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# *Yersinia enterocolitica* infection in children

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The clinical presentation, course and outcome of *Yersinia enterocolitica* infection was studied prospectively in 125 children. Enteric forms occurred in 114 children (92 enteritis, 20 pseudoappendicitis, 2 chronic ileitis), of whom 17 also had extramesenteric manifestations; 11 children had one or more extramesenteric forms without enteric disease. Enteritis occurred more frequently in young children whereas serious forms and extramesenteric forms were more common in children older than 6 years of age ( $P < 0.001$ ). Arthritis was observed in 13 children and extensive lymphadenopathy in 11; 1 child had septicemia with pleurisy, 1 had vasculitis, 1 had cholecystitis and 4 had erythema nodosum. Diagnosis was established by positive culture in 100 (80%) children and by agglutinin test in 11 of 45 (24%), demonstration of circulating specific anti-IgA and anti-IgG to *Yersinia* outer membrane proteins in 47 of 48 (98%) and detection of antigen in biopsies in 28 of 33 (85%) children. The 2 latter methods were superior to the agglutinin test. Serotype O3 and O9 predominated. The frequency and seriousness of complications may justify the use of antibiotics for *Yersinia* enteritis in children 6 years of age or older.

## INTRODUCTION

The predominant form of *Yersinia enterocolitica* infection in children is an enteric illness with or without mesenteric lymphadenitis.<sup>1,2</sup> Systemic infections including extramesenteric lymphadenitis, metastatic infections of various organs and septicemia can also occur.<sup>3</sup> Postinfectious complications such as arthritis and erythema nodosum are uncommon in children.<sup>4</sup> Diagnosis of the chronic and more complicated forms of disease can be difficult<sup>3,5</sup> because routine diagnostic methods such as stool culture and serology can become

negative after the acute phase.<sup>5</sup> This may contribute to an underestimation of the disease. In 1986 we introduced other diagnostic methods: determination of class specific serum IgA and IgG antibodies against outer membrane proteins<sup>4</sup> and antigen detection in biopsies.<sup>6,7</sup> This study reports the incidence, course, effects of treatment and outcome of yersiniosis in children during a 9-year surveillance period in an endemic area.

## MATERIALS AND METHODS

Patients were identified from specimens sent to the Public Health Laboratory in Friesland (the Netherlands). This laboratory serves a province of 700 000 inhabitants with 8 general hospitals (2100 beds) and 260 general physicians. The study period was from July 1, 1982, to January 1, 1991. *Y. enterocolitica* infection was diagnosed in 887 patients (adults and children) by culture, serology and antigen detection. A physician was asked to observe each patient with proved *Yersinia* infection for at least 2 months to obtain information on specific symptoms (diarrhea, nausea, vomiting, fever, weight loss, lymphadenitis, erythema nodosum, arthritis, icterus, complications, duration of disease, family history), clinical course, effects of treatment and outcome. A standard form to record all data was used. Complete record forms were obtained from 125 children. These patients are presented in this study.

Feces were inoculated onto *Yersinia* selective agar (Oxoid CM653), onto deoxycholate citrate agar (Oxoid CM35) and into Rappaport broth.<sup>8</sup> Plates were incubated at 22°C for 48 h; broth was incubated at 22°C for 72 h and then subcultured onto the same agar media. Colonies suspected to be *Yersinia* were isolated and identified. Other materials (pus, blood) were routinely cultured for aerobic and anaerobic bacteria and in particular for *Yersinia* on selective agar and in Rappaport broth, incubated at 22°C for 72 h. Pus was also stored for cold enrichment in phosphate buffer (pH 7.0) at 4°C for 3 weeks and then subcultured onto *Yersinia*-selective agar and sheep's blood agar.

The *in vitro* susceptibilities to amoxicillin, amoxicillin-clavulanate, cefuroxime, ceftazidime, gentamicin, trimethoprim-sulfamethoxazole, doxycycline and chloramphenicol was determined for all strains by microtiter broth dilution according to the recommenda-

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tions of the National Committee for Clinical Laboratory Standards.<sup>9</sup>

Serum samples were titrated against unheated suspensions (optical density, MacFarland Standard 5) of *Y. enterocolitica* serotype O3, O5.27, O6.30, O7.8, O8 and O9 and *Yersinia pseudotuberculosis* serotypes I to VI grown at 22°C for 48 hours, with a standard agglutination test.<sup>5,7</sup> A titer of antibody >1:160 was significant.

Specific IgA and IgG antibodies against purified plasmid-encoded virulence-associated *Yersinia* outer membrane proteins (Yops) of *Y. enterocolitica* serotype O8 (strain WA-314; Dr. C. Pai, Montreal, Canada) were demonstrated in sera by immunoblotting techniques.<sup>6</sup> Antigens ( $M_r$  25 kilodaltons (kDa) = Yop E, 36 kDa = Yop D, 38 kDa = V antigen, 46 kDa = Yop H and 58 kDa = Yop M) were blotted onto a nitrocellulose filter. Sera were diluted 1:100 in phosphate-buffered saline-Tween (150 mM NaCl, 20 mM Na<sub>2</sub>HPO<sub>4</sub> (pH 7.0) and 0.5% Tween 20) and incubated with the antigen-coated nitrocellulose strips overnight at 22°C. The IgG and IgA antibody-antigen complexes formed were visualized according to the methods of Blake et al.<sup>10</sup> Control strips were reacted with human acute sera (culture-positive *Y. enterocolitica* infection) containing antibodies to the Yops. Positive reaction (IgA and IgG) in immunoblotting with at least two identical Yops was judged significant.

Specimens from patients (lymph nodes, biopsy materials, pus, enteric preparations) were investigated with serotype-specific antisera against serotypes O3, O5.27, O8 and O9 and monospecific antisera against YadA (another plasmid-encoded virulence-associated outer membrane protein,  $M_r$  220 kDa<sup>6</sup>) and Yop H for the presence of *Y. enterocolitica* bacilli by indirect immunofluorescence.<sup>7</sup>

## RESULTS

A total of 125 children (61 females, 64 males) were evaluated. The age distribution and the various forms of yersiniosis are shown in Table 1. Eighty-four children had uncomplicated enteritis and 41 had complicated forms. Uncomplicated enteritis occurred more often in young children; serious and extramesenteric forms more often occurred in children above the age of 6 years ( $P < 0.001$ ).

Enteritis was characterized by diarrhea in 68%, abdominal pain in 40% and fever in 33%; 26 (30%) children were hospitalized because of dehydration (Table 2). Diarrhea was rather mild, the stool was not bloody and the duration was about 1 week in 22%, 2 to 4 weeks in 55 and 23% of the children had 3 to 4 periods of diarrhea for 2 to 12 months. Abdominal pain, localized in the right lower quadrant, was usually accompanied by diarrhea and was prominent. In 9 (10%) patients pain was the only symptom. Enteritis was

**TABLE 1.** Demographic data of 125 children with yersiniosis

Disease	No.	Age Range (Years)		
		0-5	6-10	11-15
Enteric forms				
No.	114	66	20	28
Enteritis	84	61	11	12
Enteritis with complications	8	4	2	2
Appendicular syndrome	20	1	7	12
Ileitis	2			2
Extramesenteric forms				
No.	28	5	8	15
(+ enteric)	17	4	5	8
Septicemia	1	1		
Vasculitis	1			1
Lymphadenopathy	11	1	3	7
Hepatitis	1	1		
Arthritis	13	1	4	8
Erythema nodosum	4	3	1	
Total no.	125	67	23	35

**TABLE 2.** Age distribution, symptoms and need for antibiotic treatment in 84 children with uncomplicated enteritis by *Yersinia enterocolitica*

Clinical Data	Age (Years)		
	0-5	6-10	11-15
No. of patients	61	11	12
Symptoms			
Diarrhea	44	9	5
Fever	22	2	4
Abdominal pain	21	7	6
Vomiting	16	5	2
Weight loss	11	4	
Hospitalization	20	3	3

complicated by extramesenteric manifestations in 8 children, including septicemia with vasculitis, pleurisy and hepatosplenomegaly (1), lymphadenopathy (1), erythema nodosum (2), arthritis (2) and arthritis and erythema nodosum (2).

Pseudoappendicular syndromes, presenting as "acute abdomen," occurred in 20 patients, all but 1 older than 6 years of age. The most important symptoms were acute abdominal pain, especially tenderness of the right lower quadrant; 5 children had fever and 3 had diarrhea. Surgery was performed in 13 patients for suspected "acute abdomen." At laparotomy mesenteric lymphadenitis with terminal ileitis was found with a normal appendix in 9 and an inflamed appendix in 4 children; the latter 4 had also mesenteric lymphadenitis. Seven of 13 operated children had extensive intra-abdominal lymphadenitis with pseudotumorous forms; 1 also had cholecystitis. Histology on ileocecal biopsies showed nonspecific inflammation with diffuse lymphoplasmacellular infiltrates and destruction of epithelial cells. The lymph nodes also showed nonspecific chronic inflammation, and *Y. enterocolitica* bacilli could be demonstrated in or between the epithelial cells of the ileocecal biopsies and in the lymph nodes.

Two patients had arthritis simultaneously.

Eighteen of 20 patients improved without complications, and 2 kept complaints of abdominal pain during the follow-up for 1 to 12 months. Both had undergone surgery; they were treated with antibiotics subsequently and recovered.

Ileitis was present in a 15-year-old girl and a 14-year old boy, who had chronic and relapsing, sometimes bloody, diarrhea with abdominal pain, fever and weight loss. The duration of complaints at the time of diagnosis was 10 months. At endoscopy the intestinal mucosa appeared diffusely swollen and erythematous; chronic inflammation and infiltration of submucosal and muscular areas were observed with nonspecific lymphocytic infiltrates, necrosis and some granulomas. Giant cells and fistulae were not found. Bacilli were found in the mucosa and submucosa and deeper in the infiltrates and granulomas, often associated with macrophages. The girl had also polyarthritis and mediastinal lymphadenopathy.

Eleven patients had only extramesenteric manifestations, including arthritis (7 patients), arthritis with erythema nodosum (1), arthritis with vasculitis (1), generalized lymphadenopathy with splenomegaly, high fever and weight loss (2 children) and liver abscesses (1).

The diagnosis of *Y. enterocolitica* infection was established by positive culture in 100 patients (Table 3). The age distribution of these patients is shown in Figure 1. Most culture-positive patients were found in the younger age group presenting with enteritis, both uncomplicated and complicated (89 (92) = 97%). Ten of 20 (50%) patients with pseudoappendicitis and 1 patient with arthritis and erythema nodosum without an enteric form were culture-positive.

Culture-negative patients were diagnosed (Table 4) by agglutination (6 patients), immunoblotting (22 patients) and antigen detection (11 patients).

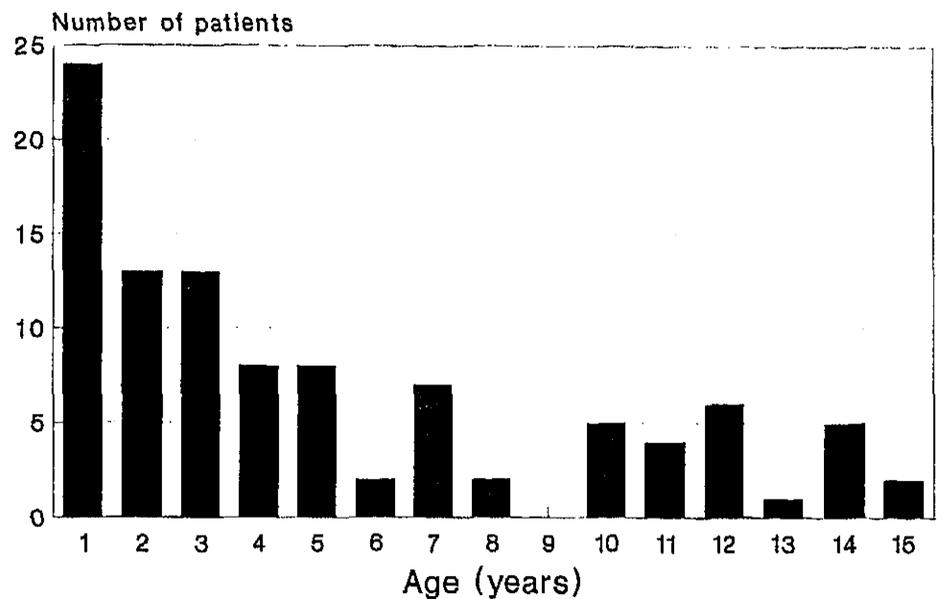


FIG. 1. Age distribution of 100 culture-positive children with *Y. enterocolitica* infection.

TABLE 4. Positive diagnostic tests in 25 culture-negative children with yersiniosis, related to clinical disease

Disease	No. Positive			
	IgA anti-Yops	IgG* anti-Yops	Agglutinin test	Antigen detection
Enteritis	3	3 (3)†	1 (3)	
Appendicular syndrome	10	9 (9)	3 (8)	7 (7)
Ileitis	2	1 (1)	0 (1)	2 (2)
Extramesenteric forms	10	9 (9)	3 (8)	2 (2)

\* Serum anti-IgA and IgG to Yops by immunoblotting, agglutinin test and antigen detection in biopsies.

† Numbers in parentheses, number of tests performed.

Overall agglutinins were found in 11 (24%) of 45 sera tested and specific IgA and IgG antibodies to Yops in 47 (98%) of 48 sera (98%) tested. Antigen detection in biopsies or surgical materials diagnosed 28 of 33 (85%) patients tested. Serum specimens were available from 11 culture-positive children. In a comparison of sensitivity and specificity of the serologic tests, it was found that 3 of 11 culture-positive children had circulating agglutinins *vs.* 10 with anti-IgA and anti-IgG to Yops. Seven of these children had complicated forms of disease.

TABLE 3. Positive diagnostic tests in 125 children with yersiniosis, related to clinical disease

Disease		Culture	No. Positive		
			IgA anti-Yops*	IgG anti-Yops*	Agglutinin test
Enteric forms					
Enteritis	92	89	13 (14)	4 (16)	4 (5)
Appendicular syndrome	20	10	12 (12)	2 (12)	14 (16)
Ileitis	2		1 (1)	0 (1)	2 (2)
Extramesenteric forms	28	9	21 (21)	5 (61)	9 (11)
Septicemia	1	1	1 (1)	0 (1)	1 (1)
Vasculitis	1		1 (1)	1 (1)	1 (1)
Lymphadenopathy	11	5	7 (7)	2 (6)	7 (8)
Hepatitis	1		1 (1)	0 (1)	1 (1)
Arthritis	13	3	10 (10)	2 (7)	2 (3)
Erythema nodosum	4	4	2 (2)	0 (2)	
Total no.	125	100	47 (48)	11 (45)	28 (33)

\* Serum anti-IgA and IgG to Yops by immunoblotting, agglutinin test and antigen detection in biopsies.

† Numbers in parentheses, number of tests performed.

The strains isolated belonged to serotypes O3 (61), O5.27 (2), O6.30 (3), O8-like (5) and O9 (21); eight strains were not typable.

All strains were resistant to amoxicillin and amoxicillin-clavulanate (90% minimal inhibitory concentration ( $MIC_{90}$ ) >32 mg/liter) and 90% were susceptible to cefuroxime ( $MIC_{90} \leq 4$  mg/liter), doxycycline ( $MIC_{90} \leq 2$  mg/liter) and chloramphenicol ( $MIC_{90} \leq 4$  mg/liter). All strains were susceptible to trimethoprim-sulfamethoxazole ( $MIC_{90}$  0.125 mg/liter), ceftazidime ( $MIC_{90}$  1 mg/liter) and gentamicin ( $MIC_{90}$  1 mg/liter).

A total of 48 children were treated with antibiotics: 32 received trimethoprim-sulfamethoxazole, 4 received amoxicillin, 4 received cefaclor, 4 received doxycycline, 2 received ciprofloxacin, 1 received chloramphenicol and 1 received gentamicin. Three clinical failures were observed: trimethoprim-sulfamethoxazole (2) and amoxicillin (1).

## DISCUSSION

The incidence of yersiniosis in this part of the Netherlands is estimated to be 20% that of salmonellosis.<sup>3</sup> This seems high compared with most European countries,<sup>11</sup> although recent data are missing. This high incidence may be partly explained by better diagnosis by use of more sensitive and specific diagnostic methods and in particular by an increasing alertness of physicians participating in this prospective study.

In common with others<sup>1, 5</sup> we found the enteric form predominant, with the highest incidence in young children. Probably the incidence in this age group is even higher inasmuch as *Yersinia* enteritis is often a mild disease at that age; probably because of this many children do not visit a doctor and fecal cultures are not made. This finding is in contrast with that of Naqvi et al.<sup>2</sup> concluding that *Yersinia* enteritis in children is a serious disease with fever and bloody stools. We found bloody stools to be an exception in young children and only one-third of them had fever. We think that Naqvi's study group was a selection of the more serious cases and not representative of the usual *Yersinia* enteritis in children. Naqvi et al. reviewed only patients seen in the children's hospital from whose stools *Yersinia* has been isolated at the pediatrician's request. Most of our children with enteritis were evaluated by general practitioners and did not require hospitalization. Severity of disease may also be associated with certain serotypes. Serotype O3 and O9 strains predominated in our patients and they are known to be less virulent than the American O8 strain. Because we do not know which serotypes were isolated in the Naqvi study, we cannot evaluate this aspect. Serotype O3, however, is also emerging as cause of pediatric gastroenteritis in the United States as reported by Lee et al.<sup>12</sup> They observed 34 children with serious enteritis with bloody stools from serotype O3 infection, but they also studied only

patients referred to the hospital, who were the more serious cases.

Characteristic for *Yersinia* enteritis was its long duration and tendency to chronicity with relapses, which was also reported by Lee et al.<sup>12</sup> Abdominal pain was a major complaint of many children and often more pronounced than diarrhea.

The more serious forms, pseudoappendicular syndrome and systemic infections, affected mainly older children and the frequency in that age group (40%) is higher than that reported by others,<sup>13</sup> but in the same range as we reported earlier for adults.<sup>14</sup>

Patients with complicated forms were often culture-negative; only a minority had significant agglutinin titers. Using more specific and novel techniques we could recognize *Yersinia*-associated disease in 21 patients otherwise missed. Comparing the agglutination reaction with determination of specific anti-IgA and anti-IgG to Yops in culture-positive patients, it was clear that the anti-Yops are far more sensitive and reliable for diagnosis of *Yersinia*-associated disease. This was previously shown.<sup>6, 7, 14, 15</sup>

We could not associate the systemic forms with predisposing factors such as malignancy, hematologic disorders and iron overload.<sup>16</sup>

We were impressed by the severity of the lymphadenopathy, which resembled that of disorders such as malignant lymphoma. Seven children had an extensive intraabdominal mass found at operation for acute abdomen and four children had generalized lymphadenitis, all accompanied by high fever and weight loss. This syndrome existed for several weeks before the diagnosis was made and disappeared completely after appropriate antibiotic therapy.

*Yersinia*-associated arthritis is a complication often reported in adults. A sporadic case has been reported in pediatrics.<sup>17</sup> We found it a complication in 10% of our children. The youngest child with arthritis in our series was 2 years of age, the other children were 7 years or older. Three children had positive stool cultures and six developed arthritis after an enteric form of yersiniosis, but seven children had arthritis without preceding diarrhea, although one of them had a positive stool culture. This means that yersiniosis is in the differential diagnosis of patients with arthritis, in particular when erythema nodosum is a concomitant finding as it was in two of our children.

Why some patients cannot recover from *Yersinia* enteritis and develop a chronic form is unclear. Persistence of infection and antigens may be responsible for or involved in these complications.<sup>15</sup>

Antibiotic treatment does not influence the course and duration of uncomplicated enteritis,<sup>14, 18</sup> and currently we do not advise antibiotic treatment in children younger than 6 years of age with enteritis in our area.

Whether antibiotics might be effective in preventing complications is difficult to establish, but their frequency and seriousness in children 6 years of age and older may justify their use in this age group. The strains cultured belonged to the serotypes common in Europe,<sup>19</sup> and their antibiotic susceptibility pattern was consistent with the findings of others.<sup>20</sup> The treatment results with trimethoprim-sulfamethoxazole, the drug most often used, were satisfactory.

This study, in which novel diagnostic methods were used, showed that *Y. enterocolitica* infection may be underdiagnosed if one is limited to standard techniques. The novel tests provided insight into conditions associated with yersiniosis. The clinical significance of the etiologic diagnosis of yersiniosis is an important one, to avoid primarily inappropriate therapy. Too often unnecessary surgical procedures or treatment with ineffective antibiotics, antiinflammatory agents, corticosteroids or even cytotoxic drugs are used for yersiniosis in the absence of the correct diagnosis.<sup>3, 14</sup> Complicated forms of enteritis and the extramesenteric forms must be treated with appropriate antibiotics. Because these antibiotics are different from those routinely used in severe infections of unknown origin<sup>14, 21</sup> it is very important to make the right diagnosis. Wrong therapy may lead to prolonged hospitalization and occurrence of late complications, especially in older children.

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