Infection Caused by *Yersinia enterocolitica* Serotype O:21

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*Yersinia enterocolitica* serotype O:21, biotype 3 (Wauters) was isolated from the appendix and stool of a 7-year-old girl. The same organism was later isolated in pure culture from pus from a postoperative wound infection in this patient. She developed a significant serological response (titer of 800). There was thus strong clinical evidence of pathogenicity associated with this rather uncommon human serotype. Laboratory studies of *in vitro* and *in vivo* pathogenicity showed that the organism was autoagglutinable, Serény test positive, and HeLa cell invasive; when given orally to mice, it produced diarrhea and subsequently death. The results of laboratory studies of virulence correlated closely with the clinical evidence of pathogenicity in this case.

Although over 50 different O serotypes of *Yersinia enterocolitica* are now known (22), the vast majority of clinical isolates belong to a relatively few serotypes. In Scandinavia and parts of Europe, for example, over 80% of clinical isolates belong to serotype O:3, and a further 10 to 15% belong to serotype O:9 (13). Serotypes O:3 and O:9 are rarely found in the United States, the commonest serotype in that country being serotype O:8 (15). An analysis of about 1,500 strains isolated from clinical specimens in Canada over a 12-year period (19) showed that about 76% of the strains were of the indole-negative serotype O:3. The next most frequent serotypes were the indole-positive types O:5,27, O:6,30, and O:8, each accounting for 3 to 5% of the isolates. Of the remaining 12% of the strains, about half were nontypable and half belonged to at least 20 different serotypes. One such infrequent human serotype is O:21, whose association with human disease has not been well defined.

The best evidence of human pathogenicity in a given bacterial serotype is its ability to cause human disease. Since isolates of *Y. enterocolitica* belonging to serotypes O:3, O:8, and O:9 are commonly recovered from humans with typical clinical syndromes, there may be justification in correlating such serotypes with human pathogenicity. On the other hand, no such correlations may be made with respect to those serotypes that have, if ever, only infrequently been isolated from clinical material. Included in the latter category are serotypes such as O:4, O:6, and O:17 that are often found in food and the environment (15, 19). The extent to which environmental isolates represent a human health hazard is unclear. A better understanding of the pathogenic potential of such isolates would clarify the need for controlling them at their sources.

Attempts have been made in recent years to predict human pathogenic potential in strains of *Y. enterocolitica* on the basis of a number of laboratory tests of *in vivo* and *in vitro* pathogenicity (6–8, 12, 14, 17, 18, 21). The results of such tests are likely to be valid only if they can be correlated closely with satisfactory clinical evidence of infection. Such correlations have been made for some of the more common human serotypes such as O:3 and O:8 (12, 18, 21). We describe here a case of human infection associated with *Y. enterocolitica* serotype O:21 in which the results of laboratory tests of virulence correlated closely with strong clinical evidence of infection.

CASE REPORT

A 7-year-old female was seen at the emergency department with a 3-day history of general malaise, headache, intermittent fever, and increasing periumbilical and lower abdominal pain. She had not had a bowel movement for 2 days.

On examination, the child looked flushed, but was not in acute distress. The temperature was 39.2°C, and the heart rate was 100 beats/min. Examination of the abdomen showed tenderness on deep palpation in the right lower quadrant. There was no guarding or rebound tenderness. Normal bowel sounds were heard. No other abnormalities were noted on examination. The leukocyte count was slightly elevated at 13.7 x 10³ cells/liter.

The patient was admitted for observation with a diagnosis of possible appendicitis. Subsequently,
when her signs and symptoms worsened, she was operated on through a McBurney incision for acute appendicitis. A small quantity of clear peritoneal fluid was present. The appendix looked grossly normal, but the terminal ileum was red and inflamed with some nearby enlarged lymph nodes. An appendectomy was performed. The child had an eventful postoperative course and was discharged well 7 days later. Bacteriological culture of the appendix stump yielded a heavy growth of \textit{Y. enterocolitica}. Histology of sections of the appendix showed "a focus of neutrophil infiltrate in the lamina propria which extends through the epithelium and is associated with exudate of neutrophils in the lumen. The appearance is that of mild early appendicitis." Five days after discharge from the hospital, the patient was readmitted with a complaint of soreness and swelling around the abdominal incision site, and fever with a temperature of 38.1°C. Examination showed redness around the incision site and a fluctuant swelling beneath the skin. The wound was reopened, and a collection of pus was noted. This was drained and examined bacteriologically. Culture of the pus gave a heavy pure growth of \textit{Y. enterocolitica}. The wound was managed by drainage and local irrigation with a sodium hypochlorite solution (Hygeol). Antibiotic therapy was withheld. The wound infection resolved satisfactorily, and the patient was discharged 4 days later.

A stool sample was obtained during the second admission. Culture yielded a heavy growth of \textit{Y. enterocolitica}. The patient lived in a small rural community about 150 miles north of Toronto. She had arrived in Toronto 2 days before the onset of her symptoms. An 8-year-old cousin and a 10-year-old brother had also developed abdominal pain and fever at around the same time as our patient. However, it was not possible to perform early bacteriological studies on these patients.

The two isolates of \textit{Y. enterocolitica} from the appendix stump and wound were obtained in pure culture on blood agar and bile salts medium. The isolate from the stool was found to be the predominant colony type among aerobic fecal flora on blood agar, MacConkey medium, and salmonella-shigella agar. All three isolates had features typical of \textit{Y. enterocolitica} biotype 3 (G. Wauters, Ph.D. thesis, Vander, Louvain, Belgium, 1970) and were negative for salicin and esculin. They were found to be serotype O:21. A serum sample obtained from the patient about 3 weeks after the onset of symptoms had an agglutinating titer of 800 against the strain of \textit{Y. enterocolitica} isolated from the appendix.

The strains were examined by one of us (D.A.S.) in various laboratory in vivo and in vitro systems used for evaluating pathogenicity. Representative colonies from two strains were autoagglutinable by the method of Laird and Cavanaugh (8). Further tests of pathogenicity were performed only on the stool isolate of \textit{Y. enterocolitica}. Representative colonies showed a high index of infectivity in a HeLa cell culture system (5, 7, 20), produced heat-stable enterotoxin in the infant mouse assay system (14), produced conjunctivitis in guinea pigs (17, 18), and were unable to grow on magnesium oxalate agar at 35°C (17). When tested in the mouse diarrhea model (8), the stool isolate produced diarrhea and subsequently death (18).

\section*{DISCUSSION}

The clinical data in this case strongly implicate \textit{Y. enterocolitica} serotype O:21 as the cause of the acute appendicular syndrome and postoperative wound infection in our patient. The acute appendicular syndrome is a common clinical manifestation of infection caused by \textit{Y. enterocolitica} (11, 13). Only seven cases of postoperative wound infection associated with this organism have been recorded (11).

The association of serotype O:21 with human disease has been limited. In one case report (9), the organism was isolated from a furuncle. In a prospective study on Montreal children with \textit{Y. enterocolitica}-associated gastroenteritis (10), the serotype isolated from 90% of the patients was O:3. Serotype O:21 was isolated from only 1 of 181 cases, but its clinical significance as well as that of 17 other nonserotype O:3 isolates was uncertain. Serotype O:21 accounted for only 0.4\% (seven strains) of all human isolates of \textit{Y. enterocolitica} in Canada over a 12-year period from 1966 to 1978 (19). These strains of human origin were similar to three isolates from our index case, i.e., \textit{Y. enterocolitica sensu stricto} (1). They were salicin and esculin negative, but their indole reaction varied from indole positive (biotype 1; five cultures) to a late positive indole reaction (biotype 2; one isolate) or an indole-negative reaction (biotype 3; one isolate). During the same 12-year-period, serotype O:21 accounted for about 25\% of all environmental isolates (10 strains), 7 of which came from water sources and 3 of which came from food products. All the environmental strains of serotype O:21 were \textit{Y. enterocolitica}-like organisms, being L-rhamnose, melibiose, raffinose, and α-methyl-D-glucoside positive. Such strains were recently defined as a new species, \textit{Y. intermedia} (3). The global distribution of \textit{Y. enterocolitica} serotype O:21 remains to be established. Schieumann et al. (18) recently examined surveys of human isolates of \textit{Y. enterocolitica} from the United States, Hungary, Israel, Belgium, Czechoslovakia, Germany, and The Netherlands, and found that none of the surveys reported the isolation of serotype O:21.

The correlation of clinical evidence of pathogenicity with laboratory tests of virulence in serotypes O:3 and O:8 (12, 18), and now also in O:21, supports the validity of such tests in predicting human virulence. However, it is not known which of the various laboratory tests of pathogenicity best reflect human virulence. It should be noted that human isolates of serotypes O:3 and O:8 behave differently in some of these tests. For example, both O:3 and O:8 are HeLa cell invasive (7, 12, 21) and produce heat-stable enterotoxin (14); both tend to be autoagglutinable (18), and both fail to grow on magnesium agar.

\section*{References}

oxalate agar at 35°C (18). On the other hand, serotype O:8 produces marked conjunctivitis in guinea pigs (17, 18), whereas only a weak conjunctival reaction is produced by serotype O:3; when given orally to mice, serotype O:3 induces a self-limiting diarrheal illness, whereas serotype O:8 produces diarrhea and subsequently death (18).

The inability of strains of Y. enterocolitica to grow on magnesium oxalate agar at 35°C is considered to reflect the presence in such strains of V and W antigens (17). These antigens were found to be identical immunologically to the virulence antigens of Y. pestis and Y. pseudotuberculosis (4). Schiemann and Devenish (17) attempted to correlate the presence of the VW antigen (as determined by the inability of strains to grow on magnesium oxalate agar at 35°C) with virulence in gerbils and in the guinea pig conjunctiva. They found that strains of serotype O:8 possessing the VW antigens were lethal for gerbils and produced marked conjunctivitis in guinea pigs, whereas strains of serotype O:8 that lacked these antigens failed to produce death in gerbils or conjunctivitis in guinea pigs. On the other hand, strains of serotype O:3 that also possessed the VW antigens were not lethal to gerbils and produced at best only mild conjunctivitis in guinea pigs. The reasons for the differences between serotypes O:3 and O:8 in their ability to produce disease in the mice, the gerbils, and guinea pigs remain to be elucidated. It should be noted that the reactions of Y. enterocolitica serotype O:21 in laboratory tests of virulence in the present study resembled those of serotype O:8 rather than those of serotype O:3 (18).

The reason for the rarity of serotype O:21 in clinical specimens is not known. The fact that this organism was readily cultured and identified from clinical specimens in our laboratory by using routine media suggests that it would be recognized in the same manner as would the other more common serotypes, such as O:3 and O:8. The latter serotypes have been found in swine (16), but no nonhuman reservoir of Y. enterocolitica sensu stricto (1) serotype O:21 is yet evident. It is possible that serotype O:21 belongs to a restricted ecological niche that has, as yet, only infrequently been encroached upon by humans.

LITERATURE CITED