Journal of Food Protection, Vol. 79, No. 8, 2016, Pages 1304–1315
doi:10.4315/0362-028X.JFP-16-054
Copyright ©, International Association for Food Protection

Prevalence and Amounts of *Salmonella* Found on Raw California Inshell Pistachios

LINDA J. HARRIS,[1,2]* VANESSA LIEBERMAN,[1] RUPINDER P. MASHIANA,[1] EDWARD ATWILL,[2,3] MAI YANG,[4] JEFFREY C. CHANDLER,[5]† BLEDAR BISHA,[5] AND THOMAS JONES[4]

[1]*Department of Food Science and Technology,* [2]*Western Center for Food Safety, and* [3]*Department of Population, Health, and Reproductive Medicine, University of California, Davis, One Shields Avenue, Davis, California 95616;* [4]*DFA of California, 1855 South Van Ness Avenue, Fresno, California 93721; and* [5]*Department of Animal Science, University of Wyoming, 1000 East University Avenue, Laramie, Wyoming 82071, USA*

MS 16-054: Received 3 February 2016/Accepted 18 March 2016

ABSTRACT

After harvest, pistachios are hulled with mechanical abrasion and then separated in a float tank containing water; the nuts that float (~15%; floaters) and those that sink (~85%; sinkers) are dried and stored separately. To determine the prevalence of *Salmonella* in pistachios, a total of 3,966 samples (1,032 floaters and 2,934 sinkers) were collected within 4 months of the 2010, 2011, and 2012 harvests from storage silos (12 samples from each silo, in most cases) and were stored at 4°C; 100-g subsamples were enriched for the presence of *Salmonella*. Twenty-one of the floater samples and 11 of the sinker samples were positive for *Salmonella*: 2.0% prevalence (95% confidence interval [CI], 1.3 to 3.1%) and 0.37% prevalence (95% CI, 0.21 to 0.67%), respectively, for a weighted average prevalence of 0.61%. Levels of *Salmonella* were determined for positive samples using a most-probable-number (MPN) method with multiple 50-g, three 5.6-g, and three 0.56-g subsamples. Geometric mean levels of *Salmonella* in floaters and sinkers were 0.66 MPN/100 g (0.14 to 5.3 MPN/100 g) and 0.18 MPN/100 g (0.10 to 0.62 MPN/100 g), respectively. Seven different serovars were identified among the isolates, with nine pulsed-field gel electrophoresis fingerprints; as many as four serovars were isolated from some samples. *Salmonella* serovars Montevideo (44%), Enteritidis (19%), Senftenberg (16%), Worthington (12%), and Liverpool (9.4%) were most commonly isolated from the initial 100-g samples. The prevalence and levels of *Salmonella* in pistachios are within those observed for other tree nuts, but the limited number of serovars isolated suggests a narrow and persistent contamination source.

Key words: Nuts; Pistachios; *Salmonella* concentration; *Salmonella* levels; Salmonella prevalence; *Salmonella* survey

Pistachios (*Pistacia vera*) are an important nut crop, with worldwide production of over 503,000 metric tons in 2013 to 2014 *(33)*. Although U.S. pistachios are grown in Arizona and New Mexico, most (98%) of the acreage is in California. In 1976, the first commercial crop (680 metric tons) was harvested *(3)*. Since then, California production volume has grown to 233,000 metric tons in 2014, and further significant increases are projected over the next 5 years on the basis of the number of hectares of nonbearing plantings *(3)*.

Pistachios were recalled after *Salmonella* serovars Montevideo, Newport, and Senftenberg (single recall 2009) *(15, 34)* and *Salmonella* Senftenberg (2013) *(35)* were isolated from commercial product. In 2009, pulsed-field gel electrophoresis (PFGE) fingerprints of *Salmonella* Montevideo from pistachios and environmental samples matched isolates from cases of salmonellosis, including one from a patient who had reported consuming pistachio-containing product *(15)*. In 2013, eight cases of salmonellosis were identified; the case isolate PFGE patterns for *Salmonella* Senftenberg matched those isolated from pistachios and from the environment in which the pistachios were processed *(35)*. Because the PFGE pattern for *Salmonella* Senftenberg isolated from both cases and pistachios was relatively rare, pistachios were considered the likely vehicle *(35)*. In 2016 in the United States, 11 cases of *Salmonella* Montevideo in nine states were associated with consumption of pistachios *(16)*.

With the exception of almonds *(8, 17)*, pecans *(10)*, and walnuts *(18)*, very little is known about the prevalence, levels, and distribution of *Salmonella* in tree nuts collected before retail distribution; these data are important for development of quantitative microbial risk assessments and of scientifically sound product sampling schemes for verification of food safety plans. Three retail market surveys have included pistachios of unknown origin. Two 25-g samples of raw whole pistachios collected in Scotland were negative for *Salmonella (12)*, 1 of 25 25-g samples of roasted pistachios was positive for *Salmonella* Havana in the United Kingdom *(26)*, and of 73 25-g samples of kernels and 111 25-g samples of inshell pistachios, none were positive for *Salmonella* in the United Kingdom *(27)*.

* Author for correspondence. Tel: 530-754-9485; Fax: 530-752-4759; E-mail: ljharris@ucdavis.edu.
† Present address: U.S. Department of Agriculture, National Wildlife Research Center, 4101 LaPorte Avenue, Fort Collins, CO 80521, USA.

In the United States, pistachios are mechanically shaken onto a catch frame and are then transported from the orchard to a facility where the hulls are removed by mechanical abrasion. Immediately after hulling, pistachios pass through a float tank filled with water that separates nuts on the basis of density. Smaller, less mature nuts and those with insect damage or adhering hull tend to float ("floaters"), whereas mature nuts (i.e., those with larger, fully developed nutmeats) are heavier and sink ("sinkers"). Floaters and sinkers make up ~15 and ~85% by weight, respectively, of the typical annual production (Fig. 1). After initial separation, floater and sinker nuts are handled independently during drying and storage. In this study, the prevalence, concentration, and distribution of *Salmonella* in U.S. floater and sinker pistachios were determined for crop years 2010, 2011, and 2012.

MATERIALS AND METHODS

Pistachios. Until very recently, 'Kerman' was the predominant (>95%) variety of pistachios grown in California and was the variety exclusively sampled in this study. Samples of raw inshell floater and sinker pistachios (approximately 1 kg each) from the 2010, 2011, and 2012 harvests were collected by DFA of California (Fresno) from storage silos located at seven pistachio handlers (processors) that represented approximately 98% of California production. Pistachios were sampled in approximate proportion to the volume produced by each of these processors. Samples were collected from the silo ports using a stainless steel nut sample probe (YWD Purchasing, Fresno, CA) that was sprayed with 70% ethanol and was air dried between sample collections. These sampling devices were custom-made products similar to a grain trier. The devices are cylinders (~1.2 m in length by ~7 to 10 cm in diameter); there is an inner and outer sleeve that can be rotated so that openings along the cylinder are open or closed. The devices are inserted into the silo in the closed position, the sleeve is then rotated, and pistachios move into the trier. After filling, the trier is again closed, removed from the silo, and then opened to allow the pistachios to be transferred into sample bags.

In general, 12 samples were collected from each silo (from the available ports on that silo). In six cases, however, sample collection varied—in 2012, 24 samples were taken from each of three silos, 20 samples from one silo, and 11 samples from another; in 2010, 11 samples were taken from one silo. Samples were collected within 4 months of harvest, were coded to blind the identity of the processor, and then were stored at 4°C for up to 6 months before analysis. Subsamples (100 g) of pistachios were analyzed for the presence of *Salmonella* by DFA (see below). The remaining pistachios from the initially positive samples, as well as several of the initially negative samples, were shipped on ice to the University of California, Davis, laboratory for further testing, including determination of most probable number (MPN) of *Salmonella*.

Presence of *Salmonella* in raw inshell pistachios. A single 100-g subsample from each pistachio sample was analyzed for the presence of *Salmonella* by DFA in accordance with AOAC official method 2001.9 *(7)*. Samples were mixed 1:10 with buffered peptone water and were shaken for 120 s. Samples were loosely capped and incubated at 35 ± 2°C for 18 to 24 h. The preenrichment culture (800 μl) was processed on an immunoconcentration *Salmonella* (ICS) test strip (bioMérieux, Hazelwood, MO), and the resulting concentrate was used to inoculate 2 ml of ICS broth, which was then incubated at 41 ± 1°C for 5 h. After incubation, the ICS broth culture (1 ml) was boiled for 15 min and cooled to room temperature, and then 500 μl was added to an SLM (*Salmonella*) test strip (bioMérieux). A relative fluorescence value >0.23 was considered a positive result when testing for *Salmonella* by this mini-VIDAS system (an enzyme-linked fluorescence assay). Presumptive-positive results were confirmed by streaking the remaining unboiled portion of the ICS broth culture onto the following *Salmonella*-selective agars: CHROMagar Salmonella, xylose lysine desoxycholate agar (XLD), and Hektoen enteric agar (HE). Three random colonies that were characteristic of *Salmonella* on one or more of the selective agars were picked from each of the positive samples and were streaked onto MacConkey agar; then their identity was confirmed with API 20E test strips (bioMérieux) according to the manufacturer's instructions. Unless otherwise specified, media were obtained from BD (Franklin Lakes, NJ).

Additional microbiological analysis of survey pistachios. Some of the samples were screened for *Escherichia coli* (2011 and 2012), aerobic plate count (APC), and yeasts and molds, as described by Bansal et al. *(8)* for almonds. Briefly, inshell pistachios (90 g) were combined with 90 ml of sterile Butterfield's phosphate buffer (Biotrace International, Bothell, WA) to achieve a 10^0 dilution and then were shaken 50 times through a 30-cm arc. Mixed samples were allowed to stand for 3 to 5 min and then were shaken an additional five times before serial dilution in Butterfield's phosphate buffer and transfer to broth or plating media. *E. coli* was quantified by MPN analysis with a modified version of AOAC official method 966.24 *(6)*; API 20E test strips were used to confirm positive *E. coli* colonies. APC was determined by AOAC official method 966.23 *(5)*. Counts of yeasts and molds were determined by the U.S. Food and Drug Administration *Bacteriological Analytical Manual* (FDA BAM) method *(31)*, modified by the use of tempered agar pour plates rather than spread plates of dichloran rose bengal chloramphenicol agar. This modified method is commonly used in the dried fruit and nut industry.

Determining levels of *Salmonella* in pistachio samples. In addition to the initial 100-g subsamples, each of four 50-g subsamples was placed into 1,600-ml Whirl-Pak bags (Nasco, Modesto, CA) containing 450 ml of lactose broth. Each bag was vigorously shaken by hand through a 30-cm arc for 1 min. The liquid from one of the bags was portioned into tubes as follows: 50 ml into each of three tubes (equivalent to 5.6 g of pistachios each) and 5 ml into each of another three tubes (equivalent to 0.56 g of pistachios each). In some cases, additional or only 50-g samples were enriched for *Salmonella* in the same way. All samples (tubes and remaining bags) were incubated at 37°C for 24 h. Enrichment was carried out using a modification of the FDA BAM method *(4)*.

After incubation, 0.1- and 1-ml aliquots of each lactose broth sample were transferred into 9.9 ml of Rappaport-Vassiliadis R10 broth and 9 ml of tetrathionate broth, respectively, and were incubated at 42°C in a water bath and at 37°C in an incubator, respectively, for 24 h. Each enrichment was streaked onto XLD, HE, and bismuth sulfite agar and was incubated at 37°C for 24 h (XLD, HE) or 48 h (bismuth sulfite agar). Each enrichment (tetrathionate and Rappaport-Vassiliadis) was evaluated separately; a single colony that displayed typical characteristics of *Salmonella* on one of those three plates was selected and restreaked onto both XLD and HE. A single isolated colony that displayed typical characteristics of *Salmonella* (from either XLD or HE) was selected; then it was streaked onto tryptic soy agar (TSA) and was

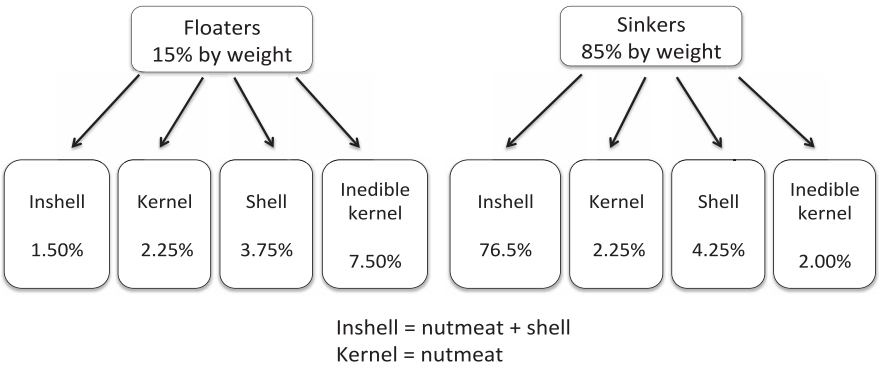

FIGURE 1. *Proportion of edible product in sinker and floater streams of pistachios (adapted from Administrative Committee for Pistachios (1)).*

stabbed and streaked onto triple sugar iron agar and lysine iron agar slants and was incubated at 37°C for 24 h. Isolates on TSA that were characteristic of *Salmonella* on triple sugar iron agar or lysine iron agar were confirmed with a *Salmonella* latex test (Oxoid Ltd, Basingstoke, Hampshire, UK). Confirmed *Salmonella* isolates were stored at –80°C in tryptic soy broth supplemented with 15% glycerol. MPN values were calculated according to the FDA BAM method *(9),* on the basis of the following dilution series: one 100-g replicate (the original enrichment), three 50-g replicates (or total number of 50-g replicates), three 5.6-g replicates, and three 0.56-g replicates.

To better understand the distribution of *Salmonella* in pistachios, multiple 50-g subsamples of initially negative samples were enriched using the method described above. In 2010, this was done for 11 samples after processing and discarding most of the samples. In 2011 and 2012, 10 and 20 initially negative samples, respectively, were selected using a random number generator, and 10 50-g subsamples from each were enriched for *Salmonella* as described above.

Serotyping and phage typing. A single isolated colony for each confirmed *Salmonella* isolate was transferred to TSA slants, which were submitted to the California Animal Health and Food Safety Laboratory (Davis, CA) for serotyping. Isolates that were serotyped as *Salmonella* Enteritidis were submitted to the National Veterinary Services Laboratory (Ames, IA) for phage typing.

PFGE. Molecular subtyping for each isolate was determined using *Xba*I macrorestriction, followed by PFGE using the standard protocol recommended by the Centers for Disease Control and Prevention's PulseNet *(13)*. Also included in the analysis were *Salmonella* Enteritidis phage type (PT) 9c (LJH1024; RM4635, a clinical isolate from a 2004 outbreak associated with consumption of raw almonds *(14)*), *Salmonella* Enteritidis PT 30 (LJH608; ATCC BAA-1045, an almond isolate from a 2001 outbreak associated with consumption of raw almonds *(22)*), and *Salmonella* Montevideo (LJH1234; FDA_2010_149_Pistachio-2, a pistachio isolate associated with a 2009 recall *(34)*). The PulseNet S*almonella* Braenderup reference standard (H9812) was used as the reference marker. Gel images were analyzed as described previously *(28)*. Gel images were captured using a MultiDoc-It imaging system (UVP, Upland, CA), and images were analyzed with Bionumerics software, version 6.6 (Applied Maths NV, Austin, TX). Similarity of PFGE patterns was assessed by the unweighted pair group method with arithmetic averages, with 2.0% band position tolerances. Dice coefficients had 1.5% optimization values. PFGE patterns that were >80% similar were considered to be in the same genetic cluster; similarity coefficients were obtained by calculating Dice coefficients.

Statistical analysis. Asymptotic *(11)* and exact *(37)* homogeneity tests were used to determine whether the odds of testing positive for *Salmonella* for inshell floaters compared with sinkers did not differ across year. If this assumption is rejected based on a P value ≤ 0.05, there is evidence that the odds ratio (OR) varies by year and that the association between testing positive for *Salmonella* for 100-g grab samples of floaters compared with sinkers needs to be summarized for each year separately, using a Fisher's exact test and associated 95% confidence intervals.

Statistical tests were performed with JMP 11 software (SAS Institute Inc., Cary, NC). The 95% Wilson (score) confidence intervals were determined with JMP 11 software. Data were transformed to calculate geometric mean and median values; when counts were <10 CFU/g or >250,000 CFU/g, we used 5 CFU/g or 250,001 CFU/g, respectively.

RESULTS

Sampling. Most of the pistachios produced in the United States are stored in cylindrical silos that are several stories tall. An initial discussion with survey participants led to the assumption that every storage silo had 12 evenly spaced sampling ports (four at each of three heights). DFA was thus instructed to obtain 12 samples per silo, one from each port. However, when analyzing the data at the end of the 3-year survey, we recognized that this assumption was incorrect and that there was actually significant variability in the number of sampling ports per silo. An informal survey of the seven participating handlers indicated that the number of ports per silo ranged from 3 to 10; ~90% of the silos had 5 to 8 sampling ports. In most cases, 12 samples were collected from each storage silo; in addition, sampling included the collection of 24 (from three silos), 20 (from one silo), and 11 (from two silos) samples. To obtain enough samples per silo, more than one sample was taken from some of the silo ports; the number of ports per silo and the number of samples collected per sampling port was not recorded at the time of sampling.

A total of 3,966 raw inshell pistachio samples were collected as follows: 1,151 samples in 2010, 1,380 samples in 2011, and 1,435 samples in 2012. These samples were obtained from 14, 40, and 32 floater silos, respectively, and 82, 75, and 84 sinker silos, respectively. In 2010, the proportion of floater (168 [15%]) and sinker (983 [85%]) samples was roughly equivalent to the proportions by weight that are typical for California pistachios (Fig. 1). An initial evaluation of data from the 2010 samples indicated a potentially higher prevalence of *Salmonella* in the floater

TABLE 1. *Prevalence of* Salmonella *in 100-g samples of inshell floaters and sinkers collected from the 2010, 2011, and 2012 California harvests*

Harvest yr	Floaters			Sinkers			Total			Odds of testing positive		
	No. of 100-g samples	No. of samples positive for *Salmonella*	Prevalence of *Salmonella* (%) (95% CI [%])[a]	No. of 100-g samples	No. of samples positive for *Salmonella*	Prevalence of *Salmonella* (%) (95% CI [%])	No. of 100-g samples	No. of samples positive for *Salmonella*	Weighted prevalence of *Salmonella* (%)[b]	OR[c]	Exact *P* values	95% CI (%)
2010	168	4	2.4 (0.93–6.0)	983	7	0.71 (0.34–1.5)	1,151	11	0.96	3.4	0.13	0.72–13.5
2011	480	3	0.62 (0.21–1.8)	900	3	0.33 (0.11–0.98)	1,380	6	0.37	1.9	0.70	0.25–14.1
2012	384	14	3.6 (2.2–6.0)	1,051	1	0.10 (0.017–0.54)	1,435	15	0.62	39.7	<0.001	5.99–1,681
Total	1,032	21	2.0 (1.3–3.1)	2,934	11	0.37 (0.21–0.67)	3,966	32	0.61			

[a] *Salmonella* prevalence, 95% Wilson (score) confidence intervals (CI), lower limit to upper limit.
[b] Assumption of 15% floaters and 85% sinkers in an average year.
[c] OR, odds ratio. The odds of testing positive for *Salmonella* was significantly higher for inshell floaters compared to sinkers for 2012 (OR = 39.7), but not significantly different for years 2010 and 2011 (i.e., OR not significantly different from 1.0).

samples. Therefore, in 2011 and 2012, a disproportionate number of floater samples (35 and 27%, respectively) were evaluated to facilitate statistical evaluation of the difference in prevalence between the two streams.

Prevalence of *Salmonella* in survey pistachios. *Salmonella* was isolated from a total of 32 of 3,966 100-g pistachio samples collected in 2010, 2011, and 2012 (weighted prevalence 0.61%; Table 1). The average 3-year prevalences in floater and sinker samples were 2.0% (95% CI, 1.3 to 3.1%) and 0.37% (95% CI, 0.21 to 0.67%), respectively. The OR was significantly different across years for testing positive for *Salmonella* in 100 g of floaters compared with sinkers on the basis of homogeneity tests for a common OR (asymptotic P value = 0.02; exact P value = 0.0123). After analyzing the data separately by year, the OR was not significantly different from 1.0 for years 2010 (OR = 3.4; exact P value = 0.13; 95% CI, 0.72 to 13.5) and 2011 (OR = 1.9; exact P value = 0.70; 95% CI, 0.25 to 14.1). In contrast, for 2012, the odds for testing positive for *Salmonella* in 100 g of floaters was ~40 times greater than the odds of testing positive in 100 g of sinkers (OR = 39.7; exact P value <0.001; 95% CI, 5.99 to 1,681).

Levels of *Salmonella* in positive pistachio samples. Levels of *Salmonella* were determined for each of the initially positive samples, using a modified MPN method that included results from additional 50-g subsamples (Table 2). In 2010, the number of additional 50-g subsamples evaluated (4 to 17) was dependent on the amount of available remaining sample. In 2011 and 2012, a total of 10 additional subsamples were enriched for *Salmonella* when sufficient material was available. For 19 (90%) of 21 floater samples, one or more of the subsequent 50, 5.6, or 0.56-g subsamples were positive on enrichment. The median and geometric mean of the levels of *Salmonella* in the 21 positive floater samples was 0.63 and 0.66 MPN/100 g, respectively; levels ranged from 0.14 (95% CI, 0.020 to 0.99) to 5.3 (95% CI, 2.8 to 10) MPN/100 g. All but 2 of the 11 initially positive sinker samples were negative by enrichment of MPN subsamples (Table 2); in each of two samples from 2010, there were four positive 50-g subsamples (4 of 20; 4 of 17). The median and geometric mean of the levels of *Salmonella* in the 11 positive sinker samples was 0.14 and 0.18 MPN/100 g, respectively, with a range of 0.10 (95% CI, 0.014 to 0.73) to 0.62 (95% CI, 0.26 to 1.5) MPN/100 g.

Distribution of *Salmonella*-positive samples among silos. A silo was considered positive when *Salmonella* was isolated from any of the initial 100-g subsamples from any of the samples collected from that silo. For most silos (302 of 327), none of the samples were positive for *Salmonella* (Table 3). Positive samples were isolated from 25 different silos. For 18 silos, 1 of 12 samples was positive, and for 7 silos, 2 of 12 samples were positive. Two positive samples each were identified in one floater silo and in one sinker silo in 2010, in one floater silo in 2011, and in four floater silos in 2012.

TABLE 2. *Levels of* Salmonella *in initially positive floater and sinker pistachio samples by harvest year*

Pistachio type	Harvest yr	No. of samples positive for *Salmonella*/total no. of samples enriched at each wt				MPN/100 g (95% CI)[a]
		100 g	50 g	5.6 g	0.56 g	
Floater	2010	1/1	9/11	3/3	1/3	5.3 (2.8–10)
		1/1	6/10	0/3	0/3	1.8 (0.84–4.0)
		1/1	1/11	0/3	0/3	0.34 (0.08–1.4)
		1/1	2/16	0/3	0/3	0.37 (0.12–1.1)
	2011	1/1	1/9	0/3	0/3	0.41 (0.10–1.6)
		1/1	4/13	0/3	0/3	0.83 (0.34–2.0)
		1/1	1/13	0/3	0/3	0.29 (0.072–1.2)
	2012	1/1	3/13	0/3	0/3	0.63 (0.23–1.7)
		1/1	2/13	0/3	0/3	0.45 (0.14–1.4)
		1/1	2/13	0/3	0/3	0.45 (0.14–1.4)
		1/1	1/13	0/3	0/3	0.29 (0.072–1.2)
		1/1	3/12	0/3	0/3	0.68 (0.25–1.8)
		1/1	5/13	0/3	0/3	1.0 (0.46–2.4)
		1/1	0/13	0/3	0/3	0.14 (0.020–0.99)
		1/1	2/11	0/3	0/3	0.53 (0.17–1.7)
		1/1	7/12	1/3	0/3	2.0 (1.0–4.0)
		1/1	5/13	0/3	0/3	1.0 (0.46–2.4)
		1/1	4/13	0/3	0/3	0.83 (0.34–2.0)
		1/1	0/12	0/3	0/3	0.15 (0.021–1.1)
		1/1	8/13	3/3	0/3	2.7 (1.5–4.9)
		1/1	4/13	1/3	0/3	1.0 (0.44–2.2)
	All years GM[b]					0.66
Sinker	2010	1/1	0/7	0/3	0/3	0.24 (0.034–1.7)
		1/1	0/9	0/3	0/3	0.19 (0.027–1.4)
		1/1	0/14	0/3	0/3	0.13 (0.018–0.92)
		1/1	0/15	0/3	0/3	0.12 (0.017–0.87)
		1/1	4/20	0/3	0/3	0.52 (0.22–1.2)
		1/1	0/18	0/3	0/3	0.10 (0.014–0.73)
		1/1	4/17	0/3	0/3	0.62 (0.26–1.5)
	2011	1/1	0/13	0/3	0/3	0.14 (0.020–0.99)
		1/1	0/13	0/3	0/3	0.14 (0.020–0.99)
		1/1	0/13	0/3	0/3	0.14 (0.020–0.99)
	2012	1/1	0/13	0/3	0/3	0.14 (0.020–0.99)
	All years GM					0.18

[a] MPN/100 g and 95% CIs were calculated with the Excel spreadsheet provided in the FDA BAM method *(9),* using the initial positive 100-g sample and all additional subsamples.
[b] GM, geometric mean.

Pistachio orchards may be shaken one time, but, when substantial crop remains on the trees after the first harvest, a grower may return 1 to 3 weeks later to shake the trees a second and, more rarely, a third time. On the advice of one of the collaborating handlers, in 2012 the DFA recorded whether the sample came from a silo containing "first shake," "second shake," or a "mix" of first and second shake pistachios (Table 3). First shake floater and sinker silos made up 7 and 34%, respectively, of the 116 silos sampled in 2012. All of the *Salmonella*-positive floater samples were collected from silos that held second shake or mixed pistachios. Ten (31%) of 32 floater silos yielded one or more *Salmonella*-positive samples, and one (1.2%) of 84 sinker silos (a second shake silo) yielded a positive sample.

Distribution of *Salmonella* in initially negative pistachio samples. Additional 50-g subsamples of initially negative pistachio samples were retested in 50-g aliquots. In 2010, the decision to retest initially negative samples was made at the end of the survey after most of the samples had been discarded. Ten sinker samples were each retested by enrichment of 10 50-g portions. Of the 10 sinker samples retested, nine were negative for all 10 subsamples, and one yielded a single positive.

A total of 10 50-g subsamples were enriched for *Salmonella* from each of 10 or 20 randomly selected initially negative samples in 2011 and 2012, respectively. In 2011, four of these samples were floaters and six were sinkers. Of the four floater subsamples tested, one was *Salmonella* positive; none of the six sinker subsamples were positive on retesting. In 2012, four of the initially negative randomly selected samples were floaters and 16 were sinkers. Of the four floater subsamples tested, one was positive; none of the 16 sinker subsamples were positive on retesting.

TABLE 3. *Number of silos with 0, 1, or 2* Salmonella-*positive samples as determined from initial 100-g samples*

Harvest yr	Silo type	Shake	No. of positive samples/silo 0	1	2	Total silos
2010	Floater	Unknown	11	2	1	14
	Sinker	Unknown	76	5	1	82
2011	Floater	Unknown	38	1	1	40
	Sinker	Unknown	72	3	0	75
2012	Floater	First shake	8	0	0	8
		Second shake	4	5	2	11
		Mixed	8	1	2	11
		Unknown	2	0	0	2
		(Total floater)	(22)	(6)	(4)	(32)
	Sinker	First shake	39	0	0	39
		Second shake	29	1	0	30
		Mixed	11	0	0	11
		Unknown	4	0	0	4
		(Total sinker)	(83)	(1)	(0)	(84)
All	Total		302	18	7	327

Distribution of *Salmonella* in positive silos. In 2011, a more extensive evaluation was made for the single floater silo that was positive for 2 of 12 samples on initial testing (Table 4). Multiple (6 to 10) additional 50-g subsamples were enriched for *Salmonella* for all 12 of the samples originally drawn from the silo. Thirty-six (24%) of the 150 50-g subsamples evaluated were positive for *Salmonella* (all serovar Montevideo); 10 of the 12 silo samples were positive at least once (Table 4). Combining the data for all of the samples tested, the calculated level of *Salmonella* in the silo was 0.58 MPN/100 g.

In 2012, there were four floater silos that were positive for 2 of 12 samples on initial testing. The MPN was determined for five (two silos) and six (two silos) of these samples. Calculated levels of *Salmonella* for the silos based on these samples were 0.27, 0.21, 1.1, and 0.37 MPN/100 g (Table 4).

***Salmonella* serovars isolated from pistachios.** Three isolated colonies from each initially positive enrichment were purified and serotyped. With one exception, all three initial isolates from a sample were the same serovar. A second serovar was identified in one floater sample in 2010. Isolates from each positive MPN or secondary 50-g subsample enrichment were also serotyped. The serovars isolated from MPN subsamples were often, but not always, the same as the initial isolate. More than one *Salmonella* serovar was isolated from 14 of the 32 positive samples; three or four different serovars of *Salmonella* were isolated from eight of the 32 positive samples (Table 5). All isolates over the 3 years were one of seven different serovars (Table 5). *Salmonella* Montevideo was isolated from 62% of the positive samples over the 3 years (45, 50, and 80% of positive samples in 2010, 2011, and 2012). *Salmonella* Montevideo was isolated from 18 (86%) of 21 of the positive floater samples and from 2 (18%) of 11 of the positive sinker samples. Three phage types (RDNC [routine dilution, no conformity], 9c, and 37) were identified among the six *Salmonella* Enteritidis isolates (9, 6, and 3% of positive samples, respectively).

PFGE fingerprints for *Salmonella* isolates. To provide further information on the diversity of the *Salmonella* isolates, *Xba*I PFGE fingerprints were determined for the isolates from initially positive samples. When a second, third, or fourth *Salmonella* serovar was isolated during the MPN analysis (Table 5), these isolates were also included. Also included in the analysis was a *Salmonella* Montevideo isolate from pistachios associated with a 2009 recall *(34)* and *Salmonella* Enteritidis PT 30 and PT 9c isolated from almonds associated with raw almond outbreaks in 2001 and 2004, respectively *(14, 22)*. The similarity analysis generated nine different genotypic clusters with a Dice coefficient index cutoff point of 80% (Fig. 2). There was a single PFGE cluster (similarity index value 82.5%) of *Salmonella* Montevideo that included 20 isolates with representatives from each year of the survey. Fifteen of these isolates had identical PFGE patterns (similarity index value of 100%) with the *Salmonella* Montevideo isolated in 2009 from recalled pistachios. With the exception of a single outlier (LJH 1292-1), isolates of *Salmonella* Senftenberg (recovered in 2010 and 2012) separated into a single cluster (similarity index value 96.3%). Cluster analysis differentiated (similarity index value 79.6%) *Salmonella* Enteritidis PT 9c and PT 30 (almond outbreak strains and isolates) from *Salmonella* Enteritidis RDNC and PT 37 isolates. *Salmonella* Liverpool separated into a single cluster (similarity index value 82.0%). All 11 isolates of *Salmonella* Worthington recovered in 2010 and 2012 clustered together (similarity index value of 87.4%). The two *Salmonella* Agona strains isolated in 2011 and 2012 had identical PFGE patterns (similarity index value of 100%).

Native microbial populations of 2010 to 2012 survey pistachios. A subset of samples was tested for levels of *E. coli* (2011 and 2012), coliforms (2010), APC, yeasts, and molds. Levels of *E. coli* were below the limit of detection (0.3 MPN/g) for all 113 samples evaluated (Table 6). Coliform levels ranged from below the limit of detection (<0.3 MPN/g) in 16 (70%) of 23 samples to >110 MPN/g in two samples. APC counts ranged from ≤100 (40 samples; 29%) to >250,000 CFU/g (two samples), with a geometric mean of 292 and median of 230 CFU/g for all samples. Geometric mean and median APC levels were 267 and 175 CFU/g and 392 and 305 CFU/g in *Salmonella*-negative and *Salmonella*-positive samples, respectively. Yeast counts were below the limit of detection (<10 CFU/g) in 131 (96%) of 136 samples. Mold levels ranged from below the limit of detection (<10; one of 136 samples) to 11,000 or 12,000 CFU/g (two samples).

DISCUSSION

In California, the pistachio harvest occurs from early September through mid-October; nuts are mechanically shaken from the trees onto catch frames. At maturity, the pistachio shell is split along the suture, but the hull remains fully intact. Although the shell is split, kernels are not

TABLE 4. *Levels and serovars of* Salmonella *in selected pistachio silos*[a]

Harvest yr	Silo	Silo sample date (mo/day/yr)	No. of samples positive for *Salmonella*/total no. of samples enriched at each wt[b]				MPN/100 g (includes additional samples) (95% CI)[c]	Serovars isolated
			100 g	50 g	5.6 g	0.56 g		
2011	1	Unknown	0/1	1/13	0/3	0/3	0.13 (0.019–0.95)	Mo
		(Table 2)[d]	1/1	1/9	0/3	0/3	0.41 (0.10–1.6)	Mo
			0/1	3/11	0/3	0/3	0.51 (0.16–1.6)	Mo
			0/1	1/13	0/3	0/3	0.13 (0.019–0.95)	Mo
			0/1	7/13	0/3	0/3	1.22 (0.57–2.6)	Mo
			0/1	0/13	0/3	0/3	<0.13 (0.019–0.95)	NA
			0/1	0/13	0/3	0/3	<0.13 (0.019–0.95)	NA
			0/1	2/13	0/3	0/3	0.28 (0.070–1.1)	Mo
			0/1	8/13	3/3	3/3	2.7 (1.6–4.7)	Mo
			0/1	4/13	0/3	0/3	0.60 (0.22–1.6)	Mo
		(Table 2)	1/1	4/13	0/3	0/3	0.83 (0.34–2.0)	Mo
			0/1	5/13	2/3	0/3	1.1 (0.53–2.4)	Mo
	Total		2/12	36/150	5/36	3/36	0.58 (0.44–0.78)	
2012	1	12/7/12	0	0/12	0/3	0/3	<0.14 (0.020–1.0)	NA
			0	2/13	0/3	0/3	0.28 (0.070–1.1)	Mo
		(Table 2)	1	3/13	0/3	0/3	0.63 (0.24–1.7)	Ag, Mo
		(Table 2)	1	2/13	0/3	0/3	0.45 (0.14–1.4)	Mo
			0	0/10	ND	ND	<0.17 (0.024–1.2)	NA
	Total		2/5	7/61	0/12	0/12	0.27 (0.14–0.52)	
	2	12/7/12	0	0/13	0/3	0/3	<0.13 (0.019–0.95)	NA
			0	3/13	0/3	0/3	0.43 (0.14–1.3)	Mo
			0	0/13	0/3	0/3	<0.13 (0.019–0.95)	NA
			0	1/13	0/3	0/3	0.13 (0.019–0.95)	Mo
		(Table 2)	1	2/13	0/3	0/3	0.45 (0.14–1.4)	Mo, Se
		(Table 2)	1	1/13	0/3	0/3	0.29 (0.07–1.2)	En RDNC, Mo
	Total		2/6	7/78	0/18	0/18	0.21 (0.11–0.40)	
	3	1/3/13	0	4/13	0/3	0/3	0.60 (0.22–1.6)	Lp, Wo
		(Table 2)	1	8/13	3/3	0/3	2.7 (1.5–4.9)	Lp, Mo, Se, Wo
			0	1/13	0/3	0/3	0.13 (0.019–0.95)	Lp
		(Table 2)	1	4/13	1/3	0/3	1.0 (0.44–2.2)	Lp, Se, Wo
			0	6/12	3/3	2/3	1.9 (0.98–3.5)	Lp, Mo, Wo
	Total		2/5	23/64	7/15	2/15	1.1 (0.80–1.6)	
	4	1/4/13	0	1/13	0/3	0/3	0.13 (0.019–0.95)	Wo
		(Table 2)	1	0/13	0/3	0/3	0.14 (0.020–0.99)	Wo
			0	4/13	0/3	0/3	0.60 (0.22–1.6)	Lp, Mo, Wo
			0	2/13	1/3	0/3	0.42 (0.14–1.3)	Wo
		(Table 2)	1	2/11	0/3	0/3	0.53 (0.17–1.7)	Lp, Mo, Wo
			0	3/13	0/3	0/3	0.43 (0.14–1.3)	Lp, Mo, Wo
	Total		2/6	12/76	1/18	0/18	0.37 (0.22–0.61)	

[a] Mo, Montevideo; NA, not applicable; Ag, Agona; ND, not done; Se, Senftenberg; En RDNC, Enteritidis phage type routine dilution, no conformity; Lp, Liverpool; Wo, Worthington.
[b] The number of enrichments that were positive for *Salmonella* in one 100-g, three 50-g, three 5.6-g, and three 0.56-g samples, respectively.
[c] MPN/100 g and 95% CIs were calculated with the Excel spreadsheet provided in the FDA BAM method *(9),* using the initial positive 100-g sample and all additional subsamples.
[d] (Table 2), data that are also shown in Table 2.

usually exposed to the environment until the hull comes off. Depending on the level of maturity, it is possible for some or part of the hull to slip off as a result of the abrasion that occurs with shaking of the trees, conveyance to bins or trailers, and transport to the huller; it is also possible for the majority of product (especially when harvested earlier in the season) to arrive at the huller with fully intact hulls.

After hulling, pistachios are separated in a float tank. Separately, floater and sinker pistachios are partially dried (minimum <15% moisture, and often <9%) in forced air driers at temperatures ranging from 75 to 95°C (initial) to 50°C (final) for 4 to 8 h. Microbial populations (APC and coliform counts) decrease by ~2 log CFU/g during drying *(23)*. The partially dried nuts are moved into large storage silos (450 to 680 metric tons) where they are further dried to <7% moisture with ambient forced air. Once the nuts have reached the target moisture level, the silo is closed and fumigated for insect control and storage of up to 14 months.

Over 98% of U.S. pistachios are grown in California *(3)*. This survey examined nearly 4,000 100-g raw inshell

TABLE 5. Salmonella *serotypes isolated from positive pistachio samples (2010 through 2012)*

Pistachio type	Harvest yr	Sample no.	Serovar[a]: Ag	En RDNC	En 9c	En 37	Lp	Mo	Se	Te	Wo
Floater	2010	1						1, 2, 3			3
		2				1		1, 2, 3			3
		3									1, 3
		4	3					1, 3			
	2011	1						1, 3			
		2						1, 2, 3			
		3						1, 3			
	2012	1	3					1, 3			
		2						1, 2, 3			
		3						3	1		
		4		1				3			
		5						1, 2, 3			
		6					3	2, 3	2, 3		1, 2, 3
		7									1
		8					3	1			3
		9					3	2, 3			1, 3
		10					1, 3	3	2, 3		3
		11					1, 2, 3	3			3
		12						1			
		13					2, 3	1, 2, 3	3		2, 3
		14					2, 3		1, 2, 3		2, 3
Sinker	2010	1		1							
		2			1						
		3								1	
		4						1			
		5						1, 3			3
		6							1		
		7							1, 3		
	2011	1			1						
		2					1				
		3		1							
	2012	1							1		
No. of samples (of 32)			2	3	2	1	8	20	8	1	12
% total samples with serovar			6	9	6	3	25	62	25	3	38

[a] Ag, Agona; En RDNC, Enteritidis phage type routine dilution, no conformity; En 9c, Enteritidis PT 9c; En 37, Enteritidis PT 37; Lp, Liverpool; Mo, Montevideo; Se, Senftenberg; Te, Tennessee; Wo, Worthington. 1, 2, and 3, serotype isolation during initial 100-g enrichment, traditional MPN, and subsequent MPN (secondary) isolation, respectively.

pistachio samples that were collected over three separate harvests from storage silos of seven processors representing 98% of the volume of pistachios grown in California. Pistachio samples were collected within 4 months of harvest and were stored at 4°C. In previous studies, no significant reductions of inoculated *Salmonella* were observed on inshell pistachios over 1 year of storage at 4°C *(24)*.

Pistachios are unique among the tree nuts in that they are sorted early after harvest into two distinct streams on the basis of their density. The average prevalence of *Salmonella* in sinker pistachios of 0.37% (100 g; 95% CI, 0.21 to 0.67%) was significantly lower than that determined in raw almond kernels and raw inshell pecans and was not significantly different from that in raw inshell walnuts. The average prevalence of *Salmonella* was 0.98% (100 g; 95% CI, 0.83 to 1.2%; 137 of 13,972) in raw almond kernels *(8, 17, 25)*, 0.95% (100 g; 95% CI, 0.70 to 1.3%; 44 of 4,641) in raw inshell pecans *(10)*, and 0.14% (375 g; 95% CI, 0.054 to 0.35%; 4 of 2,903) in raw inshell walnuts *(18)*.

The average prevalence of *Salmonella* in floater pistachios of 2.0% (100 g; 95% CI, 1.3 to 3.1%) was significantly higher than prevalence data published for raw almond kernels, inshell pecans, or inshell walnuts but was not significantly different from the prevalence in raw inshell almonds (6 [1.3%] of 455; 100 g; 95% CI, 0.61 to 2.8%) *(8)*. Floaters generally have small kernels due to immaturity or insect damage or have no kernels (blanks) *(1)*; they may also include fully formed nuts that have tightly adhering hull material. The inedible portion of the floater stream can be 75% or more of the product weight in contrast to 7% of the sinker stream (Fig. 1).

The majority of U.S. pistachios are sold as inshell product (~10 and ~90% of the floater and sinker streams, respectively; Fig. 1); the naturally split pistachios are separated from those with closed shells. Inshell product that

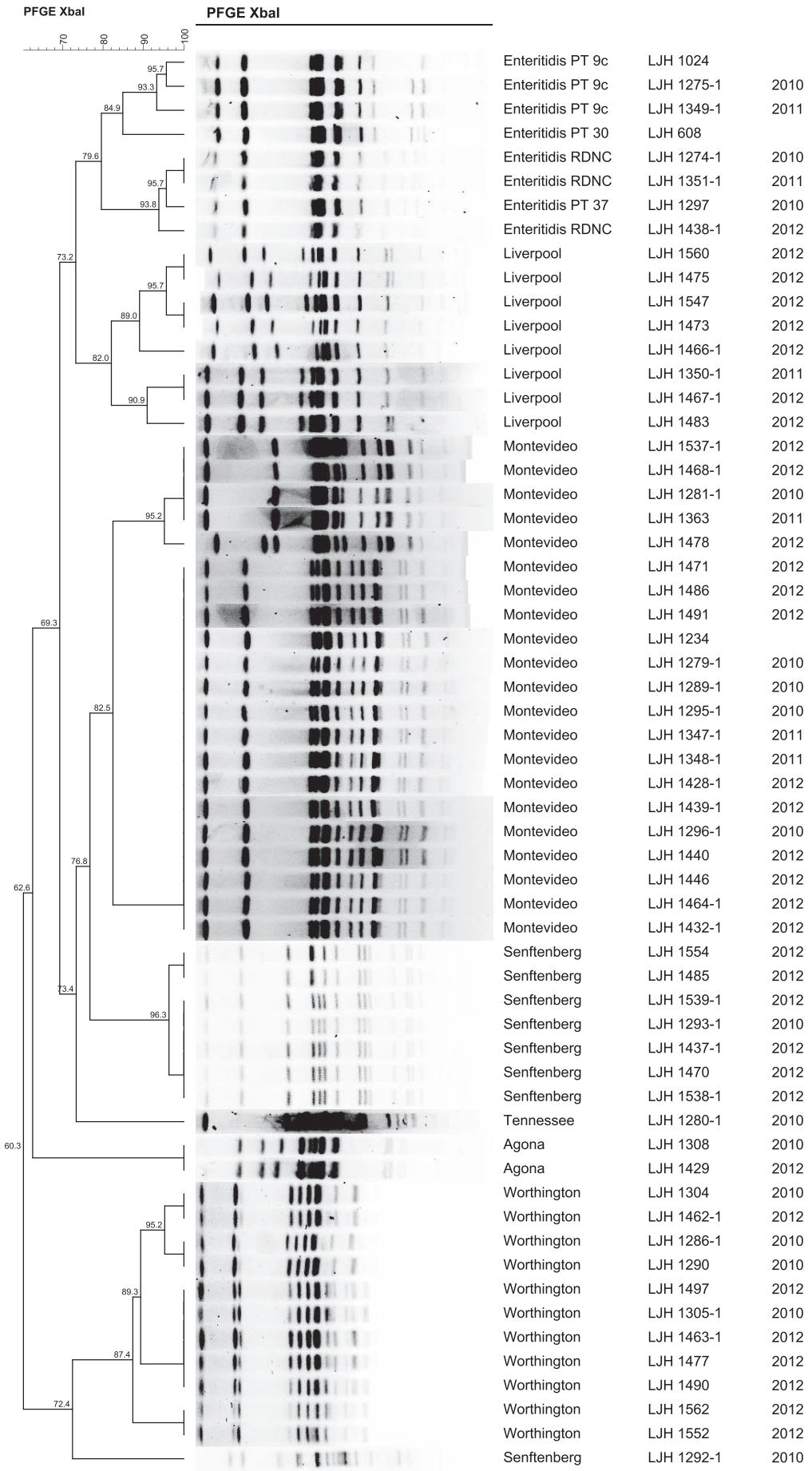

FIGURE 2. *Dendrogram of* Salmonella *isolates recovered from raw pistachios determined by PFGE-*XbaI *fingerprinting*.

TABLE 6. *Frequency of* E. coli *and coliforms and total aerobic plate, yeast, and mold counts for survey pistachios*

Harvest yr	Sample type	No. of samples	No. of samples in count range														
			E. coli (MPN/g)	Coliforms (MPN/g)			APC (CFU/g)				Yeasts (CFU/g)			Molds (CFU/g)			
			<0.3	<0.3	0.3–110	>110	10–100	101–10,000	10,001–250,000	>250,000	<10	10–300		<10	10–100	101–10,000	>10,000
2010	Positive	11	ND[a]	7	3	1	2	9	0	0	11	0		0	5	6	0
	Negative	12	ND	9	2	1	3	9	0	0	10	2		0	4	8	0
	Total	23	ND	16	5	2	5	18	0	0	21	2		0	9	14	0
2011	Positive	6	6	ND	ND	ND	3	2	1	0	4	2		0	4	2	0
	Negative	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		0	ND	ND	ND
	Total	6	6	ND	ND	ND	3	2	1	0	4	2		0	4	2	0
2012	Positive	15	15	ND	ND	ND	1	14	0	0	15	0		0	2	13	0
	Negative	92	92	ND	ND	ND	31	53	6	2	91	1		1	43	46	2
	Total	107	107	ND	ND	ND	32	67	6	2	106	1		1	45	59	2
Total	Positive	32	21				6	25	1	0	30	2			11	21	0
	Negative	104	92				34	62	6	2	101	3		1	47	54	2
	Total	136	113				40	87	7	2	131	5		1	58	75	2

[a] ND, not done.

meets quality standards (light colored shell that is open) is considered "edible inshell"; edible inshell material from the floater and sinker streams may be combined. Both sinker and floater streams go through additional mechanical and electronic sorting for size, color, and shell split after they are removed from silo storage. In most cases, the floater stream requires extensive sorting; some processors will sell their entire floater stream to companies that specialize in this type of sorting. Pistachios with closed shells from either floater or sinker streams, or those with shells that are dark or have other cosmetic defects, are shelled. The extracted kernels are sorted into inedible kernels (free kernels that are damaged by insects, shriveled, overly dark; ~50 and ~2.4% of floater and sinker streams, respectively), shell pieces (~25 and ~5.0% of floater and sinker streams, respectively), and edible kernels (~15 and ~2.6% of floater and sinker streams, respectively). In this study, no attempt was made to sort the samples as they were received at the laboratory. It is not known whether the *Salmonella* was equally distributed among the edible and inedible portions of the floater and sinker samples or among inshell or kernel fractions.

When measured, levels of *Salmonella* in tree nuts are typically <1 MPN/100 g *(8, 10, 17, 20)* and are similar to the levels reported here (Table 2). Lambertini et al. *(25)* used MPN data from recalled almonds and calculated rates of reduction during storage to estimate *Salmonella* levels of 120 MPN/100 g during a 2001 almond outbreak. Even when there was a higher prevalence of *Salmonella* in a single silo (Table 4), the calculated levels of *Salmonella* were below 1 MPN/100 g. A standard serving of inshell pistachios is ~50 g (approximately half of which is shell).

None of the authors were present during sampling. The possibility that the trier may not have been adequately sanitized in between samples or that small amounts of pistachios remained in the trier and were transferred from one sample to the next was considered. For the 2012 harvest year, the 14 positive floater samples were collected on three of 24 separate sample dates (7 December 2012, 3 January 2013, and 4 January 2013). Three of five, three of three, and five of eight floater silos, respectively, had one or two positive samples on those days. On 3 January 2013, five sinker silos were also sampled; none of the 60 sinker samples were positive on that date. The single positive sinker sample from the 2012 harvest was isolated from one of nine sinker silos that were sampled on 2 January 2013. Although it is possible that some cross-sample contamination occurred within the floater samples, it is also possible that several contaminated silos were clustered at a single processor location.

Pistachio orchards can be harvested a single time, but often a second or third shake one or more weeks later may be warranted if the first shake fails to remove all of the nuts and if it is economical to do so. The "second shake" nuts usually have softer hulls because it is later in the season and the fruit is more mature. In 2012, samples were categorized as first shake, second shake, or mixed; all of the positive samples were sourced from either a second shake or mixed silo. Because data about time of harvest were only available for one of the 3 years of the survey, it is not known whether

this variable has a predictable influence on *Salmonella* prevalence.

The limited number of *Salmonella* serovars and PFGE fingerprints for the pistachio isolates from all 3 years is unique among other published tree nut surveys. Forty-two different serovars of *Salmonella* were observed among 139 raw almond isolates recovered over 7 years *(8, 17)*. Likewise, in a 4-year survey of inshell pecans, 31 different serovars were identified in 42 positive samples *(10)*.

The isolation of *Salmonella* Montevideo from 62% of all pistachio samples either initially or in subsequent sampling is strongly suggestive of a resident population of this organism at one or more processors. A whole-genome single nucleotide polymorphism analysis was applied to 47 *Salmonella* Montevideo isolates with the *Xba*I PFGE pattern JIXX01.0011 *(19),* including the 2009 pistachio isolate analyzed here. The single nucleotide polymorphism analysis identified six strains (one from pistachios and five from humans) isolated in 2007, 2008, and 2009 that clustered away from the rest of the 2004 to 2010 isolates, and the authors denoted this group as a putative "pistachio" clade. *Salmonella* Montevideo was also associated with a 2016 pistachio outbreak *(16)*. Single nucleotide polymorphism analysis of the *Salmonella* Montevideo isolates from the current survey would provide more conclusive evidence of the long-term persistence of this organism in the pistachio environment.

Eight (25%) of the samples were positive for *Salmonella* Senftenberg (seven isolates with PFGE patterns in one cluster and a single outlier). This serovar was also isolated from recalled pistachios in 2009 (2008 harvest) and 2013 (2012 harvest) *(15, 34, 35)*. The *Salmonella* Senftenberg isolates from the 2009 and 2013 pistachio recalls were not included in our PFGE analysis. The facility associated with the 2013 recall was not included in the present survey because it was not in operation prior to 2012 *(2)*.

Salmonella Enteritidis PT 9c was isolated from single sinker samples in 2010 and 2011. This is a rare phage type associated with a 2004 salmonellosis outbreak traced to the consumption of raw almonds *(14, 30)*; it was isolated from a raw almond survey sample in 2005 *(17)*. *Salmonella* Enteritidis PT 30 was isolated from almond orchards for 6 years *(32)* and persisted in almond hulling and processing environments for several months *(22)*.

Salmonella can multiply on harvested, prehulled pistachios as well as on hulled product *(29)*. Multiplication is more rapid on floaters, in part because they have higher amounts of attached hull material. Delays between harvest and hulling or between hulling and drying might be one explanation for the higher prevalence and levels of *Salmonella* observed in floater samples. Because this was a blind study we were unable to further evaluate the source(s) of *Salmonella* in the contaminated pistachios.

Isolation of a pathogen from a positive lot of product depends on the analytical method used and the distribution and levels of the pathogen in the sample *(21)*. Analytical methods are typically assumed to detect one or more cells of *Salmonella*; and these cells are often assumed to be Poisson distributed throughout the lot, even though this may not be the case *(36)*. The combination of low prevalence (<1%) and low levels of *Salmonella* (<1 MPN/100 g) in pistachios contributed to the frequent failure to reisolate *Salmonella* from separate subsamples of initially positive pistachios and, likewise, the isolation of *Salmonella* from separate subsamples of initially negative pistachios (Tables 2 and 4). These factors should be considered when establishing sampling plans and when interpreting *Salmonella* test results for pistachios.

The majority of pistachios in North America are sold as roasted inshell product, and most of these are derived from the sinker stream. The prevalence and levels of *Salmonella* in pistachios measured here are similar to those observed for other tree nuts, but the limited range of isolated serovars suggests a more narrow contamination source. Identification and elimination or control of this contamination source may be one strategy to reduce the prevalence of *Salmonella* in U.S. pistachios.

ACKNOWLEDGMENTS

Funds were provided by the Center for Produce Safety at the University of California, Davis, in partnership with the California Department of Food and Agriculture's Specialty Crop Block Grant Program, and the California Pistachio Research Board. The contributions made by Lillian Khan, Chris Theofel, and Sylvia Yada in technical support and during the writing process are greatly appreciated.

REFERENCES

1. Administrative Committee for Pistachios. 2014. Comment re docket no. FDA-2013-N-0747: assessment of the risk of human salmonellosis associated with the consumption of tree nuts; request for comments, scientific data and information. Available at: http://www.regulations.gov/#!documentDetail;D=FDA-2013-N-0747-0031. Accessed February 2016.
2. Administrative Committee for Pistachios. 2016. Personal communication. Available from the author at: ljharris@ucdavis.edu.
3. American Pistachio Growers. 2015. Commercial cultivation of American pistachios. Available at: http://americanpistachios.org/power-of-pistachios/history. Accessed September 2015.
4. Andrews, W. H., A. Jacobson, and T. S. Hammack. 2014. *Salmonella,* chap. 5. *In* U.S. Food and Drug Administration Bacteriological analytical manual. Available at: http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm070149.htm. Accessed September 2015.
5. AOAC International. 2000. AOAC official method 966.23. Microbiological methods. *In* Official methods of analysis, 17th ed. AOAC International, Gaithersburg, MD.
6. AOAC International. 2000. AOAC official method 966.24. Coliform group and *Escherichia coli* in tree nut meats. *In* Official methods of analysis, 17th ed. AOAC International, Gaithersburg, MD.
7. AOAC International. 2000. AOAC official method 2001.09. *Salmonella* in selected foods—immuno-concentration *Salmonella* (ICS) and enzyme-linked immunofluorescent assay (ELFA) screening method. *In* Official methods of analysis, 17th ed. AOAC International, Gaithersburg, MD.
8. Bansal, A., T. M. Jones, S. J. Abd, M. D. Danyluk, and L. J. Harris. 2010. Most-probable-number determination of *Salmonella* levels in naturally contaminated raw almonds using two sample preparation methods. *J. Food Prot.* 73:1986–1992.
9. Blodgett, R. 2010. Most probable number from serial dilutions, appendix 2. *In* U.S. Food and Drug Administration Bacteriological analytical manual. Available at: http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm109656.htm. Accessed September 2015.
10. Brar, P. K., L. K. Strawn, and M. D. Danyluk. 2016. Prevalence, concentration, and types of *Salmonella* isolated from North American inshell pecans over four harvest years. *J. Food Prot.* 79:352–360.

11. Breslow, N. E., and N. E. Day (ed.). 1980. Statistical methods in cancer research, vol. 1. The analysis of case-control studies. IARC Scientific Publications no. 32. International Agency for Research on Cancer, Lyon, France.
12. Candlish, A. A. G., S. M. Pearson, K. E. Aidoo, J. E. Smith, B. Kelly, and H. Irvine. 2001. A survey of ethnic foods for microbial quality and aflatoxin content. *Food Addit. Contam.* 18:129–136.
13. Centers for Disease Control and Prevention. 2004. One-day (24–28 h) standardized laboratory protocol for molecular subtyping of *Escherichia coli* O157:H7, non-typhoidal *Salmonella* serotypes, and *Shigella sonnei* by pulsed field gel electrophoresis. Available at: http://www.cdc.gov/pulsenet/protocols/ecoli_salmonella_shigella_protocols.pdf. Accessed September 2015.
14. Centers for Disease Control and Prevention. 2004. Outbreak of *Salmonella* serotype Enteritidis infections associated with raw almonds—United States and Canada, 2003–2004. *Morb. Mortal. Wkly. Rep.* 53:484–487.
15. Centers for Disease Control and Prevention. 2009. Multistate outbreak of *Salmonella* infections linked to pistachio nuts, final update. Available at: www.cdc.gov/salmonella/pistachios/. Accessed September 2015.
16. Centers for Disease Control and Prevention. 2016. Multistate outbreak of *Salmonella* Montevideo infections linked to Wonderful pistachios. Available at: http://www.cdc.gov/salmonella/montevideo-03-16/index.html. Accessed March 2016.
17. Danyluk, M. D., T. M. Jones, S. J. Abd., F. Schlitt-Dittrich, M. Jacobs, and L. J. Harris. 2007. Prevalence and amounts of *Salmonella* found on raw California almonds. *J. Food Prot.* 70:820–827.
18. Davidson, G. R., J. C. Frelka, M. Yang, T. M. Jones, and L. J. Harris. 2015. Prevalence of *Escherichia coli* O157:H7 and *Salmonella* on inshell California walnuts. *J. Food Prot.* 78:1547–1553.
19. den Bakker, H. C., A. I. Moreno Switt, G. Govoni, C. A. Cummings, M. L. Ranieri, L. Degoricija, K. Hoelzer, L. D. Rodriguez-Rivera, S. Brown, E. Bolchacova, M. R. Furtado, and M. Wiedmann. 2011. Genome sequencing reveals diversification of virulence factor content and possible host adaptation in distinct subpopulations of *Salmonella enterica*. *BMC Genomics* 12:425.
20. Harris, L. J., M. Palumbo, L. R. Beuchat, and M. D. Danyluk. 2015. Prevalence and levels of foodborne pathogens on naturally-contaminated nuts and edible seeds, tables 1 to 4 and references. *In* Surveys for foodborne pathogens on nuts. Available at: http://ucfoodsafety.ucdavis.edu/Nuts_and_Nut_Pastes. Accessed February 2016.
21. Hoelzer, K., and R. Pouillot. 2013. Practical considerations for the interpretation of microbial testing results based on small numbers of samples. *Foodborne Pathog. Dis.* 10:907–915.
22. Isaacs, S., J. Aramini, B. Ciebin, J. A. Farrar, R. Ahmed, D. Middleton, A. U. Chandran, L. J. Harris, M. Howes, E. Chan, A. S. Pichette, K. Campbell, A. Gupta, L. Y. Lior, M. Pearce, C. Clark, F. Rodgers, F. Jamieson, I. Brophy, and A. Ellis. 2005. An international outbreak of salmonellosis associated with raw almonds contaminated with a rare phage type of *Salmonella* Enteritidis. *J. Food Prot.* 68:191–198.
23. Kimber, M. 2011. Changes in total aerobic, coliform, and presumptive *Escherichia coli* counts on pistachios during postharvest handling and survival of *Salmonella* spp., *Escherichia coli* O157:H7, and *Listeria monocytogenes* on almond kernels and inshell pistachios. M.S. thesis. University of California, Davis.
24. Kimber, M. A., H. Kaur, L. Wang, M. D. Danyluk, and L. J. Harris. 2012. Survival of *Salmonella, Escherichia coli* O157:H7, and *Listeria monocytogenes* on inoculated almonds and pistachios stored at −19, 4, and 24°C. *J. Food Prot.* 75:1394–1403.
25. Lambertini, E., M. D. Danyluk, D. W. Schaffner, C. K. Winter, and L. J. Harris. 2012. Risk of salmonellosis from consumption of almonds in the North American market. *Food Res. Int.* 45:1166–1174.
26. Little, C. L., W. Jemmott, S. Surman-Lee, L. Hucklesby, and E. de Pinna. 2009. Assessment of the microbiological safety of edible roasted nut kernels on retail sale in England, with a focus on *Salmonella*. *J. Food Prot.* 72:853–855.
27. Little, C. L., N. Rawal, E. de Pinna, and J. McLauchlin. 2010. Survey of *Salmonella* contamination of edible nut kernels on retail sale in the UK. *Food Microbiol.* 27:171–174.
28. McEgan, R., J. C. Chandler, L. D. Goodridge, and M. D. Danyluk. 2014. Diversity of *Salmonella* isolates from central Florida surface waters. *Appl. Environ. Microbiol.* 80:6819–6827.
29. Morales, V. M., H. Kaur, I. Y. Zhao, and L. J. Harris. 2011. Behavior of inoculated *Salmonella* spp. in postharvest pistachio handling, P3-115. Abstr. Annu. Meet. IAFP 2011, Milwaukee, WI, 31 July to 3 August.
30. Parker, C. T., S. Huynh, B. Quiñones, L. J. Harris, and R. E. Mandrell. 2010. Comparison of genotypes of *Salmonella enterica* serovar Enteritidis phage type 30 and 9c strains isolated during three outbreaks associated with raw almonds. *Appl. Environ. Microbiol.* 76:3723–3731.
31. Tournas, V., M. E. Stack, P. B. Mislivec, H. A. Koch, and R. Bandler. 2001. Yeasts, molds and mycotoxins, chap. 18. *In* U.S. Food and Drug Administration Bacteriological analytical manual. Available at: http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm071435.htm. Accessed September 2015.
32. Uesugi, A. R., M. D. Danyluk, R. E. Mandrell, and L. J. Harris. 2007. Isolation of *Salmonella* Enteritidis phage type 30 from a single almond orchard over a 5-year period. *J. Food Prot.* 70:1784–1789.
33. U.S. Department of Agriculture, Foreign Agricultural Service. 2015. Pistachio summary. Available at: http://apps.fas.usda.gov/psdonline/psdReport.aspx?hidReportRetrievalName=Pistachio+Summary&hidReportRetrievalID=2364&hidReportRetrievalTemplateID=8. Accessed September 2015.
34. U.S. Food and Drug Administration. 2009. Setton Pistachio of Terra Bella, Inc. announces nationwide recall of pistachios because of possible health risk. Recall—firm press release. Available at: http://www.fda.gov/safety/recalls/archiverecalls/2009/ucm128913.htm. Accessed September 2015.
35. U.S. Food and Drug Administration. 2014. FDA investigation summary—multistate outbreak of *Salmonella* Senftenberg infections associated with pistachios from a California roaster. Available at: http://www.fda.gov/Food/RecallsOutbreaksEmergencies/Outbreaks/ucm386377.htm. Accessed September 2015.
36. Van Doren, J. M., R. J. Blodgett, R. Pouillot, A. Westerman, D. Kleinmeier, G. C. Ziobro, Y. Ma, T. S. Hammack, V. Gill, M. F. Muckenfuss, and L. Fabbri. 2013. Prevalence, level and distribution of *Salmonella* in shipments of imported capsicum and sesame seed spice offered for entry to the United States: observations and modeling results. *Food Microbiol.* 36:149–160.
37. Zelen, M. 1971. The analysis of several 2×2 contingency tables. *Biometrika* 58:129–137.