Prevalence and Amounts of *Salmonella* Found on Raw California Almonds

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MS 06-427: Received 7 August 2006/Accepted 24 November 2006

**ABSTRACT**

Data on the prevalence and populations of pathogens in individual foods are critical to the development of product-specific quantitative microbial risk assessments. An outbreak of salmonellosis associated with the consumption of raw almonds in 2000 to 2001 provided an opportunity to evaluate the levels of *Salmonella* in the recalled product. Duplicate 100-g samples from each of fifty 22.7-kg boxes of recalled almonds were enriched by one of two methods. *Salmonella* was isolated by at least one method from 42 boxes (84% positive). The levels of *Salmonella* determined by a three-tube most-probable-number (MPN) method were 8.5 ± 1.3 MPN/100 g. In a subsequent study, raw almonds that arrived at almond processors were sampled from 2001 through 2005 to determine the overall prevalence and levels of *Salmonella* and to characterize the *Salmonella* isolates obtained. Aerobic plate counts, coliform counts, and MPN levels of *Escherichia coli* were also determined on positive samples. An isolation frequency for *Salmonella* of 81 (0.87% ± 0.2%) of 9,274 samples tested (100 g) was determined for raw almonds sampled from throughout California over the 5-year period. *Salmonella* was not isolated upon retesting in 59 of 65 positive samples. When detected, levels were 1.2 to 2.9 MPN/100 g. Of the 81 total isolates, 35 different serotypes of *Salmonella* were represented. Aerobic plate counts, coliform counts, and *E. coli* levels did not correlate with the presence of *Salmonella*.

Nuts (broadly classified to include nuts, seeds, legumes, and drupes) have traditionally been considered microbiologically safe because of their low water activity (generally less than 0.70). Although nut-associated outbreaks are relatively uncommon, they have been documented for almonds (11, 23), coconut (30), sesame seeds (31), and peanuts or peanut products (26, 34). *Salmonella* cannot multiply on nuts or in nut products, but the organism can survive on and in these products for extended periods (greater than 1 year), especially when held in cold storage (8, 10, 24, 27, 28, 39).

A number of studies have documented the presence of *Salmonella* on nuts, including sesame seeds (31), macadamia nuts (36), walnuts (32), cashew and Brazil nut kernels (19), and dried coconut meat (33). *Salmonella* was not found in 29 almond samples in the only published survey for this nut (25).

Outbreaks of salmonellosis from the consumption of raw almonds occurred in Canada and the United States in 2000 to 2001 (23) and in 2003 to 2004 (11). *Salmonella* Enteritidis phage type 30 (PT 30) was isolated from multiple opened and unopened boxes of raw almonds that had been epidemiologically linked to the 2000 to 2001 outbreak (23). In 2004, *Salmonella* Enteritidis PT 9c, matching the outbreak strain, was isolated from a single almond sample (unknown sample size) from an infected consumer’s open container of almonds (17). In both outbreaks, the almonds had been grown and processed in California, the source of virtually all almonds produced in North America.

Given the lack of information on the prevalence and levels of *Salmonella* in raw almonds, this study was undertaken to (i) analyze the microbiological data gathered on naturally contaminated raw almonds associated with the 2000 to 2001 outbreak of salmonellosis; (ii) determine the prevalence of *Salmonella* on raw almonds arriving at almond handlers (processors) throughout the almond-producing regions of California over a 5-year period; (iii) determine aerobic plate counts (APCs), coliform counts, and the most probable number (MPN) of *Escherichia coli* and *Salmonella* on positive samples; and (iv) characterize the *Salmonella* isolates obtained from prevalence and MPN studies.

**MATERIALS AND METHODS**

Recalled almonds from 2000 to 2001. Approximately 1,000 unopened 22.7-kg boxes of raw almonds (Carmel variety; Market Classification: Natural California Supreme; 23 kernels per 28 g) representing four lots of almonds associated with a 2000 to 2001 outbreak of salmonellosis (23) were obtained from the products recovered in the outbreak recall. These almonds were harvested in the fall of 2000, and from mid-February through early April 2001, they were transported to Ontario, Canada, at ambient temperature and subsequently stored at ambient temperature (40). The
almonds used in the study were then transported back to California at ambient temperature beginning in April 2001. Of these 1,000 boxes, 50 were randomly sampled and tested for the presence of *Salmonella* by Silliker Laboratories (Modesto, Calif.), following both U.S. Food and Drug Administration *Bacteriological Analytical Manual* (FDA-BAM) and the Canadian Health Protection Branch (HPB) methods (see below). When samples were positive by the FDA-BAM method, the corresponding box was resampled to determine the MPN of the *Salmonella* on the almonds by the FDA-BAM MPN technique (see below).

**Survey almonds.** Almonds from the 2001 through 2005 harvests were collected by seven almond handlers (processors) representing small, medium, and large processing facilities located throughout the almond-growing regions of California. The number of samples that a processor was asked to collect was proportional to the amount of almonds processed by that facility. The identities of the processors were kept confidential, and the samples were coded so that the laboratory technicians did not know the origin of the samples they were analyzing. However, the farms linked to the 2000 to 2001 outbreak, and the processors who handled their products, were purposely excluded from the survey. The rare *Salmonella* Enteritidis PT 30 phage type, associated with the outbreak and traced back to these farms, would have compromised the confidentiality of the survey had these farms been included in the study.

As processors receive deliveries from growers, each lot is sampled, either by automatic sampling devices or by the probing of individual bins, to establish a sample that is representative of the entire lot. Lot sizes can vary greatly with grower and handler but generally range from approximately 2,000 lb (900 kg) on the lower end to 30,000 to 1 million lb (13,000 to 450,000 kg) on the higher end. These samples are visually analyzed by U.S. Department of Agriculture inspectors to determine the total kernel weight and the percent that is inedible or defective and are used by the processor to determine the grade of the grower’s delivery and thereby the payment. For this survey, the processors removed approximately 500 g of almonds from the representative samples taken as grower deliveries were received. Samples were stored at 4°C before and after testing. Almond processors were instructed to select lots throughout the harvest season (July through November) and to sample the almond varieties proportionally; if Nonpareil was 50% of their variety mix, then 50% of their samples should have been Nonpareil.

The collected samples were tested for *Salmonella* by the American Council for Food Safety and Quality (ACFSQ; Fresno, Calif.) by AOAC Official Method 2001.09 (see below). Samples positive for *Salmonella* were shipped on ice to the University of California at Davis, where the FDA-BAM MPN technique was used to determine the MPN of the *Salmonella* (see below).

**Enrichment for *Salmonella* from almonds.** Enrichment and isolation of the *Salmonella* were conducted by the methods described below. Unless otherwise noted, all media and antisera were obtained from Difco, Becton Dickinson (Sparks, Md.).

(i) **FDA-BAM method.** For the FDA-BAM method (2), recalled almonds (100 g) and 400 ml of lactose broth were blended at low speed for 2 min and transferred to a sterile 500-ml screw-cap jar; these jars were incubated at 35 ± 2°C for 24 ± 2 h. Following preenrichment, the samples were enriched in both Rapaport-Vassiliadis R10 broth (42 ± 0.2°C for 24 ± 2 h) and tetrathionate broth (35 ± 2°C for 24 ± 2 h). Enrichments were streaked onto bismuth sulfite agar (35 ± 2°C for 48 ± 2 h), xylose lysine deoxycholate agar, and Hektoen Enteric agar (35 ± 2°C for 24 ± 2 h). Two typical *Salmonella* colonies were selected from each enrichment and confirmed by stabbing and streaking into lysine iron agar and triple sugar iron slants (35 ± 2°C for 24 ± 2 h). Presumptive *Salmonella* results from triple sugar iron slants were confirmed with a *Salmonella* latex test (Oxoid, Ogdensburg, N.Y.).

(ii) **HPB-OMH method.** Alternatively, enrichment for *Salmonella* was conducted by the Canadian HPB method (MFHPB-20) (14), with Ontario Ministry of Health (OMH) modifications. Recalled almonds (100 g) were added to a sterile bag containing 900 ml of 1% buffered peptone water. The bag was sealed and vigorously shaken by hand for approximately 15 s to thoroughly wash the surface of the almonds. After an incubation of the almonds in 1% peptone water at 35°C for 18 to 24 h, a 1.0-ml portion of the preenrichment broth was transferred into 10 ml of tetrathionate brilliant green broth and incubated at 42°C for 48 ± 2 h. The selective tetrathionate brilliant green broth was streaked onto brilliant green sulfa agar and novobiocin brilliant green glucose agar, prepared as described by Devenish et al. (16). Plates were incubated at 35°C for 24 ± 2 h. Suspect colonies were stabbed and streaked into triple sugar iron and lysine iron agar slants and incubated at 35°C for 24 ± 2 h. On the basis of biochemical patterns, presumptive *Salmonella* isolates were tested with polyvalent O group somatic antisera. Isolates with a positive agglutination reaction were further identified as belonging to the genus *Salmonella* with API 20E test strips (bioMérieux, St. Laurent, Quebec, Canada).

(iii) **ACFSQ method.** In accordance with AOAC Official Method 2001.09 (5), survey almonds were mixed, and subsamples (100 g) were combined with 900 ml of buffered peptone water (BD Diagnostic Systems, Sparks, Md.) in a sterile 946-ml plastic jar (Bel-Art Products, Pequannock, N.J.) and blended at 4,500 rpm for 2 min with an Omni Mixer Homogenizer (Omni International, Marietta, Ga.). Following blending, the samples were loosely capped and incubated at 35 ± 2°C for 18 to 24 h. The overnight preenrichment culture was then subjected to immunocentrifugation by the automated mini-VIDAS system (bioMérieux, Hazelwood, Mo.). Preenrichment broth (800 μl) was processed on an immunocentrifugation *Salmonella* (ICS) test strip (bioMérieux), and the resulting concentrate was used to inoculate a 2-ml vial of ICS broth (bioMérieux); vials were incubated at 41°C for 5 h. After incubation, 1 ml of the ICS broth culture was boiled for 15 min and then cooled to room temperature. To screen for *Salmonella*, 500 μl of the boiled ICS culture was added to an SLM (*Salmonella*) test strip (bioMérieux) and tested for *Salmonella* by the mini-VIDAS system. The VIDAS screening system is based on an enzyme-linked fluorescent assay; a relative fluorescence value greater than 0.23 was considered a positive result.

If a sample was positive for *Salmonella* by the VIDAS system, the remaining (unboiled) portion of the ICS broth culture from the vial was streaked onto three selective agars: bismuth sulfite, Hektoen Enteric, and xylose lysine desoxycholate. Plates were examined for typical *Salmonella* colonies after incubation at 35°C for 24 h (Hektoen Enteric and xylose lysine desoxycholate agars) or 48 h (bismuth sulfite agar). Suspect colonies were checked for purity by restreaking them onto plates of MacConkey agar (BD Diagnostic Systems). MacConkey agar plates were incubated at 35°C for 24 h and examined for typical *Salmonella* colonies. The identity of *Salmonella*-suspect colonies was confirmed with API 20E test strips according to the manufacturer’s instructions.

**FDA-BAM MPN technique.** A three-tube MPN analysis was performed for those samples of recalled almonds and survey
almonds (from 2002 to 2005) found to be positive for *Salmonella*. The MPN was not determined for the 2001 survey almonds. For recalled almonds, the initial MPN was determined for all 50 boxes of almonds with a detection limit of 0.3 MPN/g (three-tube MPN, 1-g samples). Subsequently, five boxes were retested at a lower limit of detection, which was also used for the survey almonds. Almonds (four 25-g samples) were combined with 225 ml of lactose broth and blended. One sample was portioned into 3 × 25 ml (2.5 g each) and 3 × 2.5 ml (0.25 g each), and all samples were enriched as described above for the FDA-BAM method.

Populations of *Salmonella* were estimated with the Thomas approximation of MPN per gram (37) for the three-tube *Salmonella* MPN:

\[
\text{MPN/g} = \frac{P}{\sqrt{NT}}
\]

where \(P\) is the number of positive tubes, \(N\) is the total quantity of sample (expressed in grams) in all negative tubes, and \(T\) is the total quantity of sample (expressed in grams) in all tubes. An estimation of the 95% confidence intervals was also calculated by the following equation:

\[
\log(\text{MPN/g}) = (1.96)(0.55)\sqrt{\log(an)}
\]

where \(a\) is the dilution ratio, and \(n\) is the number of tubes per dilution.

**Microbiological analysis of survey almonds.** For each survey year, samples that were confirmed positive for *Salmonella* were also screened for APC, *E. coli*, yeasts, and molds. For these tests, 90 g of almonds was combined with 90 ml of sterile Butterfield’s phosphate buffer (Biotrace International, Bothell, Wash.) to obtain a 10\(^6\) dilution and then shaken 50 times for 30 cm arc. The mixture was allowed to stand for 3 to 5 min and then shaken five times through a 30-cm arc to resuspend the sample before serial dilution in Butterfield’s phosphate buffer and inoculation of media. The APCs were carried out by AOAC Official Method 966.23 (3). *E. coli* was quantified via MPN by a modified version of AOAC Official Method 966.24 (4); the modification was to use API 20E test strips to confirm positive *E. coli* colonies. Yeast and mold counts were determined by following the FDA-BAM and HPB-OMH enrichment methods. Within lots, the frequency of *Salmonella* isolates that were serotyped as *Salmonella* Enteritidis and some of *Salmonella* Typhimurium were submitted to the National Veterinary Services Laboratory (Ames, Iowa) for phage typing.

**Identification of *Salmonella* isolates.** Confirmed *Salmonella* isolates from survey almonds were stored at −80°C in tryptic soy broth (Difco, Becton Dickinson) containing 15% glycerol. All cultures were submitted to the California Animal Health and Food Safety Laboratory System (Davis, Calif.) for serotyping. All isolates that were serotyped as *Salmonella* Enteritidis and some of the isolates that were serotyped as *Salmonella* Typhimurium were submitted to the National Veterinary Services Laboratory (Ames, Iowa) for phage typing.

**Antimicrobial susceptibility testing.** *Salmonella* isolates were assayed for susceptibility to 15 antibiotics by the calibrated dichotomous sensitivity method (7), and results were interpreted according to test standards. Antimicrobial susceptibilities were determined by adding one overnight colony from a tryptic soy agar (Difco, Becton Dickinson) plate to 1 ml of sterile saline (0.85% NaCl) and spread plating 330 μl of inoculum onto Sensitest agar (Oxoid). Sensitest agar plates were then allowed to dry (maximum, 30 min) before the application of antibiotic discs (BBL Sensi-Disc, BD Diagnostic Systems). No more than five antibiotic discs were applied per plate. Zones of inhibition were measured after incubation at 35°C for 18 h. The following antibiotics were tested: amikacin, amoxicillin–clavulanic acid, ampicillin, cefoxitin, ceftriaxone, cephalothin, chloramphenicol, ciprofloxacin, gentamicin, imipenem, kanamycin, nalidixic acid, streptomycin, sulfamethoxazole-trimethoprim, and tetracycline.

**RESULTS**

**Frequency of *Salmonella* isolation from recalled almonds.** On 2 July 2001, a total of 50 samples representing four lots (A, B, C, and D) of almonds implicated in the 2000 to 2001 outbreak were tested for *Salmonella* by both the FDA-BAM and HPB-OMH enrichment methods. *Salmonella* was isolated from 42 (84%) of the 50 boxes when data from the FDA-BAM and HPB-OMH methods were combined (Table 1). However, only 20% of the 50 boxes were positive by both methods; 40% tested positive by the HPB-OMH method, and 64% were positive by the FDA-BAM method. Within lots, the frequency of *Salmonella* isolation also varied with sample method. When data from the two methods were combined, the numbers of *Salmonella*-positive boxes for lots A, B, C, and D were 6 (87%) of 7, 3 (100%) of 3, 18 (72%) of 25, and 15 (100%) of 15, respectively.

**Concentration of *Salmonella* in recalled almonds.** Each of the 32 boxes that tested positive for *Salmonella* by the FDA-BAM method were further sampled for MPN analysis on 26 July 2001. All 32 samples were below the initial detection limit of 0.3 MPN/g of almonds. Five of the boxes (two from lot C and three from lot D) were then retested on 3 August 2001 by a three-tube MPN method capable of quantifying levels per 100-g sample. The MPN levels for four of the five samples were 9.1 MPN/100 g (95% confidence levels of 1.4 and 38 MPN/100 g), and the MPN level for the remaining sample was 6.1 MPN/100 g (95% confidence levels of 1.2 and 18 MPN/100 g), for an

**TABLE 1. Salmonella isolation from 22.7-kg boxes of naturally contaminated recalled almonds by FDA-BAM enrichment methods or HPB-OMH enrichment methods (single 100-g samples per method per box)**

<table>
<thead>
<tr>
<th>Lot</th>
<th>No. of boxes in lot</th>
<th>FDA-BAM</th>
<th>HPB-OMH</th>
<th>Both(a)</th>
<th>Either(b)</th>
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<td>5</td>
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<td>Total</td>
<td>50</td>
<td>32</td>
<td>20</td>
<td>10</td>
<td>42</td>
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\(a\) Boxes positive by both FDA-BAM and HPB-OMH enrichment methods.

\(b\) Boxes positive by either FDA-BAM or HPB-OMH enrichment method.
average of 8.5 ± 1.3 MPN/100 g in the naturally contaminated product.

**Characterization of Salmonella from recalled almonds.** Every Salmonella serovar isolated from recalled almonds by enrichment and subsequent MPN testing was identified as *Salmonella* serogroup D1 (O):G complex (H) by Silliker. One of these isolates was selected for further characterization and was identified as *Salmonella* Enteritidis by the California Animal Health and Food Safety Laboratory—the same serovar and PT isolated from cases associated with the 2000 to 2001 outbreak.

**Prevalence of Salmonella in survey almonds.** A total of 9,274 almond samples (100 g) were collected and tested over the 2001 to 2005 harvest seasons as follows: 2,003 samples in 2001, 2,012 samples in 2002, 1,764 samples in 2003, 1,643 samples in 2004, and 1,852 samples in 2005. *Salmonella* was isolated from 81 samples by the ACFSQ enrichment method, representing a yearly isolation frequency of 0.87% ± 0.2% from all samples over the 5 harvest years (Table 2). The prevalence of *Salmonella* was lowest during the 2001 harvest (0.60%) and highest during the 2002 harvest (1.1%). In most cases, the frequency of *Salmonella* isolation that was based on almond variety was low (<2.5%; Table 2). Isolation frequencies were high for uncommon varieties, Ballico and Rosetta (100 and 25%, respectively), when the sampling frequency was low (one and four samples in 5 years, respectively). For more common varieties, such as Carmel and Nonpareil, greater numbers of samples were evaluated (1,621 and 2,683, respectively), and isolation frequencies were similar to the overall frequencies (0.68 and 0.78%, respectively).

**Concentration of Salmonella serovars on survey almonds.** The MPN was determined for 61 of the 69 almonds. MPN analysis was not performed.

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**TABLE 2. Prevalence of Salmonella in different varieties of almonds surveyed over five harvests**

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<td>1,764</td>
<td>1,643</td>
<td>1,852</td>
<td>9,274</td>
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a *Salmonella*-positive samples.
b A marketing designation that includes many varieties.
c Contains two or more varieties.
d Other varieties include Avalon, Bluegum, Carrion, Chips Special, Dotte-Won, Hashem, Lagier, Legrand, Livingston, Merced, Mono, Morley, Ostrom, Peerless, Romain, Royal Kern, Ruby, Sauret, Savana, Thompson, Tokyo, and Wood Colony.

d| Harvest yr | No. of samples | <1.2 | 1.2 | 1.4 | <2.4 | 2.9 | E | ND | Avg (MPN/100 g) |
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<td>2005</td>
<td>16</td>
<td>16</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.2</td>
</tr>
<tr>
<td>Total</td>
<td>69</td>
<td>52</td>
<td>1</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td></td>
<td>1.3</td>
</tr>
</tbody>
</table>

a Average calculated with a value of 1.2 or 2.4 for those samples that were <1.2 or <2.4, respectively.
b Enriched sample.
c ND, not determined.
d Single samples (25 g) negative for *Salmonella*.
e Insufficient sample size.
f Sample discarded prior to MPN testing.
g Single sample (48 g) negative for *Salmonella*. 
TABLE 4. Frequency of E. coli, total aerobic plate, yeast, and mold counts for Salmonella-positive survey almonds

<table>
<thead>
<tr>
<th>Harvest yr</th>
<th>No. of samples</th>
<th>E. coli (MPN/g)</th>
<th>APC (CFU/g)</th>
<th>Yeast (CFU/g)</th>
<th>Mold (CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>&lt;0.3</td>
<td>0.3–0.9</td>
<td>1–10</td>
<td>&gt;10</td>
</tr>
<tr>
<td>2001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2002&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24</td>
<td>23</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2003</td>
<td>15</td>
<td>13</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>2004</td>
<td>12</td>
<td>9</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2005</td>
<td>18</td>
<td>16</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>81</td>
<td>73</td>
<td>1</td>
<td>4</td>
<td>2</td>
</tr>
</tbody>
</table>

<sup>a</sup> One sample was not tested for yeast and mold.
<sup>b</sup> One sample was not tested.

greater than 10 MPN/g (ranging from 15 to 46 MPN/g) were observed in five Salmonella-negative samples.

The APCs for Salmonella-positive survey almonds ranged from 100 to >250,000 CFU/g, with an average count of 1,800 CFU/g (Table 4). Yeasts were not detected (<10 CFU/g) in 89% of the samples. Mold levels ranged from <10 to >150,000, with average counts of 2,900 CFU/g.

Serotyping and phage typing of Salmonella from survey almonds. Table 6 provides the frequency (in descending order) of the 35 different serotypes of Salmonella isolated from almonds collected during the survey; two isolates were untypeable Salmonella. Salmonella Montevideo was the most frequently isolated Salmonella serovar (12%), followed by Thompson (10%), Enteritidis (7%), and Typhimurium (7%). The PT was determined for the six identified Salmonella Enteritidis strains by the National Veterinary Services Laboratory. The Salmonella Enteritidis isolate obtained in 2003 was determined to be PT 30, the same PT involved in a 2000 to 2001 outbreak (23). Of the five Salmonella Enteritidis isolates obtained in 2005, four were PT 8, and one was PT 9c; Salmonella Enteritidis PT 9c was associated with a 2004 outbreak in raw almonds (11). One Salmonella Typhimurium and one Salmonella Typhimurium var. Copenhagen were also typed because of their noted resistance to antibiotics (see below). A 2004 isolate of Salmonella Typhimurium var. Copenhagen was determined to be definitive type DT104, and a Salmonella Typhimurium (one of three isolates from 2002) was determined to be PT 208.

Serospecies of Salmonella isolated by MPN did not always correspond to the serotype of the original isolate. Salmonella Typhimurium was isolated in one 2002 sample, but Salmonella Infantis and Salmonella Zerefin were identified in the MPN analysis. Both Salmonella Reading and Salmonella Sandiego were isolated by MPN from a 2003 sample originally found to contain Salmonella Sandiego, and Salmonella Tennessee was isolated by MPN from a 2005 sample originally containing Salmonella Typhimurium.

Antibiotic-resistant Salmonella isolates. Of the 83 isolates characterized for antibiotic resistance (including MPN isolates), 52 were not resistant to any of the 15 antibiotics screened, 9 were resistant to one antibiotic, and 12
TABLE 6. Salmonella serotypes isolated from survey almond samples in 2001 to 2005

<table>
<thead>
<tr>
<th>Salmonella serotypes</th>
<th>No. of each serotype isolated per harvest yr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2001</td>
</tr>
<tr>
<td>Montevideo</td>
<td>4</td>
</tr>
<tr>
<td>Thompson</td>
<td>1</td>
</tr>
<tr>
<td>Enteritidis</td>
<td>1</td>
</tr>
<tr>
<td>Typhimurium</td>
<td>3</td>
</tr>
<tr>
<td>Senftenberg</td>
<td>1</td>
</tr>
<tr>
<td>Anatum</td>
<td>1</td>
</tr>
<tr>
<td>Brandenburg</td>
<td>2</td>
</tr>
<tr>
<td>Newport</td>
<td>1</td>
</tr>
<tr>
<td>Agona</td>
<td>2</td>
</tr>
<tr>
<td>Braenderup</td>
<td>1</td>
</tr>
<tr>
<td>Heidelberg</td>
<td>1</td>
</tr>
<tr>
<td>Muenchen</td>
<td>1</td>
</tr>
<tr>
<td>Tennessee</td>
<td>2</td>
</tr>
<tr>
<td>Typhimurium var. Copenhagen</td>
<td>1</td>
</tr>
</tbody>
</table>

Type II C1;gmt 2003 An, Ck, Gm

Give var. O34+ 1 1 1

Horsham 1 1

Istanbul 1 1

Kentucky 1 1

Liverpool 1 1

Lomalinda 1 1 1

Mbandaka 1 1

Nancy 1 1

Newbrunswick 1 1

Othmarschen 1 1

Saintpaul 1 1

Sandiego 1 1

Schwarzengrund 1 1

Worthington 1 1

Rough “O” 1 1

Y;3A,48; Z;2,5Z23 1

B:4,5;i;= no phase 2 1 1

C1;7;gmt;enx 1 1

Total isolation 12 (23 + 1)a (14 + 1)a 12 18 79 + 2

a One slant missing in total of 24 and 15 isolations for 2002 and 2003.

were resistant to two of the antibiotics. Table 7 provides the antibiotic-resistant profiles of 10 Salmonella isolates found to be resistant to three or more of the antibiotics screened. One Salmonella Typhimurium PT 208 and one Salmonella Typhimurium var. Copenhagen DT104 were resistant to four antibiotics.

None of the six Salmonella Enteritidis isolates in the survey were resistant to any of the antibiotics screened. The Salmonella Enteritidis PT 30 isolated from the recalled 2000 to 2001 almonds was also found to be sensitive to these antibiotics (data not shown).

DISCUSSION

Almonds that were recalled in April 2001 had been harvested in the fall of 2000 and shipped to Ontario, Canada, beginning in mid-February through early April 2001 (40). Product recalls were initiated in mid-April. By the FDA-BAM and HPB-OMH methods, Salmonella was isolated from 84% of the recalled boxes of almonds; however, of these 42 boxes determined to be positive by either method, only 10 were positive by both methods. In particulate foods such as almonds, it is probable that Salmonella is unevenly distributed within the product, and this alone may explain the difference in isolation rates between the two methods. Although the FDA-BAM and HPB-OMH methods have been internally validated for use with tree nuts, a study that directly compares these methods to each other or to more rapid methods has not, to our knowledge, been published. Further studies are necessary to determine if there are significant differences in sensitivity between the methods, ideally one that would use both an inoculated and a naturally contaminated product. However, the data suggest that the HPB-OMH method is not superior to the FDA-BAM method for raw almonds and that the selection of the FDA-BAM method for MPN analysis was appropriate. The AOAC (VIDAS system) method used for the survey was not compared to the FDA-BAM method for isolating Salmonella from raw almonds. However, it is a standard method used in the dried fruit and nut industry.

Given the likely uneven distribution of Salmonella in naturally contaminated almonds, routine testing for this organism is of limited value. Production lot samples that are held for future testing, or samples that are collected during outbreak investigations, should be refrigerated or frozen to stabilize the Salmonella population (39). Further studies to evaluate the distribution of Salmonella throughout naturally...
contaminated almonds would be useful to refine sampling programs and model risks.

Cases of salmonellosis in the 2000 to 2001 outbreak were documented through the first week of July 2001 (23). It is likely that per serving levels of *Salmonella* in the almonds were very low. Other outbreaks in which low levels of *Salmonella* have led to illness include chocolate (*Salmonella* Eastbourne, 20 to 90 cells per 100 g or <2 to 9 cells per chocolate ball (13), and *Salmonella* Napoli, <50 cells per serving (20)); Cheddar cheese (*Salmonella* Typhimurium, 1 to 9 cells per 100 g (15)); ice cream (*Salmonella* Typhimurium, <113 cells per 75-g serving (6), and *Salmonella* Enteritidis <25 cells per 65-g serving (42)); and paprika-powdered potato chips (*Salmonella* Saintpaul, *Salmonella* Javiana, and *Salmonella* Rubislaw, 4 to 45 cells per 100-g serving (29)). In each of these foods, *Salmonella* was restricted from multiplying because of low water activity or temperature. The high fat content of these foods may have protected the *Salmonella* cells from stomach acidity, but it is also possible that the cells are more resistant to environmental stresses when in a dry state (15).

The incidence of *Salmonella* in 100-g samples of the raw survey almonds was 0.87%, as determined over a 5-year period. Published information on the prevalence of *Salmonella* in other nuts is limited to macadamia nuts (93 samples [2.1%, unknown weight (36)] and walnuts (50 kernels tested [2%] (32)). A relatively high isolation rate (11 [9.4%] of 117 samples) was noted for sesame seeds and ready-to-eat sesame seed products (halva and tahini) in Germany (9). However, these products were sampled from retail and delicatessen stores during an outbreak investigation, which may have skewed the results.

Large sample-size surveys of dried foods for the presence of *Salmonella* are limited. Isolation frequencies of 1% were reported for wheat flour (4,360 samples) (35) and 3.2% for dried salted seafood (792 samples) (22). The prevalence of *Salmonella* in dried animal feed stuffs ranged from 2.7% in poultry compound feed (14,256 samples) to 4.9% in oilseed meals and other ingredients sampled at feed mills (12,460 samples) (1).

The occasional presence of salmonellae is not considered a public health hazard in products that are subsequently heat treated prior to consumption. Almonds are routinely dry- or oil-roasted or blanched, which significantly reduces populations of *Salmonella*. However, there is a limited but important market for raw or rawlike almonds, and the documented almond outbreaks were associated with the consumption of raw products. There is significant interest in processes that are capable of reducing *Salmonella* in almonds without affecting the sensory characteristics of the raw nut.

Of the survey samples initially determined to be positive for *Salmonella*, 11% were positive in a subsequent MPN analysis. When detected, the levels were below 3 MPN/100 g, which was much lower than the levels predicted for almonds involved in the 2000 to 2001 outbreak. Very few data exist on the levels of *Salmonella* in naturally contaminated dry products, other than outbreak investigations. In one other published report, a *Salmonella* concentration of 0.7 MPN/100 g of cocoa was noted (12).

The MPN analysis in this study was performed by initially dividing 100 g of almonds into four 25-g subsamples prior to blending. When a positive tube was identified, it was most frequently in a single 25-g subsample. However, it is unknown if the levels of *Salmonella* in that subsample were low (represented by the MPN value) or if one or several almonds within that subsample had much higher levels of *Salmonella*. Sample heterogeneity, including clumping of cells, can greatly influence MPN, especially for accessing small levels of *Salmonella* in a contaminated product. For future MPN analyses of particulate foods such as almonds, it would be useful to compare the current method of blending subsamples with blending the entire 100-g sample prior to division into subsamples. Such a comparison would help determine which proposed model of contamination is a better estimate of reality.

The wide variety of serotypes identified from almonds was not unexpected. The National Enteric Pathogen Surveillance Scheme in Australia isolated 17 different *Salmonella* serovars from sesame seeds and sesame seed products, including halva, tahini, and hummus (ground chick peas with tahini), tested on 30 occasions between 1985 and 2001 (31). Large-scale environmental surveys have identified similarly large numbers of serovars (18). The presence of multidrug-resistant *Salmonella* within almond isolates is of concern, as these strains have been associated with increased morbidity and mortality in humans (21).

*E. coli* MPN levels did not correlate with the presence or absence of *Salmonella* on almonds and would not be a useful indicator organism for this purpose. Some samples exceeded the FDA regulatory action guidance level for *E. coli* in finished tree nuts (0.36 MPN/g). However, the almonds sampled in this study were not finished products. Under normal circumstances, almonds would have undergone further handling steps, including sorting and sizing before sampling and testing. The impact of these additional handling steps on microbial counts is unknown.

**ACKNOWLEDGMENTS**

This study was funded by the Almond Board of California and USDA-CSREES 2002-03886. The project would not have been possible without the support of the almond production and processing industries. We are thankful for the editing skills of Sylvia Yada.

**REFERENCES**


