Incidence of *Clostridium botulinum* in Commercial Bacon

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**ABSTRACT**

Of 416 75-g samples of commercial bacon cultured with or without prior heating, only one gave rise to formation of botulinal toxin. The most probable number of *Clostridium botulinum* was estimated at 0.064 per kg, with a 99% confidence limit of 0.0004 to 0.478 per kg.

The probability of outgrowth and toxigenesis from experimentally introduced botulinal spores in temperature-abused bacon may be estimated from pack studies (2,3,10). On the assumption that such spores are equivalent to naturally contaminating spores, these estimates may then be used to calculate the probability of commercial packages of bacon becoming toxic if abused, provided the incidence of *Clostridium botulinum* in the product is known.

British surveys (14-16) indicated about 1-2 botulinal spores per kg of bacon in the UK. Since no such data are available for North America, we conducted a survey for *C. botulinum* in bacon at the Canadian retail level.

**MATERIALS AND METHODS**

A total of 104 one-lb packages of bacon were analyzed for *C. botulinum*. Fifty-two samples of two packages each were obtained at the retail level; 24 in Ottawa and 14 each in St. John, N.B. and Vancouver, B.C. Up to six different brands were sampled per store, but no effort was made to avoid duplication of brands between stores. Altogether, 36 different brands were analyzed.

Four 75-g subsamples per package were cultured, two for botulinal spores and two for both spores and vegetative cells. For a total of 416 cultures.

For recovery of *C. botulinum* spores, the bacon slices were cut, at a right angle to the meat fiber, into strips of about 5 g. These were weighed to 75 ± 5 g into Mason jars containing 400 ml of freshly prepared TPGY medium (11), tempered to 75 C. The manipulations were done aseptically and in a laminar flow cabinet. The Mason jars were kept at 75 C for 20 min, incubated at 35 C for 1 week and assayed for botulinal toxin (7). The recovery procedure for spores plus vegetative cells was the same, except that the bacon was added to the medium at ambient temperature, and the jars were incubated without prior heating.

Additional bacon samples were cultured as controls. Negative controls were prepared with autoclaved bacon and incubated along with each group of experimental cultures at a ratio of about 1:7.

For positive controls, spore suspensions (7) and 48-h-old vegetative TPGY cultures of *C. botulinum* type A (strain A-6) or B (strain 13983 H2) B were diluted in distilled water to a target number of 10-20 per ml. Counts were made subsequently by a pour plate method (9). Bacon samples of 75 g were inoculated with 0.1 ml of diluted suspensions of spores or vegetative cells, transferred to TPGY medium in Mason jars, and processed as above. Four different brands were used for each group of 16 bacon samples (Table 1).

Most probable numbers (MPN) were calculated by the formula MPN = 2.303 log n/q (6), where n = No. of replicates and q = No. of nontoxic replicates. The same formula was applied in estimating the incidence of *C. botulinum* in commercial bacon.

**RESULTS AND DISCUSSION**

**Efficacy of *C. botulinum* recovery from bacon**

With averages of 2.0 and 3.0 vegetative cells added per 75-g bacon sample, each of 16 cultures contained botulinal toxin (Table 1). Cultures of the same bacon lots without added botulinal cells remained nontoxic. About 40% of the spores were recovered in unheated cultures and 80% in the heated cultures. The differences are consistent with the requirement of heat activation for clostridial spores. It is concluded that our method allows essentially complete recoveries of vegetative *C. botulinum* cells in unheated cultures and of spores in heated cultures, while about 40% of the spores are recovered additionally in the unheated cultures.

**Clostridium botulinum in naturally contaminated bacon**

Toxin was found in 0/208 heated cultures and in 1/208 unheated cultures. A third 75-g portion from the package that had given rise to toxicity was cultured subsequently without heating, but no toxin was formed. The degree of contamination, therefore, was low. The toxic culture was neutralized with a combination of botulinal type A and B antiserum, but not by any of the two monospecific antisera alone. This would indicate contamination of the bacon sample with both type A and B, but in view of the extremely low incidence of *C. botulinum* in the bacon, such a coincidence would be most unlikely. It is possible that we encountered one of the rare strains that produce more than one kind of botulinal toxin (4), but we no longer have the culture to test this.

**TABLE 1. Efficacy of *C. botulinum* recovery from bacon.**

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>Type</th>
<th>Mean No. per sample inoculated</th>
<th>Toxic cultures</th>
<th>MPN</th>
<th>Recovery (%)</th>
<th>Toxic cultures</th>
<th>MPN</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veg. cells</td>
<td>A</td>
<td>2.0</td>
<td>16/16</td>
<td>&gt; 2.77</td>
<td>&gt; 138</td>
<td>9/16</td>
<td>0.83</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>3.0</td>
<td>16/16</td>
<td>&gt; 2.77</td>
<td>&gt; 92</td>
<td>10/16</td>
<td>0.98</td>
<td>78</td>
</tr>
<tr>
<td>Spores</td>
<td>A</td>
<td>1.0</td>
<td>5/16</td>
<td>0.38</td>
<td>38</td>
<td>9/16</td>
<td>0.83</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>1.25</td>
<td>7/16</td>
<td>0.58</td>
<td>46</td>
<td>10/16</td>
<td>0.98</td>
<td>78</td>
</tr>
</tbody>
</table>

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Our MPN estimate of *C. botulinum* was based on (a) complete recovery of spores in the heated cultures and of vegetative cells (including germinated spores) in the unheated cultures, (b) analysis of 150 g of bacon per package (two 75-g samples) in both the heated and unheated sets of cultures and (c) 0/104 and 1/104 packages of the two sets positive for *C. botulinum*. Although outgrowth in the single toxic culture may have originated from a spore, the 40% recovery of spores in unheated cultures was not considered in the estimate. The MPN of *C. botulinum* in commercial bacon was estimated at 0.064 per g, with a 99% confidence limit of 0.0004 to 0.478 per kg (12). Our MPN estimate is thus 1-2 logs lower than that of the British surveys (17-16), but it is consistent with the inability of Greenberg et al. (5) to detect *C. botulinum* spores in raw pork from processing plants in the U.S. and Canada; however, a total of only 2 kg of pork were examined in the latter survey.

Other North American surveys involving a variety of semipreserved and raw meats (5,9,18) indicated in the order of 0.1 to 1 botulinal spore per kg. In a recent survey of semipreserved meats at the Canadian retail level (Hauschild and Hilsheimer, unpublished), we detected *C. botulinum* type A spores in two out of 132 75-g samples each representing one product. The samples were made up of five 15-g subsamples and cultured in heated TPGY medium at 35 C for 1 week. Assuming even distribution of botulinal spores between the products analyzed, the MPN of spores was estimated at 0.2 per kg.

**Botulinus hazards from bacon**

Of the common human types of *C. botulinum* (A, B and E) only the proteolytic strains of types A and B are sufficiently salt tolerant to present a potential health risk in bacon, but these strains are incapable of growing below 10 C (13). Properly refrigerated bacon, therefore, is safe. For bacon subjected to certain temperature abuse, the pack studies of Christiansen et al. (2), Collins-Thompson et al. (3), Ivey et al. (10) and Pierson (unpublished report of March 14, 1978) allowed us to estimate the proportions of botulinal spores capable of outgrowth and toxigenesis (2.303 × log n/q per No. of spores introduced per package). For bacon prepared without nitrite and abused to the equivalent of 1 week at 27 or 30 C, the estimated proportions varied from about 10^-4 to < 10^-7 (or 1 in 104 to < 1 in 107). The variations are largely attributable to differences in bacon formulation and methods of adding spores. For both low-nitrite bacon (40-60 ppm) and bacon currently prepared in the U.S. (120 ppm) and Canada (150 ppm), the estimates varied from 10^-5 to < 10^-7. Differences between low-nitrite and current bacon became apparent, however, after prolonged abusive storage.

On the assumption that the experimentally introduced spores are equivalent to naturally contaminating spores, the above estimates and our survey data suggest that in the order of 10^4 to 10^6 one-lb packages of nitrite-free bacon or 10^4 to 10^9 packages of low-nitrite bacon would be required for one package to become toxic during abusive storage equivalent to about 1 week at 27 C. An additional safety factor is provided by the destruction of botulinal toxin in the cooking or frying process.

**ACKNOWLEDGMENTS**

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**REFERENCES**


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