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# Efficacy of Cold Enrichment Techniques for Recovery of *Yersinia enterocolitica* from Human Stools

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Stool specimens from children with gastroenteritis and their household contacts were cultured for *Yersinia enterocolitica* by direct plating onto routine laboratory media. These stools were also inoculated into phosphate-buffered saline and subcultured to the same media after 1 day or 3 weeks of incubation at 4°C. *Y. enterocolitica* was isolated from 174 index cases and 34 household contacts. One hundred eighty-one isolates were of serotype O:3, and the remaining 21 belonged to other serotypes. Eighty-one percent (147/181) of O:3 isolates were recovered by direct plating, and 6.1% (11/181) and 13% (23/181) were recovered by 1-day and 3-week cold enrichment, respectively. For other serotypes, 26% (7/27), 0%, and 74% (20/27) were isolated by direct plating, 1-day cold enrichment, and 3-week cold enrichment, respectively. The efficacy of the cold enrichment for the recovery of serotype O:3 varied according to the patient's clinical condition. When patients were still symptomatic, 94 and 6% of *Y. enterocolitica* were identified by direct plating and cold enrichment, respectively. Isolation rates were 66% by direct plating and 34% by cold enrichment when stools were obtained from asymptomatic carriers or from those convalescing from *Y. enterocolitica* gastroenteritis. These results indicate that the cold enrichment methods increase the sensitivity of *Y. enterocolitica* culture methods considerably in convalescent and asymptomatic subjects but only minimally in patients with diarrhea caused by serotype O:3.

*Yersinia enterocolitica* infections in humans have been reported with increasing frequency over the last decade (2, 15). Although the clinical spectrum of infections is diverse (24), gastroenteritis and other forms of abdominal illness are the most frequent manifestations, particularly in children (1, 6, 12). In Montreal, *Campylobacter coli/jejuni*, *Salmonella*, and *Y. enterocolitica* are the most frequent causes of bacterial gastroenteritis in infants and children (M. I. Marks, C. H. Pai, L. Lafleur, O. Hammerberg, L. Lackman, and L. Chicoine, Pediatr. Res. **12**:496, 1978; 17a).

The isolation of *Y. enterocolitica* from stool specimens is often difficult due to its slow growth and the marked similarity of its biochemical reactions to those of other enteric bacteria. Small colonies that develop after 1 to 2 days of incubation can be easily missed or overlooked when plates are overgrown with normal flora. To overcome this problem, cold-temperature enrichment methods have been used (7, 8, 21, 23, 25). A portion of a stool specimen is inoculated into an enrichment broth and subcultured after up to 3 weeks of incubation at 4°C. This takes advantage of the ability of *Y. enterocolitica* to survive and multiply at refrigerator temperature while other microbial species often cannot (7, 10, 14). Phosphate-buffered saline (8, 21, 25) and Rappaport broth (7, 23) have been used as enrichment broths. Using these techniques, Eiss (7) reported a 44% increase in the isolation rate of *Y. enterocolitica* from stool specimens submitted to a clinical laboratory. However, these data were not examined in relation to the clinical condition of patients. *Y. enterocolitica* isolation rates were also enhanced by the cold enrichment of fecal content collected from swine (21) and of raw milk (19).

We attempted to evaluate the usefulness of cold enrichment in the isolation of *Y. enterocolitica* from stools by including both conventional and cold enrichment methods in a prospective study of *Y. enterocolitica* gastroenteritis in children. (This paper was presented in part at the 18th Interscience Conference on Antimicrobial

Agents and Chemotherapy, Atlanta, Ga., 2 October 1978 [Abstr. no. 124].)

## MATERIALS AND METHODS

**Stool specimens.** All stool samples submitted for bacteriological examination at both Montreal pediatric hospitals (The Montreal Children's Hospital and l'Hôpital Ste. Justine) between 1 June 1977 and 31 July 1978 were cultured for enteric bacterial pathogens including *Y. enterocolitica*. Stools or rectal swabs were inoculated onto MacConkey or salmonella-shigella agar and into selenite broth, followed by isolation and identification of enteric pathogens by standard procedures (20). All stool specimens were also examined for *Y. enterocolitica* after cold enrichment. A portion of stool or feces collected by rectal swab was inoculated into a tube containing 5 ml of phosphate-buffered saline ($KH_2PO_4$-$Na_2HPO_4$, 0.067 M, and NaCl, 0.085%, pH 7.6) and subcultured onto MacConkey and salmonella-shigella agar after 1 day or 3 weeks of incubation at 4°C.

When *Y. enterocolitica* was identified in a child's stool, the physician and family were contacted, the study procedures were explained, and informed consent was obtained. Stool specimens were obtained from index cases and their household contacts, and clinical and bacteriological monitoring was continued weekly until all family members, including the index case, had three consecutive negative stool cultures for *Y. enterocolitica*.

**Identification of *Y. enterocolitica*.** Plates (primary inoculated media and those after subculture from cold enrichment broth) were examined after 1 to 2 days of incubation at 35°C, and small lactose-negative colonies were screened for *Y. enterocolitica* by inoculating onto Kliger iron agar and Christensen urea slants. During the first 3 months of this study, plates were incubated at room temperature for 2 additional days, but this practice was discontinued because the yield of isolates was no greater. Biochemical identification was carried out by methods described by Sonnenwirth (20). Serotyping was done by slide agglutination using 34 absorbed and unabsorbed O-antisera prepared in rabbits and kindly provided by S. Toma, Public Health Laboratory, Ontario Ministry of Health, Toronto.

## RESULTS

During the 14-month period from June 1977 to July 1978, *Y. enterocolitica* was isolated from 174 children with diarrhea (index cases) and 34 household contacts. One hundred and eighty-one isolates were of serotype O:3, and the remaining 27 belonged to nine different serotypes and nontypable (one O:1,2,3, one O:2, two O:5, four O:5,27, six O:6,30, two O:6,31, four O:7,8, one O:21, one O:34, and three nontypable; two remaining isolates were not of serotypes O:3, O:8, or O:9, but complete serotyping has not been done). Those serotypes other than O:3 and nontypable will be referred to as non-O:3. Serotypes O:8 and O:9, which are frequently isolated from human infections in the United States and Scandinavian countries, respectively, were not encountered during the study.

**Recovery of serotype O:3.** The efficacy of the cold enrichment techniques for the recovery of *Y. enterocolitica* serotype O:3 is shown in Table 1. Eighty-five percent of index cases were identified by the isolation of *Y. enterocolitica* from direct plating of initial stool specimens and 15% by the cold enrichment method only. However, *Y. enterocolitica* was recovered by direct plating from only 54% of culture-positive household contacts; the remaining 46% were identified from the cold enrichment buffer only. Thirteen of the 24 culture-positive household contacts were asymptomatic. The influence of diarrhea on the isolation rate was compared for the direct plating and cold enrichment methods (Table 2). *Y. enterocolitica* was cultured by direct plating in 94% of diarrheal stools but in only 66% of normal stool specimens.

During the first 2 weeks after the onset of symptoms, more than 90% of positive stools yielded *Y. enterocolitica* by direct plating (Fig. 1). The recovery by direct plating became in-

TABLE 1. *Isolation of Y. enterocolitica serotype O:3 by direct plating and by cold enrichment*

| Study population | No. of persons positive | No. (%) isolated by: | | |
|---|---|---|---|---|
| | | Direct plating | Cold enrichment only | |
| | | | 1 day[a] | 3 weeks[a] |
| Index cases | 157 | 134 (85) | 10 (6.4) | 13 (8.3) |
| Household contacts | 24 | 13 (54) | 1 (4.2) | 10 (42) |
| Total | 181[b] | 147 (81) | 11 (6.1) | 23 (13) |

[a] Time at which *Yersinia* was first isolated from cold enrichment broth.

[b] First positive stools from patients or their contacts.

TABLE 2. *Isolation of Y. enterocolitica serotype O:3 from stool specimens from symptomatic and asymptomatic patients*

| Symptoms when stools were obtained | No. of stools positive | No. (%) isolated by: | | |
|---|---|---|---|---|
| | | Direct plating | Cold enrichment only | |
| | | | 1 day[a] | 3 weeks[a] |
| Symptomatic | 67 | 63 (94) | 3 (4.5) | 1 (1.5) |
| Asymptomatic | 184 | 122 (66) | 13 (7.1) | 49 (27) |
| Total | 251[b] | 185 (74) | 16 (6.4) | 50 (20) |

[a] Time at which *Yersinia* was first isolated from cold enrichment broth.

[b] From 79 symptomatic and asymptomatic patients, from whom 251 weekly stool specimens were obtained.

creasingly more difficult after that; this figure was only 60% by 7 weeks and 50% by 8 weeks. The duration of excretion (defined as the period from the onset of symptoms to the first negative stool culture as determined by weekly stool cultures) of *Y. enterocolitica* serotype O:3 in the stool ranged from 14 to 97 days (mean, 42 days).

**Isolation of *Y. enterocolitica* non-O:3.** The recovery of *Y. enterocolitica* serotypes other than O:3 was much more difficult by direct plating than for serotype O:3 (Table 3). As many as 74% of those infected or colonized with non-O:3 serotypes would have been missed if the cold enrichment had not been used. The clinical condition of the patients at the time of stool culture did not affect the recovery rate of non-O:3 serotypes. From 4 index cases and 10 household contacts whose clinical information was available, the organism was recovered by direct plating in only 1 out of 11 nondiarrheal stools and in none of four diarrheal stools. All of the remaining 14 stools yielded non-O:3 serotypes of *Y. enterocolitica* only after 3 weeks of cold enrichment. Unlike serotype O:3, in only one case were stools positive more than once for the same individual in spite of multiple weekly stool cultures.

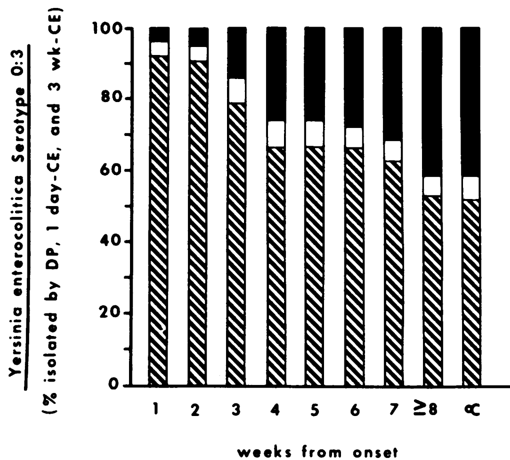


FIG. 1. *Enhancement of the recovery of Y. enterocolitica serotype O:3 by cold enrichment from stool specimens taken at different times from the onset of symptoms. The numbers of culture-positive stools were: 26, 48, 38, 47, 24, 18, 16, 17, and 16 at 1, 2, 3, 4, 5, 6, 7, ≥8, and α weeks from the onset of symptoms, respectively. Striped, open, and solid bars represent the recovery (%) of the organism by direct plating (DP), 1-day cold enrichment (CE), and 3-week cold enrichment, respectively. The sign, α, denotes those stool specimens taken from asymptomatic carriers, whose onset of symptoms, therefore, could not be determined.*

TABLE 3. *Isolation of Y. enterocolitica non-O:3 by direct plating and by cold enrichment*

| Study population | No. of persons positive | No. (%) isolated by: | | |
|---|---|---|---|---|
| | | Direct plating | Cold enrichment only | |
| | | | 1 day[a] | 3 weeks[a] |
| Index cases | 17 | 6 (35) | 0 | 11 (65) |
| Household contacts | 10 | 1 (10) | 0 | 9 (90) |
| Total | 27 | 7 (26) | 0 | 20 (74) |

[a] Time at which *Yersinia* was first isolated from cold enrichment broth.

## DISCUSSION

The data presented in this paper indicate that the efficacy of cold enrichment procedures for the isolation of *Y. enterocolitica* from stools varies considerably depending on the clinical condition of patients from whom stools have been obtained and the serotype of the infecting organisms. For serotype O:3, the recovery of the organism was enhanced significantly by cold enrichment techniques in stools obtained from asymptomatic carriers or from patients convalescing from gastroenteritis. Of these stool specimens, more than one-third of positive cultures were identified only after cold enrichment. However, these techniques were found to have little value in enhancing the recovery of *Y. enterocolitica* serotype O:3 when stools were obtained from patients during the early diarrheal stage of their illness. The recovery of *Y. enterocolitica* serotypes other than O:3 was extremely poor without cold enrichment. A similar observation was reported by Eiss (7).

The etiological association of *Y. enterocolitica* non-O:3 with gastroenteritis is uncertain. The period of excretion in the stools was brief, and, unlike O:3 serotype, no intrafamilial spread was demonstrated nor was there any serological response in the host (Marks et al., Pediatr. Res. **12**:496, 1978; O. Hammerberg, L. Lafleur, M. I. Marks, L. Lackman, J. Auclair, and C. H. Pai, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 18th, Atlanta, Ga., abstr. no. 124, 1978). The isolation of "atypical" serotypes from human infections has been reported by others (3, 4). Non-O:3 serotypes are frequently isolated from environmental sources (9, 10, 11, 13, 19), and, although many of these strains produce heat-stable enterotoxin (16, 17), its in vivo role has not been established.

*Y. enterocolitica* serotypes O:8 and O:9, other predominant human types, were not isolated in this study, and it remains to be seen whether or not our findings with serotype O:3 are also ap-

plicable to these serotypes. Animal experiments, however, have demonstrated that *Y. enterocolitica* serotypes O:3, O:8, and O:9 are excreted in stools in a large number for several weeks, whereas strains of other serotypes disappear from stools in a few days (5, 18, 22). These findings, coupled with the excretion pattern of O:3 and non-O:3 serotypes observed in our patients, suggest to us that the recovery of O:8 and O:9 serotypes from diarrheal stools may also be enhanced only minimally by the cold enrichment methods.

Based on the findings from this study, we conclude that routine use of cold enrichment techniques, with their attendant expense, is not indicated for the culture of *Y. enterocolitica* from diarrheal stools in a hospital laboratory located in the area where the human isolates of the organism are predominantly of serotype O:3. Since the majority of stool specimens will be submitted by patients during the acute stage of illness, direct plating alone will be sufficient for the optimum recovery of the O:3 serotypes of *Y. enterocolitica*. Recovery of the non-O:3 serotypes of *Y. enterocolitica* will be increased by cold enrichment, but the clinical significance of these isolates is uncertain. Cold enrichment procedures should be used selectively, however, for diagnosis during the convalescent stage of illness, surveillance of asymptomatic carriage, and investigation of possible postinfection complications.

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