A Research Note

Clostridium Botulinum in Honey, Syrups and Dry Infant Cereals

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ABSTRACT

A total of 150 honey, 43 syrup and 40 dry cereal samples were analyzed for Clostridium botulinum spores, each in triplicate quantities of 25 g. The foods were sampled randomly, except for two lots of honey which were potentially associated with illness.

Botulinal spores were detected in a sample of honey associated with infant botulism and in a single sample of rice cereal. Honey has been identified as a source of botulinal spores resulting in infant botulism (1). Spores of Clostridium botulinum have been isolated repeatedly from leftover honey fed to afflicted infants, and their toxin type (A or B of group I) consistently matched that of the isolates from the infants' stools. By 1984, 20 such cases had been reported in the United States (4).

Some incriminated honey samples have been analyzed quantitatively for C. botulinum spores. Midura et al. (14) reported concentrations of $5 \times 10^3$ to $8 \times 10^4$ per kg. A product implicated in Quebec (10) contained $10^3 - 10^4$ spores per kg.

Surveys for spores in honey not related to illness have also been conducted. Five surveys reported from the U.S. indicate spore levels in the order of $<1$ to 20 per kg (6,12,13,14,18), whereas surveys of European honey were all negative for botulinal spores (2,3,5,11,15). These results are consistent with the predominance of C. botulinum group II and a scarcity of group I strains in the European environment (17). The surveys all included a heat process for the elimination of the vegetative microflora ranging in severity from 65°C for 30 min to 80°C for 25 min which likely resulted in the destruction of most or all spores of group II (16).

Infant foods other than honey have also been examined (6,13). Of these, only corn syrup showed any detectable contamination with C. botulinum. Kautter et al. (13) detected C. botulinum in 13 out of 1001 25-g syrup samples. Assuming even spore distribution among the products, this result would indicate one spore in about 2 kg (7).

Four cases of infant botulism have been recorded in Canada to-date, with only one of them linked conclusively to honey (10). The honey-associated incident prompted the present survey in which we examined a number of Canadian infant foods as potential sources of C. botulinum spores. The results are consistent with the low incidence of infant botulism in Canada.

MATERIALS AND METHODS

Two surveys were conducted. Survey I was carried out in the five Regional Laboratories of the Health Protection Branch and involved the analysis of three types of nonsterile foods: honey, dry cereals and syrups. The foods were randomly sampled at retail in 9 provinces and were all of domestic origin, except for 2 lots of syrup and 3 lots of cereal. Each sample was analyzed in triplicate.

The procedure for detecting botulinal spores in honey and syrups was described before (8). For the analysis of cereals, 25-g replicates were added to 600 ml of TPGYB medium (8) in Mason jars tempered to 65°C. The jars were kept at 65°C for 30 min, and the media covered with 40-45 ml of sterile paraffin oil (8). Incubation was at 35°C for 7 d. Toxin was analyzed as described (9).

Survey II involved two groups of honey. The first consisted of 51 retail samples of which 49 were collected at random; two were received in connection with one case each of sudden infant death syndrome (SIDS) and infant botulism. Except for six imported samples, all the honey originated from 9 Canadian provinces. The second group consisted of 56 samples representing individual production lots from a total of 50 Canadian producers in 9 provinces. Six manufacturers each supplied two samples from separate lots.

In survey II, the procedure for spore detection in honey (8) was slightly modified in order to facilitate the filtration process. Suspension of 25 g of honey in 100 ml of sterile distilled water with 1% Tween 80 were placed in 300-ml stainless-steel centrifuge bottles, held at 65°C for 30 min and centrifuged at 15,000 x g for 20 min. Each sediment was carefully transferred to a dialysis bottle containing 110 ml of TPGYB broth (14). The super-

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nate was filtered through a 0.45 μm Millipore membrane filter (HAWP 4700). The funnel was rinsed with distilled water, and the membrane combined with the sediment in TPGYB broth.

The method for the recovery of challenge spores was as described (8) except that type A and B spores were combined in equal numbers, and that 6 replicate bottles per challenge dose were used.

RESULTS AND DISCUSSION

The recovery of challenge spores by the modified procedure used in survey II was essentially complete (Table 1). Similar recovery data were reported also for the original method (8). The modified procedure facilitated the filtration process considerably; of 107 honey samples analyzed in survey II, only two required a second membrane filter to complete the filtration.

Survey I showed a single sample (rice cereal) with C. botulinum (Table 2). Its toxin was of type B. The contamination of the sample was light because only 1/3 replicates was positive for C. botulinum, and three additional replicates from the same lot were all negative. MPN calculations (7) for honey, dry cereals and syrups were all below one spore per kg.

Of the 107 honey samples analyzed in survey II, only the product associated with a case of infant botulism developed toxin. A 5-bottle MPN count indicated $8 \times 10^3$ C. botulinum spores per kg. Concentrations in the same order (9) for honey, dry cereals and syrups were all below one spore per kg.

This work confirms that honey generally contains few botulinial spores, and suggests that their numbers are probably insignificant, in comparison to the infant's exposure to spores from other sources. However, the results are not fully consistent with reports from the United States (6,12,13,14,18) because all our general-survey samples which comprised a total of over 10 kg, were negative for C. botulinum, whereas the U.S. surveys showed 31 positive identifications from a total of about 20 kg honey cultured and analyzed. On the other hand, the results (Table 2) are in accord with the low incidence of general and honey-associated infant botulism in Canada. Whether or not they also reflect the number of botulinial group I spores in the environment is uncertain because, apart from coastal regions, the distribution of C. botulinum spores in Canadian soils remains to be investigated.

The presence of single serotypes in honey associated with illness (10,14) indicates that the large spore numbers are not the result of some haphazard contamination. They rather point to a build-up of spores from perhaps a single spore. Huhtanen et al. (12) suggested external environmental foci as spore sources. Alternatively, multiplication of C. botulinum may take place in the hive itself, e.g. in contaminated dead larvae which are frequently encountered in honeycombs and likely present a good environment for anaerobic growth.

Exposure of infants to botulinial spores via other foods seems to be minimal. Although corn syrup (13) and now rice cereal have been shown to occasionally contain spores, their potential for causing illness is not comparable to that of honey because contaminating spores are unlikely to multiply during manufacture. In addition, no food other than honey has yet been implicated in infant botulism.

ACKNOWLEDGMENTS

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REFERENCES


C. BOTULINUM IN HONEY, SYRUPS AND CEREAL

TABLE 1. Recovery of C. botulinum challenge spores from honey by a modified procedure.

<table>
<thead>
<tr>
<th>Inoculated spores (mean No./sample)</th>
<th>Toxic bottles/total bottles</th>
<th>MPN/sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5</td>
<td>6/6</td>
<td>&gt;1.8</td>
</tr>
<tr>
<td>2.0</td>
<td>6/6</td>
<td>&gt;1.8</td>
</tr>
<tr>
<td>1.0</td>
<td>3/6</td>
<td>0.7</td>
</tr>
</tbody>
</table>

As ln n/q, where n = No. of samples and q = No. of nontoxic samples.

TABLE 2. Clostridium botulinum spores in infant foods.

<table>
<thead>
<tr>
<th>Survey</th>
<th>Food</th>
<th>Food origin</th>
<th>Food sampling associated with illness</th>
<th>Samples analyzed</th>
<th>No. with C. botulinum spores</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Honey</td>
<td>Retail</td>
<td>No</td>
<td>43</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Dry cereals&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Retail</td>
<td>No</td>
<td>40</td>
<td>1</td>
</tr>
<tr>
<td>II</td>
<td>Honey</td>
<td>Retail</td>
<td>No</td>
<td>43</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>SIDS</td>
<td></td>
<td>No</td>
<td>49</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Infant botulism</td>
<td></td>
<td>No</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup>Three 25-g replicates each.

<sup>b</sup>Oat (7), barley (6), rice (4) and mixed (23).

<sup>c</sup>Corn (14), maple (14), cane (11) and unidentified (4).