Journal of Food Protection, Vol. 82, No. 6, 2019, Pages 1022–1027
https://doi.org/10.4315/0362-028X.JFP-18-345
Published 2019 by the International Association for Food Protection

Research Paper

Occurrence and Levels of *Salmonella,* Enterohemorrhagic *Escherichia coli,* and *Listeria* in Raw Wheat

SAMUEL P. MYODA,[1] STEFANIE GILBRETH,[2] DEANN AKINS-LEWENTHAL,[3] SEANA K. DAVIDSON,[1] AND MANSOUR SAMADPOUR[1]*

[1]*IEH Laboratories & Consulting Group, 15300 Bothell Way N.E., Lake Forest Park, Washington 98155;* [2]*The WhiteWave Foods Company, 1225 Seventeenth Street, Suite 1000, Denver, Colorado 80202; and* [3]*ConAgra Foods, Inc., One ConAgra Drive, Omaha, Nebraska 68102, USA*

MS 18-345: Received 22 July 2018/Accepted 29 January 2019/Published Online 23 May 2019

ABSTRACT

Wheat flour has been implicated in several outbreaks of foodborne illness in recent years, yet little information is available regarding microbial pathogens in wheat and wheat flour. Information about microbial pathogens in wheat is needed to develop effective methods to prevent foodborne illnesses caused by wheat products. From 2012 to 2014, we conducted a baseline study to determine the prevalence and levels of pathogens in wheat samples taken before milling. A total of 5,176 wheat samples were tested for enterohemorrhagic *Escherichia coli* (EHEC), *Salmonella* spp., *Listeria* spp., and *L. monocytogenes*. Positive samples were assayed for most probable numbers (MPNs), and isolates were fingerprinted by pulsed-field gel electrophoresis (PFGE). The rate of detection of each pathogen tested was as follows: *Salmonella* was in 1.23% of the samples (average level of 0.110 MPN/g), EHECs occurred in 0.44% of the samples (0.039 MPN/g), and *Listeria* spp. occurred in 0.08% of samples (0.020 MPN/g), but *L. monocytogenes* was not detected. The PFGE assessment found a high diversity for all organisms. All EHEC PFGE patterns (22 of 22) were unique, and 39 of 47 *Salmonella* patterns (83%) were unique. These results indicate a diverse background of naturally occurring organisms. These findings suggest that the microbial contamination is coming from diverse sources and provide no evidence in support of a specific pathogen load. Altogether, our surveillance study shows that contamination of wheat with pathogens is clearly evident and poses a foodborne illness risk.

HIGHLIGHTS

- Prevalence of *Salmonella* and *E. coli* in raw wheat emphasizes the need to cook wheat products.
- 3,891 grain samples were tested for *E. coli* and *Salmonella*; 1,285 were tested for *Listeria*.
- Of wheat berries sampled, 0.44% were positive for *E. coli* and 1.23% were positive for *Salmonella*.
- *Salmonella* diversity was high, indicating various animal sources that are difficult to prevent.
- Cooking wheat products is the best preventative measure against foodborne illness from wheat.

Key words: *Escherichia coli*; Foodborne illness; *Salmonella*; Raw dough; Raw wheat

Throughout the production process chain, wheat is exposed to multiple sources of microbial contamination, including soil, water, insects, and animal feces *(3, 7, 16)*. Wheat berries may host both spoilage and pathogenic organisms; the number and types depend on environmental factors including weather, field treatments, and animal activity *(20, 22)*. The levels of microbial load can vary and include coliforms, *Escherichia coli, Salmonella,* and other potential pathogens.

In recent years, wheat flour has been implicated as the root cause of several recalls and outbreaks of foodborne illness. Suspected vehicles include cookie dough, cake batter, and raw wheat flour *(12, 24)*. For example, in 2005, 26 people in several states across the United States were infected by a single strain of *Salmonella enterica* serotype Typhimurium after eating cake batter ice cream *(25)*. The cake mix used to prepare the cake batter in the ice cream was implicated by epidemiologic investigation as the source of *Salmonella*. In 2008, a cluster of salmonellosis cases emerged in New Zealand. These illnesses were attributed to consumption of an uncooked baking mixture containing flour contaminated with *S. enterica* serotype Typhimurium phage type 42 (STM42) *(17)*. In 2015 and 2016, there was suspected *Salmonella* contamination in bags of wheat flour distributed in the United States *(24)* and Germany *(10)*. Although illnesses were not reported in these last two incidents, they resulted in large-scale recalls and significant losses of manufactured products and revenue.

An *E. coli* O157:H7 outbreak in 2009 resulted from consumption of raw cookie dough *(18)*. This was the first reported enterohemorrhagic *E. coli* (EHEC) outbreak associated with consuming ready-to-bake commercial

* Author for correspondence. Tel: 206-522-5432; Fax: 206-306-8883; E-mail: ms@iehinc.com.

TABLE 1. *Detection of* E. coli, Salmonella, *and* Listeria *in four types of wheat*

		E. coli O157		EHEC		*Salmonella*		*Listeria* spp.		*Listeria monocytogenes*	
	n	No. positive	% positive	No. positive	% positive	No. positive	% positive	No. positive	% positive	No. positive	% positive
Hard red spring	653	0	0	1	0.15	2	0.31	0	0	0	0
Hard red winter	1,353	0	0	5	0.37	21	1.55	0	0	0	0
Soft red winter early	1,168	0	0	4	0.34	12	1.03	1	0.16	0	0
Soft red winter late	717	0	0	7	0.98	13	1.81	0	0	0	0
2012–2014	3,891	0	0	17	0.44	48	1.23	1	0.08	0	0

prepackaged cookie dough *(18)*. Multistate outbreaks of EHEC serotype O121, *E. coli* O121, or *E. coli* O26 were attributed to eating flour produced at a Missouri facility *(5)* and resulted in multiple recalls as a result of investigations. An investigation of a cluster of patients infected with EHEC serotype O121 in Canada (2017) linked that outbreak to contaminated wheat flour *(12)*. These cases illustrate that wheat products contaminated with foodborne pathogens pose risks for both human health and loss of revenue and consumer confidence following recalls.

The outbreaks and recalls associated with wheat flour show that a raw agricultural commodity expected to be subjected to a kill step (e.g., baking), can still result in outbreaks (e.g., when consumers eat raw dough made from contaminated flour). Contaminations can occur during growth, harvesting, storage, and processing *(20)*. Sources may include animal activity, irrigation water, handling, harvesting equipment, and processing into finished products, including flour. Here, we present the results of a 3-year screening study to determine a baseline presence of common foodborne pathogens (*Salmonella,* EHEC, and *Listeria*) in wheat before storage in grain silos and milling at flour mills. It is important to establish such baselines to enable evaluation of variables leading to contamination of wheat flours.

MATERIALS AND METHODS

Wheat sampling. Over 3 years (2012 to 2014), four types of wheat were tested: hard red spring, hard red winter, soft red winter early, and soft red winter late (Table 1). Truckloads of grain were sampled using autosamplers before the grain was offloaded into grain elevators, following Federal Grain Inspection Service protocols *(23)*. Sampling from truckloads was used to prevent contamination from the grain elevators and is a better indicator for contamination during growth in the field and during harvest. Depending on the size of the grain trailer, 44 or 77 samples per trailer were taken and composited (per trailer) for 3,891 samples taken for detection of *Salmonella* and *E. coli* (EHEC) and 1,285 samples tested for *Listeria* spp. and *L. monocytogenes* (the sample number for each is presented in Table 1). The composite samples were shipped to the IEH Laboratories & Consulting Group for analysis (5,176 samples). Each 1,500-g sample was divided into 375-g portions to test for each pathogen (EHEC, *E. coli* O157, *Salmonella,* and *Listeria*), and 750 g was retained for background microbiota analysis.

Sample enrichment. Three volumes (1,125 mL) of prewarmed IEH media were added per 375-g sample; stomached for 60 s, followed by visual confirmation of homogeneity; and then incubated for at least 18 h at 42°C before sampling for screening by multiplex PCR.

PCR screening and confirmation of presumptive-positive detection. Initial screening was done using IEH multiplex PCR methods: AOAC International Performance Tested Method (PTM) 100701 for *Salmonella* and EHEC (defined as Shiga toxin–producing *E. coli* with intimin) and AOAC PTM 021201 for *Listeria* spp. and *L. monocytogenes (14, 15)*. An aliquot of enriched sample was used for cell lysis and amplification of specific bacterial DNA fragments by multiplex PCR using *Taq* DNA polymerase. Portions were retained for all presumptive positives and quantified using a 12-tube PCR most probable number (MPN) (3 at 100 g, 3 at 10 g, 3 at 1 g, and 3 at 0.1 g). All presumptive-positive enrichments were also confirmed following appropriate U.S. Food and Drug Administration *Bacteriological Analytical Manual* protocols to identify *Salmonella (1),* EHEC *(11),* and *Listeria (13)*.

Statistical analysis. Statistical differences were determined using Fisher's exact test (http://www.langsrud.com/fisher.htm) for all 2×2 comparisons within EHEC and within *Salmonella* to establish two-tailed P values.

PFGE analysis of *Salmonella* and EHEC isolates. To establish diversity of strains, *Salmonella* and EHEC genomic DNA were isolated from the wheat samples and digested with the *Xba*I restriction enzyme (Invitrogen), and then fragments separated by pulsed-field gel electrophoresis (PFGE) following Centers for Disease Control and Prevention PulseNet protocols *(6)*.

RESULTS

Incidence of pathogens in wheat samples. Of the 3,891 samples tested for *Salmonella* and EHEC, 48 were confirmed positive for *Salmonella* (1.23%), and levels of *Salmonella* were 0.110 ± 0.448 MPN/g; 17 samples in total were confirmed positive for EHEC (0.44%), with levels of 0.039 ± 0.175 MPN/g, but none of the EHEC positives were confirmed as *E. coli* O157 (Tables 1 and 2). Using Fisher's exact test using the two-tailed test for statistical differences between incidence of *Salmonella* and incidence of EHEC among wheat types, the only statistically significant differences were found for the incidence of *Salmonella* between hard red winter and hard red spring wheat ($P \leq 0.01$) and hard red spring and soft red winter late wheat ($P < 0.01$) (2012 to 2014). Only 1 of 1,285 *Listeria*-targeted samples tested positive

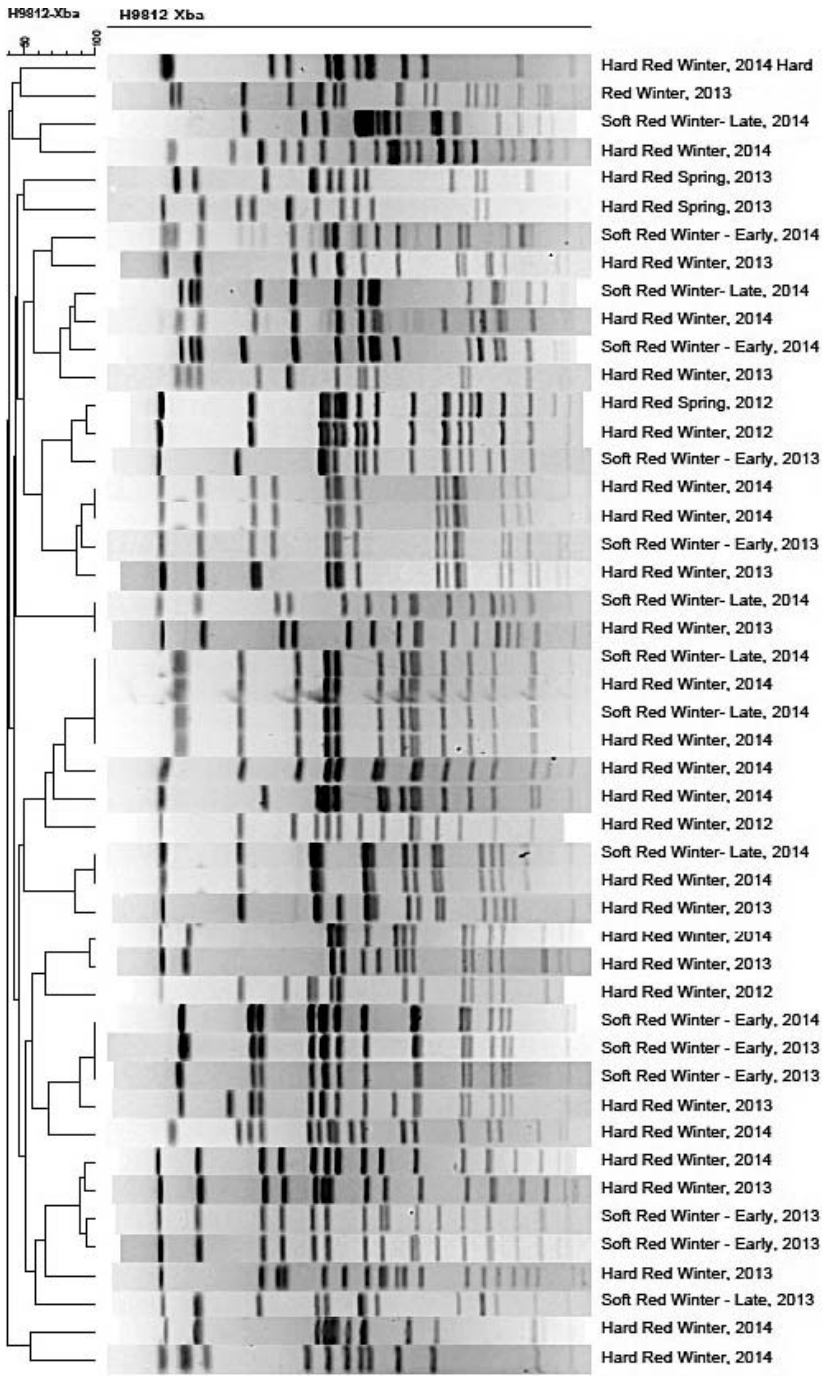

FIGURE 1. *PFGE analysis results of* Salmonella *isolates from wheat. Among 47* Salmonella *isolates subjected to PFGE analysis, there were 39 unique patterns.*

for *Listeria* spp. (0.08%) in the first year of sampling, but all other samples were negative for *L. monocytogenes*; the level in the one positive *Listeria* spp. sample was 0.020 MPN/g.

TABLE 2. *Numbers of pathogens in wheat samples*

	MPN/g		
Wheat variety	EHEC	*Salmonella*	*Listeria* spp.
Hard red spring	0.042	0.003	0
Hard red winter	0.135	0.181	0
Soft red winter early	0.004	0.002	0.02
Soft red winter late	0.006	0.011	0
2012–2014	0.039	0.11	0.02

PFGE as an indicator of genetic diversity. The PFGE fingerprints of the isolated pathogens were diverse (Figs. 1 and 2). Multiple EHEC isolates from the same sample were fingerprinted and found to be different; 22 isolates pulled from 17 positive EHEC samples had unique patterns (Fig. 2). Analysis of *Salmonella* isolate diversity found that 39 (83%) of 47 isolates tested were unique. These results indicate naturally occurring pathogen strains in the field are diverse.

DISCUSSION

Outbreaks and recalls involving wheat products highlight a need for better understanding of risks associated with wheat and wheat flour containing products. The

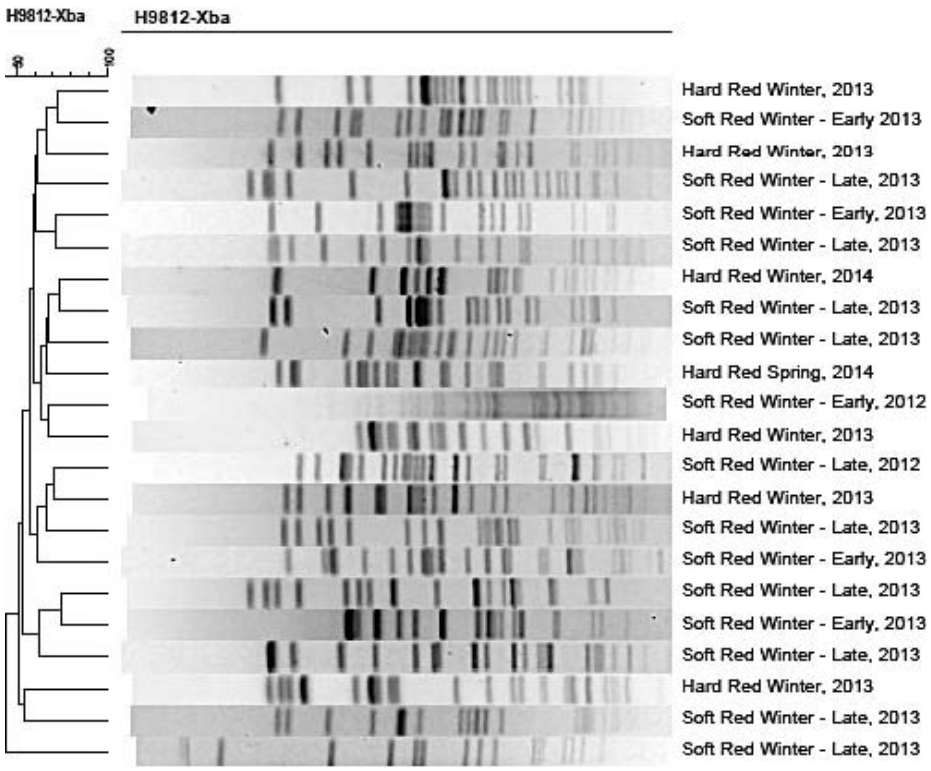

FIGURE 2. *PFGE analysis of enterohemorrhagic* E. coli *isolated from wheat. Of 22 EHEC isolates from 17 positive samples, 22 unique PFGE patterns appeared, indicating diversity in naturally present pathogen strains.*

purpose of this wheat survey was to evaluate a baseline level of contamination in raw wheat before processing wheat berries into flour. The levels of *Salmonella,* EHEC, *Listeria* spp., and *L. monocytogenes* were measured in wheat from 2012 to 2014 before loading into silos. Testing for *Listeria* was completed only for samples from 2012 because of the low incidence observed (1 in 1,285, 0.08%). Overall, *Salmonella* was detected in 1.23% of samples tested and EHEC appeared in 0.28% of samples. The pathogen levels in samples testing positive were *Salmonella* at 0.110 ± 0.448 MPN/g, EHEC at 0.039 ± 0.175 MPN/g, and *Listeria* spp. at 0.020 MPN/g, which equated to approximately 41, 15, and 7.5 MPN/375-g sample, respectively. Given the low infective dose for *Salmonella* and EHEC, these pose significant threats to public health.

Sabillón et al. *(22)* surveyed wheat in Nebraska through the growing seasons from 2012 to 2013 to evaluate changes in pathogen load under varied environmental conditions. They detected organisms using three plating methods (aerobic plate counts, coliforms, and *Enterobacteriaceae*). Coliforms were detected in wheat from one district only, and *Enterobacteriaceae* were found in all batches at a level between 2.87 and 5.09 CFU/g. However, neither *E. coli* nor *Salmonella* was detected, likely because their sample size was small ($n = 27$ for each growing season, spread over several locations). They did detect differences in the bacterial loads depending on location, which varied with the amount of rainfall *(22)*.

Contamination sources are diverse and vary with several conditions, including weather, temperature, precipitation, time of flowering, and time of harvest *(22)*.

TABLE 3. *Results of Fisher's exact test using two-tailed test for statistical differences between incidence of* Salmonella *and EHEC among wheat types*

	Hard red spring	Hard red winter	Soft red winter early	Soft red winter late
EHEC—two-tailed *P* values				
Hard red spring	X	0.67	0.66	0.072
Hard red winter		X	1	0.124
Soft red winter early			X	0.116
Soft red winter late				X
Salmonella—two-tailed *P* values				
Hard red spring	X	0.0126	0.10	0.008
Hard red winter		X	0.29	0.72
Soft red winter early			X	0.15
Soft red winter late				X

Postharvest contamination was not examined in our study and will be discussed in reference to other studies. Overall, the diversity of the strains detected from harvested wheat in this study is consistent with the expectation that organisms are coming from diverse sources. The only significant differences in the number of contaminated samples detected seems associated with the season of planting and harvest. The highest incidences of *Salmonella* were found in winter wheats, both soft and hard (Tables 2 and 3), with the lowest positive sample number detected in hard red spring wheat. Winter wheat is planted in late fall and overwinters in the field, growing in spring and being harvested in early summer. Spring wheat is planted in spring and is harvested in late summer and early autumn. The higher *Salmonella* incidence among samples may be associated with seasonal conditions. However, given the low number of positive samples over all, relative to the total tested, it is difficult to conclude there is a seasonal effect without further research designed to study seasonal influences on pathogen load.

If wheat heads are contaminated with pathogens in the field, pathogens on the surfaces of the wheat berries are redistributed throughout the final products during milling. Microbial growth on dry wheat heads in the field or during transport is unlikely given the low water activity (a_w) of intact wheat berries *(4)*. The microbial load may increase during the production process through cross-contamination, notably during the tempering step required for milling flour, when wheat berries are sprayed with water *(2)*. This step raises the moisture level of the grain to between 12 and 17% and increases the a_w of dry wheat berries from 0.4 to 0.5 a_w to nearly 0.7 to 0.85 a_w over a 16- to 24-h period, which is still too low for growth of bacteria within the wheat berries *(4)*. Although the total increase in water content of the wheat berries may not be sufficient for bacterial growth, growth may occur on the surfaces and in wet pockets between the wheat berries, and on the surfaces of the bins in organic residue that may have accumulated *(2, 8, 9, 20)*. The possible support of growth of pathogens in these residues may be an important factor to evaluate. Tempering steps often incubate at ambient temperatures, which vary with location and season, and at temperatures that allow microbial growth. The a_w of flour remains well below levels needed for growth of common foodborne pathogens, including *E. coli, Salmonella,* and *Listeria (4)*. Water activities required for propagation are likely reached only when the flour is mixed into dough.

Concern for limiting growth of organisms during tempering has led to several studies testing methods for inhibiting microbial growth in tempering bins. Using ozonated water or adding a mild acid with salt (acetic, lactic, and propionic acids) at different levels have both been found to decrease microbial loads *(8, 9, 21)*. However, ozonated water detrimentally changed the behavior of the dough *(19)*. Incubation with mild acid together with salt seemed to both reduce bacterial load and maintain performance of the flour *(21)*. These methods may offer promising preventative measures for flour contamination as long as the final product remains at a low cost to consumers.

The levels of samples positive for EHEC and *Salmonella* detected in our study indicate that the presence of these pathogens in wheat pose a significant food safety hazard. Among the four types of wheat tested (hard red spring, hard red winter, soft red winter early, and soft red winter late), there may have been higher incidence of *Salmonella* contamination in wheat planted in late autumn to overwinter in the field. However, given the low numbers of positive samples, the difference between types or years was inconclusive. The genetic diversity suggests that microbial pathogens were varied and transient. This supports the conclusion that contamination occurs in the field from various sources and that their presence will not likely be eliminated. Although the overall pathogen numbers were low, the possibility for pathogens to be carried through the milling process and end up in the finished flour is a risk factor for foodborne illness in uncooked products.

There are two main pathways in which the presence of pathogens in wheat flour can cause human illness: (i) by the consumption of raw dough and (ii) through cross-contamination. The widespread use of wheat products in foods and the occurrence of outbreaks associated with wheat products confirm that eating raw wheat products poses a risk for foodborne illnesses. While the pathogen hazard from industrial production can be mitigated through testing of lots, in reality not every lot is tested, and hazards associated with mishandling or improper cooking of products by the consumers are a reality. People will likely eat raw dough regardless of warnings on labels to cook the dough first. To reduce risk in wheat flour production, interventions may be possible during tempering to reduce microbial load, such as treatment with mild acids and salt *(21)*. With these factors in consideration, the best recommendations to prevent foodborne illnesses caused by wheat products is to cook the products, inform people to avoid eating raw dough, and hope they heed this warning.

REFERENCES

1. Andrews, W. H., H. Wang, A. Jacobson, and T. S. Hammack. 2007. *Salmonella,* chap. 5. *In* Bacteriological analytical manual. U.S. Food and Drug Administration, Silver Spring, MD.
2. Berghofer, L. K., A. D. Hocking, D. Miskelly, and E. Jansson. 2003. Microbiology of wheat and flour milling in Australia. *Int. J. Food Microbiol*. 85:137–149.
3. Bullerman, L. B., and A. Bianchini. 2009. Food safety issues and the microbiology of cereals and cereals products, chap. 15, p. 315–335. *In* N. Heredia, I. Wesley, and S. Garcia (ed.), Microbiologically safe foods. John Wiley & Sons, Hoboken, NJ.
4. Carter, B. P., C. F. Morris, G. L. Weaver, and A. H. Carter. 2015. The case for water activity as a specification for wheat tempering and flour production. *Cereal Foods World* 60:166–170.
5. Centers for Disease Control and Prevention (CDC). 2016. Multistate outbreak of Shiga toxin–producing *Escherichia coli* infections linked to flour (final update). Available at: https://www.cdc.gov/ecoli/2016/o121-06-16/index.html. Accessed 18 December 2017.
6. Centers for Disease Control and Prevention (CDC). 2017. Standard operating procedure for PulseNet PFGE of *Escherichia coli* O157:H7, *Escherichia coli* non-O157 (STEC), *Salmonella* serotypes, *Shigella sonnei* and *Shigella flexneri*. Available at: https://www.cdc.gov/pulsenet/pdf/ecoli-shigella-salmonella-pfge-protocol-508c.pdf. Accessed 22 March 2019.

7. Deibel, K. E., and K. M. J. Swanson. 2001. Cereal and cereal products, p. 549–553. *In* P. F. Downes and K. Ito (ed.), Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, DC.
8. Dillhon, B., D. Wiesenborn, H. Dhillon, and C. Wolf-Hall. 2010. Development and evaluation of a fluidized bed system for wheat grain disinfection. *J. Food Sci*. 75:E372–E378.
9. Dillhon, B., D. Wiesenborn, C. Wolf-Hall, and F. Manthey. 2009. Development and evaluation of an ozonated water system for antimicrobial treatment of durum wheat. *J. Food Sci*. 74:E396–E403.
10. European Commission. 2016. *Salmonella* (presence/25g) in wheat flour from Germany. Available at: https://webgate.ec.europa.eu/rasff-window/portal/?event=notificationDetail&NOTIF_REFERENCE=2016.0101. Accessed 16 December 2017.
11. Feng, P., S. D. Weagant, and K. Jinneman. 2018. Diarrheagenic *Escherichia coli,* chap. 4A. *In* Bacteriological analytical manual. U.S. Food and Drug Administration, Silver Spring, MD.
12. Government of Canada. 2017. Public health notice: outbreak of *E. coli* public health notice—outbreak of *E. coli* infections linked to various flours and flour products (final update). Available at: https://www.canada.ca/en/public-health/services/public-health-notices/2017/public-health-notice-outbreak-e-coli-infections-linked-various-flours-flour-products.html. Accessed 18 December 2017.
13. Hitchins, A. D., K. Jinneman, and Y. Chen. 2017. *Listeria monocytogenes,* chap. 10. *In* Bacteriological analytical manual. U.S. Food and Drug Administration, Silver Spring, MD.
14. IEH Laboratories & Consulting Group. IEH *E. coli* O157, Stx-producing *E. coli* (STEC) with intimin and *Salmonella* test system. Microbiologique, Seattle, WA.
15. IEH Laboratories & Consulting Group. IEH *Listeria* spp. & *Listeria monocytogenes* test system. Microbiologique, Seattle, WA.
16. Laca, A., A. Mousia, M. Diaz, C. Webb, and S. S. Pandiella. 2006. Distribution of microbial contamination within cereal grains. *J. Food Eng*. 72:332–338.
17. McCallum, L., S. Paine, K. Sexton, M. Dufour, K. Dyet, M. Wilson, D. Campbell, D. Bandaranayake, and V. Hope. 2013. An outbreak of *Salmonella* Typhimurium phage type 42 associated with the consumption of raw flour. *Foodborne Pathog. Dis*. 10:159–164.
18. Neil, K. P., G. Biggerstaff, J. MacDonald, E. Trees, C. Medus, K. A. Musser, S. G. Stroika, D. Zink, and M. J. Sotir. 2012. A novel vehicle for transmission of *Escherichia coli* O157:H7 to humans: multistate outbreak of *E. coli* O157:H7 infections associated with consumption of ready-to-bake commercial prepackaged cookie dough—United States. 2009. *Clin. Infect. Dis*. 54:511–518.
19. Rose, D. J., A. Bianchini, B. Martinez, and R. A. Flores. 2012. Methods for reducing microbial contamination of wheat flour and effects on functionality. *Cereal Foods World* 57:104–109.
20. Sabillón, L., and A. Bianchini. 2015. From field to table: a review on the microbiological quality and safety of wheat-based products. *Cereal Chem*. 93:105–216.
21. Sabillón, L., J. Stratton, D. J. Rose, R. A. Flores, and A. Bianchini. 2016. Reduction in microbial load of wheat by tempering with organic acid and saline solutions. *Cereal Chem*. 93:638–646.
22. Sabillón, L., J. Stratton, D. J. Rose, T. H. Regassa, and A. Bianchini. 2016. Microbial load of hard red winter wheat produced at three growing environments across Nebraska, USA. *J. Food Prot*. 79:646–654.
23. U.S. Department of Agriculture, Federal Grain Inspection Service. 1995. Grain inspection handbook—book I sampling. Available at: https://www.gipsa.usda.gov/fgis/handbook/BK1/BookI_2015-09-18.pdf. Accessed 22 March 2019.
24. U.S. Food and Drug Administration. 2015. Voluntary recall for Navajo Pride bleached flour due to possible health risk. Available at: https://wayback.archive-it.org/7993/20170723005828/https://www.fda.gov/Safety/Recalls/ArchiveRecalls/2015/ucm445036.htm. Accessed 16 December 2017.
25. Zhang G., L. Ma, N. Patel, B. Swaminathan, S. Wedel, and M. P. Doyle. 2007. Isolation of *Salmonella* Typhimurium from outbreak-associated cake mix. *J. Food Prot*. 70:997–1001.