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Isolation of *Clostridium botulinum* from Honey

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Methods for the isolation of *Clostridium botulinum* from honey samples are described. A total of 9 of 90 honey samples were positive for *C. botulinum*; 6 of the positive samples had been fed to babies who developed infant botulism.

Infant botulism has been under investigation in California since it was identified in late 1976 as a distinct clinical entity that results from intestinal colonization and toxin production by *Clostridium botulinum* (1, 5). During these investigations, honey was the only food item from which *C. botulinum* organisms could be isolated (2, 4). Honey cannot be treated in the usual manner (3) for detecting *C. botulinum* organisms because of its viscosity and high sugar content.

The purpose of this report is to describe two methods to isolate *C. botulinum* from honey and to relate our experiences in the use of these methods in testing 90 honey samples.

Honey samples were obtained from the following sources: the homes of infant botulism cases, homes of healthy infants, grocery stores, small apiaries, and a commercial honey processing plant.

Following is the initial method, designated as MDL-10, for the demonstration of *C. botulinum* organisms in honey. A 10-g amount of honey was diluted 1:3 with sterile 1% peptone-water in a sterile 50-ml polycarbonate centrifuge tube. The mixture was then centrifuged at 12,000 × *g* for 0.5 h in a 5°C refrigerated centrifuge. The supernatant was removed carefully and used to assay for botulinal toxin with the mouse neutralization test (3). The sediment was resuspended in about 2 ml of peptone-water and was inoculated into two tubes of freshly steamed and cooled cooked meat enrichment medium. The cooked meat enrichment tubes were then incubated at 30°C for up to 10 days and were checked daily for evidence of growth and gas production. Tubes showing this evidence were screened for the presence of toxin by animal testing with the use of trypsinized and untrypsinized supernatant fluids. Isolation of the organism was also attempted. The laboratory techniques for the identification of botulinal toxin by the mouse neutralization test and the isolation of *C. botulinum* organisms from the positive tubes have been described elsewhere (3, 4).

To explore the influence of sample size on the detection of *C. botulinum*, the same honey samples were tested by a modified method, designated as MDL-20. The samples of honey were warmed in a 37°C water bath. A 20-g sample of honey was weighed into a 200-ml sterile polycarbonate centrifuge bottle and mixed with 150 ml of warm sterile distilled water. The mixture was centrifuged at 3,300 × *g* in a 5°C refrigerated centrifuge for 2 h. The supernatant was decanted; the sediment was resuspended in about 2 ml of sterile water, and an equal amount was inoculated into each of two tubes of cooked meat enrichment broth. One tube was heated in a 70°C water bath for 15 min and then rapidly cooled. Both tubes were incubated at 30°C for up to 10 days as previously described.

Since the diets of some infants were supplemented with corn syrup (Karo brand) instead of honey, it was decided to examine this food item by these methods. Corn syrup, like honey, is a nonlactose, carbohydrate-containing food. Samples of corn syrup were obtained from the homes of ill infants, the homes of healthy infants, and from grocery stores. Fifteen samples were examined.

Studies were conducted also to evaluate the sensitivity of the two methods. Frozen suspensions (−20°C) of *C. botulinum* type B spores were thawed and serial dilutions were prepared in sterile 1% peptone-water. The number of viable organisms in each serial dilution was determined by heating the suspension at 70°C for 15 min and performing plate counts using egg yolk agar, which were incubated at 30°C for 48 h in a Brewer GasPak jar (BBL). Also, 1 ml of each serial dilution was well mixed into several 10-g and 20-g portions of honey. Each of the two procedures previously described (MDL-10 and MDL-20) was followed. With both methods it was found that as few as seven to nine viable *C. botulinum* spores per gram of honey could be detected.

A total of 90 honey samples were examined by both procedures. Table 1 shows the samples that were positive for *C. botulinum* organisms by the

TABLE 1. *Comparison of two procedures yielding C. botulinum isolates from honey*

Honey sample	Method		
	MDL-10 unheated	MDL-20	
		Unheated	Heated
1	+	−	−
2	+	−	−
3	−	+	+
4	−	+	+
5	−	+	+
6	−	+	+
7	+	+	+
8	+	−	−
9	+	−	−

two methods. Each method gave the same total number of positive (five out of nine); sample 7 was the only one positive by both methods. The nine samples that contained *C. botulinum* organisms represented 10.0% of the 90 samples we examined by both methods. From the results in Table 1, it would appear that the *C. botulinum* organisms, when present, are unevenly distributed in honey samples. Since it is difficult to obtain a homogeneous sample with honey, the examination of multiple portions by both unheated and heated treatments probably offers the best opportunity for recovery of *C. botulinum* in honey. An estimation of the number of *C. botulinum* organisms in the positive honey samples ranged between 5 and 25 per g; interestingly, sample 7—the only one positive by both methods—had 70 to 80 per g. From the seeding studies previously described, the MDL-10 and MDL-20 procedures could detect seven to nine *C. botulinum* spores per gram of honey. Samples containing spores at or below the detectable levels would be expected to give variable results.

We have demonstrated that honey is a source of *C. botulinum* organisms for infants. More studies are needed to determine not only the prevalance of *C. botulinum* in this food but also how *C. botulinum* becomes incorporated into honey. In our laboratory we will be using the MDL-20 procedure in duplicate as the reference technique to compare other methods for isolating *C. botulinum* from honey.

Table 2 shows the positive honey samples associated with five cases of infant botulism. In each instance the toxin type of *C. botulinum* that was isolated from the honey was the same toxin type as that isolated from the stools of the ill infant. Six of the nine positive honey samples were obtained from the same container as that of the honey actually fed to the infants. The

TABLE 2. *Positive honey samples associated with cases of infant botulism*

Honey sample	Type isolated from:		
	Honey	Infant	Honey fed to infant
1	B	B	Yes
2	B	B	Yes
3	B	B	Yes
4	B	B	No[a]
5	B	B	Yes
6	B	B	Yes
7	B	B	Yes
8	A	A	No[a]
9	A		No[b]

[a] None of the honey that was fed to the infant was available. A sample of the same brand and size was purchased in the same store for test purposes.

[b] An unprocessed sample that was obtained at a commercial processing plant.

others were from different containers of the same brand in those cases when the original container was not available. Seventeen actual containers from which honey had been fed to the baby were available for examination.

C. botulinum could not be detected in the 15 corn syrup (Karo brand) samples. Ten were tested by both methods and the additional five were tested by the MDL-10 only. Eight of these corn syrup samples had been fed to infants who developed infant botulism.

Because infant botulism is considered an infectious disease that results from in vivo toxin production by *C. botulinum* in the intestinal tract (1, 4), a potential health hazard may exist for some infants that are fed honey. This possibility should be recognized by public health officials, physicians, and parents.

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