Prevalence of *Yersinia enterocolitica* Bioserotype 3/O:3 among Children with Diarrhea, China, 2010–2015

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*Yersinia enterocolitica* is thought to not significantly contribute to diarrheal disease in China, but evidence substantiating this claim is limited. We determined the prevalence of *Y. enterocolitica* infection and strain types present among children <5 years of age with diarrhea in China. The overall prevalence of pathogenic isolates was 0.59%. Prevalence of pathogenic bioserotype 3/O:3 varied geographically. In this population, the presence of fecal leukocytes was a characteristic of *Y. enterocolitica* infection and should be used as an indication for microbiological diagnostic testing, rather than for the diagnosis of bacillary dysentery. In contrast with *Y. enterocolitica* isolates from adults, which were primarily biotype 1A, isolates from children were primarily bioserotype 3/O:3. Most pathogenic isolates from children shared pulsed-field gel electrophoresis patterns with isolates from pigs and dogs, suggesting a possible link between isolates from animals and infections in children. Our findings underscore the need for improved diagnostics for this underestimated pathogen.

*Yersinia enterocolitica* is an emerging infectious pathogen that has caused wide public health concern since the 1980s. After campylobacteriosis and salmonellosis, yersiniosis ranks third most common among the notifiable bacterial zoonoses in the European Union (1,2). The incidence of human yersiniosis was 1.92 cases/100,000 population in 2013 in Europe (3); in the United States, incidence decreased from 1.0 cases/100,000 population in 1996 to 0.3 cases/100,000 population in 2009 (4). Gastroenteritis and enteritis are among the most common clinical signs. Autoimmune complications such as reactive arthritis sometimes occur (2,5). Deadly hemorrhagic septicemic yersiniosis occurs in immune-compromised patients. Strains of *Y. enterocolitica* biotype 1A (1 of the 6 biotypes) lack the pYV plasmid and the major chromosomal determinants of virulence and, thus, have been regarded as avirulent (2). However, this avirulent biotype has also been implicated in foodborne and nosocomial outbreaks and has reportedly produced disease symptoms indistinguishable from those produced by the known pathogenic biotypes (6–8).

In most countries in Europe, the bioserotype 4/O:3 accounts for ≈80% of human infections; 4/O:3 is also dominant in North America, where 3/O:3 infection is rarely reported (9). Conversely, 3/O:3 is the most prevalent bioserotype in China (10–15). Studies have shown that the prevalence of pathogenic strains among pigs in China is higher than that in countries of Europe (15,16). However, except for 2 outbreaks reported in the 1980s (10), we have little data concerning human infections in China. Because yersiniosis is not notifiable through the national surveillance systems in China, most hospitals do not routinely tested for *Y. enterocolitica*. In China, infectious diarrhea is primarily diagnosed on the basis of clinical examination rather than microbiological diagnostic testing (except for rotavirus, norovirus, and a few types of bacteria in some large hospitals). For example, shigellosis is often diagnosed in persons with signs such as tenesmus after leukocytes are found in their fecal samples. These diagnostic criteria render *Shigella, Salmonella*, enteroinvasive *Escherichia coli*, Campylobacter, and *Y. enterocolitica* infections indistinguishable.

According to surveys around the world, most *Yersinia* infections have occurred in infants and young children (17,18). In the United States, ≈100,000 episodes

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of foodborne illness caused by *Y. enterocolitica* occur annually, and risk for disease is higher among infants (4,19). In Germany, the average annual incidence of *Y. enterocolitica* infection among children <5 years of age was ~12-fold higher than the average incidence among persons ≥5 years of age (3,20). Thus, in 10 regions of China, we performed microbiological diagnostic tests for children ≤5 years of age with diarrhea to determine the prevalence of *Y. enterocolitica* infection in this population and the need for improved diagnosis of yersiniosis. We also investigated possible links between strains from animals and humans.

**Methods**

**Population Design and Collection of Case Information and Samples**

During 2010–2015, we invited all patients with diarrhea from 17 hospitals to participate in this study. Diarrhea was defined according to the Global Enteric Multicenter Study: ≥3 loose stools within the previous 24 h (21). The study participants provided informed consent, fecal samples, and completed questionnaires. We followed the same protocol for all cases and excluded cases if either sample or questionnaire was lost.

**Sampling from Children**

We recruited children ≤5 years of age with diarrhea at sentinel pediatric hospitals in different parts of China: Henan in central China; Beijing and Tianjin in northern China; Jiangsu, Shandong, and Anhui in eastern China; Guangxi in southern China; Sichuan and Yunnan in southwestern China; and Ningxia in northwestern China. Within each region, we gave primary hospitals (such as community hospitals in cities and village clinics in the countryside) the opportunity to become sentinel sites for this study. The staff of sentinel hospitals were capable of collecting case information and specimens and taking into account patients’ environment, folk customs, and eating habits during treatment. The same procedures were performed at each site to avoid bias in sampling procedures and in storing and handling samples. In some village clinics, fecal microscopy could not be conducted.

To compare the *Y. enterocolitica* prevalence between children and adults, we collected samples from 2 sites in central Beijing. We recruited adults from a general hospital and children from a pediatric hospital 5 km away that was also 1 of the sentinel hospitals for this study.

**Questionnaire**

The questionnaire included questions about demographics (name, sex, birth date, address, and contact information) and clinical features (date of onset, date of visiting doctor, diarrhea frequency, body temperature, vomiting, fecal characteristics, and results of routine fecal sample inspection). Fecal samples were routinely examined for the presence of leukocytes and erythrocytes. Doctors wrote the primary diagnosis on the patient’s questionnaire.

**Sample Collection**

Fresh fecal samples were collected from patients after enrollment in the study. Fecal samples were stored in peptone sorbitol bile broth (Fluka, Everett, WA, USA) at 4°C.

**Y. enterocolitica Isolation and Identification**

During the study, we conducted 2 technical trainings for sentinel hospital staff on *Y. enterocolitica* isolation and identification. *Y. enterocolitica* was isolated from samples by following the procedures described previously (15). To ensure laboratory capacity, we sent for assessment samples to the sentinel hospital staff who were blinded to sample identity. Hospital staff enriched the strains in peptone sorbitol bile broth at 4°C for 21 d and then amplified 2 *Y. enterocolitica* genes: *foxA* (conserved) and *ail* (pathogenic) (22). Samples positive for either or both of these genes were inoculated onto Yersinia Selective Agar (Schiemann’s CIN [Cefsulodin, Irgasan, Novobiocin] agar; Oxoid, Basingstoke, UK). To obtain pure cultures, staff then inoculated the presumptive *Y. enterocolitica* colonies having a typical bull’s-eye appearance on CIN agar onto brain–heart infusion agar plates and incubated them at 25°C for 24 h (10). Hospital staff performed the biochemical test Analytical Profile Index (API) 20E (bioMérieux, Marcy l’Etoile, France) and bioserotype identification methods reviewed by Wang et al. with all isolates (13). The Wauters’ biotype method was used (23).

**Identification of Pathogenic Strains and Cluster Analysis**

We amplified virulence genes (*ail*, *ystA*, *ystB*, *virF*, and *yadA*) from the chromosomes and plasmids for all *Y. enterocolitica* isolates. We used the PCR method, including primer sequences and annealing temperatures, described by Liang et al. (15).

For the analysis of identified pathogenic isolates, we used the pulsed-field gel electrophoresis (PFGE) method described by Wang et al., with the following modifications: the DNA samples were digested with 25 U NotI and electrophoresed with pulse times from 2 to 20 s over 18 h at 200 V (13). For data analysis, we imported the images of gels into the PFGE database of *Y. enterocolitica* strains from China and performed a cluster analysis for the serotypes O:3 and O:9. The clustering of band patterns was performed by using BioNumerics software version 5.1 (http://www.applied-maths.com/bionumerics) and the Pearson algorithm. We visually inspected all patterns after computer analysis. For patterns that were indistinguishable by computer and visual inspection, we assigned a pattern designation.
Results

Characteristics of Pathogenic Y. enterocolitica Infection among Children

Prevalence and Demographics
We recruited a total of 7,304 patients ≤5 years of age with diarrhea from 10 regions of China. Fecal samples and answered questionnaires were collected for each patient, but 18 were excluded because either sample or questionnaire was lost. In total, we found 43 patients with pathogenic Y. enterocolitica infection. The average prevalence of Y. enterocolitica disease in all 10 regions was 0.59% (43/7,304); prevalence was highest in Anhui Province (2.29%, 3/131). Y. enterocolitica prevalence among young children with diarrhea was generally classified into 3 levels: 0.01%–0.50% (Shandong, Ningxia, and Henan); 0.51%–1.00% (Beijing, Guangxi, Tianjin, and Jiangsu); and 1.01%–2.29% (Anhui, Yunnan, and Sichuan) (Figure 1). Through year-round collection, we found that cases of pathogenic Y. enterocolitica infection occurred during January–November. The prevalence calculated for southern China (0.80%) was slightly higher than that for northern China (0.53%), when the northern and southern regions were defined by the Huaihe River, the natural border. Cases occurred more often in boys than in girls (1.63:1) (Figure 2). We found the largest proportion of Y. enterocolitica infections among children >0.5–2 years of age; among children in this age group, more cases also occurred in boys than in girls (1.45:1).

Fecal Characteristics
Fecal samples from children ≤5 years of age infected with pathogenic Y. enterocolitica had the following characteristics: mucous (37%), watery (30%), pasty (22%), and loose (4%) (Figure 3, panel A). Fecal microscopy was performed with fecal samples from all children; leukocytes were detected in samples from 85% (23/27) of children ≤5 years of age with diarrhea. A higher proportion of the fecal samples from those in the >0.5–2 years age group had leukocyte counts ≥15 cells/high-power field (HPF). Fecal leukocyte counts were >30 cells/HPF only among patients in this age group, and in 2 cases the concentration reached as high as 45 cells/HPF and 84 cells/HPF.

Bioserotypes of Isolates from Patients with Acute Diarrhea and Prolonged Shedding
The predominant cause of acute Yersinia infection among children ≤5 years of age was bioserotype 3/O:3 (Table); 41 of 43 patients were infected with this bioserotype. The other 2 patients were infected with 4/O:3 or 2/O:9, both found in Beijing. Except for one 3/O:3 infection, all isolates harbored the Yersinia virulence plasmid and virulence genes ail, ystA, virF, and yadA. In addition to the acute diarrhea cases, 3 cases from different regions involved prolonged Y. enterocolitica 3/O:3 shedding that had progressed from acute diarrhea. These patients were 1–1.5 years of age. Once pathogen shedding stopped, the diarrhea ceased as well. The period of shedding could be as long as ≈3 months.

Difference in Y. enterocolitica Prevalence between Children and Adults
A total of 2,127 children and 1,904 adults with diarrhea were enrolled at the Beijing sites. Pathogenic Y. enterocolitica infection accounted for 0.61% (13/2,127) of the children and 0.11% (2/1,904) of the adults tested. One child and 1 adult had 2/O:9 Y. enterocolitica infections; the other 13 patients had 3/O:3 infections. Leukocytes were detected in the fecal samples of all 13 children and 1 of the 2 adults.

Figure 1. Prevalence of pathogenic Yersinia enterocolitica infection among children ≤5 years of age with diarrhea, by region, China, 2010–2015. Inset shows the islands of China in the South China Sea. AH, Anhui; BJ, Beijing; GX, Guangxi; HA, Henan; JS, Jiangsu; NX, Ningxia; SC, Sichuan; SD, Shandong; TJ, Tianjin; YN, Yunnan.
The overall prevalence of *Y. enterocolitica* biotype 1A was 0.28% among children (6/2,127) and 1.52% among adults (29/1,904) (Figure 4). Among the 35 patients with biotype 1A infections, we found leukocytes in the fecal samples of 33% (2/6) of children and 31% (9/29) of adults. Regardless of whether the samples had leukocytes or not, all isolates (6/6) from children and most isolates (20/29) from adults carried the ystB gene.

**Discussion**

*Y. enterocolitica* is a zoonotic pathogen widely distributed throughout China. However, yersiniosis, predominantly a diarrheal illness, is not notifiable through the national surveillance systems of China. Our large-scale investigation of *Y. enterocolitica* infection among children ≤5 years of age with diarrhea in China found *Y. enterocolitica* bioserotype 3/O:3 is a major pathogen (prevalence 0.56%; 41/7,304). According to reports in various years from Finland, Canada, Chile, Holland,
Italy, New Zealand, and the United States, the prevalence of *Y. enterocolitica* among patients with diarrhea was ≈0.6%–2.9% (24–28).

Most hospitals in China do not routinely test for *Y. enterocolitica*; diagnosis of diarrhea is mainly based on signs, symptoms, and fecal microscopy results. We found that a predominant characteristic of feces from young children with *Y. enterocolitica* infection was the presence of leukocytes (Figure 4), which were detectable despite the consistency of the fecal samples (Figure 3, panel A). However, the presence of fecal leukocytes is often regarded as a diagnostic feature of bacillary dysentery, a term that is used interchangeably with shigellosis, and consequently diagnosed as such, leading to confusion over which pathogen is the causative agent (*Shigella*, *Salmonella*, enteroinvasive *Escherichia coli*, *Campylobacter*, or *Yersinia*) (29). A decade (2004–2013) of surveillance in Beijing indicates that bacillary dysentery consistently ranked as the infectious disease of the highest incidence, except for a second place ranking in 2013, in which bacillary dysentery was 3–6-fold the national average incidence (29). The primary reason for the overdiagnosis of shigellosis has been the lack of microbiological diagnostic testing. In this study, according to the primary diagnoses listed on the questionnaires, quite a few cases among children were regarded as shigellosis. Conversely, diarrhea cases without fecal leukocytes tended not to be diagnosed as infectious diarrhea, which delayed administration of the correct and best treatments.

A limitation of our study was that fecal microscopy could not be conducted in some village clinics. Whether these children without fecal microscopy results were overlooked requires further investigation.

In countries where clinical signs guide diagnosis, a case of diarrhea with persistent abdominal pain and fever would prompt culture for *Y. enterocolitica* and cold enrichment (30). Using microbiological diagnostic techniques, we found that the prevalence of pathogenic *Y. enterocolitica* among children ≤5 years of age with diarrhea was ≈6-fold higher than that among adults with diarrhea, and the prevalence of infection with biotype 1A was the reverse (≈6-fold higher among adults than among children ≤5 years of age with diarrhea). Besides other possible explanations, such as incidental infection or acquired immunity, misuse of antimicrobial drugs by adults might play a substantial role in limiting infection with pathogenic strains among adults in China; isolation of pathogenic strains from adult patients is generally difficult. However, a typical family in China would not readily administer antimicrobial drugs to young children. In this study, primary hospitals given the opportunity to be sentinel sites for *Y. enterocolitica* isolation were instructed to avoid giving patients antimicrobial drugs before enrollment as much as possible.

Biotype 1A is a *Y. enterocolitica* strain widely distributed throughout the natural environment that serves as a source of infection and food contamination (32). The diets of adults are not as restricted as that of children, which potentially explains why a higher percentage of adults have diarrhea attributable to biotype 1A. Biotype 1A isolates have generally been regarded as avirulent, but some isolates harboring genes such as *ystB*, which encodes a heat-stable enterotoxin, have been implicated in foodborne and nosocomial outbreaks (6–8). In this study, *ystB* was present in most biotype 1A isolates found from adults, suggesting possible pathogenicity of these isolates as well.

This study had another limitation. The diagnostics protocol included a cold enrichment step, which made identifying nonpathogenic strains and inapparent infections more likely and diagnosis more time-consuming (33). Consequently, early treatment decisions could not be guided by our diagnostic test results. However, cold enrichment did improve overall recovery of *Y. enterocolitica*, especially when the bacteria density of the fecal

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**Table.** Bioserotype and virulence genes of pathogenic *Yersinia enterocolitica* isolates from children ≤5 years of age with diarrhea, China, 2010–2015

| Bioserotype | No. cases | ail | ystA | ystB | yadA | virF |
|-------------|-----------|-----|------|------|------|------|
| 3/O:3       | 40        | +   | +    | –    | +    | +    |
| 4/O:3       | 1         | +   | –    | –    | –    | –    |
| 2/O:9       | 1         | +   | +    | –    | +    | –    |

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![Figure 4](image-url)
sample was low, such as during the convalescent phase or long-term shedding. Diarrhea is often considered to be mild and self-limiting in patients with \textit{Y. enterocolitica} infection (5), but we found 3 cases of long-term bacterial shedding of \textit{Y. enterocolitica} 3/O:3 among children. Low acquired immunity among children might be a possible explanation, and a timely and accurate diagnosis is greatly needed to prevent these types of cases from occurring. Although cold enrichment has its limitations, we included it in the protocol to more accurately and completely diagnose \textit{Y. enterocolitica} infection in the study population. This method has been used in multiple surveillance studies around the world (12,13,34–36).

Generally, only a subset of bioserotypes are pathogenic, mainly 1B/O:8; 4/O:3; 2/O:5; 27; 2/O:9; and 3/O:3 (mostly found in China). In recent decades in most countries and regions, the pathogenic bioserotypes of highest prevalence and incidence shifted from strain 1B/O:8 to 4/O:3. In China, the shift was from 2/O:9 to 3/O:3; as of July 2017, the 1B/O:8 strain has not been detected yet in China. Strain 4/O:3, having limited PFGE pattern diversity and high similarity with reference strains abroad (data not shown), has rarely been isolated in China. Only a single 4/O:3 isolate was found in this study, even though this strain is the predominant bioserotype found in other parts of the world. Whether this strain was acquired domestically or from travelers to China is not known. According to our previous research (37), the susceptibilities of strains 3/O:3 and 4/O:3 to 0:3-specific phage are similar; thus, O:3-specific phage susceptibility cannot explain the rarity of 4/O:3 in China, but susceptibility to 4/O:3-specific phage might.

When comparing pathogenic isolates from different sources, isolates from children shared PFGE patterns with isolates from local pigs and dogs, suggesting a link between isolates from animals and human infection. Pigs have been shown to be a source of \textit{Y. enterocolitica} infection (20,38–41). In correlation studies in Belgium and Norway, human infections have been associated with ingestion of raw or undercooked pork (38,39). In Germany, the state with the highest consumption of meat showed the highest incidence of yersiniosis (20). The prevalence of pathogenic \textit{Y. enterocolitica} was even higher in China than in Europe, potentially because the population of China is a big consumer of pork (15). However, persons in China seldom eat undercooked pork; a more likely route of transmission is cross-contamination (12). Lee et al. described cases in which \textit{Yersinia} seemed to have been transferred from raw tripe to infants on the unwashed hands of caregivers (42). Whether transmission is aided by transportation of pork products between regions needs further investigation. Pigs from multiple regions are slaughtered in Beijing, the location where we found the highest number of isolates from children with PFGE patterns indistinguishable from isolates from pigs. Researchers in Japan reported isolation of \textit{Y. enterocolitica} of different bioserotypes from imported meat products (i.e., pork, beef, and chicken) from Europe, the United States, and other regions of Asia (43). PFGE patterns of some isolates from children in our study were not indistinguishable from those from animals, perhaps because our surveillance of isolates from animals is not complete.

The results of this nationwide investigation in China emphasize that \textit{Y. enterocolitica} bioserotype 3/O:3 is a prominent pathogen of children ≤5 years of age with diarrhea and that microbiological diagnostic testing should be considered for patients who have leukocytes in their feces. Children might acquire infection from contaminated food, and to establish an epidemiologic link between the illness and the consumption of or contact with pork, a case–control study comparing exposures of ill and healthy children is needed. Geographic or seasonal differences in prevalence should also be examined in the future. Our team will continue its surveillance of \textit{Y. enterocolitica} infection among children with diarrhea. We suggest that hospitals routinely test for \textit{Y. enterocolitica} and report laboratory-confirmed cases to public health authorities.

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Technical Appendix

Technical Appendix Figure 1. Cluster analysis of pulsed-field gel electrophoresis patterns of 3/O:3 *Yersinia enterocolitica* isolates obtained from children ≤5 years of age, pigs, and dogs by region, China,
2010–2015. Gels depict DNA digests with \textit{Not}I. Clustering trees indicate relatedness of strains. The proportion of each pattern detected per region for children, dogs, and pigs are indicated.

\textbf{Technical Appendix Figure 2.} Cluster analysis of pulsed-field gel electrophoresis patterns show that 3/O:3 \textit{Yersinia enterocolitica} isolates from 6 children ≤5 years of age from Guangxi and Beijing share patterns indistinguishable from isolates found in pigs from other regions. Gels depict DNA digests with \textit{Not}I, and clustering trees indicate the relatedness of the isolates shown.