1–3) (*4*).

Hospitalizations and Deaths

For most pathogens, numbers of hospitalizations and deaths were estimated by determining (from surveillance data) the proportion of persons who were hospitalized and the proportion who died and applying these proportions to the estimated number of laboratory-confirmed illnesses (online Appendix Table; online Technical Appendixes 1, 3). Rates of hospitalization and death caused by *G. intestinalis* and *T. gondii* were based on the 2000–2006 Nationwide Inpatient Sample. Because some persons with illnesses that were not laboratory confirmed would also have been hospitalized and died, we doubled the number of hospitalizations and deaths to adjust for underdiagnosis, similar to the method used by Mead et al. (*3*) but applied an uncertainty distribution (online Technical Appendix 3). For diarrheagenic *E. coli* other than STEC and ETEC, total numbers of hospitalizations and deaths were assumed to be the same as those for ETEC. For rotavirus, we used previous estimates (*14*). For astrovirus and sapovirus, we assumed that the number was 25% that of rotavirus (*19,20*). Numbers of norovirus hospitalizations and deaths were determined by multiplying the estimated number of hospitalizations and deaths caused by acute gastroenteritis, estimated by using national data on outpatient visits resulting in hospitalization, hospital discharge surveys, and death certificates (online Appendix Table; online Technical Appendixes 1–3)

(*4*), by the same norovirus proportion (11%) used to estimate illnesses (*15–18*).

Domestically Acquired Foodborne Illnesses

Data from published studies and surveillance were used to determine, for each pathogen, the proportion of illnesses acquired while the person had been traveling outside the United States (online Technical Appendixes 1, 3). The remaining proportion was considered domestically acquired. We based our estimates of the proportion of domestically acquired foodborne illnesses caused by each pathogen on data from surveillance, risk factor studies, and a literature review (online Technical Appendixes 1, 3).

Uncertainty Analysis

We used empirical data, when available, to define entire distributions or parameters of distributions (online Technical Appendix 3). When data were sparse, we made reasoned judgments based on context, plausibility, and previously published estimates. The parametric distribution used for almost all multipliers was a 4-parameter beta (modified PERT) distribution (*21*). The first 3 parameters are low, modal, and high. The fourth parameter is related to the variability of the distribution. We typically fixed this last parameter at 4, which yields the simple PERT distribution (*21*). However, when describing the outbreak reporting multiplier, we used a value of 20 (online Technical Appendix 4).

Uncertainty in the estimates is the cumulative effect of uncertainty of each of the model inputs. We iteratively generated sets of independent pathogen-specific adjustment factors and used these multipliers to estimate illnesses, hospitalizations, and deaths (Figure; online Technical Appendix 2). On the basis of 100,000 iterations, we obtained empirical distributions of counts corresponding to Bayesian posterior distributions and used these posterior distributions to generate a point estimate (posterior mean) and upper and lower 5% limits for 90% CrIs. Because incidence of illnesses differed by location and over time, we included these variations in the models, which led to wider CrIs than if we had assumed that inputs represented independent random samples of a fixed US population. We used SAS version 9.2 (SAS Institute, Cary, NC, USA) for these analyses.

Results

Foodborne Illnesses

We estimate that each year in the United States, 31 pathogens caused 37.2 million (90% CrI 28.4–47.6 million) illnesses, of which 36.4 million (90% CrI 27.7–46.7 million) were domestically acquired; of these, 9.4 million (90% CrI 6.6–12.7 million) were foodborne (Table 2; expanded version available online, www.cdc.gov/EID/content/17/1/7-T2.htm). We estimate that 5.5 million (59%) foodborne illnesses were caused by viruses, 3.6 million (39%) by bacteria, and 0.2 million (2%) by parasites. The pathogens that caused the most illnesses were norovirus (5.5 million, 58%), nontyphoidal *Salmonella* spp. (1.0 million, 11%), *C. perfringens* (1.0 million, 10%), and *Campylobacter* spp. (0.8 million, 9%).

Hospitalizations

We estimate that these 31 pathogens caused 228,744 (90% CrI 188,326–275,601) hospitalizations annually, of which 55,961 (90% CrI 39,534–75,741) were caused by contaminated food eaten in the United States (Table 3; expanded version available online, www.cdc.gov/EID/content/17/1/7-T3.htm). Of these, 64% were caused by bacteria, 27% by viruses, and 9% by parasites. The leading causes of hospitalization were nontyphoidal *Salmonella* spp. (35%), norovirus (26%), *Campylobacter* spp. (15%), and *T. gondii* (8%).

Deaths

We estimate that these 31 pathogens caused 2,612 deaths (90% CrI 1,723–3,819), of which 1,351 (90% CrI

$$Count \times Year \times Sub \times Ob \times \left\{ \begin{array}{c} CS(Severe) \times SS(Severe) \times PS \\ + \\ CS(Mild) \times SS(Mild) \times (1 - PS) \end{array} \right\} \times LT \times LS \times \left[\begin{array}{c} 1 \\ \text{or} \\ H \\ \text{or} \\ D \end{array} \right] \times [1 \text{ or } F] \Rightarrow$$

Illnesses	Illnesses, foodborne
Hospitalizations	Hospitalizations, foodborne
Deaths	Deaths, foodborne

Figure. Example schematic diagram of the estimation and uncertainty model used to estimate episodes of illness, hospitalizations, and deaths in the United States. *Count*, data (empirical distribution); *Year,* factor to standardize non-2006 counts to 2006 (constant); *Sub*, expansive factor to scale area surveillance to the entire US population (constant); *Ob,* expansive factor to scale outbreak counts up to outbreak plus sporadic counts (beta distribution); *CS*, expansive factor to scale care seekers to all ill, with severe and mild illness versions (PERT distribution); *SS,* expansive factor to scale submitted samples to all visits, with severe and mild illness versions (PERT distribution); *PS*, estimated proportion of illnesses that are severe (PERT distribution); *LT,* expansive factor to scale tests performed up to samples submitted (PERT distribution); *LS,* expansive factor to scale positive test results up to true positive specimens (PERT distribution); *H,* contractive factor to scale illnesses down to hospitalized illnesses (PERT distribution); *D*, contractive factor to scale illnesses down to deaths (PERT distribution); *F*, contractive factor to scale illnesses down to foodborne illnesses (PERT distribution).

712–2,268) were caused by contaminated food eaten in the United States (Table 3). Of these, 64% were caused by bacteria, 25% by parasites, and 12% by viruses. The leading causes of death were nontyphoidal *Salmonella* spp. (28%), *T. gondii* (24%), *L. monocytogenes* (19%), and norovirus (11%).

Table 2. Estimated annual number of episodes of domestically acquired foodborne illnesses caused by 31 pathogens, United States*

Pathogen	Laboratory confirmed	Multipliers: Under-reporting	Multipliers: Under-diagnosis	Travel related, %	Foodborne, %†	Domestically acquired foodborne, mean (90% credible interval)
Bacteria						
Bacillus cereus, foodborne	85‡	25.5	29.3	<1	100	63,400 (15,719–147,354)
Brucella spp.	120§	1.1	15.2	16	50	839 (533–1,262)
Campylobacter spp.	43,696¶	1.0	30.3	20	80	845,024 (337,031–1,611,083)
Clostridium botulinum, foodborne	25§	1.1	2.0	<1	100	55 (34–91)
Clostridium perfringens, foodborne	1,295‡	25.5	29.3	<1	100	965,958 (192,316–2,483,309)
STEC O157	3,704¶	1.0	26.1	4	68	63,153 (17,587–149,631)
STEC non-O157	1,579¶	1.0	106.8	18	82	112,752 (11,467–287,321)
ETEC, foodborne	53‡	25.5	29.3	55	100	17,894 (24–46,212)
Diarrheagenic *E. coli* other than STEC and ETEC	53	25.5	29.3	<1	30	11,982 (16–30,913)
Listeria monocytogenes	808¶	1.0	2.1	3	99	1,591 (557–3,161)
Mycobacterium bovis	195¶	1.0	1.1	70	95	60 (46–74)
Salmonella spp., nontyphoidal	41,930¶	1.0	29.3	11	94	1,027,561 (644,786–1,679,667)
S. enterica serotype Typhi	433¶	1.0	13.3	67	96	1,821 (87–5,522)
Shigella spp.	14,864¶	1.0	33.3	15	31	131,254 (24,511–374,789)
Staphylococcus aureus, foodborne	323‡	25.5	29.3	<1	100	241,148 (72,341–529,417)
Streptococcus spp. group A, foodborne	15‡	25.5	29.3	<1	100	11,217 (15–77,875)
Vibrio cholerae, toxigenic	8§	1.1	33.1	70	100	84 (19–213)
V. vulnificus	111§	1.1	1.7	2	47	96 (60–139)
V. parahaemolyticus	287§	1.1	142.4	10	86	34,664 (18,260–58,027)
Vibrio spp., other	220§	1.1	142.7	11	57	17,564 (10,848–26,475)
Yersinia enterocolitica	950¶	1.0	122.8	7	90	97,656 (30,388–172,734)
Subtotal						3,645,773 (2,321,468–5,581,290)
Parasites						
Cryptosporidium spp.	7,594¶	1.0	98.6	9	8	57,616 (12,060–166,771)
Cyclospora cayetanensis	239¶	1.0	83.1	42	99	11,407 (137–37,673)
Giardia intestinalis	20,305§	1.3	46.3	8	7	76,840 (51,148–109,739)
Toxoplasma gondii		1.0	0.0	<1	50	86,686 (64,861–111,912)
Trichinella spp.	13§	1.3	9.8	4	100	156 (42–341)
Subtotal						232,705 (161,923–369,893)
Viruses						
Astrovirus	NA	NA	NA	0	<1	15,433 (5,569–26,643)
Hepatitis A virus	3,576§	1.1	9.1	41	7	1,566 (702–3,024)
Norovirus	NA	NA	NA	<1	26	5,461,731 (3,227,078–8,309,480)
Rotavirus	NA	NA	NA	0	<1	15,433 (5,569–26,643)
Sapovirus	NA	NA	NA	0	<1	15,433 (5,569–26,643)
Subtotal						5,509,597 (3,273,623–8,355,568)
Total						9,388,075 (6,641,440–12,745,709)

*All estimates based on US population in 2006. Modal or mean value shown unless otherwise stated; see online Technical Appendix 3 (www.cdc.gov/EID/content/17/1/7-Techapp3.pdf) for the parameters of these distributions. STEC, Shiga toxin–producing *Escherichia coli*; ETEC, enterotoxigenic *E. coli*; NA, not applicable. An expanded version of this table is available online (www.cdc.gov/EID/content/17/1/7-T2.htm).
†Percentage foodborne among domestically acquired illnesses.
‡Passive surveillance data on outbreak-associated illnesses from the Foodborne Disease Outbreak Surveillance System. Estimates based on the number of foodborne illnesses ascertained in surveillance and therefore assumed to reflect only foodborne transmission.
§Passive surveillance data from Cholera and Other *Vibrio* Illness Surveillance or the National Notifiable Disease Surveillance System.
¶Active surveillance data from Foodborne Diseases Active Surveillance Network, adjusted for geographic coverage; data from the National Tuberculosis Surveillance System for *M. bovis*.

Discussion

We estimate that foods consumed in the United States that were contaminated with 31 known agents of foodborne disease caused 9.4 million illnesses, 55,961 hospitalizations, and 1,351 deaths each year. Norovirus caused the most illnesses; nontyphoidal *Salmonella* spp., norovirus, *Campylobacter* spp., and *T. gondii* caused the most hospitalizations; and nontyphoidal *Salmonella* spp., *T. gondii*, *L. monocytogenes*, and norovirus caused the most deaths. Scarce data precluded estimates for other known infectious and noninfectious agents, such as chemicals. Foodborne diseases are also caused by agents not yet recognized as being transmitted in food and by unknown agents (*22*). The numbers of illnesses caused by these unspecified agents are estimated elsewhere (*4*).

Studies estimating the overall number of foodborne illnesses have been conducted in England and Wales and in Australia (*23,24*). Similar to our findings, in Australia norovirus was the leading cause of foodborne illness, accounting for 30% of illnesses caused by known pathogens.

Table 3. Estimated annual number of domestically acquired foodborne hospitalizations and deaths caused by 31 pathogens, United States*

Pathogen	Hospitalization rate, %†	Hospitalizations, mean (90% credible interval)	Death rate, %†	Deaths, mean (90% credible interval)
Bacteria				
Bacillus cereus, foodborne‡	0.4	20 (0–85)	0	0
Brucella spp.	55.0	55 (33–84)	0.9	1 (0–2)
Campylobacter spp.	17.1	8,463 (4,300–15,227)	0.1	76 (0–332)
Clostridium botulinum, foodborne‡	82.6	42 (19–77)	17.3	9 (0–51)
Clostridium perfringens, foodborne‡	0.6	438 (44–2,008)	<0.1	26 (0–163)
STEC O157	46.2	2,138 (549–4,614)	0.5	20 (0–113)
STEC non-O157	12.8	271 (0–971)	0.3	0 (0–0)§
ETEC, foodborne	0.8	12 (0–53)	0	0
Diarrheagenic *E. coli* other than STEC and ETEC	0.8	8 (0–36)	0	0
Listeria monocytogenes	94.0	1,455 (521–3,018)	15.9	255 (0–733)
Mycobacterium bovis	55.0	31 (21–42)	4.7	3 (2–3)
Salmonella spp., nontyphoidal	27.2	19,336 (8,545–37,490)	0.5	378 (0–1,011)
S. enterica serotype Typhi	75.7	197 (0–583)	0	0
Shigella spp.	20.2	1,456 (287–3,695)	0.1	10 (0–67)
Staphylococcus aureus, foodborne‡	6.4	1,064 (173–2,997)	<0.1	6 (0–48)
Streptococcus spp. group A, foodborne‡	0.2	1 (0–6)	0	0
Vibrio cholerae, toxigenic	43.1	2 (0–5)	0	0
V. vulnificus	91.3	93 (53–145)	34.8	36 (19–57)
V. parahaemolyticus	22.5	100 (50–169)	0.9	4 (0–17)
Vibrio spp., other	37.1	83 (51–124)	3.7	8 (3–19)
Yersinia enterocolitica	34.4	533 (0–1,173)	2.0	29 (0–173)
Subtotal		35,796 (21,519–53,414)		861 (260–1,761)
Parasites				
Cryptosporidium spp.	25.0	210 (58–518)	0.3	4 (0–19)
Cyclospora cayetanensis	6.5	11 (0–109)	0.0	0
Giardia intestinalis	8.8	225 (141–325)	0.1	2 (1–3)
Toxoplasma gondii	2.6	4,428 (2,634–6,674)	0.2	327 (200–482)
Trichinella spp.	24.3	6 (0–17)	0.2	0 (0–0)
Subtotal		4,881 (3,060–7,146)		333 (205–488)
Viruses				
Astrovirus	0.4	87 (32–147)	<0.1	0
Hepatitis A virus	31.5	99 (42–193)	2.4	7 (3–15)
Norovirus	0.03	14,663 (8,097–23,323)	<0.1	149 (84–237)
Rotavirus	1.7	348 (128–586)	<0.1	0
Sapovirus	0.4	87 (32–147)	<0.1	0
Subtotal		15,284 (8,719–23,962)		157 (91–245)
Total		55,961 (39,534–75,741)		1,351 (712–2,268)

*All estimates were based on US population in 2006. STEC, Shiga toxin–producing *Escherichia coli*; ETEC, enterotoxigenic *E. coli*. An expanded version of this table is available online (www.cdc.gov/EID/content/17/1/7-T3.htm).
†For laboratory-confirmed illnesses. Unadjusted hospitalization and death rates are presented here. These rates were doubled to adjust for underdiagnosis before being applied to the number of laboratory-confirmed cases to estimate the total number of hospitalizations and deaths. The hospitalization and death rates for astrovirus, norovirus, rotavirus, and sapovirus presented here are the percentage of total estimated illness and were not subject to further adjustment.
‡Estimates based on the number of foodborne illnesses ascertained in surveillance, therefore assumed to reflect only foodborne transmission.
§We report median values instead of means for the distributions of deaths caused by STEC non-O157 because of extremely skewed data.

In England and Wales, norovirus accounted for only 8% of known foodborne illnesses; however, stool sample reexamination using molecular techniques documented higher rates (*18*). Nontyphoidal *Salmonella* spp. and *Campylobacter* spp. were leading causes of foodborne illnesses in all 3 countries (England and Wales, Australia, and the United States), although nontyphoidal *Salmonella* spp. accounted for a greater proportion of illness in the United States. Recent serologic data from Europe suggest that *Salmonella* spp. infections are more common than estimated by our methods; however, many infections may be asymptomatic (*25*). Our estimates did not capture mild illnesses associated with some pathogens. For example, mild cases of botulism are often recognized as part of outbreaks, but affected persons seldom seek medical care and are not captured by surveillance except during outbreaks (*26,27*). Likewise, *L. monocytogenes* is rarely diagnosed as the cause of gastroenteritis and fever, partly because this organism is not detected by routine stool culture (*28*). Early spontaneous abortion or miscarriage associated with listeriosis may also be underdiagnosed.

Accurately estimating hospitalizations and deaths caused by foodborne pathogens is particularly challenging. National data on outpatient visits resulting in hospitalization, hospital discharges, and death certificates probably substantially underestimate pathogen-specific cases because for pathogen-specific diagnoses to be recorded, health care providers must order the appropriate diagnostic tests and coding must be accurate. Particularly in vulnerable populations, dehydration or electrolyte imbalance from a gastrointestinal illness may exacerbate a chronic illness, resulting in hospitalization or death well after resolution of the gastrointestinal illness; thus, the gastrointestinal illness may not be coded as a contributing factor. Moreover, if a pathogen is not detected, infections may be coded as noninfectious illnesses (*29*). For norovirus, we estimated the number of hospitalizations and deaths by applying the estimated proportion of acute gastroenteritis illnesses caused by norovirus to overall estimates of hospitalizations and deaths from acute gastroenteritis; this choice is supported by studies of hospitalizations for norovirus (*30,31*). For most other pathogens, we used data from surveillance to estimate pathogen-specific hospitalizations and deaths and doubled the numbers to adjust for underdiagnosis. More precise information about the degree of undercounting of hospitalizations and deaths for each pathogen would improve these estimates.

Our methods and data differed from those used for the 1999 estimates (*3*). Our estimate of medical care seeking among persons with a diarrheal illness, derived from the 3 most recent FoodNet Population Surveys conducted during 2000–2007, was higher than that estimated from the 1996–1997 FoodNet Population Survey used for the 1999 estimates (35% and 18% among persons reporting bloody and nonbloody diarrhea, respectively, compared with 15% and 12% in the earlier [1999] study) (*8*). These data resulted in lower underdiagnosis multipliers, which contributed to lower estimates of number of illnesses. The biggest change from the earlier estimate was the estimated number of norovirus illnesses, which decreased for 2 reasons. First, the number of acute gastrointestinal illnesses estimated from the FoodNet Population Survey and used in the current study was lower than the estimated number of acute gastrointestinal illnesses used in the 1999 assessment. The earlier study used data from 1996–1997; the sample size was one fifth as large as ours and incorporated data from US studies conducted before 1980 (*32,33*). Both estimates excluded persons reporting concurrent cough or sore throat, but the proportion of persons reporting these signs and symptoms was higher in the FoodNet Population Surveys we used than that in the older US studies (38% vs. 25%), contributing to a lower estimated prevalence of acute gastroenteritis (0.60 vs. 0.79 episodes/person/year) (*4,32,33*). Additionally, the current study excluded persons with vomiting who were ill for <1 day or whose illness did not result in restricted daily activities, whereas the earlier study included all vomiting episodes. These factors contributed to the new estimate of acute gastroenteritis being 24% lower than the earlier estimate, more likely the result of increased accuracy than a true decrease in illnesses (*4*). Second, the lower current estimate for norovirus illnesses resulted from a lower proportion of norovirus estimated to be foodborne (decreased from 40% to 26%); this lower proportion is similar to that estimated in recent studies from other countries (*23,24*). Because of these reasons and use of other data sources and methods, our estimate cannot be compared with the 1999 estimate for the purpose of assessing trends. FoodNet provides the best data on trends over time (*34*).

Data used in the current study came from a variety of sources and were of variable quality and representativeness. FoodNet sites, from which we used data for 10 pathogens, are not completely representative of the US population, but 1 study indicated that demographic data from FoodNet and from the 2005 US census did not differ much (*6*). For 5 pathogens, only data on foodborne outbreak–related cases were available. No routine surveillance data were available for most viruses, forcing us to use a different modeling approach for viruses than for most other pathogens. Given the large number of norovirus illnesses in these estimates, the paucity of supporting data is a major limitation. Moreover, combining different methods is not optimal because methods themselves may affect the estimates. We chose our modeling approach and used the PERT distribution for many inputs because data were sometimes limited and subjective decisions were required. Other investigators could

have chosen other distributions, for good reasons, and arrived at different estimates.

Our assumptions about the proportion of illnesses transmitted by food profoundly affect our estimates, but data on which to base these estimates were often lacking. We used data from surveillance, risk factor studies, and the current literature to estimate the proportion of pathogen-specific illnesses caused by consumption of contaminated food (*35*), but it is not known how representative these data are of total illnesses and whether the foodborne proportion is similar across age groups. For example, the proportion of some illnesses acquired from animals (e.g., STEC O157) may be higher among children than adults (*36*), and the proportions that spread person-to-person (e.g., norovirus) may be higher among institutionalized elderly persons (*37*). Because a higher proportion of cases are reportedly associated with hospitalization or death in these vulnerable groups, we may have overestimated the total contribution of foodborne transmission for these outcomes.

The methods used for this study could be adapted to estimate the proportion of illnesses attributable to other modes of transmission, such as waterborne and direct animal contact. The estimates from this study can be used to help direct policy and interventions; to conduct other analyses (e.g., evaluation of economic cost of these diseases and attribution to various food commodities); and as a platform for developing estimates of effects of disease caused by sequelae of foodborne infections.

Acknowledgments

We thank Tracy Ayers, Michael J. Beach, John Besser, Elizabeth Bosserman, Christopher R Braden, Anthony E. Fiore, Kelly A. Jackson, Rebecca Hall, Erin Hedican, Barbara L. Herwaldt, Scott D. Holmberg, Martha Iwamoto, Philip LoBue, Michael F. Lynch, Eric D. Mintz, John A. Painter, Ian T. Williams, Patricia A. Yu, and David L. Swerdlow for providing data and expert advice for these estimates. We also thank Barbara Mahon for reviewing the manuscript and the anonymous reviewers for helpful suggestions.

Dr Scallan is assistant professor in the Department of Epidemiology at the Colorado School of Public Health. Her research focuses on the burden and attribution of foodborne diseases.

References

1. Flint JA, Van Duynhoven YT, Angulo FJ, DeLong SM, Braun P, Kirk M, et al. Estimating the burden of acute gastroenteritis, foodborne disease, and pathogens commonly transmitted by food: an international review. Clin Infect Dis. 2005;41:698–704. DOI: 10.1086/432064
2. Kuchenmuller T, Hird S, Stein C, Kramarz P, Nanda A, Havelaar AH. Estimating the global burden of foodborne diseases—a collaborative effort. Euro Surveill. 2009;14:pii:19195.
3. Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, et al. Food-related illness and death in the United States. Emerg Infect Dis. 1999;5:607–25. DOI: 10.3201/eid0505.990502
4. Scallan E, Griffin PM, Angulo FJ, Tauxe RV, Hoekstra RM. Foodborne illness acquired in the United States—unspecified agents. Emerg Infect Dis. 2011;17:16–22.
5. Powell M, Ebel E, Schlosser W. Considering uncertainty in comparing the burden of illness due to foodborne microbial pathogens. Int J Food Microbiol. 2001;69:209–15. DOI: 10.1016/S0168-1605(01)00495-0
6. Scallan E. Activities, achievements, and lessons learned during the first 10 years of the Foodborne Diseases Active Surveillance Network: 1996–2005. Clin Infect Dis. 2007;44:718–25.
7. US Census Bureau. Population estimates [cited 2010 March 9]. http://www.census.gov/popest/states/NST-ann-est.html
8. Jones TF, McMillian MB, Scallan E, Frenzen PD, Cronquist AB, Thomas S, et al. A population-based estimate of the substantial burden of diarrhoeal disease in the United States; FoodNet, 1996–2003. Epidemiol Infect. 2007;135:293–301.
9. Scallan E, Jones TF, Cronquist A, Thomas S, Frenzen P, Hoefer D, et al. Factors associated with seeking medical care and submitting a stool sample in estimating the burden of foodborne illness. Foodborne Pathog Dis. 2006;3:432–8. DOI: 10.1089/fpd.2006.3.432
10. Voetsch AC, Angulo FJ, Rabatsky-Ehr T, Shallow S, Cassidy M, Thomas SM, et al. Laboratory practices for stool-specimen culture for bacterial pathogens, including *Escherichia coli* O157:H7, in the FoodNet sites, 1995–2000. Clin Infect Dis. 2004;38(Suppl 3):S190–7. DOI: 10.1086/381586
11. Jones JL, Lopez A, Wahlquist SP, Nadle J, Wilson M. Survey of clinical laboratory practices for parasitic diseases. Clin Infect Dis. 2004;38(Suppl 3):S198–202. DOI: 10.1086/381587
12. Jones JL, Kruszon-Moran D, Sanders-Lewis K, Wilson M. *Toxoplasma gondii* infection in the United States, 1999–2004, decline from the prior decade. Am J Trop Med Hyg. 2007;77:405–10.
13. World Health Organization. Toxoplasmosis. Technical report series no. 431. Geneva: The Organization; 1969.
14. Widdowson MA, Meltzer MI, Zhang X, Bresee JS, Parashar UD, Glass RI. Cost-effectiveness and potential impact of rotavirus vaccination in the United States. Pediatrics. 2007;119:684–97.
15. Wheeler JG, Sethi D, Cowden JM, Wall PG, Rodrigues LC, Tompkins DS, et al. Study of infectious intestinal disease in England: rates in the community, presenting to general practice, and reported to national surveillance. The Infectious Intestinal Disease Study Executive. BMJ. 1999;318:1046–50.
16. de Wit MA, Koopmans MP, Kortbeek LM, Wannet WJ, Vinje J, van Leusden F, et al. Sensor, a population-based cohort study on gastroenteritis in the Netherlands: incidence and etiology. Am J Epidemiol. 2001;154:666–74. DOI: 10.1093/aje/154.7.666
17. Marshall JA, Hellard ME, Sinclair MI, Fairley CK, Cox BJ, Catton MG, et al. Incidence and characteristics of endemic Norwalk-like virus–associated gastroenteritis. J Med Virol. 2003;69:568–78. DOI: 10.1002/jmv.10346
18. Amar CF, East CL, Gray J, Iturriza-Gomara M, Maclure EA, McLauchlin J. Detection by PCR of eight groups of enteric pathogens in 4,627 faecal samples: re-examination of the English case–control Infectious Intestinal Disease Study (1993–1996). Eur J Clin Microbiol Infect Dis. 2007;26:311–23.
19. Dennehy PH, Nelson SM, Spangenberger S, Noel JS, Monroe SS, Glass RI. A prospective case–control study of the role of astrovirus in acute diarrhea among hospitalized young children. J Infect Dis. 2001;184:10–5.
20. Klein EJ, Boster DR, Stapp JR, Wells JG, Qin X, Clausen CR, et al. Diarrhea etiology in a children's hospital emergency department: a prospective cohort study. Clin Infect Dis. 2006;43:807–13. DOI: 10.1086/507335

21. Vose D. Risk analysis. A quantitative guide. 2nd ed. Chichester (UK): John Wiley & Sons; 2000.
22. Tauxe RV. Emerging foodborne pathogens. Int J Food Microbiol. 2002;78:31–41. DOI: 10.1016/S0168-1605(02)00232-5
23. Adak GK, Meakins SM, Yip H, Lopman BA, O'Brien SJ. Disease risks from foods, England and Wales, 1996–2000. Emerg Infect Dis. 2005;11:365–72.
24. Hall G, Kirk MD, Becker N, Gregory JE, Unicomb L, Millard G, et al. Estimating foodborne gastroenteritis, Australia. Emerg Infect Dis. 2005;11:1257–64.
25. Simonsen J, Teunis P, Van Pelt W, Van Duynhoven Y, Krogfelt KA, Sadkowska-Todys M, et al. Usefulness of seroconversion rates for comparing infection pressures between countries. Epidemiol Infect. 2010; Apr 12:1–8 [Epub ahead of print].
26. St Louis ME, Peck SH, Bowering D, Morgan GB, Blatherwick J, Banerjee S, et al. Botulism from chopped garlic: delayed recognition of a major outbreak. Ann Intern Med. 1988;108:363–8.
27. Sobel J, Malavet M, John S. Outbreak of clinically mild botulism type E illness from home-salted fish in patients presenting with predominantly gastrointestinal symptoms. Clin Infect Dis. 2007;45:e14–6. DOI: 10.1086/518993
28. Dalton CB, Austin CC, Sobel J, Hayes PS, Bibb WF, Graves LM, et al. An outbreak of gastroenteritis and fever due to *Listeria monocytogenes* in milk. N Engl J Med. 1997;336:100–5. DOI: 10.1056/NEJM199701093360204
29. Pinner RW, Teutsch SM, Simonsen L, Klug LA, Graber JM, Clarke MJ, et al. Trends in infectious diseases mortality in the United States. JAMA. 1996;275:189–93.
30. Patel MM, Widdowson MA, Glass RI, Akazawa K, Vinje J, Parashar UD. Systematic literature review of role of noroviruses in sporadic gastroenteritis. Emerg Infect Dis. 2008;14:1224–31.
31. Jansen A, Stark K, Kunkel J, Schreier E, Ignatius R, Liesenfeld O, et al. Aetiology of community-acquired, acute gastroenteritis in hospitalised adults: a prospective cohort study. BMC Infect Dis. 2008;8:143. DOI: 10.1186/1471-2334-8-143
32. Badger GF, Curtiss C, Dingle JH, Hodges RG, Jordan WS Jr, McCorkle LP. A study of illness in a group of Cleveland families. XI. The occurrence of gastrointestinal symptoms. Am J Hyg. 1956;64:349–56.
33. Monto AS, Koopman JS. The Tecumseh Study. XI. Occurrence of acute enteric illness in the community. Am J Epidemiol. 1980;112:323–33.
34. Centers for Disease Control and Prevention. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food—10 states, 2009. MMWR Morb Mortal Wkly Rep. 2010;59:418–22.
35. Pires SM, Evers EG, van Pelt W, Ayers T, Scallan E, Angulo FJ, et al. Attributing the human disease burden of foodborne infections to specific sources. Foodborne Pathog Dis. 2009;6:417–24.
36. Crump JA, Sulka AC, Langer AJ, Schaben C, Crielly AS, Gage R, et al. An outbreak of *Escherichia coli* O157:H7 infections among visitors to a dairy farm. N Engl J Med. 2002;347:555–60. DOI: 10.1056/NEJMoa020524
37. Blanton LH, Adams SM, Beard RS, Wei G, Bulens SN, Widdowson MA, et al. Molecular and epidemiologic trends of caliciviruses associated with outbreaks of acute gastroenteritis in the United States, 2000–2004. J Infect Dis. 2006;193:413–21. DOI: 10.1086/499315

Address for correspondence: Elaine Scallan, Department of Epidemiology, Colorado School of Public Health, Mailstop B119, Rm W3146, UCD-AMC Bldg 500, 13001 E 17th Pl, Aurora, CO 80045, USA; email: elaine.scallan@ucdenver.edu

Risk Analysis, Vol. 35, No. 1, 2015 DOI: 10.1111/risa.12235

Listeria monocytogenes Dose Response Revisited—Incorporating Adjustments for Variability in Strain Virulence and Host Susceptibility

Régis Pouillot*, Karin Hoelzer, Yuhuan Chen, and Sherri B. Dennis

Evaluations of *Listeria monocytogenes* dose-response relationships are crucially important for risk assessment and risk management, but are complicated by considerable variability across population subgroups and *L. monocytogenes* strains. Despite difficulties associated with the collection of adequate data from outbreak investigations or sporadic cases, the limitations of currently available animal models, and the inability to conduct human volunteer studies, some of the available data now allow refinements of the well-established exponential *L. monocytogenes* dose response to more adequately represent extremely susceptible population subgroups and highly virulent *L. monocytogenes* strains. Here, a model incorporating adjustments for variability in *L. monocytogenes* strain virulence and host susceptibility was derived for 11 population subgroups with similar underlying comorbidities using data from multiple sources, including human surveillance and food survey data. In light of the unique inherent properties of *L. monocytogenes* dose response, a lognormal-Poisson dose-response model was chosen, and proved able to reconcile dose-response relationships developed based on surveillance data with outbreak data. This model was compared to a classical beta-Poisson dose-response model, which was insufficiently flexible for modeling the specific case of *L. monocytogenes* dose-response relationships, especially in outbreak situations. Overall, the modeling results suggest that most listeriosis cases are linked to the ingestion of food contaminated with medium to high concentrations of *L. monocytogenes*. While additional data are needed to refine the derived model and to better characterize and quantify the variability in *L. monocytogenes* strain virulence and individual host susceptibility, the framework derived here represents a promising approach to more adequately characterize the risk of listeriosis in highly susceptible population subgroups.

KEY WORDS: Dose response; *Listeria monocytogenes*; risk assessment

1. INTRODUCTION

Listeria monocytogenes is one of the leading causes of hospitalization, fetal loss, and death due to foodborne illnesses in the United States.[1] Derivations of *L. monocytogenes* dose-response relationships, though crucially important for risk assessment and risk management, are impaired by the difficulties of collecting adequate data from outbreak investigations or sporadic cases, by the lack of appropriate animal models, and by the inability to use volunteer studies due to ethical and practical concerns.[2,3]

Two well-accepted *L. monocytogenes* dose-response models have been developed by U.S. agencies[4] and an international expert panel,[5] both scaled to epidemiological data. In 2003, the Food and Drug Administration (FDA) of the U.S. Department of Health and Human Services and the Food Safety and Inspection Service (FSIS) of the U.S.

*Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, 5100 Paint Branch Pkwy, HFS-005 MD 20740, USA; Regis.Pouillot@fda.hhs.gov.

Department of Agriculture published a joint risk assessment for *L. monocytogenes* in 23 selected categories of ready-to-eat (RTE) foods.[4] The risk assessment evaluated the risk of invasive listeriosis and death due to listeriosis for the total U.S. population as well as for three separate population subgroups: (i) neonates infected in utero through contaminated food consumed by their mothers; (ii) the intermediate-age population; and (iii) older adults. One dose-response relationship (i.e., modeling mortality in humans following the ingestion of *L. monocytogenes)* was initially developed and different multipliers were subsequently applied to generate models for invasive listeriosis for each of the population subgroups. To derive the dose-response relationship for mortality in humans, five dose-response models (i.e., probit, exponential, logistic, multihit, and Gompertz-log) were initially fitted to data obtained in mice challenged with a single *L. monocytogenes* strain. These models were weighted and used simultaneously to characterize uncertainty in the shape of the dose-response curve, with the best-fitting exponential model receiving the greatest weight. A distribution of median lethal dose values (LD_{50}) observed in mice challenged with different *L. monocytogenes* strains was subsequently incorporated in the dose-response model to characterize *L. monocytogenes* strain variability in virulence and its uncertainty. Variability and uncertainty in host susceptibility within the three population subgroups were estimated based on observations in mice and epidemiological data, and incorporated in the dose-response model as well. Finally, because the derived model considerably overestimated the expected number of invasive listeriosis cases, surveillance data on the incidence of listeriosis in the United States were used to scale the dose-response relationship to reflect differences in susceptibility between humans and mice.[4]

In 2004, an international expert panel of the Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) developed another dose-response model based on a data subset extracted from the exposure estimates and the estimated annual number of cases used to derive the draft FDA/FSIS dose-response model published in 2001. The FAO/WHO dose-response model for invasive listeriosis is an exponential dose-response model.[6] The exponential dose-response model is a "single-hit" model:[6,7] it assumes that the probability of a given bacterial cell causing the adverse effect is independent of the number or characteristics of other ingested pathogens, so that a single ingested microorganism is sufficient to cause the adverse effect with some probability greater than zero. The exponential dose-response model further assumes that the bacterial cells are randomly distributed in the food, hence the dose per portion follows a Poisson distribution, and that the average probability, r, that one pathogen, within a given exposure of a particular consumer to a specific population of pathogens, will survive the host-pathogen interaction to initiate infection and cause illness is constant.[8]

If the virulence of pathogens or the susceptibility of consumers varies from exposure to exposure, then r may vary and may be represented by a random variable with distribution $f(r)$. [8] Challenges remain regarding how best to quantify the distribution of r in relation to the host, the bacterial strain, and the exposure scenario. To account for differences in host susceptibility for *L. monocytogenes*, the FAO/WHO group of experts assumed the existence of two distinct values for r, applicable to the general population and population subgroups with increased susceptibility, respectively. The two r parameters (i.e., one value for each of the two population subgroups) were estimated from epidemiological[9] and food exposure[10] data obtained in the United States. The estimated r parameters were extremely low (i.e., approximately 10^{-12}–10^{-13} for the population with increased susceptibility and 10^{-13}–10^{-15} for the general population), translating into a very low probability of illness following the ingestion of a low dose of bacteria. This dose-response model or some adaptations of the model have been used in various risk assessments.[11-14]

Since 2004, new scientific data have become available, demonstrating the considerable variability in virulence among *L. monocytogenes* strains and molecular subtypes.[15–18] New data have, for example, shown that the entry of *L. monocytogenes* into certain human epithelial cells is primarily receptor mediated, depending on specific interactions between internalins on the bacterial surface and their respective host cell receptors.[19–22] Therefore, point mutations in the *inlA* gene can lead to virulence attenuation of *L. monocytogenes* strains.[16,23,24] New data are also available regarding the variability in susceptibility among individuals with different predisposing conditions such as pregnancy, old age, or other underlying conditions.[25–28] The relative risk of listeriosis for pregnant women, for example, has been estimated to be approximately 100 times higher than

that for nonpregnant women.[25–27] Relative risks higher than 1,000 have been reported for individuals with chronic lymphocytic leukemia when compared to a reference population of individuals <65-year old without any known underlying conditions.[26]

Because of the challenges in developing adequate dose-response models of listeriosis, an interagency expert workshop was held in the United States in 2011, with the goal of identifying new data, strategies, and insights for *L. monocytogenes* dose-response modeling. Short-term strategies identified during this workshop included updating the dose-response model developed by FDA/FSIS[4] by incorporating new data and insights about differences in strain virulence and *L. monocytogenes* pathophysiology. A key-events approach to dose-response modeling[29] was identified as a promising though extremely challenging, data-intensive, and potentially unachievable framework for future microbial dose-response models.[2]

Current dose-response models linked to epidemiological data tend to agree that a low dose of *L. monocytogenes* leads to an average low probability of invasive listeriosis in the general population as well as in broadly defined populations with heightened susceptibility.[4,5,30] However, a more nuanced evaluation of *L. monocytogenes* dose response for *L. monocytogenes* strains with different virulence and for different human population subgroups at heightened risk of listeriosis is needed to adequately characterize the listeriosis risk in different population subgroups, including those with highest susceptibility. Such nuanced models would allow for more in-depth inference about the listeriosis risk posed to highly susceptible population subgroups by highly virulent *L. monocytogenes* strains, and may become instrumental for evaluating key risk management issues such as the potential public health threat associated with the ingestion of a given dose of *L. monocytogenes*.

In this article, the existing exponential *L. monocytogenes* dose-response model[5] for invasive listeriosis is being revisited. A mathematical framework for considering variability in *L. monocytogenes* virulence and in host susceptibility is derived and applied to currently available epidemiological data, including data from one well-documented listeriosis outbreak.[4,5,31] Unlike other foodborne pathogens such as *Salmonella*,[32–34] *Campylobacter*,[35] or norovirus,[36,37] *L. monocytogenes* is characterized by an extremely low probability of illness at low exposure doses when averaging across the total population or broadly defined population subgroups[4,5,30] and by extreme variability in the probability of infection among population subgroups with different predisposing risk factors.[5,26,27,38] Two dose-response models are evaluated and compared here in light of the unique challenges associated with modeling *L. monocytogenes* dose response.[2,4,5,29] The first evaluated model uses beta distributions to characterize variability in r from exposure to exposure, resulting in an "exact beta-Poisson" dose-response relation[6] (also known as "hypergeometric"[7] or "actual beta-Poisson"[8] dose-response relation), which may be simplified to an approximate "beta-Poisson" model if certain conditions are met.[7,39] The second model, a newly developed "lognormal-Poisson" model, characterizes variability in r due to variability in strain virulence and host susceptibility using lognormal distributions. As will be illustrated in this article, the lognormal distribution was found appropriate and useful for modeling the special case of *L. monocytogenes* dose response whereas the beta-Poisson model showed insufficient flexibility to adequately model one of the well-described *L. monocytogenes* outbreaks.

2. FRAMEWORK, MODEL, AND DATA

2.1. General Derivation of the Evaluated Dose-Response Models

A single-hit model is assumed.[6,7] The probability of acquiring the adverse effect under study (i.e., invasive listeriosis) if a dose of d bacterial cells is ingested in a certain serving is given by:

$$P(\text{ill}; d, r) = 1 - (1 - r)^d, \tag{1}$$

where "ill" stands for "illness" (here, invasive listeriosis) and r is the probability of developing invasive listeriosis from the ingestion of a bacterial cell in a given, specific serving. Note that r may be seen as constant for that serving,[6] or as an average probability that one cell of the specific population of pathogens present in the meal will survive and initiate the infection and illness of this specific consumer.[8] Assume that each serving is specific to a given context, determined by the individual i (characterized by the presence of a given set of predisposing risk factors at the time of consumption) consuming the food and by the *L. monocytogenes* strain s present in the ingested food (with a certain set of given virulence determinants at

the time of consumption). In this study, r is considered constant for this particular serving, but variable across servings, with its variability determined by the variation in susceptibility across individuals and the variation in virulence across strains.

Assume further that the *L. monocytogenes* dose in a given serving is Poisson distributed and the distribution of r across a given population of servings is described by a random variable with density function $f(r;\boldsymbol{\theta})$. Then the marginal probability of infection for an average dose d is described by:[6]

$$P(\text{ill};d,\boldsymbol{\theta}) = \int_0^1 (1-\exp(-rd))\, f(r;\boldsymbol{\theta})\, \mathrm{d}r. \quad (2)$$

Any probability density function with practical domain [0; 1] can be chosen for f. A beta distribution is a convenient choice for modeling variability in r because its domain is restricted to [0,1], it provides flexibility over the domain, and the simplified beta-Poisson model is easy to implement.[6] The exact and simplified beta-Poisson dose-response models have been repeatedly used for modeling illnesses from other foodborne pathogens such as norovirus,[37,40] *Salmonella*,[33,34] or *Campylobacter jejuni*.[8] The beta-Poisson model was also used to model *L. monocytogenes* dose-response from animal data.[41] If a lognormal (base 10) distribution is chosen for f, that is, $\log_{10}(r) \sim \text{normal}(\mu,\sigma)$, with negligible probability that $r \geq 1$, Equation (2) leads to:

$$P(\text{ill};d,\mu,\sigma) = \frac{\log_{10}(e)}{\sigma\sqrt{2\pi}} \int_0^1 \left(\frac{1}{r}(1-\exp(-rd)) \times \exp\left(-\frac{(\log_{10}(r)-\mu)^2}{2\sigma^2}\right)\right)\mathrm{d}r. \quad (3)$$

Equation (3) has no closed form and requires numerical integration. However, it simplifies to an exponential dose-response model for any given value r.

$$P(\text{ill};d,r) = 1-\exp(-rd) \quad (4)$$

In this study, we investigated a beta distribution and a lognormal distribution to characterize the distribution of r from meal to meal, using data from multiple sources, including human surveillance and food survey data. The derivation using the beta-Poisson model can be found in the Appendix, which shows that this model is inappropriate for the special case of modeling *L. monocytogenes* dose response in humans, most notably because it could not adequately model extreme situations such as outbreaks. The lognormal distribution was eventually chosen because its heavy-tail property was deemed useful for modeling the special case of *L. monocytogenes* dose response, and because its infinitively divisional property allowed for mathematically relatively simple separation of different sources of variability in dose response.

2.2. Dose-Response Model Within Populations Subgroups

The probability of developing listeriosis after ingesting a given dose of *L. monocytogenes* is highly variable from meal to meal, and considerably impacted by the *L. monocytogenes* strain and the presence and nature of underlying host conditions such as pregnancy, old age, or certain diseases and conditions.[25–27] The variability in r may be separated into three sources: variability in susceptibility across mutually exclusive population subgroups with a shared predisposing risk factor, variability in susceptibility across individuals within a given population subgroup, and variability in virulence among *L. monocytogenes* strains with different virulence determinants.

For a given population subgroup g, the marginal dose response can be rewritten as:

$$P(\text{ill};d,\boldsymbol{\theta}_g) = \int_0^1 (1-\exp(-rd))\, f(r;\boldsymbol{\theta}_g)\, dr,$$

where $\boldsymbol{\theta}_g$ is characteristic of the subgroup g. The distribution $f(r;\boldsymbol{\theta}_g)$ represents the remaining individual (within group) susceptibility variability and strain virulence variability in r.

The resulting distribution of r across all population subgroups can be expressed as a mixture of distributions for individual population subgroups, weighted by the relative size of each population subgroup in the total population:

$$g(r) = \sum_g \pi_g f(r;\boldsymbol{\theta}_g), \quad (5)$$

where π_g, $\sum_g \pi_g = 1$, is the proportional size of the population subgroup g within the total population.

Substituting $f(r)$ by $g(r)$ in Equation (2) leads to the dose response for the total population. This dose-response relationship integrates, in addition to those factors accounted for by the subpopulation-specific dose-response model, the variability in mean susceptibility across population subgroups.

2.3. Specification of μ_g and σ_g from Surveillance Data

Let c_g equal the number of invasive listeriosis cases in a given population subgroup g and $M_{d,g}$ equal the number of servings with a given mean dose d ingested by the population subgroup g. Then, the expected value of c_g is given by:

$$E[c_g] = \int_0^\infty M_{d,g} P(\text{ill}; d, \boldsymbol{\theta}_g)\, \mathrm{d}d. \tag{6}$$

Estimating c_g from epidemiological data and $M_{d,g}$ from food exposure data generates an infinite number of solutions for the ordered pair (μ_g, σ_g). However, if a measure of variability of r_g is known, the problem simplifies to a root-finding problem. As an example, if we are able to characterize Q_{90}, the $\log_{10}$ of the ratio between the 5th and the 95th percentile of $f(r; \boldsymbol{\theta}_g)$, we can estimate $\boldsymbol{\theta}_g$ for estimated $E[c_g]$ and Q_{90} using some iterative solver routine.

2.4. Characterization of Variability

2.4.1. Specification of σ_g

Under limited assumptions, the infinitively divisional property of lognormal distributions allows for a characterization and separation of interindividual and interstrain variability. The potential of a given *L. monocytogenes* strain to cause disease (i.e., strain virulence determined by a given set of transient and fixed virulence factors) may be considered independent of the susceptibility of a given host to listeriosis (i.e., host susceptibility due to a given set of comorbidities and other factors impacting individual susceptibility such as genetic predisposition).

In this study, r is defined as the probability of infection for a given individual following the ingestion of one given *L. monocytogenes* cell during a given serving. Note that r may be considered for our purpose as the product of two independent probabilities: the probability p_i, linked to events controlled by host factors that ultimately lead to a failure to stop infection, and p_s, which reflects bacterial factors that control virulence and pathogenicity:

$$r = p_i \times p_s. \tag{7}$$

We assume that p_s and p_i follow lognormal distributions. Because the product of two independent lognormally distributed random variables is itself a lognormal random variable, r is also lognormally distributed. Let $p_i \sim \text{lognormal}(\mu_i, \sigma_i)$ for all $i \in g$, and let $p_s \sim \text{lognormal}(\mu_s, \sigma_s)$ for strains s. Based on Equation (7) we see that for a given population subgroup and strain,

$$r \sim \text{lognormal}\left(\mu_i + \mu_s, \sqrt{\sigma_i^2 + \sigma_s^2}\right), \tag{8}$$

and the marginal density across all strains can therefore be found by $\mu_g = \mu_i + \mu_s$ and $\sigma_g = \sqrt{\sigma_i^2 + \sigma_s^2}$.

$Q_{90,i}$ is defined as the $\log_{10}$ of the 90% individual within-group susceptibility variability range. Note that σ_i can be estimated as $\sigma_i = (Q_{90,i}/2)/\Phi^{-1}(0.95)$ where Φ^{-1} denotes the inverse of the standard normal cumulative density function. Here, σ_s can be estimated using the same rationale for the interstrain variability. If $Q_{90,s}$ is the $\log_{10}$ difference between the 5th and the 95th percentile, $\sigma_S = (Q_{90,s}/2)/\Phi^{-1}(0.95)$.

The subroutine must find (μ_g, σ_g) solution of:

$$E[c_g] = \int_0^\infty M_{d,g} P(\text{ill}; \mu_g, \sigma_g)\, \mathrm{d}d, \tag{9}$$

where

$$\sigma_g = \frac{\sqrt{{Q_{90,i}}^2 + {Q_{90,s}}^2}}{2\Phi^{-1}(0.95)}. \tag{10}$$

2.4.2. Intragroup Variability $Q_{90,i}$

Due to a variety of factors, such as genetic predisposition, susceptibility to infection differs across individuals, even after accounting for underlying comorbidities, albeit with considerably decreased variability. To derive estimates for our model, we used the estimates of variability in susceptibility presented in FDA/FSIS.[4] In FDA/FSIS,[4] three distributions that encompass the range of susceptibility observed in animal studies were used to adjust the $\log_{10}$ cfu of the effective dose for populations with low, medium, and high variability.[4] Assuming exponential dose response in animal studies, the range of variation in the $\log_{10}$ LD_{50} translates into the range of variation in the $\log_{10}$ r parameter.[1] Therefore, we represented the variability in the probability of illness from a single cell (in $\log_{10}$ r) using the variability in the $\log_{10}$ cfu that had been used to modify the effective dose in FDA/FSIS.[4] According to FDA/FSIS (Table IV-8 in Ref. 4), 90% of the individual variability within the population group with low, medium, and high

[1] We have, for an exponential dose response, $r = \frac{-\ln(.5)}{\text{LD}_{50}}$. The LD_{50} is inversely proportional to r. A variation of $\pm x$ $\log_{10}$ in $\log_{10}$ LD_{50} corresponds to a similar variation of $\pm x$ $\log_{10}$ in $\log_{10}$ r.

variability in susceptibility may be contained within a range of 0.8 $\log_{10}$, 1.8 $\log_{10}$, and 2.9 $\log_{10}$, respectively. FDA/FSIS[4] used the medium variability distribution for neonatal populations and high variability for intermediate-age and elderly subpopulations. In this study, we divided the population into 11 population subgroups with similar underlying conditions (Table I), essentially as described previously.[11,42] Assuming that our 11 subpopulations would be more precisely defined with regard to predisposing risk factors and therefore less variable in susceptibility than the broadly defined "elderly" and the "intermediate-age" population subgroups defined by FDA/FSIS,[4] we used FDA/FSIS[4] "medium variability" estimates for all of the 11 groups, that is, $Q_{90,i} = 1.8 \log_{10}$.

2.4.3. Interstrain Virulence Variability $Q_{90,S}$

In the FDA/FSIS assessment, variations in host susceptibility and in strain virulence were represented by distributions that modified the effective dose for individual servings.[4] The distribution for strain virulence was estimated notably by the observed variation in LD_{50} (in $\log_{10}$ cfu) among different *L. monocytogenes* strains in mouse experiments.[4] According to FDA/FSIS (Table IV-6 in Ref. 4), 90% of the strain variability ranges within a 5 $\log_{10}$, leading to $Q_{90,s} = 5 \log_{10}$.

Substituting these values in Equation (10) generates $\sigma_g = 1.62 \log_{10}$.

2.5. Integration of the dose-response Models

2.5.1. Exposure Data

The *L. monocytogenes* concentration distribution reported by Chen *et al.*[30] was used for exposure estimates. This distribution was obtained by fitting data from a survey of more than 31,000 RTE retail food samples, representing eight RTE categories sampled in the years 2000 and 2001 in two states of the United States.[43] *L. monocytogenes* was not detected in 98.2% of the samples. The $\log_{10}$ concentration ($\log_{10}$ cfu/g) in the remaining contaminated products followed a four-parameter beta distribution[2] with parameters $\alpha = 0.29$, $\beta = 2.68$, $a = -1.69$, and $b = 6.1$.[30] A 50 g serving size was assumed in this study. The number of servings of these eight RTE categories consumed by the U.S. population was estimated at 1.23×10^{11} servings per year based on the FDA/FSIS risk assessment.[4] As considered in previous risk assessments,[4,5] we made the assumption of an identical distribution of *L. monocytogenes* doses and strains for all population subgroups.

[2] x follows a four-parameter beta distribution with parameters (α, β, a, b) if $(x\text{-}a)/(b\text{-}a) \sim \text{Be}(\alpha, \beta)$

2.5.2. Epidemiological Data

To allow comparisons across population subgroups g with similar underlying conditions, we identified population subgroups with specific predisposing risk factors (e.g., different types of illness, old age, pregnancy), and evaluate variability in susceptibility within and across these subgroups.

Goulet *et al.*[26] published data on the relative risk of listeriosis in France for 36 mutually exclusive susceptible population subgroups, each consisting of individuals sharing a specific underlying condition. Because the data were too scarce to derive dose-response models separately for 36 mutually exclusive subgroups, the 36 subgroups identified by Goulet *et al.*[26] were combined (where appropriate) and regrouped into 11 subgroups based on underlying pathophysiology and expected degree of T-cell inhibition, essentially using a grouping scheme as previously described.[11,42]

We assumed that the relative risk of listeriosis for a given population subgroup and the relative size of each evaluated population subgroup would be comparable between France and the United States. The number of cases in each subgroup had to be normalized to the listeriosis burden estimates from the United States to allow extrapolation of the data (Table I). We evaluated two estimates of the total listeriosis cases in the United States, the first based on 1996–1997 data[9] and the second on 2005–2008 data[1] from FoodNet surveillance. We chose the latter, i.e., 1,591 cases per year, as input to derive the dose-response relationship because the 2000–2001 timeframe for the food survey[43] corresponded to the timeframes for the listeriosis estimates and, more importantly, the latter listeriosis estimate was based on new and improved methods for estimating overall foodborne illness in the United States.[1]

2.5.3. Sensitivity Analysis

As will be discussed below, we identified two major assumptions needed to use the data described above. To evaluate the impact of these assumptions

Table I. Number of Persons with Underlying Conditions and Number of Cases of Invasive Listeriosis Observed in France, 2001–2008;[26] Expected Number of Invasive Listeriosis Cases per Subgroups in the United States; See Text for Underlying Assumptions and Ref. 26 for a More In-Depth Description of the Population Subgroups

Population Subgroup	Number of Individuals in France (from and Adapted from Ref. 26)	Listeriosis Cases During an 8-Year Period in France (from and Adapted from Ref. 26)	Relative Risk (CI 95%)[a]	Expected Number of Listeriosis Cases in the United States (Based on 1,591 Cases from Ref. 1)
Less than 65 years old, no known underlying condition (i.e., "healthy adult")	48,909,403	189	Reference group	153
More than 65 years old, no known underlying condition	7,038,068	377	13.9 (8.6, 23.1)	306
Pregnancy	774,000	347	116 (71, 194.4)	282
Nonhematological cancer	2,065,000	437	54.8 (34.2, 90.3)	355
Hematological cancer	160,000	231	373.6 (217.3, 648.9)	188
Renal or liver failure (dialysis, cirrhosis)	284,000	164	149.4 (82, 270.1)	133
Solid organ transplant	25,300	16	163.7 (26.3, 551.5)	13
Inflammatory diseases (rheumatoid arthritis, ulcerative colitis, giant cell arteritis, Crohn's disease)	300,674	68	58.5 (25.2, 123.4)	55
HIV/AIDS	120,000	22	47.4 (10.5, 140.4)	18
Diabetes (type I or type II)	2,681,000	79	7.6 (3.5, 15.6)	64
Heart diseases	1,400,000	29	5.4 (1.5,14.4)	24
Total population	63,757,445	1,959		1,591

[a]Estimated using a Poisson regression without adjustment. These 95% CIs should be considered only as indicative but suggest that all those groups have a risk of listeriosis significantly higher than the reference group.

on the generated risk estimates we conducted the following sensitivity analyses for these two assumptions. (i) Due to the lack of sufficient data, we assumed equal exposure to contaminated food for all population subgroups. This assumes that outreach targeted at minimizing foodborne exposures of high-risk population subgroups is ineffective. As a sensitivity analysis, the model was tested with the alternative assumption that the number of servings containing a given number of bacteria for all of the more susceptible subgroups are one-tenth of that for "healthy adults" (i.e., the <65 years of age without any known underlying conditions). (ii) The exposure data we used in deriving the dose-response model did not consider bacterial growth from retail to consumption, and considered a maximum level of contamination of 6.1 $\log_{10}$ cfu/g. Because growth in the consumer home has been identified as a potentially important risk factor in previous risk assessments, we conducted a sensitivity analysis to evaluate the impact of this assumption. The model was tested using the four-parameter beta distribution of $\log_{10}$ concentration described in Section 2.5.1, with a maximum parameter increased from $b = 6.1$ to $b = 8.1$ $\log_{10}$ cfu/g. This distribution leads to an average concentration in contaminated products of 20,545 cfu/g as compared to 390 cfu/g for the baseline scenario.

2.6. Dose-response Relationship Using Outbreak Data

It was assumed that a single food item and *L. monocytogenes* strain are involved in an outbreak affecting a specific population subgroup g, thus eliminating the impact of strain-to-strain variability in the dose-response evaluation. The virulence of the outbreak strain, $p_{s(\text{outbreak})}$, is then fixed but unknown. We used the lognormal-Poisson model (and the beta-Poisson model; see the Appendix) to analyze a well-documented listeriosis outbreak, the butter outbreak that occurred in Finland in 1998–1999,[31] as re-examined by FDA/FSIS[4] and FAO/WHO.[5] This outbreak was characterized by a relatively high attack rate among immunocompromised individuals (mostly hematological or organ transplant patients) for a relatively low dose of *L. monocytogenes*.[4,5] The FAO/WHO panel derived an r value of 3.15×10^{-7} from data collected during this outbreak.[5]

The lognormal dose-response properties help to evaluate the dose response during outbreaks. As can be inferred based on Equation (7), r is the product of a fixed value $p_{s(outbreak)}$ and a lognormally distributed variable p_i. Thus:

$$r \sim \text{lognormal}\left(\mu_g + \log_{10}\left(p_{s(outbreak)}\right), \sigma_i\right). \quad (11)$$

Given that $p_s \sim lognormal\,(\mu_S, \sigma_S)$, the jth quantile of p_s is given by $p_s\,(j) = 10^{\left(\mu_s + \Phi^{-1}(j)\times\sigma_s\right)}$. Assuming that $p_{s(\text{outbreak})} = p_s\,(j)$:

$$r \sim \text{lognormal}\left(\mu_i + \mu_s + \Phi^{-1}\,(j) \times \sigma_s, \sigma_i\right). \quad (12)$$

Substituting μ_g for $\mu_i + \mu_s$gives for r:

$$r \sim \text{lognormal}\left(\mu_g + \Phi^{-1}\,(j) \times \sigma_s, \sigma_i\right). \quad (13)$$

Percentiles of interest can now easily be estimated using the parameters derived above.

All numerical integrations and optimizations of the models were performed using the R software.[44] The code is available from the corresponding author on request.

3. RESULTS

3.1. Estimation of *r* for Different Population Subgroups Using Food Exposure and Epidemiological Surveillance Data

Solutions for the ordered pair (μ_g, σ_g) for all 11 population subgroups, based on numerical integration, are presented in Table II. Notably, estimates of $\mu_g$varied widely across population subgroups, ranging from $\mu_g = -14.1$ for those less than 65 years of age without any known underlying conditions (i.e., "healthy adults") to $\mu_g = -11.0$ for individuals with hematological cancer. These estimates translate into mean values of r equaling 7.9×10^{-12} and 9.6×10^{-9}, respectively. The corresponding 99.9th percentiles equal 7.7×10^{-10} and 9.3×10^{-7} for healthy adults and individuals with hematological cancer, respectively, indicating that the risk of illness per ingested cell generally remains relatively low for most population subgroups and most types of exposure. The variation in dose response across population subgroups is illustrated in Fig. 1, highlighting in particular the comparison among the total population, pregnant women, and healthy adults. As expected, the marginal dose-response model for the total population more closely resembles that for healthy adults than those for the most susceptible population subgroups.

The probability of illness and the expected numbers of cases for a variety of population subgroups and ingested doses are presented in Table III. For healthy adults <65 years old, the mean probability of illness remains below 1:10,000 if doses below 7.5 $\log_{10}$ cfu/serving are ingested. However, for those with hematological cancer, ingestion of doses in the range of 5.5 $\log_{10}$ cfu/serving translates into a mean probability of illness around 1:1,000. Considering this dose-response relationship and the exposure to *L. monocytogenes* through food consumption, most of the 1,591 cases analyzed in this study are expected to be due to foods contaminated with doses between 3.5 and 7.5 $\log_{10}$ cfu/serving (Table III). Notably, 20% of the 188 expected cases among those with hematological cancer are expected to be due to contamination with doses ≤5 $\log_{10}$ cfu/serving. Doses of 4 $\log_{10}$ cfu/serving or lower are estimated to be responsible for 2% of cases among healthy adults, but an estimated 4% of cases among pregnant women and an estimated 5% of cases among individuals with hematological cancer are expected to be caused by such relatively low doses.

As shown above in Equation (4), for a fixed value of r, the dose-response model simplifies to an exponential dose-response model. Fig. 2 illustrates the dose-response relationships for the total population for the 0.01st, 0.1st, 1st, 50th, 99th, 99.9$^{\text{th}}$, and 99.99th percentiles of the distribution (including group-to-group, individual within-group, and strain-to-strain variability) of r. This figure also overlays the marginal lognormal-Poisson model from this study with the exponential dose-response models reported previously by FAO/WHO[5] for the susceptible population as well as the one by Chen *et al.*[24] for *L. monocytogenes* strains with genes encoding a full-length *inlA* for the 25% higher-risk population. Notably, the dose response for the total population derived here results in a higher risk of infection for low doses than either of the two published dose-response models (Fig. 2). The dose-response model obtained in this study for the least virulent strains, however, leads to a considerably lower risk of illness at low doses than either of the published models.

3.2. Sensitivity Analyses

When the model was tested with the alternative assumption that the number of servings including a given number of bacteria for all of the more susceptible subgroups equals one-tenth of that for

Table II. Parameters of the Lognormal-Poisson Dose-response Model for Invasive Listeriosis Following the Ingestion of *L. monocytogenes* in Different Population Subgroups and Resulting Statistics for *r*, the Probability of Illness Following the Ingestion of One Cell of *L. monocytogenes*; The Distribution of *r* Includes the Individual Within-Group and the Strain Variability

	Estimates of a Log_{10} Normal Distribution[a] of *r*		Estimates of *r*			
Population Subgroup	μ	σ	Mean	50th Percentile	99th Percentile	99.9th Percentile
Less than 65 years old, no known underlying condition (i.e., "healthy adult")	−14.11	1.62	7.90×10^{-12}	7.82×10^{-15}	4.48×10^{-11}	7.68×10^{-10}
More than 65 years old, no known underlying condition	−12.83	1.62	1.49×10^{-10}	1.47×10^{-13}	8.44×10^{-10}	1.45×10^{-8}
Pregnancy	−11.70	1.62	2.01×10^{-9}	1.99×10^{-12}	1.14×10^{-8}	1.95×10^{-7}
Nonhematological cancer	−12.11	1.62	7.76×10^{-10}	7.68×10^{-13}	4.40×10^{-9}	7.54×10^{-8}
Hematological cancer	−11.02	1.62	9.60×10^{-9}	9.51×10^{-12}	5.44×10^{-8}	9.33×10^{-7}
Renal or liver failure (dialysis, cirrhosis)	−11.56	1.62	2.79×10^{-9}	2.76×10^{-12}	1.58×10^{-8}	2.71×10^{-7}
Solid organ transplant	−11.51	1.62	3.14×10^{-9}	3.11×10^{-12}	1.78×10^{-8}	3.06×10^{-7}
Inflammatory diseases (rheumatoid arthritis, ulcerative colitis, giant cell arteritis, Crohn's disease)	−12.08	1.62	8.43×10^{-10}	8.35×10^{-13}	4.78×10^{-9}	8.19×10^{-8}
HIV/AIDS	−12.19	1.62	6.50×10^{-10}	6.44×10^{-13}	3.69×10^{-9}	6.32×10^{-8}
Diabetes (type I or type II)	−13.13	1.62	7.47×10^{-11}	7.39×10^{-14}	4.23×10^{-10}	7.26×10^{-9}
Heart diseases	−13.30	1.62	5.01×10^{-11}	4.96×10^{-14}	2.84×10^{-10}	4.86×10^{-9}
Whole population	N/A[b]	N/A	1.19×10^{-10}	1.56×10^{-14}	2.47×10^{-10}	6.87×10^{-9}

[a]The log_{10} normal distribution is parameterized as $x \sim \text{lognormal}(\mu, \sigma)$ if $\log_{10}(x) \sim \text{normal}(\text{mean: } \mu, \text{standard error: } \sigma)$.
[b]Nonapplicable: the dose response for the whole population uses a mixture of log_{10} normal distribution (see text for details).
[c]The distribution of *r* includes the individual and the strain variability.

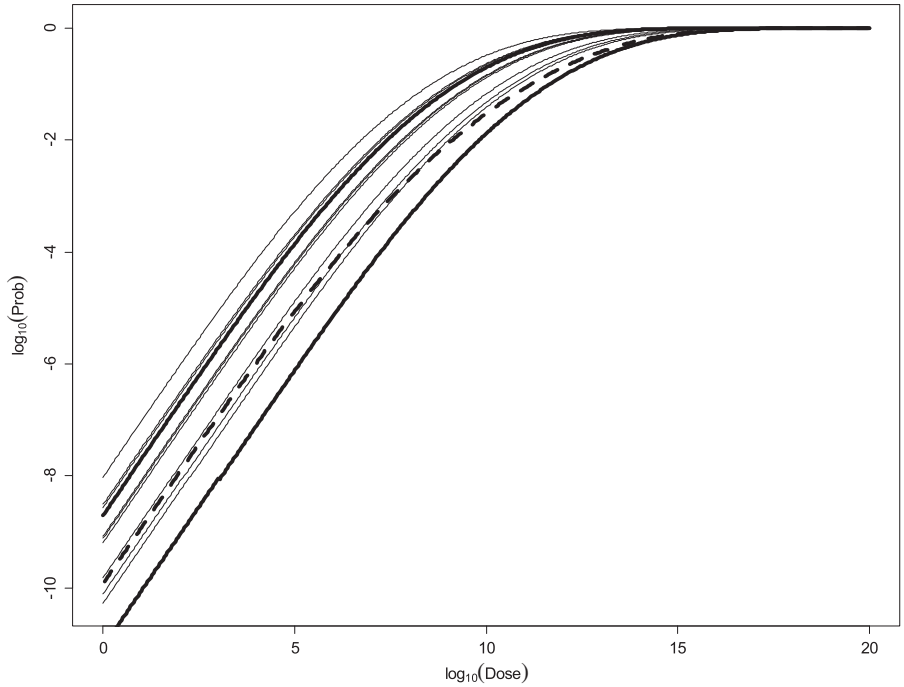


Fig. 1. Marginal (over strains and individuals within subgroups) lognormal-Poisson dose-response models for the 11 population subgroups (thin lines), emphasizing (thick lines) the dose-response relationship for those <65 years of age without known underlying conditions ("healthy adult"; bottom thick line) and for pregnant women (top thick line). Marginal (over strains and individuals) lognormal-Poisson dose response for the total population (thick dashed line).

"healthy adults," the dose response was shifted to the left for the susceptible groups. In this case, the overall expected number of cases for servings containing ≤ 4 $\log_{10}$ cfu equaled less than 6% of all cases as compared to 3% of all cases in the baseline scenario. The assumption of equal food consumption across population subgroups therefore only had a modest impact on our analysis. When the model was tested with a maximum *L. monocytogenes* level of 8.1 $\log_{10}$ cfu/g, a shift of the corresponding dose response to the right was logically obtained: with this maximum level, 0% of the cases would be predicted for a dose of 4 $\log_{10}$ cfu/g and 4% for a dose of 6 $\log_{10}$ cfu/g for the total population.

3.3. Application of the Dose-response Framework to Listeriosis Outbreaks

Fig. 3 compares the published exponential dose-response model[5] estimated from the Finnish butter outbreak data ($r = 3.15 \times 10^{-7}$)[5,31] to the dose-response model for transplant recipients derived in this study, showing both the prediction averaged across individual strains and for individual percentiles of the virulence distribution p_s. Fig. 3 suggests that the dose-response model from this study is able to predict the data observed in the Finnish outbreak, and that the strain was highly virulent, as the corresponding dose-response overlays that of a strain with a level of virulence close to the 99.9th percentile of r.

4. DISCUSSION

4.1. The New Framework for *L. Monocytogenes* Dose-response, Adjusted for Variability in Host Susceptibility and Strain Virulence

The FAO/WHO[5] dose-response model can be considered as a marginal dose-response model for a population exposed to a cross-section of *L. monocytogenes* strains. As such, this model averages across numerous individuals with differing levels of susceptibility and multiple *L. monocytogenes* strains with varying levels of virulence. While such evaluations can be highly informative for many purposes they may be inappropriate to evaluate certain rare but potentially highly relevant events, such as the

Table III. Marginal Probability of Illness and Expected Number of Cases in Selected Population Subgroups and in the General Population as a Function of the Dose, Considering Individual Susceptibility Within Groups, Strain Variability, and Dose Variability for a Given Mean Dose

	Marginal Probability of Illness for Individuals						Expected Number of Cases					
Log_{10}(Dose)	<65-year old	>65-year old	Pregnant women	Hematological cancer	Solid organ transplant	Whole population	<65-year old	>65-year old	Pregnant women	Hematological cancer	Solid organ transplant	Whole population
0.0	8.8×10^{-12}	1.5×10^{-10}	2.0×10^{-9}	9.5×10^{-9}	3.1×10^{-9}	1.2×10^{-10}	0	0	0	0	0	0
0.5	2.8×10^{-11}	4.8×10^{-10}	6.2×10^{-9}	3.0×10^{-8}	9.8×10^{-9}	3.8×10^{-10}	0	0	0	0	0	0
1.0	8.8×10^{-11}	1.5×10^{-9}	2.0×10^{-8}	9.5×10^{-8}	3.1×10^{-8}	1.2×10^{-9}	0	0	0	0	0	1
1.5	2.8×10^{-10}	4.8×10^{-9}	6.3×10^{-8}	3.0×10^{-7}	9.9×10^{-8}	3.8×10^{-9}	0	0	0	0	0	1
2.0	8.8×10^{-10}	1.5×10^{-8}	2.0×10^{-7}	9.3×10^{-7}	3.1×10^{-7}	1.2×10^{-8}	0	0	0	0	0	1
2.5	2.8×10^{-9}	4.7×10^{-8}	6.2×10^{-7}	2.8×10^{-6}	9.6×10^{-7}	3.7×10^{-8}	0	0	1	1	0	3
3.0	8.8×10^{-9}	1.5×10^{-7}	1.9×10^{-6}	8.6×10^{-6}	2.9×10^{-6}	1.1×10^{-7}	0	1	1	1	0	6
3.5	2.5×10^{-8}	4.6×10^{-7}	5.8×10^{-6}	2.5×10^{-5}	8.9×10^{-6}	3.5×10^{-7}	1	2	3	3	0	14
4.0	7.8×10^{-8}	1.4×10^{-6}	1.7×10^{-5}	7.2×10^{-5}	2.6×10^{-5}	1.0×10^{-6}	2	4	6	5	0	29
4.5	2.5×10^{-7}	4.3×10^{-6}	5.0×10^{-5}	2.0×10^{-4}	7.5×10^{-5}	3.0×10^{-6}	4	9	12	10	1	60
5.0	7.6×10^{-7}	1.3×10^{-5}	1.4×10^{-4}	5.2×10^{-4}	2.0×10^{-4}	8.6×10^{-6}	8	19	22	17	1	115
5.5	2.4×10^{-6}	3.8×10^{-5}	3.7×10^{-4}	1.3×10^{-3}	5.4×10^{-4}	2.4×10^{-5}	15	35	38	28	2	200
6.0	7.1×10^{-6}	1.1×10^{-4}	9.5×10^{-4}	3.1×10^{-3}	1.3×10^{-3}	6.3×10^{-5}	27	58	56	38	3	308
6.5	2.1×10^{-5}	2.9×10^{-4}	2.3×10^{-3}	7.0×10^{-3}	3.2×10^{-3}	1.6×10^{-4}	39	77	68	42	3	389
7.0	6.1×10^{-5}	7.5×10^{-4}	5.2×10^{-3}	1.5×10^{-2}	7.1×10^{-3}	3.9×10^{-4}	41	73	56	33	3	344
7.5	1.7×10^{-4}	1.8×10^{-3}	1.1×10^{-2}	2.9×10^{-2}	1.5×10^{-2}	9.1×10^{-4}	17	27	19	10	1	121
8.0	4.5×10^{-4}	4.3×10^{-3}	2.3×10^{-2}	5.3×10^{-2}	2.9×10^{-2}	2.0×10^{-3}	0	0	0	0	0	0
8.5	1.1×10^{-3}	9.3×10^{-3}	4.3×10^{-2}	9.2×10^{-2}	5.4×10^{-2}	4.2×10^{-3}	0	0	0	0	0	0
9.0	2.7×10^{-3}	1.9×10^{-2}	7.6×10^{-2}	1.5×10^{-1}	9.4×10^{-2}	8.5×10^{-3}	0	0	0	0	0	0
9.5	6.1×10^{-3}	3.7×10^{-2}	1.3×10^{-1}	2.3×10^{-1}	1.5×10^{-1}	1.6×10^{-2}	0	0	0	0	0	0
10.0	1.3×10^{-2}	6.6×10^{-2}	2.0×10^{-1}	3.2×10^{-1}	2.3×10^{-1}	3.0×10^{-2}	0	0	0	0	0	0
10.5	2.6×10^{-2}	1.1×10^{-1}	2.9×10^{-1}	4.3×10^{-1}	3.3×10^{-1}	5.1×10^{-2}	0	0	0	0	0	0
11.0	4.8×10^{-2}	1.8×10^{-1}	3.9×10^{-1}	5.5×10^{-1}	4.4×10^{-1}	8.5×10^{-2}	0	0	0	0	0	0
Total							153	306	282	188	13	1591

[a]No serving with >7.8 log_{10} *L. monocytogenes* cells is expected from the set of exposure data, leading mathematically to no expected cases at or above 8.0 log_{10}.

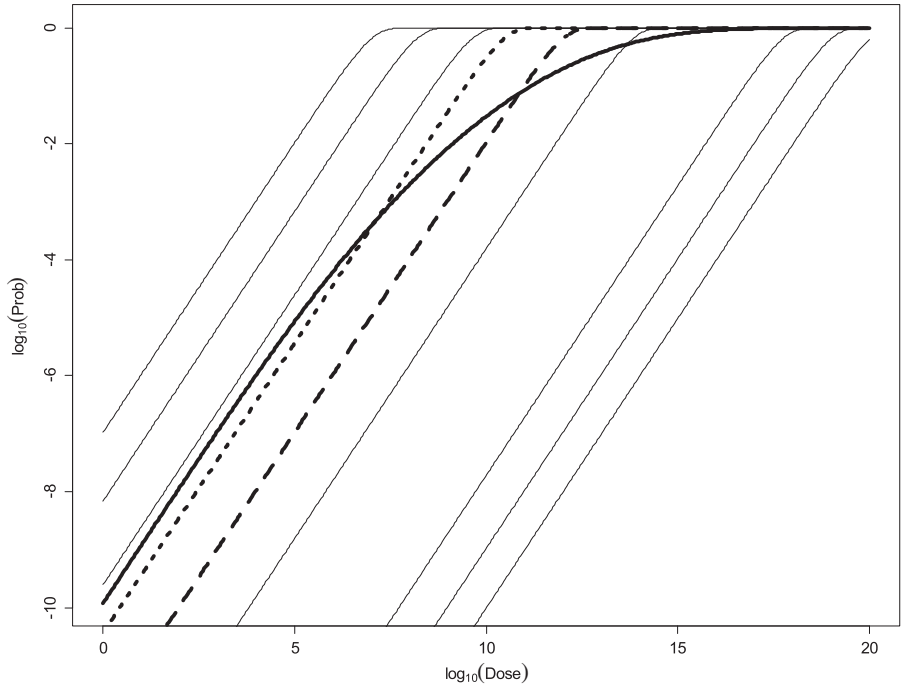


Fig. 2. Marginal lognormal-Poisson dose-response model for the total population (black solid line) and exponential dose-response model for r in the 0.01st, 0.1st, 1st, 50th, 99th, 99.9th, and 99.99th percentiles of the strain and individual distribution (thin black lines, from right to left). These estimates are compared to the dose-response relationships generated by FAO/WHO[5] for invasive listeriosis in the fraction of the population with increased susceptibility ($r = 1.06 \times 10^{-12}$; see Ref. 5, p. 56) (dashed line) and by Chen *et al.*[24] for *L. monocytogenes* with genes encoding a full-length *inlA* for the 25% higher-risk population ($\log_{10}(r) = -10.44$; dotted line).

ingestion of a highly virulent *L. monocytogenes* strain by a highly susceptible individual. Moreover, small population subgroups with extremely high susceptibility may not be adequately reflected in such dose-response relationships, potentially explaining at least in part why traditional exponential dose-response models of *L. monocytogenes* could so far not be reconciled with outbreak data.

The lognormal-Poisson dose-response models derived here extend and advance *L. monocytogenes* dose-response modeling to explicitly consider variability in strain virulence and in susceptibility across population subgroups. As such, the extended model more accurately captures the risk of listeriosis in those population subgroups at highest risk of listeriosis. Because the relative risk of listeriosis has been shown to vary by as much as 1,000-fold across population subgroups with clearly defined risk factors,[26] the ability to accurately characterize the listeriosis risk for different population subgroups is of paramount importance for risk management and for a comprehensive characterization of the listeriosis risk posed by different RTE food items. Similarly, strains differ considerably in virulence. Chen *et al.*[24] found a 2–3 $\log_{10}$ difference in the marginal exponential dose-response parameters r for *L. monocytogenes* subtypes encoding a full length or truncated version of *inlA,* respectively. In a guinea pig model, Van Stelten *et al.*[23] found more than a 1 $\log_{10}$ increase in median infectious dose for a *L. monocytogenes* strain carrying a premature stop codon (PMSC) in *inlA* compared to that for an epidemic clone. Accounting for variability in strain virulence is therefore clearly of great importance. The variation in virulence used in this study (i.e., variability of 5 $\log_{10}$ based on inter 5th–95th percentiles) is higher than the differences in strain virulence that would be expected based on the data for strains with and without PMSCs in *inlA*. However, other virulence factors likely also contribute to virulence differences among *L. monocytogenes* strains.[21,45] Therefore, the true variability in strain virulence is likely larger than that estimated solely based on differences in *inlA* alleles. In addition, food matrix effects were implicitly accounted for in the variability in strain virulence, thus likely also increasing

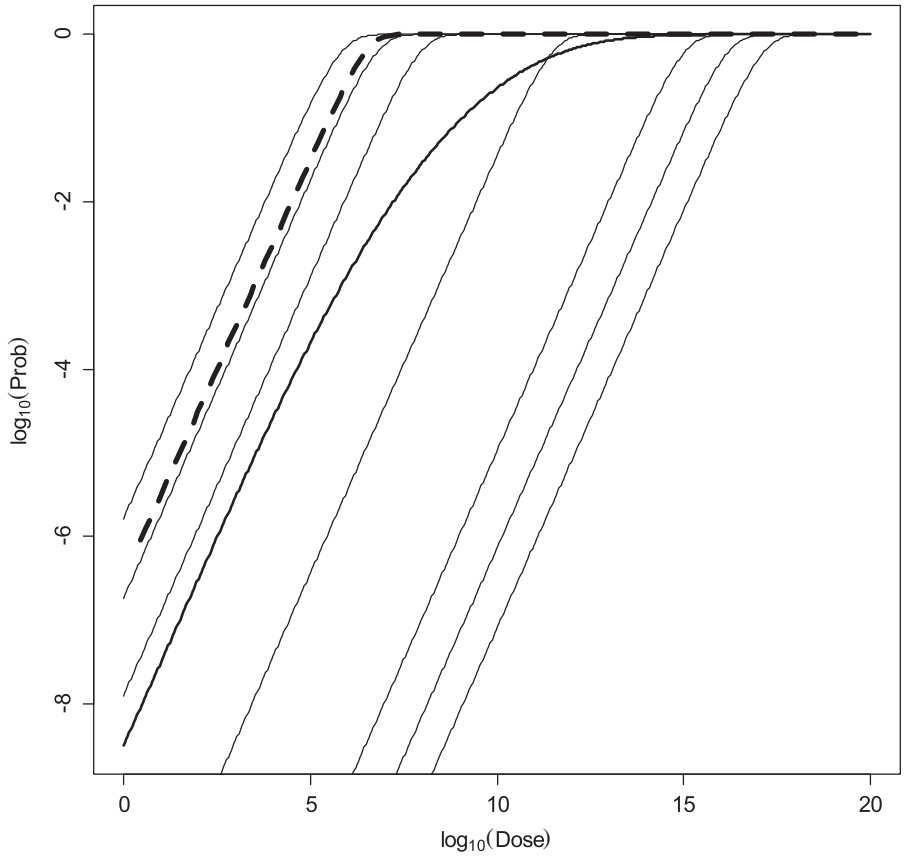


Fig. 3. Lognormal dose-response relationships for invasive listeriosis following the ingestion of *L. monocytogenes*, comparing the marginal dose response for the transplant recipient population (solid thick line), the dose response for individual strains with virulence in the 0.01st, 0.1st, 1st, 50th, 99th, 99.9th, and 99.99th percentiles of the virulence distribution (thin lines, from right to left), and the exponential dose-response model for invasive listeriosis based on a butter outbreak in Finland, 1998–1999,[31] as reexamined by FAO/WHO ($r = 3.15 \times 10^{-7}$,[5] p. 34; dashed line).

variability. Despite the progress that has been made in recent years, a better understanding of virulence differences among *L. monocytogenes* strains and, in particular, experimental data evaluating the potential impact of food matrix effects, is clearly needed to further refine *L. monocytogenes* dose-response models.

4.2. Beta-Poisson vs. Lognormal-Poisson Dose Response

The beta distribution was introduced as a pragmatic choice to model the variability in r.[6,39] It offers a great amount of flexibility on the [0; 1] domain,[6] but a mechanistic basis for the choice of beta distributions is lacking. In the case of *L. monocytogenes*, the expected value of r is extremely low when averaging over the general population or even over relatively broadly defined susceptible population subgroups, leading to extremely high values of parameter β. The shape of the beta distribution when used with such extreme parameters does not allow sufficient flexibility, making it impossible to fit the model to certain epidemiological listeriosis data, including the Finnish outbreak data, as illustrated in the Appendix. Therefore, even though the beta-Poisson represents a useful and often-used choice for modeling a number of foodborne pathogens, it appears suboptimal for the unique case of *L. monocytogenes* as evaluated here. Interestingly, if a gamma distribution with $r \sim \text{gamma}(\alpha, 1/\beta)$, with a negligible probability of $r > 1$, would be used to describe r variability, the associated probability of infection would also lead to the beta-Poisson dose-response model (Equation (12)).[8,46] Our result thus suggests that the use of a gamma distribution to model r would

similarly not be suitable for the unique case of *L. monocytogenes* dose response.

We used a lognormal distribution to model variability in host susceptibility and strain virulence, leading to a "lognormal-Poisson" dose-response relationship. Importantly, the resulting lognormal-Poisson dose-response equation does not simplify to a simple mathematical formula and requires numerical integration, thus making the use of this model mathematically more challenging. The domain of the lognormal distribution is defined as $[0; \infty)$. Yet, in this study we found that even for the most susceptible population subgroup (i.e., hematological cancer patients) the probability of r exceeding 1 is estimated at 4.5×10^{-12}, thus in the order of 1 in a trillion, and therefore *de facto* negligible. Because the probability of r exceeding 1 is *de facto* zero, r is theoretically $[0; \infty)$ but practically distributed on the domain $[0; 1]$ in the considered *L. monocytogenes* case. Importantly, this is most likely not true for pathogens other than *L. monocytogenes.* For other foodborne pathogens, the probability of illness after ingestion of a single cell is usually much higher than that for *L. monocytogenes* and the probability of $r > 1$ would be nonnegligible, which would make it incorrect to use the lognormal-Poisson dose response. The lognormal distribution is a heavy-tail distribution. Using heavy-tail distributions is an appropriate modeling assumption if the objective is to describe extreme events such as the ingestion of a highly virulent *L. monocytogenes* strain by a highly susceptible individual. Importantly, the lognormal-Poisson dose-response model was able to predict a well-described outbreak of listeriosis where traditional models of *L. monocytogenes* dose response failed to do so, indicating the potential usefulness of this model.

4.3. Limitations of the Currently Available Data

Whenever possible, health-protective assumptions that would lead to estimating a higher probability of infection for low doses were preferentially chosen in this study. However, the potential impact of some assumptions is more difficult to evaluate than for others. For instance, French data were used as the basis of extrapolations of the expected number of listeriosis cases per population subgroup in the United States. This extrapolation appears appropriate for several reasons. One key finding of the FAO/WHO[5] risk assessment of *L. monocytogenes* in RTE foods is a lack of evidence for differences in the risk of listeriosis after consumption of a given *L. monocytogenes* dose by a member of given population subgroup across countries. Similarly, epidemiological studies have shown that the relative risk of listeriosis for pregnant women appears to be comparable between France and the United States.[26,27] Unfortunately, data on the relative risk of listeriosis is currently lacking for other population subgroups in the United States.[27] It was estimated that for each case of invasive listeriosis, 1.1 cases were not diagnosed in the United States.[1] This figure might be higher in neonatal and elderly cases as compared to other subpopulations.[47] Due to a lack of information, we have not addressed this uncertainty in the partitioning of the total number of cases in the United States among the different population subgroups.

In addition, the French relative size of population subgroups was directly extrapolated to the U.S. population. Even though certain indicators, such as the proportion of individuals with diabetes, are not the same in France and in the United States,[48] some major demographic parameters relevant in this study appear comparable between these countries, such as the proportion of people under 65 year of age, the proportion of people living with cancer, the fertility rates, and life expectancies.[49,50] Actually, the estimation of the relative size of population subgroups in the French study is based on a rigorous, specific, and complicated method designed to avoid duplicated counts.[26] Therefore, it appears preferable to use the French estimates directly rather than further adjusting the estimates to the relative size of U.S. populations with similar comorbidities.

For every risk assessment anchored to human surveillance data—such as our risk assessment presented here—the assumptions used to estimate exposure data highly influence the dose-response model and prediction. If it is estimated that only a small number of bacteria are consumed, any dose-response scaled to epidemiological data will mathematically be shifted to the left (i.e., toward a higher risk at low dose). We used data from Chen *et al.*,[30] which was the most extensive food survey in the United States on record. However, even this large of a study may not capture the true variability in the numbers of *L. monocytogenes* in RTE foods, particularly for the high end of the concentration distribution, and thus may be considered as underestimating exposure. Using these data leads to three implicit assumptions: (i) all bacterial cells consumed

in the population originate from only eight RTE food categories (i.e., fresh soft cheeses, bagged salad, blue veined cheeses, mold ripened cheeses, seafood salads, smoked seafood, luncheon meats, and deli salads) even though other products, such as low acid cut fruits[51,52] or vegetables,[53] could also be nonnegligible sources of *L. monocytogenes*; (ii) no growth is considered to occur between retail and consumption even though postretail growth has been shown to be one important factor increasing the risk for listeriosis[4,5]—these data have the advantage of being actual observed *L. monocytogenes* levels originating from a market basket survey[43] and not relying on predictive modeling that may overestimate the bacterial growth in products; and (iii) the maximal achievable concentration of *L. monocytogenes* in products equals 6.1 $\log_{10}$ cfu/g. This assumption is also underestimating exposure since others assume that *L. monocytogenes* can reach a maximal population density of 8 $\log_{10}$ in a food.[4,5] Altogether, these assumptions lead to an estimated lower exposure compared to other available data sets. In our study, it is estimated that only 120 servings include *L. monocytogenes* levels at or above 10^8 cells each year in the United States; by comparison, the FDA/FSIS[4] report, considering bacterial growth at the consumer step and 23 contaminated products, estimates 70,000,000+ servings at these levels. When tested with a maximum level of *L. monocytogenes* contamination of 8.1 $\log_{10}$ cfu/g, we confirmed the shift of the corresponding dose response to the right: with this maximum level, 0% of the cases would be predicted for a dose of 4 $\log_{10}$ cfu/g. Indeed, the maximum population density in a food has been shown to be an influential parameter for the predicted risk of invasive listeriosis.[54,55] Given the same dose response, the higher the maximum population density, the higher the predicted number of cases.[54,55] In addition, assumption on the maximum population density affects dose-response model parameters based on surveillance data.[5] The FAO/WHO risk assessment of *L. monocytogenes* in RTE foods[5] shows that a shift in the maximum population density by 2 $\log_{10}$ results in approximately one order of magnitude shift in the *r* value. The resulting dose-response presented here may be overestimating the probability of illness from a given dose.

As considered in previous risk assessments,[4,5] the assumption of equal exposure to contaminated food for all population subgroups does not consider the potential effectiveness of prevention campaigns to change behavior of susceptible populations, notably for pregnant women, people with cancer, transplant recipients, for older adults, or for people with diabetes. Reported consumption estimates for certain food types suggests differences do exist in food consumption across population subgroups.[25,56] Nevertheless, the model appeared relatively insensitive to this assumption when tested with an alternative assumption of a lower exposure for the more susceptible subgroups than for "healthy adults." Refinements accounting for differences in consumption habits across population subgroups would improve the current dose-response models. However, such data are currently not available for many of the 11 population subgroups analyzed here.

4.4. Dose-response Evaluation in Highly Susceptible Groups and in Outbreak Situations

For the most susceptible population subgroup (i.e., hematological cancer patients), the marginal probability (i.e., averaged across all strains) of illness following the ingestion of 1 *L. monocytogenes* cell is estimated at 9.5×10^{-9}. It is 9.3×10^{-7} following the ingestion of 100 cells and 7.2×10^{-5} for the ingestion of 10,000 cells (e.g., 100 g of product contaminated with 100 cfu/g). These estimates are considerably higher than the ones estimated by FAO/WHO,[5] averaged over all possible risk factors. The corresponding estimates, using their *r* parameter of 5.85×10^{-12}, would be 5.9×10^{-12}, 5.9×10^{-10}, and 5.9×10^{-8}, respectively, that is, 1,610, 1,576, and 1,220 times lower, respectively.

By characterizing specifically the most susceptible individuals and the most virulent strains in this study, the lognormal-Poisson dose-response analysis reconciles data observed in outbreaks with dose response derived from epidemiological studies, as illustrated Fig. 3. The high fat content of the food vehicle in the Finish butter outbreak (~80% fat) could potentially be partially responsible for this high probability of infection. High fat content in food may actually protect bacteria from gastric acid and, possibly, enhance uptake and survival in host cells *via* interaction with cell membrane lipids.[4,57]

4.5. The Need for Better Data

Assumptions were made in the derivation of this model that lead to higher risk predictions at low dose

(higher predicted marginal probability of illness) compared to previously published dose-response models.[4,5] The estimates presented here should generally be viewed as overestimating the probability of illness. The characterization of the range of the individual susceptibility within groups and of the range of the strain virulence variability should be refined for a better characterization of these dose-response relationships. A mix of illness data from France[26] and the United States,[1] and exposure data obtained in two states from the United States,[30,43] were used, with the underlying assumptions that characteristics of listeriosis would be comparable in these areas. More current and detailed exposure data and data on the relative risk of listeriosis among different population subgroups in the United States are needed to refine this model. The primary purpose of this study was to derive a framework and to test with currently available data; to provide a definitive dose-response model is a secondary goal that would likely require refinements.

5. CONCLUSIONS

The exponential model has the oversimplifying assumption of a constant probability of infection following the ingestion of *L. monocytogenes* in a given population. This study incorporates variability in strain virulence and host susceptibility into the dose-response relationships. Additional data are needed to better understand and model the process from the ingestion of *L. monocytogenes* cells to the development of invasive listeriosis. However, several general conclusions can be made based on the available data. Overall, our model predicts the expected number of cases linked to the consumption of 10,000 cfu or less in 55 out of 1,591 cases, i.e., 3.5% of cases. Notably, these servings are expected to represent 99.96% of all RTE servings, indicating that most cases are expected to be caused by highly contaminated food items. Importantly, however, most of these cases attributable to low contamination doses are predicted to occur in the most highly susceptible population subgroups, including, for example, pregnant women. Using the model and assumptions discussed above led to the conclusion that, while most of the cases are linked to a medium to high exposure doses to *L. monocytogenes*, those at greatest risk of developing listeriosis are also at a measurable risk of illness when consuming food contaminated with relatively low doses of *L. monocytogenes,* especially if highly virulent bacterial strains are involved.

ACKNOWLEDGMENTS

This work was supported, in part, by an appointment to the Research Participation Program at the Center for Food Safety and Applied Nutrition administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the U.S. Department of Energy and the U.S. Food and Drug Administration. We thank Eric Ebel (USDA-FSIS) and the anonymous reviewers for their constructive comments on the first draft of the article.

APPENDIX: DERIVATIONS USING A BETA-POISSON MODEL

If a beta distribution $Be(\alpha, \beta)$ is chosen for f in Equation (2), this integrate leads to the "exact beta-Poisson,"[6]

$$P(\text{ill}; d, \alpha, \beta) = 1 - {}_1F_1(\alpha, \alpha + \beta, -d), \qquad \text{(A.1)}$$

in which ${}_1F_1$ is the Kummer confluent hypergeometric function. Equation (A.1) simplifies to the "beta-Poisson" dose-response model:

$$P(\text{ill}; d, \alpha, \beta) = 1 - \left(1 + \frac{d}{\beta}\right)^{-\alpha}, \qquad \text{(A.2)}$$

when $\beta \gg \alpha$ and $\beta \gg 1$.[7,39] Note that these conditions are expected to be fulfilled for *L. monocytogenes*: the average probability of infection is very low,[30] thus $E[r] = \frac{\alpha}{\alpha+\beta} \ll 1$, leading to $\beta \gg \alpha$ and $\beta \gg 1$.

Assume $Be(\alpha_g, \beta_g)$ accounts for variability in r among *L. monocytogenes* strains and individuals within a given population subgroup g. Contrary to the lognormal distribution, the beta distribution does not easily allow for separation among interstrain and interindividual variability components of this distribution as in Equation (10). An overall (i.e., interindividual and interstrain) measure of the variability in r therefore needs to be estimated. Denote Q_{90}, the $\log_{10}$ of the combined 90% individual susceptibility and strain virulence variability. Q_{90} equals the range between the 5th and the 95th percentile of $Be(\alpha_g, \beta_g)$. Using and combining FDA

FDA/FSIS[4] strain-to-strain virulence variability distributions (Table IV-5 in Ref. 4) and host susceptibility variability (Table IV-7 in Ref. 4) lead to an overall $\log_{10}$ of the inter 5%–95% variability of $Q_{90} = 5.4$ $\log_{10}$.

Equivalently to Equation (9), the subroutine must find (α_g, β_g) solutions of:

$$\begin{aligned} E[c_g] &= \int_0^{\infty} M_{d,g} \left(1 - \left(1 + \frac{d}{\beta_g}\right)^{-\alpha_g}\right) \mathrm{d}d \\ Q_{90} &= \log_{10}(q_{0.95}) - \log_{10}(q_{0.05}) \end{aligned}, \quad \text{(A.3)}$$

with q_x the xth quantile of the $Be(\alpha_g, \beta_g)$ distribution.

The quantile function of beta distributions is not available in a closed form, and a numerical routine is required. Nevertheless, a solution exists for the parameters of a beta distribution given any combination of a lower and an upper quantile constraint.[58] The 11 pairs (α_g, β_g) were evaluated numerically using R optimization subroutines. As expected, the βs were extremely high. Similar αs were obtained for all populations. The parameters for the "healthy adult population" (i.e., the less susceptible subgroup) and the "hematological cancer population" (i.e., the more susceptible subgroup) were $(0.253, 3.86 \times 10^{10})$ and $(0.253, 9.9 \times 10^{7})$, respectively.

A $Be(0.253, 2.3 \times 10^{8})$ was estimated for the "solid organ transplant" population subgroup. With this set of parameters, the probability to obtain a r parameter equal or higher than 3.15×10^{-7}, estimated from the Finnish butter outbreak data,[5,31] equals 2.7×10^{-34}. This extremely low probability proves that the Finnish outbreak cannot be predicted using the beta-Poisson dose-response model, as parameterized here. Fig. A.1 illustrates the density of the underlying beta distribution of the beta-Poisson dose-response model and the underlying lognormal distribution of the lognormal-Poisson dose-response models. The graph clearly illustrates the contrast between the very sharp decrease in the density for the beta distribution compared to the smoother decrease for the lognormal distribution. With such parameters ($\beta \to \infty$), the beta distribution converge to a degenerate distribution with a single point mass at some $x \in [0, 1]$.[58] With parameters estimated from epidemiological data, the beta distribution is not flexible enough to predict r values high enough to explain the Finnish butter outbreak.

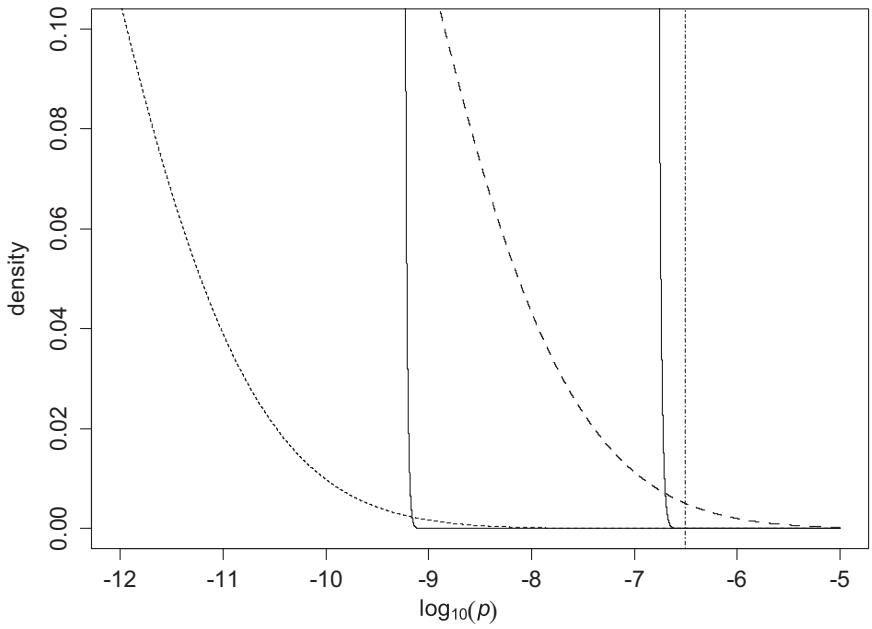


Fig. A.1. 1 Density of r according to the beta-Poisson dose response (plain) or the lognormal-Poisson dose response (dashed) for the healthy population (thin on the left) and the most susceptible population subgroup (hematological cancer population, thick on the right). The values estimated using the Finnish butter outbreak data by FAO/WHO[5] equals 3.15×10^{-7}, that is, $10^{-6.5}$ (dot-dashed vertical line).

REFERENCES

1. Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, Jones JL, Griffin PM. Foodborne illness acquired in the United States—Major pathogens. Emerging Infectious Diseases, 2011; 17(1):7–12.
2. Hoelzer K, Chen Y, Dennis S, Evans P, Pouillot R, Silk BJ, Walls I. New data, strategies, and insights for *Listeria monocytogenes* dose-response models: Summary of an interagency workshop, 2011. Risk Analysis, 2013; 33(9):1568–1581.
3. Hoelzer K, Pouillot R, Dennis S. Animal models of listeriosis: A comparative review of the current state of the art and lessons learned. Veterinary Research, 2012; 43(1):1–27.
4. FDA/FSIS. Quantitative assessment of relative risk to public health from foodborne *Listeria monocytogenes* among selected categories of ready-to-eat foods. Food and Drug Administration, U.S. Department of Agriculture, Centers for Disease Control and Prevention, 2003. Available at: http://www.fda.gov/Food/FoodScienceResearch/RiskSafetyAssessment/ucm183966.htm, Accessed March 20, 2014.
5. FAO/WHO. Risk assessment of *Listeria monocytogenes* in ready to eat foods—Technical report. Rome: Food and Agriculture Organization of the United Nations and World Health Organization Report No.: Microbiological Risk Assessment Series 5; 2004. Available at: http://www.fao.org/docrep/010/y5394e/y5394e00.htm, Accessed March 20, 2014.
6. Haas CN, Rose JB, Gerba CP. Quantitative Microbial Risk Assessment. New York: Wiley, 1999.
7. Teunis PF, Havelaar AH. The Beta Poisson dose-response model is not a single-hit model. Risk Analysis, 2000; 20(4):513–520.
8. Schmidt PJ, Pintar KD, Fazil AM, Topp E. Harnessing the theoretical foundations of the exponential and Beta-Poisson dose-response models to quantify parameter uncertainty using Markov chain Monte Carlo. Risk Analysis, 2013; 33(9):1677–1693.
9. Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, Griffin PM, Tauxe RV. Food-related illness and death in the United States. Emerging Infectious Diseases, 1999; 5(5):607–625.
10. FDA/FSIS. Draft assessment of the relative risk to public health from foodborne *Listeria monocytogenes* among selected categories of ready-to-eat foods. Food and Drug Administration, U.S. Department of Agriculture, Centers for Disease Control and Prevention, 2001.
11. Ross T, Rasmussen S, Fazil A, Paoli G, Summer J. Quantitative risk assessment of *Listeria monocytogenes* in ready-to-eat meats in Australia. International Journal of Food Microbiology, 2009; 131(2–3):128–137.
12. Sanaa M, Coroller L, Cerf O. Risk assessment of listeriosis linked to the consumption of two soft cheeses made from raw milk: Camembert of Normandy and Brie of Meaux. Risk Analysis, 2004; 24(2):389–399.
13. Pouillot R, Goulet V, Delignette-Muller ML, Mahe A, Cornu M. Quantitative risk assessment of *Listeria monocytogenes* in French cold-smoked salmon: II. Risk characterization. Risk Analysis, 2009; 29(6):806–819.
14. Gallagher D, Ebel ED, Gallagher O, Labarre D, Williams MS, Golden NJ, Pouillot R, Dearfield KL, Kause J. Characterizing uncertainty when evaluating risk management metrics: Risk assessment modeling of *Listeria monocytogenes* contamination in ready-to-eat deli meats. International Journal of Food Microbiology, 2013; 162(3):266–275.
15. Jacquet C, Doumith M, Gordon JI, Martin PM, Cossart P, Lecuit M. A molecular marker for evaluating the pathogenic potential of foodborne *Listeria monocytogenes*. Journal of Infectious Diseases, 2004; 189(11):2094–2100.
16. Nightingale KK, Ivy RA, Ho AJ, Fortes ED, Njaa BL, Peters RM, Wiedmann M. *inlA* premature stop codons are common among *Listeria monocytogenes* isolates from foods and yield virulence-attenuated strains that confer protection against fully virulent strains. Applied and Environmental Microbiology, 2008; 74(21):6570–6583.
17. Nightingale KK, Windham K, Martin KE, Yeung M, Wiedmann M. Select *Listeria monocytogenes* subtypes commonly found in foods carry distinct nonsense mutations in *inlA*, leading to expression of truncated and secreted internalin A, and are associated with a reduced invasion phenotype for human intestinal epithelial cells. Applied and Environmental Microbiology, 2005; 71(12):8764–8772.
18. Ward TJ, Evans P, Wiedmann M, Usgaard T, Roof SE, Stroika SG, Hise K. Molecular and phenotypic characterization of *Listeria monocytogenes* from U.S. Department of Agriculture Food Safety and Inspection Service surveillance of ready-to-eat foods and processing facilities. Journal of Food Protection, 2010; 73:861–869.
19. Bonazzi M, Lecuit M, Cossart P. *Listeria monocytogenes* internalin and E-cadherin: From bench to bedside. Cold Spring Harbor Perspectives in Biology, 2009; 1(4):1–5.
20. Bonazzi M, Lecuit M, Cossart P. *Listeria monocytogenes* internalin and E-cadherin: From structure to pathogenesis. Cellular Microbiology, 2009; 11(5):693–702.
21. Lecuit M. Understanding how *Listeria monocytogenes* targets and crosses host barriers. Clinical Microbiology and Infection, 2005; 11(6):430–436.
22. Lecuit M, Vandormael-Pournin S, Lefort J, Huerre M, Gounon P, Dupuy C, Babinet C, Cossart P. A transgenic model for listeriosis: Role of internalin in crossing the intestinal barrier. Science, 2001; 292(5522):1722–1725.
23. Van Stelten A, Simpson JM, Chen Y, Scott VN, Whiting RC, Ross WH, Nightingale KK. Significant shift in median guinea pig infectious dose shown by an outbreak-associated *Listeria monocytogenes* epidemic clone strain and a strain carrying a premature stop codon mutation in *inlA*. Applied and Environmental Microbiology, 2011; 77(7):2479–2487.
24. Chen Y, Ross WH, Whiting RC, Van Stelten A, Nightingale KK, Wiedmann M, Scott VN. Variation in *Listeria monocytogenes* dose responses in relation to subtypes encoding a full-length or truncated internalin A. Applied and Environmental Microbiology, 2011; 77(4):1171–1180.
25. Silk BJ, Date KA, Jackson KA, Pouillot R, Holt KG, Graves LM, Ong KL, Hurd S, Meyer R, Marcus R, Shiferaw B, Norton DM, Medus C, Zansky SM, Cronquist AB, Henao OL, Jones TF, Vugia DJ, Farley MM, Mahon BE. Invasive listeriosis in the Foodborne Diseases Active Surveillance Network (FoodNet), 2004–2009: Further targeted prevention needed for higher-risk groups. Clinical Infectious Diseases, 2012; 54 (Suppl 5):S396–404.
26. Goulet V, Hebert M, Hedberg C, Laurent E, Vaillant V, De Valk H, Desenclos JC. Incidence of listeriosis and related mortality among groups at risk of acquiring listeriosis. Clinical Infectious Diseases, 2012; 54(5):652–660.
27. Pouillot R, Hoelzer K, Jackson KA, Henao OL, Silk BJ. Relative risk of listeriosis in Foodborne Diseases Active Surveillance Network (FoodNet) sites according to age, pregnancy, and ethnicity. Clinical Infectious Diseases, 2012; 54(Suppl 5):S405–410.
28. Mook P, O'Brien SJ, Gillespie IA. Concurrent conditions and human listeriosis, England, 1999–2009. Emerging Infectious Diseases, 2011; 17(1):38–43.
29. Buchanan RL, Havelaar AH, Smith MA, Whiting RC, Julien E. The key events dose-response framework: Its potential for application to foodborne pathogenic microorganisms. Critical Reviews in Food Science and Nutrition, 2009; 49(8):718–728.

30. Chen Y, Ross WH, Scott VN, Gombas DE. *Listeria monocytogenes:* Low levels equal low risk. Journal of Food Protection, 2003; 66(4):570–577.
31. Lyytikainen O, Autio T, Maijala R, Ruutu P, Honkanen-Buzalski T, Miettinen M, Hatakka M, Mikkola J, Anttila VJ, Johansson T, Rantala L, Aalto T, Korkeala H, Siitonen A. An outbreak of *Listeria monocytogenes* serotype 3a infections from butter in Finland. Journal of infectious Diseases, 2000; 181(5):1838–1841.
32. Bollaerts K, Aerts M, Faes C, Grijspeerdt K, Dewulf J, Mintiens K. Human salmonellosis: Estimation of dose-illness from outbreak data. Risk Analysis, 2008; 28(2):427–440.
33. FAO/WHO. Risk Assessment of *Salmonella* in eggs and broiler chickens. Technical report. Rome: Food and Agriculture Organization of the United Nations and World Health Organization Report No.: Microbiological Risk Assessment Series 3; 2002. Available at: http://www.fao.org/DOCREP/005/Y4392E/Y4392E00.HTM, Accessed March 10, 2014.
34. Teunis PFM, Kasuga F, Fazil A, Ogden ID, Rotariu O, Strachan NJC. Dose response modeling of *Salmonella* using outbreak data. International Journal of Food Microbiology, 2010; 144(2):243–249.
35. Teunis P, Van den Brandhof W, Nauta M, Wagenaar J, Van den Kerkhof H, Van Pelt W. A reconsideration of the *Campylobacter* dose-response relation. Epidemiology and Infection, 2005; 133(4):583–592.
36. Teunis PF, Moe CL, Liu P, Miller SE, Lindesmith L, Baric RS, Le Pendu J, Calderon RL. Norwalk virus: How infectious is it? Journal of Medical Virology, 2008; 80(8):1468–1476.
37. Thebault A, Teunis PF, Le Pendu J, Le Guyader FS, Denis JB. Infectivity of GI and GII noroviruses established from oyster related outbreaks. Epidemics, 2013; 5(2):98–110.
38. Rocourt J. Risk factors for listeriosis. Food Control, 1996; 7(4–5):195–202.
39. Furumoto WA, Mickey R. A mathematical model for the infectivity-dilution curve of tobacco mosaic virus: Theoretical considerations. Virology, 1967; 32(2):216–223.
40. Teunis PF, Nagelkerke NJ, Haas CN. Dose response models for infectious gastroenteritis. Risk Analysis, 1999; 19(6):1251–1260.
41. Haas CN, Thayyar- Madabusi A, Rose JB, Gerba CP. Development and validation of dose-response relationship for *Listeria monocytogenes*. Quantitative Microbiology, 1999; 1(1):89–102.
42. Goulet V, Marchetti P. Listeriosis in 225 non-pregnant patients in 1992: Clinical aspects and outcome in relation to predisposing conditions. Scandinavian Journal of Infectious Diseases, 1996; 28(4):367–374.
43. Gombas DE, Chen Y, Clavero RS, Scott VN. Survey of *Listeria monocytogenes* in ready-to-eat foods. Journal of Food Protection, 2003; 66(4):559–569.
44. R Development Core Team. R: A language and environment for statistical computing 2008 [cited March 20, 2012]. Available at: http://www.R-project.org, Accessed March 20, 2014.
45. Cossart P, Pizarro-Cerda J, Lecuit M. Invasion of mammalian cells by *Listeria monocytogenes*: Functional mimicry to subvert cellular functions. Trends in Cell Biology, 2003; 13(1): 23–31.
46. Moran PA. The dilution assay of viruses. Journal of Hygiene, 1954; 52(2):189–193.
47. Painter J, Slutsker L. Listeriosis in humans. Pp. 85–109 in Ryser ET, Marth EH (eds). *Listeria*, Listeriosis and Food Safety. 3rd ed. Boca Raton: CRC Press, 2007.
48. CDC. 2011 National Diabetes Fact Sheet 2011 [cited January 17, 2014]. Available at: http://www.cdc.gov/diabetes/pubs/pdf/ndfs˙2011.pdf, Accessed March 20, 2014.
49. American Cancer Society. Cancer Facts & Figures 2012. Atlanta: American Cancer Society, 2012. Available at: http://www.cancer.org/acs/groups/content/@epidemiologysurveilance/documents/document/acspc-031941.pdf, Accessed March 20, 2014.
50. World Bank. Indicators 2014 [cited January 17, 2014]. Available at: http://data.worldbank.org/indicator, Accessed March 20, 2014.
51. Anonymous. Multistate outbreak of listeriosis associated with Jensen Farms cantaloupe—United States, August–September 2011. Morbidity and Mortality Weekly Report, 2011; 60(39):1357–1358.
52. Varma JK, Samuel MC, Marcus R, Hoekstra RM, Medus C, Segler S, Anderson BJ, Jones TF, Shiferaw B, Haubert N, Megginson M, McCarthy PV, Graves L, Gilder TV, Angulo FJ. *Listeria monocytogenes* infection from foods prepared in a commercial establishment: A case-control study of potential sources of sporadic illness in the United States. Clinical Infectious Diseases, 2007; 44(4):521–528.
53. Gaul LK, Farag NH, Shim T, Kingsley MA, Silk BJ, Hyytia-Trees E. Hospital-acquired listeriosis outbreak caused by contaminated diced celery—Texas, 2010. Clinical Infectious Diseases, 2013; 56(1):20–26.
54. Ellouze M, Gauchi JP, Augustin JC. Global sensitivity analysis applied to a contamination assessment model of *Listeria monocytogenes* in cold smoked salmon at consumption. Risk Analysis, 2010; 30(5):841–852.
55. Pouillot R, Lubran MB. Predictive microbiology models *vs*. modeling microbial growth within *Listeria monocytogenes* risk assessment: What parameters matter and why. Food Microbiology, 2011; 28(4):720–726.
56. Hoelzer K, Pouillot R, Egan K, Dennis S. Produce consumption in the United States: An analysis of consumption frequencies, serving sizes, processing forms, and high-consuming population subgroups for microbial risk assessments. Journal of Food Protection, 2012; 75(2):328–340.
57. Coleman M, Marks H. Topics in dose-response modeling. Journal of Food Protection, 1998; 61(11):1550–1559.
58. van Dorp JR, Mazzuchi TA. Solving for the parameters of a beta distribution under two quantile constraints. Journal of Statistical Computation and Simulation, 2000; 67(2): 189–201.

Journal of Food Protection, Vol. 79, No. 5, 2016, Pages 696–702
doi:10.4315/0362-028X.JFP-15-442

Fate of *Listeria monocytogenes* in Fresh Apples and Caramel Apples

JOELLE K. SALAZAR,[1] CHRISTINA K. CARSTENS,[1] VRIDDI M. BATHIJA,[2] SARTAJ S. NARULA,[2] MICKEY PARISH,[3] AND MARY LOU TORTORELLO[1]*

[1]*U.S. Food and Drug Administration, Division of Food Processing Science and Technology, Bedford Park, Illinois 60501;* [2]*Institute for Food Safety and Health, Illinois Institute of Technology, Bedford Park, Illinois 60501; and* [3]*U.S. Food and Drug Administration, Office of Food Safety, College Park, Maryland 20740, USA*

MS 15-442: Received 1 October 2015/Accepted 7 January 2016

ABSTRACT

An outbreak of listeriosis in late 2014 and early 2015 associated with caramel apples led to questions about how this product became a vector for *Listeria monocytogenes*. This investigation aimed to determine information about the survival and growth of *L. monocytogenes* in both fresh apples and caramel apples, specifically examining the effects of site and level of inoculation, inoculum drying conditions, and storage temperature. At a high inoculation level (7 log CFU per apple), *L. monocytogenes* inoculated at the stem end proliferated on Gala caramel apples at both 5 and 25°C and on Granny Smith caramel apples at 25°C by as much as 3 to 5 log CFU per apple. Fresh apples and caramel apples inoculated at the equatorial surface supported survival but not growth of the pathogen. Growth rates (μ_{max}) for apples inoculated at the stem end, as determined using the Baranyi and Roberts growth model, were 1.64 ± 0.27 and 1.38 ± 0.20 log CFU per apple per day for Gala and Granny Smith caramel apples, respectively, stored at 25°C. At a low inoculation level (3 log CFU per apple), *L. monocytogenes* inoculated at the stem end and the equatorial surface survived but did not grow on fresh Gala and Granny Smith apples stored at 25°C for 49 days; however, on caramel apples inoculated at the stem end, *L. monocytogenes* had significant growth under the same conditions. Although certain conditions did not support growth, the pathogen was always detectable by enrichment culture. The inoculation procedure had a significant effect on results; when the inoculum was allowed to dry for 24 h at 5°C, growth was significantly slowed compared with inoculum allowed to dry for 2 h at 25°C. Variation in stick materials did affect *L. monocytogenes* survival, but these differences were diminished once sticks were placed into caramel apples.

Key words: Caramel apples; Fresh apples; Growth kinetics; *Listeria monocytogenes*; Survival

Listeria monocytogenes has caused outbreaks of listeriosis that have been associated with consumption of meats, dairy products, and fresh vegetables, but few documented cases of listeriosis have been linked to fresh fruits. In the United States, *L. monocytogenes* was first involved in an outbreak associated with fresh fruit, specifically cantaloupe, in 2011 *(9)*. A total of 147 illnesses, 142 hospitalizations, and 33 deaths were attributed to this outbreak. Another unusual fruit-linked outbreak of listeriosis occurred in late 2014 and early 2015, and the vector was commercially produced prepackaged caramel apples. This outbreak resulted in a total of 35 illnesses in 12 states and included 34 hospitalizations and seven deaths; the Public Health Agency of Canada also reported one associated case *(10)*. Of the illnesses, 11 were pregnancy related, 1 of which resulted in fetal loss. Of the 31 ill individuals interviewed, 28 reported eating commercially produced prepackaged caramel apples before becoming ill *(10)*. The other three individuals who did not report eating caramel apples did report consuming sliced or whole apples. Caramel apples from three manufacturers were implicated, and further investigation led to one apple grower-packer as the source of the apples. Fresh Gala and Granny Smith apples from the apple grower-packer were shipped either to retailers for direct consumption or to manufacturers to be processed into caramel apples. Only caramel apples, not fresh apples, were associated with the outbreak. The pathogen was isolated from environmental swab samples taken in the storage room and from food contact surfaces at the apple grower-packer facility. Commercial apple contamination by *L. monocytogenes* resulted in recalls of packaged fresh-cut apples in 2001 *(18, 25)* and of packaged apple slices in 2015 *(8)*, although no illnesses were associated with either recall.

How the caramel apples became a vector for the listeriosis outbreak is not known. Apples are not an adequate medium for proliferation of this pathogen because of their low pH (<4.0) *(5)*. *L. monocytogenes* cannot penetrate into the flesh through the peel unless scars or cuts are already present on the apple surface *(4)*. Application of the hot molten caramel during the manufacture of caramel apples provides a thermal impediment to bacterial survival.

* Author for correspondence. Tel: 708-924-0645; Fax: 708-924-0690; E-mail: mary.tortorello@fda.hhs.gov.

TABLE 1. L. monocytogenes *populations on inoculated fresh apples (without sticks) stored at 5 or 25°C*[a]

Temp (°C)	Apple variety	Inoculation location	Mean ± SD log CFU/apple at[b]: 0 days	1 day	2 days	6 days	9 days	15 days
5	Gala	Stem end	5.7 ± 0.6	5.4 ± 0.8	6.1 ± 0.7	4.4 ± 0.8	5.9 ± 1.0	5.5 ± 0.6
		Surface	2.7 ± 0.3	2.7 ± 0.3	BE[c]	BE	4.7 ± 0.8	BE
25	Gala	Stem end	5.7 ± 0.6	5.4 ± 0.7	5.8 ± 0.7	3.8 ± 0.7	3.9 ± 0.7	3.9 ± 0.5
		Surface	2.7 ± 0.3	BE	BE	BE	5.7 ± 1.5	BE
25	Granny Smith	Stem end	6.7 ± 1.1	5.2 ± 0.9	BE	6.9 ± 1.7	6.0 ± 1.5	2.9 ± 0.5
		Surface	4.8 ± 0.8	BE	BE	BE	5.1 ± 0.8	3.3 ± 0.6

[a] Initial inoculation was 6.9 ± 0.6 log CFU per apple.
[b] Values are means for $n = 6$.
[c] BE, below sensitivity of place count assay (2.5 log CFU per apple). In all cases, *L. monocytogenes* was detectable by enrichment culture.

Current practices for caramel apple production may involve selection of apples based on the manufacturer's specifications, cleaning of apples using water washing, brushing, and sanitizing, dipping and coating the apples with molten caramel, drying, and packaging of the completed caramel apples. After packaging, the finished products may or may not enter the cold chain during transport to retailers, where they are stored at ambient temperature awaiting consumer purchase.

This study was conducted to determine potential factors in caramel apple production that may have influenced the survivability and growth of *L. monocytogenes*. Factors included contamination level, site of contamination, apple variety, storage temperature, presence or absence of caramel coating, and stick material.

MATERIALS AND METHODS

***L. monocytogenes* strains and culture conditions.** Three clinical outbreak isolates of *L. monocytogenes* (573-035, 576-043, and 580-060) from patients with listeriosis associated with the 2014 caramel apple outbreak were kindly provided by the Wisconsin State Laboratory of Hygiene (Madison, WI). All strains were serotype 4b with GX6A16.0012 pulsed-field gel electrophoresis *Asc*I patterns *(24)*. All strains were grown separately in brain heart infusion (BD, Sparks, MD) broth at 37°C for 16 to 18 h with shaking at 200 rpm.

Apple selection and experimental design. Whole fresh waxed Gala apples and Granny Smith apples were purchased from local retail supermarkets. Apples with obvious bruising or cuts were discarded. Average weights of apples used for experiments were 178.9 ± 7.2 and 177.0 ± 10.1 g for Gala and Granny Smith apples, respectively. Experimental variables included temperature during storage (5 and 25°C), inoculation level (10^7 or 10^3 CFU per apple), inoculation site (equatorial surface and stem end), inoculum drying conditions (5°C for 24 h or 25°C for 2 h), and caramel coating with wood stick insertion. Apples were prepared in triplicate for each variable for each timepoint of 0, 1, 2, 6, 9, and 15 days for Gala apples stored at 5 and 25°C and Granny Smith apples stored at 25°C and for each timepoint of 0, 7, 14, 21, 28, 35, 42, and 49 days for Gala and Granny Smith apples stored at 25°C. For 5°C storage studies, variables were stem end inoculation, equatorial surface inoculation, stem end inoculation with caramel coating and stick, and equatorial surface inoculation with caramel coating and stick. For 25°C storage studies, an additional variable was stick material (plastic, paper, or wood). For each caramel apple experiment, uninoculated control apples consisting of caramel coating and stick were assayed for pH and spoilage. All experiments were conducted in two independent trials.

***L. monocytogenes* inoculation of apples.** Overnight cultures of *L. monocytogenes* strains were normalized, washed with Butterfield's phosphate buffer (BPB; pH 7.4), and combined equally to make a cocktail of approximately 9 or 5 log CFU/ml. Apples were inoculated at the stem end or along the equatorial surface by pipetting 10 μl of the *L. monocytogenes* cocktail to yield final levels of 6.9 ± 0.6 or 3.1 ± 0.2 log CFU per apple, as determined by plate count assay of the cocktail on PALCAM (BD) agar. The inoculum was dried for 2 h at 25°C or for 24 h at 5°C. The *L. monocytogenes* population recovered from apples after drying and with or without caramel was approximately 2 log CFU lower than the initial inoculum (data not shown).

Preparation of caramel apples. A wood stick typically used for making caramel apples was inserted approximately 3 to 4 cm into the stem end of each apple prior to caramel coating. Where indicated for some experiments, paper or plastic sticks also were used. Caramel pieces (containing corn syrup, sugar, milk, fructose, hydrogenated coconut oil, butter, mono- and diglycerides, salt, soy lecithin, and vanillin; inherent water activity of 0.66) were purchased from local retailers and melted to 76°C in a 4211c Twin Caramel Apple Dip Warmer according to the manufacturer's instructions (Gold Medal Products Co., Bensenville, IL). Temperature was monitored with a candy thermometer inserted into the caramel. For apples on which inoculum had dried at 5°C for 24 h, apples were equilibrated to room temperature prior to dipping. Apples were dipped manually into the caramel so that approximately 3 cm of the stick was covered. Excess caramel was allowed to drip off, and the apples were placed onto wax paper to dry at ambient temperature for 2 h. After drying, all apples, with or without caramel, were placed into food-grade clamshell containers for storage at 5 or 25°C for various time periods. Fresh apple and caramel apple trials were conducted concurrently.

Enumeration of *L. monocytogenes* from apples. At the appropriate time intervals, apples were taken out of clamshells and placed into 3-liter stomacher bags. Visual and odor changes in apples were recorded. Apples were smashed five to seven times with a rubber mallet, 350 ml of buffered *Listeria* enrichment broth (BLEB, BD) was added, and the mixture was stomached for 1 min at 180 rpm in a stomacher (model 3500, Seward Laboratory Systems Inc., Davie, FL). BLEB was chosen because of its superior capacity to neutralize the acid from the apples and maintain the pH at approximately 7.0. A 10-ml sample of the

TABLE 2. L. monocytogenes *populations and growth kinetics on inoculated caramel apples stored at 5 or 25°C*[a]

Temp (°C)	Apple variety	Inoculation location	Mean ± SD log CFU/apple at:						Growth kinetics[b]		
			0 days	1 day	2 days	6 days	9 days	15 days	μ_{max} ± SE	r^2	Time to 1-log increase (h)
5	Gala	Stem end	5.4 ± 0.8	5.7 ± 0.8	7.0 ± 1.0	8.0 ± 1.3	8.4 ± 1.7	8.5 ± 2.2	0.95 ± 0.21 A	0.80	26.2 ± 0.2
		Stem end, dried[c]	3.3 ± 0.4	2.9 ± 0.3	BE[d]	3.5 ± 0.4	6.5 ± 1.3	6.4 ± 1.6	0.80 ± 0.31 A	0.81	172.8 ± 0.6
		Surface	4.7 ± 0.6	4.8 ± 0.6	4.5 ± 0.8	BE	4.4 ± 0.8	BE	ND[e]	ND	ND
25	Gala	Stem end	5.4 ± 0.8	5.6 ± 1.3	7.9 ± 2.0	9.5 ± 1.6	10.0 ± 2.5	9.6 ± 1.6	1.64 ± 0.27 B	0.89	14.9 ± 0.1
		Stem end, dried[c]	3.3 ± 0.4	BE	6.8 ± 2.4	7.8 ± 1.3	8.1 ± 1.4	8.6 ± 1.5	0.85 ± 0.19 B	0.85	29.3 ± 0.3
		Surface	4.7 ± 0.6	4.7 ± 0.6	4.4 ± 0.5	4.6 ± 0.5	4.4 ± 0.8	BE	0.38 ± 0.23 B	0.23	287.5 ± 2.3
25	Granny Smith	Stem end	5.5 ± 0.9	7.2 ± 1.2	8.1 ± 1.2	8.6 ± 1.4	8.7 ± 1.4	8.9 ± 1.5	1.38 ± 0.20 C	0.91	17.6 ± 0.2
		Stem end, dried[c]	4.4 ± 0.6	5.2 ± 0.9	6.8 ± 1.1	8.4 ± 1.4	8.5 ± 1.2	8.8 ± 1.5	1.19 ± 0.17 C	0.95	20.4 ± 0.2
		Surface	6.3 ± 1.1	4.6 ± 0.7	BE	BE	6.9 ± 1.2	6.4 ± 1.1	0.15 ± 0.09 C	0.11	219.2 ± 11.6

[a] Initial inoculation was 6.9 ± 0.6 log CFU per apple. Values are means for $n = 6$.
[b] μ_{max} ± SE, mean maximum growth rate (log CFU per apple per day) ± standard error. Means with different letters are significantly different ($P < 0.05$) for comparisons of the same inoculation locations on different apple varieties at both temperatures; r^2, coefficient of determination.
[c] Inoculum placed at the stem end was dried at 5°C for 24 h.
[d] BE, below sensitivity of plate count assay (2.5 log CFU per apple). In all cases, *L. monocytogenes* was detectable by enrichment culture.
[e] ND, not determined.

homogenate was placed into a 15-ml tube. Serial dilutions in BLEB were spread plated in duplicate onto PALCAM agar. For timepoints at which *Listeria* was expected to be below the sensitivity of the plate count assay of 3 log CFU/ml, duplicate 1-ml aliquots of homogenates were plated over three PALCAM agar plates to increase the assay sensitivity to 2.5 log CFU/ml. PALCAM plates were incubated at 37°C for 48 h. All apple homogenates in BLEB were also used for enrichment cultures. These cultures were incubated at 30°C for 4 h, supplements were added, and the cultures were incubated again at 30°C for 24 h. When no growth was present on enumeration plates, the BLEB enrichment cultures were streaked onto PALCAM plates and tested for the presence of *L. monocytogenes* using the *Listeria* Visual Immunoprecipitate Assay (BioControl Systems Inc., Bellevue, WA) according to the manufacturer's directions.

Enumeration of native microbiota from apples. Populations of native microbiota on apples were monitored at each timepoint using the control apples (no inoculation, with caramel coating and stick). Apples were stomached as previously described, and the homogenates were serially diluted and plated in duplicate onto Dichloran Rose Bengal (DRBA) and deMan Rogosa Sharpe (MRS) agars (BD) for enumeration of presumptive yeasts and molds and lactic acid bacteria, respectively. DRBA plates were incubated at 25°C for 48 h, and MRS plates were incubated anaerobically at 37°C for 72 h before enumeration.

Apple pH. Apple pH was monitored at each timepoint using the control apples (no inoculation, with caramel coating and stick). pH was measured using a PH/ORP waterproof pH spear (Oakton Instruments, Vernon Hills, IL) by inserting the tip of the spear into the stem end of the apple and allowing the pH reading to equilibrate for 2 min.

***L. monocytogenes* survival on various stick materials.** Overnight cultures of *L. monocytogenes* strains were normalized, washed with BPB, and combined equally to make a cocktail of approximately 9 log CFU/ml. Wood, paper, and plastic sticks (14 to 15 cm long) were each inoculated with five 2-μl spots, yielding a final inoculation of 7.0 ± 0.7 log CFU per stick. Sticks were stored in sterile containers at 5 or 25°C. At 0, 2, 5, 7, and 15 days, triplicate samples of each type of stick material were placed into sterile 1.2-liter stomacher bags with 100 ml of BLEB, massaged by hand for 1 min, and then stomached at 180 rpm for 1 min. A 10-ml sample of this homogenate was transferred to a 15-ml tube, and serial dilutions in BLEB were plated in duplicate onto PALCAM agar, which were incubated at 37°C for 48 h.

Modeling. The DMFit version 3.0 (Institute of Food Research, Norwich, UK) Excel (Microsoft, Redmond, WA) add-on from ComBase (www.combase.cc) was used to model the maximum growth rates (μ_{max}) and lag phases of the *L. monocytogenes* cocktail based on the Baranyi and Roberts *(3)* model. The value at time 0 was the *L. monocytogenes* recovered from the fresh apples (after inoculum drying) or from the caramel apples (after 2 h of drying of caramel). Calculation of μ_{max} was based on the *L. monocytogenes* recovered at different timepoints relative to time 0. Growth of *L. monocytogenes* on the apples was defined by the calculation of a positive growth rate using DMFit. Survival of the pathogen was defined by the detection of the pathogen after enrichment culture. Linear regression analysis with the μ_{max} values was used to determine the time to achieve 1 log CFU of growth, assuming no lag phase, at each condition and temperature.

TABLE 3. L. monocytogenes *populations on inoculated caramel apples stored long term at 25°C*[a]

Apple variety	Inoculation location	Mean ± SD log CFU/apple at[b]: 0 days	7 days	14 days	21 days	28 days	35 days	42 days	49 days
Gala	Stem end	BE[c]	8.6 ± 1.4	8.8 ± 1.0	9.1 ± 1.8	9.3 ± 1.6	8.8 ± 1.8	8.0 ± 4.0	9.4 ± 3.1
	Stem end, dried[d]	BE	6.7 ± 1.4	8.2 ± 1.1	9.8 ± 1.7	8.3 ± 1.4	7.6 ± 1.3	6.6 ± 3.3	7.5 ± 2.4
	Surface	BE	BE	BE	BE	BE	BE	BE	BE
Granny Smith	Stem end	BE	8.9 ± 1.3	8.5 ± 1.4	9.1 ± 1.5	8.8 ± 1.4	7.5 ± 1.3	8.2 ± 1.0	8.4 ± 1.4
	Stem end, dried[d]	BE	7.9 ± 1.6	8.0 ± 1.0	9.1 ± 1.5	7.7 ± 1.3	7.4 ± 2.5	6.9 ± 3.3	7.7 ± 3.7
	Surface	BE	BE	2.9 ± 0.5	BE	BE	BE	BE	BE

[a] Initial inoculation was 3.1 ± 0.2 log CFU per apple.
[b] Values are means for $n = 6$.
[c] BE, below sensitivity of plate count assay (2.5 log CFU per apple). In all cases, *L. monocytogenes* was detectable by enrichment culture.
[d] Inoculum placed on the stem end was dried at 5°C for 24 h.

Statistical analysis. Data were statistically evaluated using Tukey's adjusted one-way analysis of variance using GraphPad InStat for Windows. A P value of less than 0.05 was considered significant.

RESULTS

***L. monocytogenes* survival on fresh apples.** At an initial inoculation level of 7 log CFU per apple, *L. monocytogenes* inoculated both at the stem end and on the equatorial surface survived on Gala apples stored at both 5 and 25°C and on Granny Smith apples stored at 25°C; however, populations decreased by approximately 1 to 4 log CFU per apple (Table 1). Although *L. monocytogenes* levels were below the sensitivity of the plate count assay (2.5 log CFU per apple) at various timepoints during storage in these experiments, the pathogen was still present as determined by enrichment culture (data not shown).

At the initial inoculation level of 3 log CFU per apple, *L. monocytogenes* inoculated at the stem end and on the surface did not produce detectable growth on fresh Gala or Granny Smith apples stored at 25°C for 49 days. At most of the timepoints, the population of *L. monocytogenes* was below the sensitivity of the plate count assay. Nevertheless, the pathogen survived on the fresh apples, as determined by enrichment culture (data not shown).

***L. monocytogenes* survival and growth on caramel apples.** At an initial inoculation level of 7 log CFU per apple, *L. monocytogenes* inoculated both at the stem end and on the surface were capable of surviving and at times growing on Gala caramel apples stored at 5 and 25°C and on Granny Smith caramel apples stored at 25°C (Table 2). On Gala caramel apples inoculated at the stem end stored at 5°C, the population of *L. monocytogenes* increased by nearly 3 log CFU after 15 days of incubation. On the surface-inoculated Gala caramel apples stored at 5°C, *L. monocytogenes* remained nearly at initial inoculation levels or decreased to below the sensitivity of the place count assay; however, the presence of the pathogen was detectable by enrichment culture. The highest μ_{max} value for Gala caramel apples stored at 5°C was found for *L. monocytogenes* inoculated at the stem end, 0.95 log CFU per apple per day, leading to a 1-log increase in only 26.18 h (Table 2).

During storage at 25°C, *L. monocytogenes* inoculated at the stem end at 7 log CFU per apple had 3- to 4-log increases in population on both Gala and Granny Smith caramel apples (Table 2). The μ_{max} value was 1.64 log CFU per apple per day on the stem end-inoculated Gala caramel apples, and 1.38 log CFU per apple per day on Granny Smith caramel apples, leading to a 1-log increase in just 17.58 h. Surface-inoculated caramel apples stored at 5 and 25°C had similar results; at the various timepoints, the populations appeared to be nearly at the initial inoculation levels or were below the assay sensitivity limit (Tables 2 and 3). At weeks 6 and 7 of storage at 25°C, the quality of both the fresh apples and the caramel apples was poor, with many of the apples exhibiting surface mold growth.

For *L. monocytogenes* inoculated at 3 log CFU per apple at the stem end and stored at 25°C, approximately 7- to 8-log increases were found on both Gala and Granny Smith caramel apples (Table 3). Because of insufficient data, an accurate growth rate could not be determined for these trials; nevertheless, the increase in population within week 1 of storage was substantial. Surface-inoculated *L. monocytogenes* levels were below the sensitivity of the assay except for one timepoint (Granny Smith, 14 days); however, in all cases the pathogen was detectable by enrichment culture.

Effect of inoculum drying conditions on *L. monocytogenes* populations on caramel apples. In all cases, drying of the inoculum at 5°C for 24 h resulted in lower μ_{max} values and longer times to achieve a 1-log increase in population compared with drying of the inoculum for 2 h at ambient temperature (25°C; compare "stem end, dried" and "stem end," respectively, in Table 2). On Gala apples inoculated with 7 log CFU per apple at the stem end and dried for 2 h at ambient temperature before the addition of a stick and dipping in caramel, *L. monocytogenes* had a μ_{max} value of 0.95 log CFU per apple per day during storage at 5°C; DMFit did not predict a lag phase. However, the apples for which inocula were dried for 24 h at 5°C and then stored at 5°C, DMFit did predict a lag phase (5.8 days) and a significantly lower μ_{max} value (0.80 log CFU per apple per day). Therefore, the *L. monocytogenes* population on the apples in which the inoculum was dried for 2 h at 25°C increased by 1 log CFU in only 26.2 h, compared with 172.8

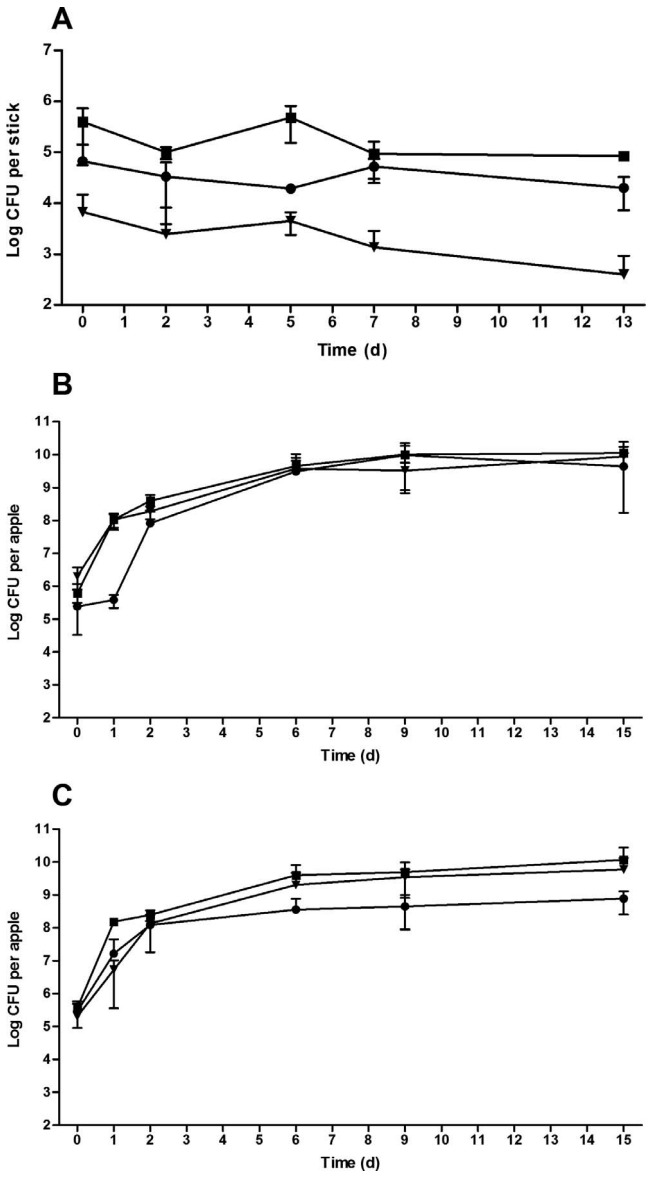


FIGURE 1. *Survival of* L. monocytogenes *on stick materials (A) during storage at 5°C: paper (filled square), wood (filled circle), and plastic (filled triangle). Each data point represents the mean ± SD log CFU per stick (*n = 6*).* L. monocytogenes *survival and growth on Gala (B) and Granny Smith (C) caramel apples with different stick materials during storage at 25°C. Apples were inoculated at the stem end, and a wood stick (closed circle), paper stick (closed square), or plastic stick (closed triangle) was inserted. Each data point represents mean ± SD log CFU per apple (*n = 6*). The sensitivity of the assay was 2.5 log CFU per apple.*

h on the apples that were dried for 24 h at 5°C. Although no other lag-phase values were determined by DMFit for apples inoculated at the stem end and dried for 24 h at 5°C, all the μ_{max} values were significantly lower than those for apples dried for 2 h at 25°C. However, the final population levels at the end of the storage periods were often similar for apples under both inoculum drying scenarios.

***L. monocytogenes* survival on stick materials.** In a comparison of *L. monocytogenes* survival on three caramel apple stick materials (paper, wood, and plastic), survival was significantly better on paper and wood than on plastic (Fig. 1A). After initial inoculation with 7 log CFU per stick, an approximately 1- to 2-log decrease occurred on both paper and wood sticks. A significantly greater decrease, i.e., approximately 3 log CFU, occurred on plastic sticks. After 13 days of incubation at 5°C, overall populations on paper and wood sticks did not decrease significantly, whereas the population on plastic sticks decreased by approximately 1 log CFU. Even though differences in *L. monocytogenes* survival on different stick materials were observed, these differences were diminished when the sticks were used in the preparation of caramel apples (Fig. 1B and 1C). Small but significant differences ($P < 0.05$) in growth of the pathogen on Gala caramel apples stored at 25°C were found when wood, paper, and plastic sticks were used for caramel apple preparation (Table 4). A 3- to 4-log increase in populations of *L. monocytogenes* on Gala and Granny Smith apples was found after 15 days compared with initial levels.

TABLE 4. L. monocytogenes *growth kinetics on caramel apples with wood, paper, or plastic sticks during storage at 25°C for 15 days*[a]

Stick material	Apple variety	$\mu_{max} \pm$ SE[b]	r^2	Time to 1 log CFU growth (h)
Wood	Gala	1.64 ± 0.27 A a	0.89	14.9 ± 0.1
	Granny Smith	1.38 ± 0.20 B a	0.91	17.6 ± 0.2
Paper	Gala	1.40 ± 0.24 A b	0.77	17.4 ± 0.1
	Granny Smith	1.23 ± 0.28 B b	0.71	20.2 ± 0.2
Plastic	Gala	1.02 ± 0.14 A c	0.87	23.9 ± 0.1
	Granny Smith	1.25 ± 0.300 B b	0.73	20.0 ± 0.2

[a] $\mu_{max} \pm$ SE, mean maximum growth rate (log CFU per apple per day) ± standard error; r^2, coefficient of determination.
[b] Means with different uppercase letters are significantly different ($P < 0.05$) for comparisons of different apple varieties with the same stick material. Means with different lowercase letters are significantly different ($P < 0.05$) for comparisons of the same apple variety with different stick materials.

Native microbiota. Populations of certain native microbiota (yeasts, molds, and lactic acid bacteria) that were monitored throughout the storage experiments increased during storage at 25°C for both apple varieties. At 5°C, only the yeast and mold populations increased (Fig. 2). A correlation could not be made between native microflora populations and pH changes in the apples during the storage periods.

DISCUSSION

Although the listeriosis outbreak evaluated here is the first to be attributed to whole caramel apples, studies have shown that *L. monocytogenes* is capable of both surviving and growing on raw fruits such as whole and cut melons *(13, 14, 20, 23, 27),* melon pulp *(26),* cut pears *(1, 11, 23),* and whole and cut berries *(1, 23).* This pathogen also can proliferate on fresh-cut apple slices when contamination occurs after processing procedures, such as peeling and cutting *(2, 4, 12).* For example, the *L. monocytogenes* population on whole Red Delicious apples increased by 0.6 log CFU per apple slice after 7 days of storage at 10°C *(18).*

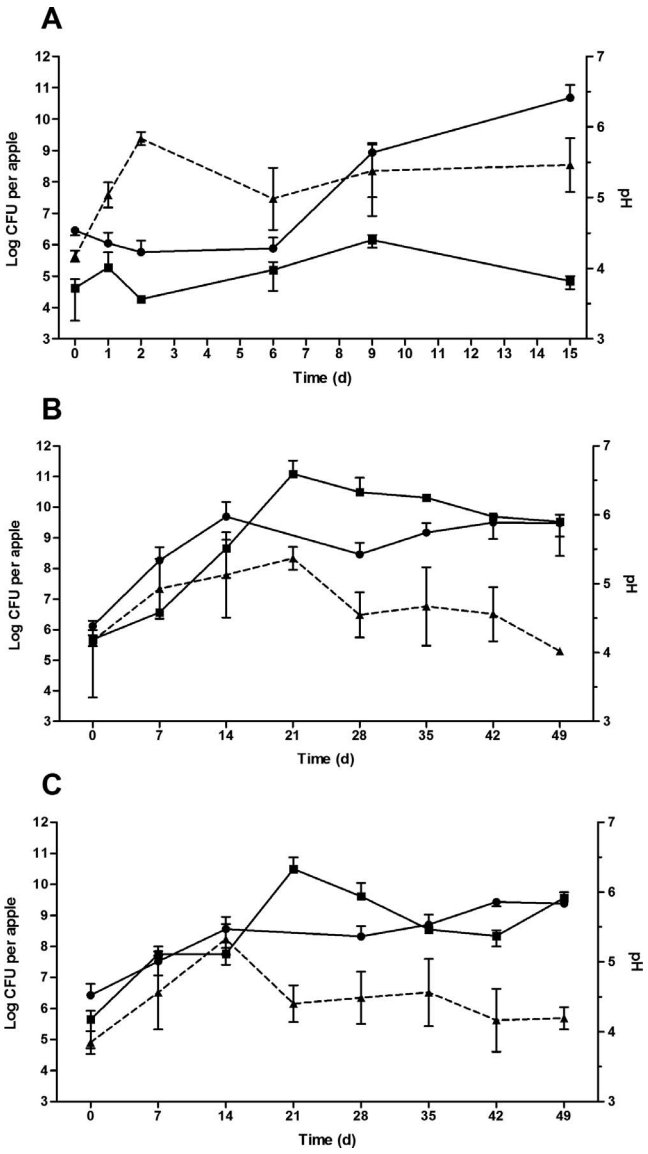


FIGURE 2. *Comparison of pH (closed triangle, dotted line) and native microflora populations (yeasts and molds, closed circle; lactic acid bacteria, closed squares) on (A) control (no inoculation, with caramel coating and stick insertion) Gala apples stored at 5°C for 15 days; (B) control Gala apples stored at 25°C for 49 days, and (C) control Granny Smith apples stored at 25°C for 49 days. Each data point represents mean ± SD log CFU per apple* (n = *6). Sensitivity of the assay was 2.5 log CFU per apple.*

Listeria innocua (a nonpathogenic surrogate for *L. monocytogenes*) increased on Granny Smith and Golden Delicious apple plugs by 2 log CFU per plug after 2 days when stored at either 20 or 25°C; at 10°C, *L. innocua* increased by 2.4 log CFU per plug after 6 days *(2)*. In addition to the increase in *L. monocytogenes* populations, the levels of general microbiota on apples can also increase during storage *(17)*. The results of the present study revealed increases in populations of lactic acid bacteria and yeasts and molds. Yeasts may aid in growth of *L. monocytogenes* and other microorganisms on caramel apples because of their saccharolytic interactions with caramel and apple sugars *(19)*.

In the present study, *L. monocytogenes* inoculation at the stem end of the apple followed by stem end stick insertion and caramel coating including 2 to 3 cm of stick resulted in an environment in which this pathogen was able to both survive and grow. The interface between the stem end of the apple and the caramel layer may produce a microenvironment with high water activity and high nutrient (apple and apple juices produced from the insertion of the stick) and sugar (caramel) concentrations *(16)*. Specific microenvironments of multicomponent foods can affect the survival and thermal behavior of pathogens such as *Salmonella enterica (21)*. In the present study, the apples were submerged in the molten caramel just long enough for the apple and approximately 2 to 3 cm of the stick to be completely covered. *L. monocytogenes* residing in the microenvironment of the stem end of the apple would be exposed to molten caramel at 71 to 88°C in a caramel apple manufacturing plant during the dipping process. The length of time the pathogen is in contact with the caramel could determine, in part, pathogen survival *(15)*. In the present study, some survival curves were highly variable, possibly because of inconsistencies in exposure of the pathogen to the molten caramel. For example, the most inconsistent recovery of *L. monocytogenes* was occurred with surface-inoculated caramel apples. Variable exposure of the pathogen to the thermal effects of the molten caramel could be attributed to location of the inoculum on the apple surface, the temperature of the caramel coating, and the amount of caramel applied, all of which may have produced different microenvironments for the pathogen. The most consistent trends observed in this study occurred with caramel apples inoculated at the stem end. In these apples, some inoculum cells may have been partially protected from thermal exposure by being pushed inside the apple during stick insertion. Nevertheless, the data indicate that manufacturers should not consider hot caramel dip a lethality step sufficient to reduce or eliminate the risk of *L. monocytogenes* contamination on caramel apples.

The mechanism of contamination may be a factor influencing pathogen survival. We studied the effects of two inoculation procedures to mimic two hypothetical contamination scenarios, i.e., contamination prior to cold storage (inoculum drying for 24 h at 5°C) and contamination as a short event at ambient temperature (inoculum drying for 2 h at 25°C). In this study, drying at 5°C slowed the growth of *L. monocytogenes* on caramel apples. In all cases, the inoculum dried for 24 h at 5°C always had a slower growth rate and took longer to achieve a 1-log increase (Table 2). Therefore, for conservative growth models and risk assessments, a 25°C inoculum drying time may be used for data generation. In all of the experiments the *L. monocytogenes* populations may be 2-log higher than indicated because of the efficiency of recovery (see "Materials and Methods"); thus, data depicting the final population levels may be estimated at approximately 2-log higher than the values actually recorded.

During washing of fresh apples, the stem and blossom ends are more difficult to clean than are the smooth surfaces, which is a significant problem *(6, 7, 22)*. Postharvest processing procedures for fresh apples include washes with sanitizers such as chlorine to reduce the total microbial load and to eliminate pathogenic organisms such as *L. monocytogenes*. Once a wound is introduced at the stem end via the

insertion of a stick during caramel apple manufacture, microorganisms may invade the core or flesh and proliferate. This scenario may explain the growth of *L. monocytogenes* during the recent caramel apple outbreak. The Baranyi and Roberts *(3)* model used in this study determined values for growth rate, lag phase, and length of time for a 1-log increase in *L. monocytogenes* on the apples (Table 2). Apples were not washed prior to the experiments to ensure that native microflora remained and would interact with the pathogen in a realistic manner. The data provided a conservative model prediction of time to a 1-log increase in population and risk assessment for *L. monocytogenes* survival and growth on the apples. Apple variety and choice of stick material did not play significant biological roles in the growth of this pathogen on caramel apples. These results provide a starting point for the development of guidelines for caramel apple manufacturers on the safe handling practices of fresh apples and caramel apple products. Many questions remain with respect to potential preventive control options for caramel apple production, which may ultimately depend on the mechanisms by which contamination occurs.

ACKNOWLEDGMENTS

The authors sincerely thank Dr. Kathleen Glass for helpful discussions, Tim Monson (Wisconsin State Laboratory of Hygiene) for providing outbreak-associated *L. monocytogenes* strains, Karl Reineke and Travis Morrissey for help with acquiring apples for experiments, Dr. Yun Wang for laboratory support, and Dr. Don Zink for motivational guidance. This work was supported by grant U19FD005322 from the U.S. Food and Drug Administration to the Illinois Institute of Technology. J. K. Salazar and C. K. Carstens were supported by a Oak Ridge Institute for Science and Education Research Participation Program grant to the U.S. Food and Drug Administration. The sponsors had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

REFERENCES

1. Adhikari, A., R. M. Syamaladevi, K. Killinger, and S. S. Sablani. 2015. Ultraviolet-C light inactivation of *Escherichia coli* O157:H7 and *Listeria monocytogenes* on organic fruit surfaces. *Int. J. Food Microbiol.* 210:136–142.
2. Alegre, I., M. Abadias, M. Anguera, M. Oliveira, and I. Viñas. 2010. Factors affecting growth of foodborne pathogens on minimally processed apples. *Food Microbiol.* 27:70–76.
3. Baranyi, J., and T. A. Roberts. 1994. A dynamic approach to predicting bacterial growth in food. *Int. J. Food Microbiol.* 23:277–294.
4. Beuchat, L. R. 1996. *Listeria monocytogenes*: incidence on vegetables. *Food Control* 7:223–228.
5. Beuchat, L. R. 2002. Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. *Microbes Infect.* 4:413–423.
6. Buchanan, R. L., S. G. Edelson, R. L. Miller, and G. M. Sapers. 1999. Contamination of intact apples after immersion in an aqueous environment containing *Escherichia coli* O157:H7. *J. Food Prot.* 62:444–450.
7. Burnett, S. L., J. Chen, and L. R. Beuchat. 2000. Attachment of *Escherichia coli* O157:H7 to the surfaces and internal structures of apples as detected by confocal scanning laser microscopy. *Appl. Environ. Microbiol.* 66:4679–4687.
8. Canadian Food Inspection Agency. 2015, Food recall warning—sliced apples and products containing sliced apples recalled due to *Listeria monocytogenes*. Available at: http://www.inspection.gc.ca/about-the-cfia/newsroom/food-recall-warnings/complete-listing/20150429b/eng/1430375161334/1430375167258. Accessed 17 September 2015.
9. Centers for Disease Control and Prevention. 2011. Multistate outbreak of listeriosis associated with Jensen Farms cantaloupe—United States, August–September 2011. *Morb. Mortal. Wkly. Rep.* 60:1357–1358.
10. Centers for Disease Control and Prevention. 2015. Multistate outbreak of listeriosis linked to commercially produced, prepackaged caramel apples made from Bidart Bros. apples (final update). Available at: http://www.cdc.gov/listeria/outbreaks/caramel-apples-12-14/. Accessed 19 March 2015.
11. Colás-Medà, P., M. Abadias, I. Alegre, J. Usall, and I. Viñas. 2015. Effect of ripeness stage during processing on *Listeria monocytogenes* growth on fresh-cut 'Conference' pears. *Food Microbiol.* 49:116–122.
12. Conway, W. S., B. Lewerents, and R. A. Saftner. 2000. Survival and growth of *Listeria monocytogenes* on fresh-cut apple slices and its interaction with *Glomerella cingulata* and *Penicillium expansum*. *Plant Dis.* 84:177–181.
13. Danyluk, M. D., L. M. Friedrich, and D. W. Schaffner. 2014. Modeling the growth of *Listeria monocytogenes* on cut cantaloupe, honeydew and watermelon. *Food Microbiol.* 38:52–55.
14. Fang, T., Y. Liu, and L. Huang. 2013. Growth kinetics of *Listeria monocytogenes* and spoilage microorganisms in fresh-cut cantaloupe. *Food Microbiol.* 34:174–181.
15. Farber, J. M. 1989. Thermal resistance of *Listeria monocytogenes* in foods. *Int. J. Food Microbiol.* 8:285–291.
16. Glass, K. A., M. C. Golden, B. J. Wanless, W. Bedale, and C. Czuprynski. 2015. Growth of *Listeria monocytogenes* within a caramel-coated apple microenvironment. *MBio* 6(5):e01232-15.
17. Leff, J. W., and N. Fierer. 2013. Bacterial communities associated with the surfaces of fresh fruits and vegetables. *PLoS One* 8:e59310.
18. Leverentz, B., W. S. Conway, M. J. Camp, W. J. Janisiewicz, T. Abuladze, M. Yang, R. Saftner, and A. Sulakvelidze. 2003. Biocontrol of *Listeria monocytogenes* on fresh-cut produce by treatment with lytic bacteriophages and a bacteriocin. *Appl. Environ. Microbiol.* 69:4519–4526.
19. Leverentz, B., W. S. Conway, W. Janisiewicz, M. Abadias, C. P. Kurtzman, and M. J. Camp. 2006. Biocontrol of the food-borne pathogens *Listeria monocytogenes* and *Salmonella enterica* serovar Poona on fresh-cut apples with naturally occurring bacterial and yeast antagonists. *Appl. Environ. Microbiol.* 72:1135–1140.
20. Leverentz, B., W. S. Conway, W. Janisiewicz, and M. J. Camp. 2004. Optimizing concentration and timing of a phage spray application to reduce *Listeria monocytogenes* on honeydew melon tissue. *J. Food Prot.* 67:1682–1686.
21. Li, H., X. Fu, Y. Bima, J. Koontz, C. Megalis, F. Yang, G. Fleischman, and M. L. Tortorello. 2014. Effect of the local microenvironment on survival and thermal inactivation of *Salmonella* in low- and intermediate-moisture multi-ingredient foods. *J. Food Prot.* 77:67–74.
22. Li-Cohen, A. E., and C. M. Bruhn. 2002. Safety of consumer handling of fresh produce from the time of purchase to the plate: a comprehensive consumer survey. *J. Food Prot.* 65:1287–1296.
23. Molinos, A. C., H. Abriouel, N. Ben Omar, R. Lucas, E. Valdivia, and A. Gálvez. 2008. Inactivation of *Listeria monocytogenes* in raw fruits by enterocin AS-48. *J. Food Prot.* 71:2460–2467.
24. Monson, T. (Wisconsin State Laboratory of Hygiene). 2015. Personal communication.
25. Ozturk, I., F. Tornuk, O. Sagdic, and O. Kisi. 2012. Application of non-linear models to predict inhibition effects of various plant hydrosols on *Listeria monocytogenes* inoculated on fresh-cut apples. *Foodborne Pathog. Dis.* 9:607–616.
26. Penteado, A. L., and M. F. Leitão. 2004. Growth of *Listeria monocytogenes* in melon, watermelon and papaya pulps. *Int. J. Food Microbiol.* 92:89–94.
27. Rodgers, S. L., J. N. Cash, M. Siddiq, and E. T. Ryser. 2004. A comparison of different chemical sanitizers for inactivating *Escherichia coli* O157:H7 and *Listeria monocytogenes* in solution and on apples, lettuce, strawberries, and cantaloupe. *J. Food Prot.* 67:721–731.

Growth of *Listeria monocytogenes* within a Caramel-Coated Apple Microenvironment

Kathleen A. Glass, Max C. Golden, Brandon J. Wanless, Wendy Bedale, Charles Czuprynski

Food Research Institute, University of Wisconsin—Madison, Madison, Wisconsin, USA

ABSTRACT **A 2014 multistate listeriosis outbreak was linked to consumption of caramel-coated apples, an unexpected and previously unreported vehicle for *Listeria monocytogenes*. This outbreak was unanticipated because both the pH of apples (<4.0) and the water activity of the caramel coating (<0.80) are too low to support *Listeria* growth. In this study, Granny Smith apples were inoculated with approximately 4 $\log_{10}$ CFU of *L. monocytogenes* (a cocktail of serotype 4b strains associated with the outbreak) on each apple's skin, stem, and calyx. Half of the apples had sticks inserted into the core, while the remaining apples were left intact. Apples were dipped into hot caramel and stored at either 7°C or 25°C for up to 11 or 28 days, respectively. Data revealed that apples with inserted sticks supported significantly more *L. monocytogenes* growth than apples without sticks under both storage conditions. Within 3 days at 25°C, *L. monocytogenes* populations increased >3 $\log_{10}$ in apples with sticks, whereas only a 1-$\log_{10}$ increase was observed even after 1 week for caramel-coated apples without sticks. When stored at 7°C, apples with sticks exhibited an approximately 1.5-$\log_{10}$ increase in *L. monocytogenes* levels at 28 days, whereas no growth was observed in apples without sticks. We infer that insertion of a stick into the apple accelerates the transfer of juice from the interior of the apple to its surface, creating a microenvironment at the apple-caramel interface where *L. monocytogenes* can rapidly grow to levels sufficient to cause disease when stored at room temperature.**

IMPORTANCE **Neither caramel nor apples are a food where the pathogenic bacterium *Listeria monocytogenes* should grow, as caramel does not contain enough free water and apples are too acidic. Caramel-coated apples, however, were recently linked to a deadly outbreak of listeriosis. We hypothesized that inserting a stick into the apple releases juice to the interface between the apple and caramel, providing a more hospitable environment than either component alone. To test this hypothesis, apples were inoculated with *L. monocytogenes* prior to caramel dipping. Some apples had sticks inserted into them before dipping, while others did not. No growth of *L. monocytogenes* occurred on refrigerated caramel apples without sticks, whereas slow growth was observed on refrigerated caramel apples with sticks. In contrast, significant pathogen growth was observed within 3 days at room temperature on caramel apples with sticks inserted. Food producers should consider interfaces between components within foods as potential niches for pathogen growth.**

Received 21 July 2015 **Accepted** 14 September 2015 **Published** 13 October 2015

Citation Glass KA, Golden MC, Wanless B, Bedale W, Czuprynski C. 2015. Growth of *Listeria monocytogenes* within a caramel-coated apple microenvironment. mBio 6(5): e01232-15. doi:10.1128/mBio.01232-15.

Editor Stefan H. E. Kaufmann, Max Planck Institute for Infection Biology

Address correspondence to Kathleen Glass, kglass@wisc.edu.

This article is a direct contribution from a Fellow of the American Academy of Microbiology.

The 2014 caramel apple listeriosis outbreak infected 35 people across the United States and one additional person in Canada; seven deaths were reported, with listeriosis directly causing three of the deaths (1, 2). The outbreak took producers, public health officials, and food safety experts by surprise: caramel-coated apples are not a food on which *Listeria monocytogenes* should grow. First, the pH of apples is too low (usually <4.0) to support growth of *L. monocytogenes* (3). Second, the caramel coating used on apples both is hot (~95°C) and has low water activity, usually <0.80 (4), and most *L. monocytogenes* strains require water activity (a_w) of at least 0.93 for growth (5). Although *Listeria* spp. are common in the produce fields (6), there are no surveys that suggest that *L. monocytogenes* is a pathogen routinely associated with apples (7). Additionally, intact apples have not been implicated previously in foodborne disease outbreaks (8), with one exception due to an unknown etiological agent (9).

The epidemiological association with caramel apples was strong, as 28 of the 31 persons interviewed reported eating them (2). Three additional patients sickened with the outbreak strains did not remember eating caramel apples but did recall eating whole or sliced green apples from an unknown source (1). At least three different caramel apple manufacturers were involved in the outbreak, although the apples were sourced from a single common apple producer. *Listeria monocytogenes* isolates from environmental samples collected from that apple producer's facility matched isolates from persons sickened in the outbreak, as determined by using whole-genome sequencing (2). These findings strongly suggested the *L. monocytogenes* originated on the apples

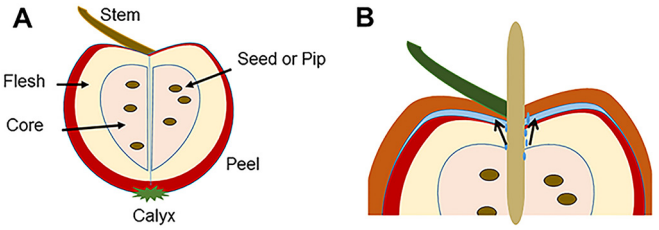


FIG 1 Key parts of the apple (A) and the caramel-apple interface microenvironment (B).

but left unanswered how the pathogen multiplied on caramel-coated apples.

L. monocytogenes is thought to have an infectious dose of about 10^5 to 10^7 CFU in high-risk individuals (10, 11). As noted above, the pathogen is common in the environment, including in soils, pastures, and decaying vegetation, and can colonize food processing plants as well. Strains that cause foodborne disease tend to be particularly adept at biofilm formation (12), making them especially difficult to eliminate in the environment once established. Importantly, *L. monocytogenes* has the ability to multiply at refrigeration temperatures.

We hypothesized that the caramel layer on the apple traps moisture next to the surface, creating a microenvironment on the surface of the apple that facilitates growth of *L. monocytogenes* cells that are already present on the apple surface (Fig. 1A). Insertion of the stick may expedite juice migrating to the surface of the apple, increasing the water activity in or just below the caramel layer. Although caramel-coated apples are typically distributed under refrigeration conditions, they may be unrefrigerated for 2 to 4 weeks by retailers or consumers. Storage at nonrefrigeration temperatures can accelerate both moisture migration and microbial growth.

Listerial growth on caramel-coated apples. To test our hypothesis, three separate caramel apple growth trials were conducted, with three apples tested for each set of conditions and time point in each trial (a total of 144 apples assayed in the study). The results reported are the means and standard errors of enumeration data across all trials. We prepared a cocktail of four *L. monocytogenes* strains associated with the outbreak (all serotype 4b and described further in "*Listeria monocytogenes* inoculum preparation" below). Apples (as purchased, without any additional sanitation procedures or removal of wax) were inoculated on the skin, stem, and calyx regions (Fig. 1A) with an average of 4.2 ± 0.7 $\log_{10}$ CFU per apple. A wooden stick was inserted through the stem of half of the apples. The other apples did not receive a stick. Dipping the apples into the hot caramel (95°C) resulted in an immediate reduction of ~0.8 to 1.2 $\log_{10}$ *L. monocytogenes* per apple. Coated apples were allowed to cool and then stored at 25°C or 7°C. On caramel apples with sticks, the mean populations of *L. monocytogenes* increased an average 3.6 $\log_{10}$ CFU by day 3 when apples were stored at room temperature (25°C) and remained at least 3.4 $\log_{10}$ CFU above baseline for the duration of the study (Fig. 2). In contrast, listerial growth was delayed on caramel apples without sticks, with populations increasing an average 0.3, 1.5, and 2.1 $\log_{10}$ CFU above baseline by days 3, 7, and 11, respectively. Levels of *L. monocytogenes* growth on caramel-coated apples without sticks were statistically significantly different from those on apples without sticks ($P < 0.05$).

Reducing the storage temperature to 7°C slowed *L. monocytogenes* growth on caramel apples, especially in the absence of sticks. No *L. monocytogenes* growth was observed on caramel apples

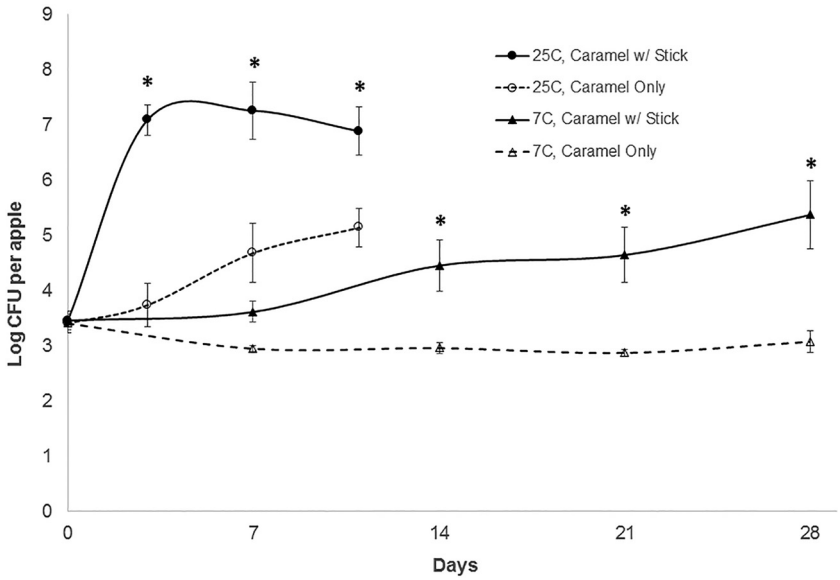


FIG 2 Changes in populations of *L. monocytogenes* in inoculated caramel-coated apples, with and without stick penetration, stored at 7 and 25°C for up to 28 days. Data are means and standard errors from three separate trials, with three apples per variable at each time interval ($n = 9$); a total of 144 apples were assayed for the data presented. Asterisks indicate values that are statistically significantly different ($P < 0.05$) from corresponding values for apples without sticks. After 3 days at 25°C, *L. monocytogenes* levels were statistically significantly different from baseline levels ($P < 0.05$) in caramel apples with a stick. In contrast, for caramel apples without sticks, *L. monocytogenes* levels did not become statistically significantly different from baseline levels until 11 days at 25°C. At 7°C, *L. monocytogenes* levels in apples with sticks did not become statistically significantly different from baseline until 28 days. In caramel apples without sticks at 7°C, no change in *L. monocytogenes* levels was observed at any time point compared to baseline.

without sticks during 4 weeks of storage at 7°C (Fig. 2). When caramel apples were penetrated with sticks and stored at 7°C, no growth was detected at 1 week, but populations increased 1.0, 1.2, and 1.9 $\log_{10}$ CFU per apple above baseline at 2, 3, and 4 weeks, respectively (Fig. 2). No *L. monocytogenes* growth (~0.4-log reduction) was observed on inoculated, uncoated apples stored at 7°C for 21 days (data not shown).

These data are consistent with the hypothesis that *L. monocytogenes* can grow in the microenvironment between the apple surface and caramel coating of contaminated caramel-coated apples that are stored at room temperature. We hypothesize that transpiration of moisture across the cuticle occurs during long-term storage of apples and that the moisture is trapped under the caramel coating, increasing the localized a_w even in the absence of a stick. *L. monocytogenes* growth was greater in apples into which a stick was inserted. Juice from the apple is expressed when the stick initially penetrates the apple core, and liquid may continue to migrate to the surface along the region where the stick was inserted (Fig. 1B) during storage. This increased amount of liquid could further raise the a_w under the caramel coating. The low pH of the juice is likely neutralized by the caramel during equilibration, resulting in conditions conducive to growth of *L. monocytogenes*.

Although we did not yet test whether *L. monocytogenes* grows on the surface of uncoated apples following stick insertion, the apple juice transported to the apple surface would evaporate quickly. This would restore a low a_w to the surface that would be unsuitable for bacterial growth. The use of wax coating on the apple reduces dehydration of the apple during storage. Wax (e.g., carnauba-shellac wax) itself does not have antimicrobial activity against *L. monocytogenes* or *Escherichia coli* O157:H7 *in vitro* (13); however, lower populations of total bacteria, molds, and yeast were recovered from waxed apples than unwaxed apples throughout 5 months of storage at 1°C (13). Therefore, using unwaxed apples may not alter the growth rate of *L. monocytogenes* on the caramel-coated apples.

In addition, we hypothesize that some *L. monocytogenes* cells harbored in the stem area might be pushed into the core when the stick is inserted, where these bacterial cells would be protected from the heat of the caramel. Liquid could carry surviving *L. monocytogenes* cells to the surface, where they would be trapped under the caramel in a region where the local a_w might be sufficient for listerial growth. Both moisture transfer (which is trapped under the caramel layer) and microbial growth are accelerated at room temperature compared to refrigeration.

We chose regions of the apple surface (calyx, stem, and peel areas) for inoculation because intact apples rarely harbor bacteria within the flesh (7), and the stem and calyx regions are common harborage sites for microbes on apples (14, 15). We also focused on these regions for microbial collection from the caramel apples by immersing them in buffer and massaging the caramel off the apple. *L. monocytogenes* present in this wash buffer was then enumerated. It is unlikely that *L. monocytogenes* was also present within the flesh of the fruit because of the surface inoculation method used in our study. In addition, the pH of the apple flesh used in our experiments was measured to be 3.2, and growth of *L. monocytogenes* below pH 4.0 has not been reported (16). A previous study reported *L. monocytogenes* inactivation in pH 3.4 apple juice but growth in Red Delicious apples slices (pH 4.7) stored at 10 or 20°C (17). Both Granny Smith and Gala apples were implicated in the 2014 listeriosis outbreak, but Granny Smith apples were chosen for these experiments because their exceptionally low pH represents a steeper hurdle for bacterial growth (3).

It is possible that other parts of the apple, such as the core or seeds, also hosted *L. monocytogenes* growth. These parts of the apple are not typically eaten completely, but may be bitten into by consumers. The pH of the core region of Granny Smith apples used in these experiments was not measured, but in other apple varieties, the core region pH may be 0.6 to 0.8 units higher than that in the apple flesh (18, 19). Future experiments are planned to investigate whether *L. monocytogenes* growth occurs in the core region.

It is unknown whether the strains of *L. monocytogenes* from this disease outbreak possess unusual resistance to low pH or exceptional virulence. Additional studies are in progress to determine the minimum pH for growth of the outbreak strains in laboratory media and apple juice and to determine if the addition of antimicrobials to the caramel dip can inhibit listerial growth. All outbreak strains tested were able to form biofilms, invade, and multiply within the human adenocarcinoma cell line Caco-2 and exhibit virulence in an established mouse model (N. G. Faith and C. Czuprynski, unpublished data), comparable to that of a different *L. monocytogenes* strain implicated in another significant foodborne disease outbreak (20).

The level of *L. monocytogenes* that was recovered from the surface of the apples following caramel dipping (3 to 3.4 $\log_{10}$ CFU per apple) represents a level that could potentially be found on produce. A review of 165 prevalence studies found a 0.17% probability for *L. monocytogenes* to be present on a fresh or minimally processed vegetable at 3 $\log_{10}$ CFU/g (21). Following 3 days of incubation at 25°C, some individual caramel apples with sticks had levels of *L. monocytogenes* as high as 7 $\log_{10}$ CFU/apple, which is sufficient to cause disease if the product is consumed by a susceptible individual.

Conclusions. Our findings suggest that the 2014 listeriosis outbreak associated with caramel-coated apples can be explained by growth of *L. monocytogenes* occurring at the interface between two foods which, by themselves, are inhibitory to pathogen growth. If *L. monocytogenes* was present on or in the apple after coating with hot caramel, the typical extended storage at ambient temperature by the retailer, and perhaps the consumer, would be sufficient to allow the pathogen to grow to infectious levels. The insertion of the stick into the apples increased the growth rate of *L. monocytogenes* in caramel-coated apples, likely by enhancing the moisture migration to the caramel-apple interface and accelerating the development of optimal growth conditions. One might suggest eliminating the stick; however, this could hinder both production and consumption of the product and therefore may not be a useful strategy for the caramel apple industry. Practical intervention strategies might include validated disinfection of the apple, addition of growth inhibitors to the caramel coating or apple wax, or temperature-time controls to inhibit growth of *L. monocytogenes* on caramel apples.

***Listeria monocytogenes* inoculum preparation.** A four-strain mixture of *L. monocytogenes* clinical isolates was used in this study. The inoculum was composed of three strains from the 2014 caramel apple outbreak (573-035, 576-043, and 580-060; all serotype 4b) plus one additional strain (548-072, also a serotype 4b strain) that was not considered responsible for an outbreak case but matched the pulsed-field gel electrophoresis (PFGE) patterns of

the outbreak strains (provided by the Wisconsin State Laboratory of Hygiene, Madison, WI). Stocks of these strains were maintained in ceramic beads (CRYO/M; Copan Diagnostics Inc., Murrieta, CA) stored at −80°C. For inoculum preparation, each individual strain bead was cultured in 10 ml of fresh Trypticase soy broth (TSB; Becton, Dickinson and Company, Sparks, MD, USA) at 37°C for 20 to 24 h. The freshly grown culture (0.1 ml) was further transferred into 10 ml of fresh TSB and incubated at 37°C for 18 to 22 h. Cells were harvested by centrifugation (4,000 × *g*, 20 min) and suspended in 4.5 ml 0.1% buffered peptone water (pH 7.1 ± 0.1). Equivalent populations of each isolate were combined to provide a four-strain mixture of *L. monocytogenes*. Purity and populations of each strain were verified by plating on Trypticase soy agar (TSA) and modified Oxford agar (MOX; *Listeria* selective agar base; Difco, BD Biosciences, Sparks, MD).

Inoculated apple preparation and testing. Waxed Granny Smith apples (1.4-kg bags) and commercially prepared caramel apple dip (ingredients included high-fructose corn syrup, skim milk, corn syrup, palm oil, sugar, butter, modified corn starch, disodium phosphate, potassium sorbate, tert-butylhydroquinone, salt, mono- and diglycerides, and artificial flavors) were purchased from a local retailer. The pH of the apple flesh (skin removed) was 3.2, and the a_w was 0.98; the caramel apple dip had a measured a_w of 0.79 and a pH of 5.85. Apples with obvious damage/bruising were not used for these experiments. Granny Smith apples were chosen for tests because this variety was implicated in the listeriosis outbreak and because their high acidity represents a higher barrier for microbial growth.

In order to simulate/prepare *L. monocytogenes*-contaminated apples, 200 μl of *L. monocytogenes* cocktail was pipetted into the bottom calyx of the apple (~22°C). The inoculum was allowed to stand for 2 min; the residual volume was removed by pipette and applied to the stem region and allowed to sit for another 2 min; finally, the residual volume was applied over the surface of the apple using a sterile cotton swab. Apples were then divided into two groups; for one set of apples, wooden sticks (either flat sticks, 11.4 cm long by 0.95 cm wide by 0.2 cm high, or round sticks, 14 cm long by 0.6 cm in diameter; there was no difference in growth rates among apples with different stick dimensions) were inserted approximately 5 cm into the core region from the stem side, whereas no sticks were inserted into the second set of apples. The sticks were not sterilized or treated in any way before use, and the moisture content of the dry sticks was not measured in this study. All apples were air dried for a minimum 5 to 10 min at room temperature (visibly dry). *L. monocytogenes* populations were determined on triplicate inoculated apples after air drying as described below.

Caramel dip was placed in a 2.5-liter double-jacketed mixer (Universal Machine UMC-5; Stephan Machinery GmbH, Hameln, Germany) and heated with agitation to 95°C (commercial caramel apple makers typically use a temperature of 104 to 116°C, but temperatures can cool to <100°C during production). The caramel was removed from the heat once it reached 95°C, and apples were then dipped into the caramel using either the stick or kitchen tongs. During the process, the caramel temperature decreased to 85°C. The dipping process resulted in a caramel coating approximately 3 mm thick.

Coated apples were placed on individual sanitized polystyrene weighing boats, transferred to household polyethylene storage containers, lidded, and then stored at 25 or 7°C (without additional humidity control); triplicate samples for each treatment were assayed before and after coating and on days 3, 7, 11, and 14 for 25°C and at weeks 1, 2, 3, and 4 at 7°C. The study was performed three times.

L. monocytogenes populations were enumerated from inoculated apples by transferring to sterile polypropylene sample bags and adding 100 ml of sterile 1% buffered peptone water to each package. The contents of the bag were massaged externally by hand for about 3 min to release the caramel and cells from the surface. Rinsates were serially diluted, and *L. monocytogenes* populations were enumerated by surface plating serial dilutions of rinse material on MOX. Typical colonies recovered on MOX were considered confirmatory.

Statistical analysis. Data were analyzed using a one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. *P* values of 0.05 or less were considered statistically significant.

ACKNOWLEDGMENTS

This study was funded by the University of Wisconsin-Madison Food Research Institute (FRI).

We thank Amanda Skarlupka, Ming Mu, Subash Shrestha, Adam Bartling, Emily Merry, Katina Fisher, Anna Spensely, Abby Dabson, and Katie Osterbauer for technical assistance during inoculation and testing, Ken Brandenburg for statistical analysis, and Nan Faith for virulence and biofilm testing. We are grateful to Tim Monson, Wisconsin State Laboratory of Hygiene, for the *L. monocytogenes* isolates and Rachel Klos, Wisconsin Division of Public Health, for helpful discussion.

REFERENCES

1. **Centers for Disease Control and Prevention.** 2015. Multistate outbreak of listeriosis linked to commercially produced, prepackaged caramel apples made from Bidart Bros. apples. http://www.cdc.gov/listeria/outbreaks/caramel-apples-12-14/. Accessed 9 March 2015.
2. **U.S. FDA.** 2015. FDA investigated *Listeria monocytogenes* illnesses linked to caramel apples. http://www.fda.gov/Food/RecallsOutbreaksEmergencies/Outbreaks/ucm427573.htm. Accessed 9 June 2015.
3. **Wu J, Gao H, Zhao L, Liao X, Chen F, Wang Z, Hu X.** 2007. Chemical compositional characterization of some apple cultivars. Food Chem **103:** 88–93. http://dx.doi.org/10.1016/j.foodchem.2006.07.030.
4. **Ergun R, Lietha R, Hartel RW.** 2010. Moisture and shelf life in sugar confections. Crit Rev Food Sci Nutr **50:**162–192. http://dx.doi.org/10.1080/10408390802248833.
5. **Ryser ET, Buchanan RL.** 2013. *Listeria monocytogenes*. *In* Doyle MP, Buchanan RL (ed), Food microbiology: fundamentals and frontiers. ASM Press, Washington, DC.
6. **Strawn LK, Grohn YT, Warchocki S, Worobo RW, Bihn EA, Wiedmann M.** 2013. Risk factors associated with *Salmonella* and *Listeria monocytogenes* contamination of produce fields. Appl Environ Microbiol **79:** 7618–7627. http://dx.doi.org/10.1128/AEM.02831-13.
7. **Doores S, Splittstoesser DF.** 1983. The microbiology of apples and apple products. CRC Crit Rev Food Sci Nutr **19:**133–149. http://dx.doi.org/10.1080/10408398309527372.
8. **Keller SE.** 2014. Tree fruits and nuts: outbreaks, contamination sources, prevention, and remediation, p 291–312. *In* Matthews KR, Sapers GM, Gerba CP (ed), Produce contamination problem: causes and solutions, 2nd ed. Elsevier, Amsterdam, The Netherlands. http://dx.doi.org/10.1016/b978-0-12-404611-5.00013-0:291-312.
9. **Sivapalasingam S, Friedman CR, Cohen L, Tauxe RV.** 2004. Fresh produce: a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. J Food Prot **67:**2342–2353.
10. **Farber JM, Ross WH, Harwig J.** 1996. Health risk assessment of *Listeria monocytogenes* in Canada. Int J Food Microbiol **30:**145–156. http://dx.doi.org/10.1016/0168-1605(96)01107-5.
11. **Williams D, Castleman J, Lee C, Mote B, Smith MA.** 2009. Risk of fetal mortality after exposure to *Listeria monocytogenes* based on dose-response data from pregnant guinea pigs and primates. Risk Anal **29:**1495–1505. http://dx.doi.org/10.1111/j.1539-6924.2009.01308.x.

12. **Chae MS, Schraft H, Hansen L, Mackereth R.** 2006. Effects of physico-chemical surface characteristics of *Listeria monocytogenes* strains on attachment to glass. Food Microbiol **23:**250–259. http://dx.doi.org/10.1016/j.fm.2005.04.004.
13. **Jo W, Song H, Song N, Lee J, Min SC, Song KB.** 2014. Quality and microbial safety of "Fuji" apples coated with carnauba-shellac wax containing lemongrass oil. LWT Food Sci Technol **55:**490–497. http://dx.doi.org/10.1016/j.lwt.2013.10.034.
14. **Buchanan RL, Edelson SG, Miller RL, Sapers GM.** 1999. Contamination of intact apples after immersion in an aqueous environment containing *Escherichia coli* O157:H7. J Food Protect **62:**444–450.
15. **Baskaran SA, Upadhyay A, Kollanoor-Johny A, Upadhyaya I, Mooyottu S, Roshni Amalaradjou MA, Schreiber D, Venkitanarayanan K.** 2013. Efficacy of plant-derived antimicrobials as antimicrobial wash treatments for reducing enterohemorrhagic *Escherichia coli* O157:H7 on apples. J Food Sci **78:**M1399–M1404. http://dx.doi.org/10.1111/1750-3841.12174.
16. **Lado BH, Yousef AE.** 2007. Characteristics of *Listeria monocytogenes* important to food processors, p 157–213. *In* Ryser ET, Marth EH (ed), Listeria, listeriosis, and food safety. CRC Press, Boca Raton, FL.
17. **Conway WS, Leverentz B, Saftner RA, Janisiewicz WJ, Sams CE, Leblanc E.** 2000. Survival and growth of *Listeria monocytogenes* on fresh-cut apple slices and its interaction with *Glomerella cingulata* and *Penicillium expansum*. Plant Dis **84:**177–181. http://dx.doi.org/10.1094/PDIS.2000.84.2.177.
18. **Niem J, Miyara I, Ettedgui Y, Reuveni M, Flaishman M, Prusky D.** 2007. Core rot development in red delicious apples is affected by susceptibility of the seed locule to *Alternaria alternata* colonization. Phytopathology **97:**1415–1421. http://dx.doi.org/10.1094/PHYTO-97-11-1415.
19. **Dingman DW.** 2000. Growth of *Escherichia coli* O157:H7 in bruised apple (Malus domestica) tissue as influenced by cultivar, date of harvest, and source. Appl Environ Microbiol **66:**1077–1083. http://dx.doi.org/10.1128/AEM.66.3.1077-1083.2000.
20. **MacDonald PDM, Whitwam RE, Boggs JD, MacCormack JN, Anderson KL, Reardon JW, Saah JR, Graves LM, Hunter SB, Sobel J.** 2005. Outbreak of listeriosis among Mexican immigrants as a result of consumption of illicitly produced Mexican-style cheese. Clin Infect Dis **40:**677–682. http://dx.doi.org/10.1086/427803.
21. **Crepet A, Albert I, Dervin C, Carlin F.** 2007. Estimation of microbial contamination of food from prevalence and concentration data: application to *Listeria monocytogenes* in fresh vegetables. Appl Environ Microbiol **73:**250–258. http://dx.doi.org/10.1128/AEM.00351-06.