Pathogenic Properties of *Yersinia enterocolitica*

V. MORS AND C. H. PAI*

Departments of Microbiology and Pediatrics, McGill University-Montreal Children's Hospital Research Institute, Montreal, Quebec, Canada

A total of 88 isolates of *Yersinia enterocolitica* was examined for heat-stable enterotoxin production and ability to penetrate HeLa cells and evoke keratoconjunctivitis in guinea pigs (Séreny test) as potential pathogenic properties. All 49 isolates belonging to serotypes O:3, O:8, O:9, and O:5,27 and only 5 of 39 strains of other serotypes were HeLa positive. Séreny-positive strains were found only in serotype O:8. Heat-stable enterotoxin production was almost ubiquitous in all serotypes. *Y. enterocolitica* strains were classified into five groups with regard to their potential pathogenic properties.

*Yersinia enterocolitica* is an important cause of bacterial gastroenteritis in children (1, 5, 7, 9a). The potential pathogenic properties of the organism as an enteric pathogen which have been reported are: (i) heat-stable enterotoxin (ST) production (2, 6, 11, 12, 14); (ii) ability to penetrate HeLa cells (8, 9, 13, 17); and (iii) ability to evoke keratoconjunctivitis in the guinea pig (2, 6). However, these three properties have never been examined simultaneously, and their relative importance in the pathogenesis of gastroenteritis has not been elucidated, although histopathological findings from human cases (3) and experimental infections (4, 10, 16) have suggested that the invasive capacity of the organism plays an essential role. The purpose of this study was to characterize a number of *Y. enterocolitica* strains of various serotypes and of different origins for their potential pathogenic properties.

Of 88 strains of *Y. enterocolitica* used in this study, 26 were isolated from the fecal specimens of children with diarrhea seen at the Montreal Children's Hospital or Ste. Justine's Hospital (L. Lafleur), Montreal, 3 were received from W. H. Lee (U.S. Department of Agriculture, Beltsville, Md.), and the remaining strains were from the stock cultures maintained at the National Reference Service for *Yersinia*, Toronto (kindly provided by S. Toma). Bacteria were suspended in brain heart infusion broth (Difco) with 20% glycerol and stored at -70°C. The ST activity of culture filtrates was assayed in the suckling mouse system as described previously (11). The enterotoxin of *Y. enterocolitica* is not active in the Y-1 adrenal assay system (6, 11, 14). The ability to penetrate HeLa cells was examined by the method described by Lee et al. (8). The Séreny test was performed with inoculum prepared from blood agar plates incubated overnight at room temperature. A paste, not a suspension, of bacteria was inoculated into guinea pig conjunctiva. *Escherichia coli* 4608-58 (kindly provided by H. L. Dupont) was used as a positive control.

The ST production, HeLa cell penetration, and Séreny reaction among 12 different serotypes and nontypable strains of *Y. enterocolitica* are shown in Table 1. All 49 strains of serotypes O:3, O:8, O:9, and O:5,27 tested were capable of penetrating HeLa cells, whereas most of them were enterotoxigenic. In contrast, the strains of serotype O:6,30 and other serotypes were only rarely HeLa cell positive, although the majority of them elaborated ST. The association of HeLa cell infectivity with serotypes O:3, O:8, O:9, and O:5,27 was reported by Une et al. (17), and the almost ubiquitous distribution of enterotoxigenicity in all *Y. enterocolitica* strains was described by Pai et al. (12). Of 34 recognized serotypes of *Y. enterocolitica*, only O:3, O:8, and O:9 are clearly associated with human infections, and serotypes O:5,27 and O:6,30 are occasionally isolated from human sources in Canada (15). The other serotypes and nontypable strains are primarily of environmental origin.

Séreny-positive strains were found only in serotype O:8. The keratoconjunctivitis caused by *Y. enterocolitica* was not as severe as that caused by an invasive strain of *E. coli*. This difference was also noted by others (2). Zink et al. (D. L. Zink, J. Feeley, J. Vickery, G. A. O' Donovan, and C. Vanderzant, Abstr. Ann. Meet. Am. Soc. Microbiol. 1978, P31, p. 191) reported the presence of a plasmid associated with invasiveness (positive Séreny test) in serotype O:8 strains of *Y. enterocolitica*. The plasmid and invasiveness were lost in some strains when cultures were incubated at 37°C. In view of these findings and the fact that all of our clinical isolates of serotype O:3 were isolated originally from stool cultures incubated at 37°C, the Séreny test was performed with an addi-
enterocolitica strains and classification into five pathogenic groups

<table>
<thead>
<tr>
<th>Serotype</th>
<th>No. tested</th>
<th>ST production</th>
<th>HeLa(^b)</th>
<th>Sereny(^c)</th>
<th>No. of strains in group(^a):</th>
</tr>
</thead>
<tbody>
<tr>
<td>O:3</td>
<td>22</td>
<td>20</td>
<td>22</td>
<td>0</td>
<td>I: 2  II: 20  III: 1  IV: 8  V: 8</td>
</tr>
<tr>
<td>O:8</td>
<td>14</td>
<td>13</td>
<td>14</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>O:9</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>O:5,27</td>
<td>9</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>O:6,30</td>
<td>10</td>
<td>7</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Other(^d)</td>
<td>29</td>
<td>18</td>
<td>3(^e)</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Grouping was based on ST, HeLa, and Sereny: I (---), II (---), III (---), IV (+++), and V (+++).
\(^b\) HeLa, Ability to penetrate HeLa cells.
\(^c\) Sereny, Ability to evoke keratoconjunctivitis in guinea pigs.
\(^d\) Included serotypes (number) O:1 (1); O:2 (1); O:6,31 (1); O:7,8 (4); O:7,13 (3); O:8,19 (1); O:16 (5); and nontypable (12).
\(^e\) The three HeLa cell-positive strains were serotype O:1 isolated from oyster and serotypes O:2 and O:7,13 isolated from human diarrheic stools.

Tional four pairs of Y. enterocolitica strains (serotype O:3) isolated at two different temperatures. Stool specimens from four culture-positive children with diarrhea were plated onto MacConkey agar in duplicate. One plate was incubated at 37°C, and the other was incubated at room temperature. Isolates were frozen immediately and stored at -70°C until tested in guinea pigs. None of the eight cultures were positive for Sérény.

Based on the above findings, Y. enterocolitica was classified into five pathogenic groups (Table 1). Group I strains possessed none of the three pathogenic properties (---); group II strains were positive for ST production only (+++); group III strains were capable of penetrating HeLa cells (+++); group IV strains were positive for ST production and HeLa cell penetration (++-); and group V strains possessed all three pathogenic properties (+++). Most of serotypes O:3, O:9, and O:5,27 belong to group IV, and none belonged to group I, II, or V, whereas serotype O:8 strains were divided between the two major groups IV and V. Other serotypes were primarily of groups I and II.

The distribution of the pathogenic groups among human and nonhuman isolates is shown in Table 2. The source of isolation was not clearly associated with a particular group, although the majority of human isolates were of group IV, and most nonhuman isolates belonged to groups I and II.

Theoretically, a total of eight pathogenic groups would be expected if the three pathogenic properties were of independent determinants. However, Sérény-positive strains are always HeLa positive (not vice versa) and, therefore, a combination of --- or +++ would not be possible. However, as ST production and invasive-

### Table 1. Enterotoxin production, HeLa cell penetration, and Sérény reaction of Y. enterocolitica strains and classification into five pathogenic groups

<table>
<thead>
<tr>
<th>Serotype</th>
<th>No. tested</th>
<th>ST production</th>
<th>HeLa(^b)</th>
<th>Sereny(^c)</th>
<th>No. of strains in group(^a):</th>
</tr>
</thead>
<tbody>
<tr>
<td>O:3</td>
<td>22</td>
<td>20</td>
<td>22</td>
<td>0</td>
<td>I: 2  II: 20  III: 1  IV: 8  V: 8</td>
</tr>
<tr>
<td>O:8</td>
<td>14</td>
<td>13</td>
<td>14</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>O:9</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>O:5,27</td>
<td>9</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>O:6,30</td>
<td>10</td>
<td>7</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Other(^d)</td>
<td>29</td>
<td>18</td>
<td>3(^e)</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Grouping was based on ST, HeLa, and Sérény: I (---), II (---), III (---), IV (+++), and V (+++).
\(^b\) HeLa, Ability to penetrate HeLa cells.
\(^c\) Sérény, Ability to evoke keratoconjunctivitis in guinea pigs.
\(^d\) Included serotypes (number) O:1 (1); O:2 (1); O:6,31 (1); O:7,8 (4); O:7,13 (3); O:8,19 (1); O:16 (5); and nontypable (12).
\(^e\) The three HeLa cell-positive strains were serotype O:1 isolated from oyster and serotypes O:2 and O:7,13 isolated from human diarrheic stools.

ness are not related, strains that are ST negative but HeLa and Sérény positive (+++) may be isolated. These strains would be classified into an additional group.

We did not examine the stability of the three pathogenic properties after repeated subcultures. Although Sérény invasiveness of Y. enterocolitica has been reported as plasmid encoded, we did not find Sérény-positive strains in any serotype other than O:8. The ST production of Y. enterocolitica appears to be stable (2), and an analysis of the plasmid DNA complement by agarose gel analysis failed to reveal any plasmids associated with ST production (D. L. Zink et al., Abstr. Ann. Meet. Am. Soc. Microbiol., 1978, P31, p. 191).

The importance of invasiveness as characterized by HeLa cell penetration in the pathogenesis of Y. enterocolitica-induced diarrheal diseases has been strongly suggested by the invasive pattern of enteritis seen in fatal cases of human infections (3) and in experimental animal models (4, 10, 16; C. H. Pai, V. Mors, and T. A. Seemayer, Infect. Immun., in press) and by the positive correlation of the pathogenic serotypes (those commonly associated with human infection) with HeLa cell infectivity (17). The latter observation was confirmed in the present study.

### Table 2. Distribution of human and nonhuman isolates of Y. enterocolitica among five pathogenic groups

<table>
<thead>
<tr>
<th>Source</th>
<th>No. tested</th>
<th>No. of isolates in group(^a):</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>60</td>
<td>I: 4  II: 15  III: 33  IV: 8  V: 1</td>
</tr>
<tr>
<td>Nonhuman</td>
<td>20</td>
<td>I: 10  II: 5  III: 5  IV: 5  V: 5</td>
</tr>
<tr>
<td>Unknown</td>
<td>8</td>
<td>I: 4  II: 4  III: 4  IV: 4  V: 4</td>
</tr>
</tbody>
</table>

\(^a\) See Table 1, footnote c.
The questions that remain to be answered are: (i) What is the role of enterotoxin in the pathogenesis of \textit{Y. enterocolitica} gastroenteritis? (ii) Do the enterotoxigenic strains of \textit{Y. enterocolitica} that lack invasive capacity cause diarrhea in humans? and (iii) Are there any differences clinically and pathologically between diarrheal diseases caused by HeLa cell-positive (group IV) and Séreny-positive (group V) strains of \textit{Y. enterocolitica}? The answers to these questions may be obtained in experimental infections with representative strains of the five different pathogenic groups of \textit{Y. enterocolitica} described in this study. An alternative and more ideal approach would be to use isogenic strains of various pathogenic groups derived from a group V strain, although the isolation of such mutants would be technically difficult because of the lack of simple in vitro tests for these pathogenic properties.

We thank Evelyn Oman for excellent technical assistance and L. Lafleur, W. H. Lee, and S. Toma for generous gifts of \textit{Y. enterocolitica} strains.

This study was supported by Medical Research Council grant MA-7108 and Natural Sciences and Engineering Research Council grant A-9433.

\textbf{LITERATURE CITED}


