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Etiology and epidemiology of diarrhea in children in Hanoi, Vietnam

Trung Vu Nguyen [a,c], Phung Le Van [a], Chinh Le Huy [a], Khanh Nguyen Gia [b], Andrej Weintraub [c,*]

[a] *Department of Medical Microbiology, Hanoi Medical University, Hanoi, Vietnam*
[b] *Department of Paediatrics, Hanoi Medical University, Hanoi, Vietnam*
[c] *Department of Laboratory Medicine, Division of Clinical Bacteriology, F-82, Karolinska Institutet, Karolinska University Hospital, Huddinge, S-141 86 Stockholm, Sweden*

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Summary
Objectives: This paper provides a preliminary picture of diarrhea with regards to etiology, clinical symptoms, and some related epidemiologic factors in children less than five years of age living in Hanoi, Vietnam.
Methods: The study population included 587 children with diarrhea and 249 age-matched healthy controls. The identification of pathogens was carried out by the conventional methods in combination with ELISA, immunoseparation, and PCR. The antibiotic susceptibility was determined by MIC following the NCCLS recommendations.
Results: Of those with diarrhea, 40.9% were less than one year old and 71.0% were less than two years old. A potential pathogen was identified in 67.3% of children with diarrhea. They were group A rotavirus, diarrheagenic *Escherichia coli*, *Shigella spp*, and enterotoxigenic *Bacteroides fragilis*, with prevalences of 46.7%, 22.5%, 4.7%, and 7.3%, respectively. No *Salmonella spp* or *Vibrio cholerae* were isolated. Rotavirus and diarrheagenic *E. coli* were predominant in children less than two years of age, while *Shigella spp*, and enterotoxigenic *B. fragilis* were mostly seen in the older children. Diarrheagenic *E. coli* and *Shigella spp* showed high prevalence of resistance to ampicillin, chloramphenicol, and to trimethoprim/sulfamethoxazole. Children attending the hospitals had fever (43.6%), vomiting (53.8%), and dehydration (82.6%). Watery stool was predominant with a prevalence of 66.4%, followed by mucous stool (21.0%). The mean episodes of stools per day was seven, ranging from two to 23 episodes. Before attending hospitals, 162/587 (27.6%) children had been given antibiotics. Overall, more children got diarrhea in (i) poor families; (ii) families where piped water and a latrine were lacking; (iii) families where mothers washed their hands less often before feeding the children; (iv) families where mothers had a low level of education; (v) families where information on health and sanitation less often reached their households.

* Corresponding author. Tel.: +46 8 585 87831; fax: +46 8 711 3918.
E-mail address: andrej.weintraub@ki.se (A. Weintraub).

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Conclusions: Group A rotavirus, diarrheagenic *Escherichia coli*, *Shigella spp*, and enterotoxigenic *Bacteroides fragilis* play an important role in causing diarrhea in children in Hanoi, Vietnam. Epidemiological factors such as lack of fresh water supply, unhygienic septic tank, low family income, lack of health information, and low educational level of parents could contribute to the morbidity of diarrhea in children.

Introduction

Infectious diarrhea is a leading cause of morbidity and mortality worldwide, affecting mainly infants.[1] Approximately 12 million children in developing countries die before the age of five years, and 70% of these deaths are due to five health problems, including diarrhea.[2] Unhygienic and unsafe environments place children at risk of death.[3,4] Ingestion of contaminated water, inadequate availability of water for hygiene, and lack of access to sanitation contribute to about 1.5 million child deaths and around 88% of deaths from diarrhea per year.[3,4] In addition, there are international studies where it has been reported that a higher prevalence of diarrhea, with higher episodes of child diarrhea, are related to a low socio-economic status of the household and community, as well as to a low educational level of the child's parents.[5–8]

Diarrhea is mainly caused by enteric pathogens including viruses, bacteria, and parasites. Rotavirus and diarrheagenic *Escherichia coli* (DEC) are considered to be the most common of the many recognized enteropathogenic organisms, the former on a global scale,[9] with DEC being particularly important in developing countries.[10] Rotavirus, especially group A rotavirus, is the leading cause of infantile gastroenteritis worldwide and is responsible for approximately 20% of diarrhea-associated deaths in children under five years of age.[11]

There are six main categories of DEC identified. These are: (i) enteroaggregative *E. coli* (EAEC); (ii) enteroinvasive *E. coli* (EIEC); (iii) enterohemorrhagic *E. coli* (EHEC); (iv) enteropathogenic *E. coli* (EPEC); (v) diffusely adherent *E. coli* (DAEC); and (vi) enterotoxigenic *E. coli* (ETEC). It has been shown that there are important regional differences in the prevalence of the different categories of DEC.[12–15]

Besides group A rotavirus and DEC, the expanding list of potential enteropathogens includes *Salmonella spp*, *Shigella spp*, *Vibrio cholerae*, enterotoxigenic *Bacteroides fragilis* (ETBF), *Campylobacter spp* and *Cryptosporidium spp*. Advances in diagnostic techniques have increased our ability to detect these pathogens. The present study was undertaken with the aim of assessing the role of the enteric pathogens in relation to clinical symptoms and epidemiological factors.

Study subjects

A total of 836 children from 0 to 60 months of age including 587 children with diarrhea attending the examination rooms of three different hospitals and 249 age-matched healthy controls were studied. The healthy children were enrolled from a daycare center and a healthcare center in Hanoi, Vietnam. They had not had any diarrheal episode for at least one month before the collection of fecal samples.

The children were enrolled in the study during a one-year period starting in March 2001 and ending in April 2002. Diarrhea was characterized by the occurrence of three or more loose, liquid, or watery stools or at least one bloody loose stool within a 24-h period. An episode was considered resolved on the last day of diarrhea followed by at least three diarrhea-free days. An episode was considered persistent if it continued for 14 or more days.[16] Vomiting was defined as the forceful expulsion of gastric contents occurring at least once in a 24-h period. Fever was defined as an under-arm temperature of $>37.2\,^{\circ}C$. Thresholds of 37.2–39 °C and $>39\,^{\circ}C$ were set for moderate and high fever, respectively. Dehydration level was assessed following the recommendations of the WHO Program for Control of Diarrheal Diseases and these assessments were carried out by the pediatricians.[17]

After informed consent was obtained, a pediatrician specifically assigned to the study examined each patient and filled out the demographic data and information on clinical symptoms and illness onset on a standardized questionnaire. The healthcare workers also obtained similar information from the controls.

Some other factors related to the demography and socioeconomic status of the children's parents were also obtained. Education of the parents was assessed as being at either a higher or lower level based on whether they were educated (persons finishing at least college or university) or workers, farmers, and laborers (persons educated up to high school). The living standard of the child's family was evaluated by monthly income of the whole family in Vietnamese Dong (VND). Five levels (very poor, poor, middle, fair, and rich) were ranked according to the Survey of the Center of Scientific Research for the Family and Woman carried out in Vietnam in 2001. Water sources were divided into hygienic (piped water) and unhygienic (pool or well, or rainy water) resources. A latrine was considered to be a hygienic convenience. The availability of information on health and sanitation from any source was assessed according to whether the child's family had access to this kind of information often (daily and weekly) or less often (monthly, rarely, or almost never).

Materials and methods

Sample collection

Fecal samples (one from each subject) from children without diarrhea were collected in a clean container by their parents when the children defecated. From the children with diarrhea, one stool specimen was collected within 24 hours of admission. All feces were collected in special containers with Cary—Blair transport medium, kept at 4 °C and transported to the microbiological laboratory within 24 hours. The residue of each sample after the first culture on media was kept at −70 °C for further work. The collection of samples stopped for two weeks for the TET holidays in Vietnam in February 2002.

Isolation and identification of diarrheal pathogens

The methods for isolation and identification of the diarrheal pathogens: rotavirus A, diarrheagenic *E. coli*, *Shigella spp*, *Salmonella spp*, *Vibrio cholerae* and enterotoxigenic *Bacteroides fragilis*, have been published in detail previously and are briefly described below.[18—20]

For group A rotavirus: stool samples were analyzed for rotavirus A by using the IDEA rotavirus enzyme-linked immunosorbent assay kit (DAKO Ltd, Ely, UK), according to the instructions of the manufacturer. This test is a qualitative enzyme immunoassay for the detection of rotavirus (group A) in human fecal samples.

For diarrheagenic *E. coli*, *Shigella spp*, *Salmonella spp*, *Vibrio cholerae*: stool samples were cultured on the surface of (i) sorbitol MacConkey agar (Labora, Stockholm, Sweden) for the selection of *Escherichia coli* isolates; (ii) thiosulfate citrate bile salt cholera medium (Labora) for the selection of *Vibrio* species; and (iii) deoxycholate citrate agar (Sigma-Aldrich, Stockholm, Sweden) for the selection of *Shigella spp* and *Salmonella spp*. The cultures were incubated overnight at 37 °C. All samples were tested for *Vibrio spp*, *Shigella spp*, and *Salmonella spp* by using colony morphology, biochemical properties, and agglutination with specific sera. A multiplex PCR using eight primer pairs specific for the virulent genes of five different pathotypes of diarrheagenic *E. coli* was used for the identification of these *E. coli*.

Enterotoxigenic *Bacteroides fragilis* was identified by immunoseparation in combination with PCR. In brief, a fecal sample suspension was inoculated into fastidious anaerobe broth (FAB) medium (LAB 71, LAB M, International Diagnostic Group, Bury, UK) and then incubated at 37 °C for 48 h. After incubation, the broth medium was centrifuged twice. The pellet was suspended and incubated with magnetic beads coated with monoclonal antibody (mAb C3) that binds specifically to a common epitope present in the inner core region of *B. fragilis* lipopolysaccharide (LPS). Bacteria bound to the coated beads were separated by magnetic separator. PCR using primers specific for enterotoxin gene and its subtypes were applied for ETBF identification.

Campylobacter spp, and parasites were not investigated due to the lack of facilities in Hanoi, Vietnam. The methods for antimicrobial susceptibility of the isolated *E. coli* and *Shigella* strains have been published elsewhere.[21]

Statistical analysis

The proportion difference was determined by the Chi-square test. In the case where the expected value for a cell was <5, Fisher's exact test was used. Multiple comparisons of mean values of groups were done by the Kruskal—Wallis H test, and the Mann—Whitney *U* test (for nonparametric data) was used for comparing two groups. A *p* value of <0.05 was considered statistically significant. Data from antibiotic susceptibility testing were analyzed by WHONET 5.1 software.

Ethical committee approval

Both the Ethical Committees at the Karolinska Institutet, Sweden and Hanoi Medical University, Vietnam approved the project.

Results

Etiology and clinical properties of diarrhea

The rates of identification of different enteric pathogens are shown in Table 1.

In 587 children with diarrhea, group A rotavirus was the most frequently identified enteric pathogen with a prevalence of 46.7%, showing a significant difference compared to the controls (3.6%). Within the diarrhea group, the detection prevalence in children less than two years of age was 51.1%, significantly different ($p < 0.001$) from that in the older children (35.9%). Rotavirus infection was most prevalent in children in the 13—24 months group. The second highest number of cases were seen in the 0—12 months and 25—36 months age groups, although cases were also seen in the older children. There was a significantly decreasing trend in rotavirus prevalence with age (Chi-square test for trend, 8.904; $p < 0.005$).

The second most common pathogen in the diarrhea group was DEC. The isolation prevalence was 22.5%. This included 68 samples (11.6%) with EAEC, 12 (2.0%) with EIEC, 39 (6.6%) with EPEC, and 13 (2.2%) with ETEC. DEC accounted for 12% in the controls ($p < 0.001$). The distribution was: 18 (7.2%) with EAEC, 11 (4.4%) with EPEC, and one (0.4%) with ETEC. Of the isolated ETEC in both groups, 7/14 (50%) produced heat-labile toxins (LT) only, 4/14 (28.6%) LT and heat-stable toxins (ST), and 3/14 (21.4%) ST only. All the isolated EPEC were atypical. In the diarrhea group, EAEC and EPEC were more frequently isolated in children less than two years of age (14.1% and 7.9%, respectively), whereas EIEC and ETEC were less frequently found (1.9% and 1%, respectively). No children were colonized with more than two DEC.

Twenty-eight *Shigella* strains (4.7% of the samples) including one *S. boydii*, seven *S. flexneri*, and 20 *S. sonnei* were isolated in the diarrhea group. The isolation prevalence of *S. sonnei* in children less than two years of age and the older ones was 1.4% and 8.2%, respectively. The difference was statistically significant ($p < 0.0001$). *Shigella ssp* were not found in the healthy controls.

Within the group of children with diarrhea, 7.3% ETBF was detected. The corresponding figure for the controls was 2.4% ($p < 0.01$). Within the diarrhea group, the prevalence was significantly higher in children older than one year. Three subtypes of ETBF isolates have been identified with prevalences of 67.4%, 18.6%, and 16% for *bft*-1, *bft*-2, and *bft*-3, respectively. In the controls, two of the subtypes were identified, five *bft*-1 and one *bft*-2.

No EHEC, *Salmonella spp*, or *V. cholerae* were identified. The occurrences of single and mixed infections of enteric

Table 1 Distribution of identified pathogens according to age group in children with diarrhea and healthy controls

Identified pathogens	Age group (months)	Group of children No. (%)[a]		*p* Value
		Diarrhea (*n* = 587)	Control (*n* = 249)	
Group A rotavirus	0—12	111 (46.3)	1 (2.1)	<0.00001
	13—24	102 (57.6)	4 (5.6)	<0.00001
	25—36	42 (44.2)	3 (6.3)	<0.00001
	37—48	12 (29.3)	1 (2.0)	<0.001
	49—60	7 (20.6)	0 (0)	<0.05
Diarrheagenic *E. coli*				
EAEC	0—12	35 (14.6)	2 (4.2)	<0.05
	13—24	24 (13.6)	5 (7.0)	>0.05
	25—36	6 (6.3)	7 (14.6)	>0.05
	37—48	2 (4.9)	3 (6.1)	>0.05
	49—60	1 (2.9)	1 (3.0)	>0.05
EIEC	0—12	2 (0.8)	0 (0)	>0.05
	13—24	6 (3.4)	0 (0)	>0.05
	25—36	2 (2.1)	0 (0)	>0.05
	37—48	0 (0)	0 (0)	See[b]
	49—60	2 (5.9)	0 (0)	>0.05
EPEC	0—12	15 (6.3)	1 (2.1)	>0.05
	13—24	18 (10.2)	5 (7.0)	>0.05
	25—36	3 (3.2)	2 (4.2)	>0.05
	37—48	2 (4.9)	2 (4.1)	>0.05
	49—60	1 (2.9)	1 (3.0)	>0.05
ETEC	0—12	2 (0.8)	0 (0)	>0.05
	13—24	2 (1.1)	1 (1.4)	>0.05
	25—36	4 (4.2)	0 (0)	>0.05
	37—48	3 (7.3)	0 (0)	>0.05
	49—60	2 (5.9)	0 (0)	>0.05
Shigella spp				
S. flexneri	0—12	0 (0)	0 (0)	See[b]
	13—24	3 (1.7)	0 (0)	>0.05
	25—36	1 (1.1)	0 (0)	>0.05
	37—48	3 (7.3)	0 (0)	>0.05
	49—60	0 (0)	0 (0)	See[b]
S. sonnei	0—12	1 (0.4)	0 (0)	>0.05
	13—34	5 (2.8)	0 (0)	>0.05
	25—36	5 (5.3)	0 (0)	>0.05
	37—48	6 (14.6)	0 (0)	<0.01
	49—60	3 (8.8)	0 (0)	>0.05
S. boydii	0—12	1 (0.4)	0 (0)	See[b]
ETBF	0—12	12 (5.0)	0 (0)	>0.05
	13—34	16 (9.0)	3 (4.2)	>0.05
	25—36	5 (5.3)	1 (2.1)	>0.05
	37—48	5 (12.2)	2 (4.1)	>0.05
	49—60	5 (14.7)	0 (0)	=0.053

[a] Percentage calculated according to total number in each age group.
[b] Could not perform statistical test.

pathogens are shown in Tables 2 and 3. Among children with diarrhea, 79 (13.5%) had infections with two or more pathogens, and among control children, two (0.8%) had a mixed infection ($p < 0.00001$). A potential enteric pathogen was identified from 395 children with diarrhea (67.3%) and 43 controls (17.3%) ($p < 0.00001$).

The seasonality of infection was analyzed for rotavirus, DEC, *Shigella spp*, and ETBF. Rotavirus infection occurred year-round but the prevalence trend was higher in September—December, the cooler autumn and winter months. Infections with other pathogens peaked during the summer time when it was warm and rainy (Figure 1).

Table 2 Occurrence of single and mixed infections of enteric pathogens

Patterns of infection	Group of children No. (%)		Total (*n* = 836)
	Diarrhea (*n* = 587)	Control (*n* = 249)	
Group A rotavirus	201 (34.2)	7 (2.8)	208 (24.9)
EAEC	37 (6.3)	17 (6.8)	54 (6.5)
EIEC	10 (1.7)	0 (0)	10 (1.2)
EPEC	19 (3.2)	10 (4.0)	29 (3.5)
ETEC	8 (1.4)	1 (0.4)	9 (1.1)
S. flexneri	5 (0.9)	0 (0)	5 (0.6)
S. sonnei	16 (2.7)	0 (0)	16 (1.9)
S. boydii	1 (0.2)	0 (0)	1 (0.1)
ETBF	19 (3.2)	6 (2.4)	25 (3.0)
Group A rotavirus + EAEC	29 (4.9)	1 (0.4)	30 (3.6)
Group A rotavirus + EPEC	19 (3.2)	1 (0.4)	20 (2.4)
Group A rotavirus + ETEC	5 (0.9)	0 (0)	5 (0.6)
Group A rotavirus + *S. flexneri*	1 (0.2)	0 (0)	1 (0.1)
Group A rotavirus + *S. sonnei*	1 (0.2)	0 (0)	1 (0.1)
Group A rotavirus + ETBF	15 (2.6)	0 (0)	15 (1.8)
ETBF + EIEC	2 (0.3)	0 (0)	2 (0.2)
ETBF + *S. flexneri*	1 (0.2)	0 (0)	1 (0.1)
ETBF + *S. sonnei*	3 (0.5)	0 (0)	3 (0.4)
Group A rotavirus + EAEC + ETBF	2 (0.3)	0 (0)	2 (0.2)
Group A rotavirus + EPEC + ETBF	1 (0.2)	0 (0)	1 (0.1)
No pathogen	192 (32.7)	206 (82.7)	398 (47.6)

Table 3 Infection with single and mixed pathogens in children with diarrhea and controls

No. of infecting pathogens	Group of children No. (%)		Total (*n* = 836)
	Diarrhea (*n* = 587)	Control (*n* = 249)	
No pathogen	192 (32.7)	206 (82.7)	398
One pathogen	316 (53.8)	41 (16.5)	357
Two pathogens	76 (13.0)	2 (0.8)	78
Three pathogens	3 (0.5)	0 (0)	3

The susceptibility of isolated DEC and *Shigella* strains was tested against eight antibiotics. Imipenem (IPM), ciprofloxacin (CIP), nalidixic acid (NAL), cefotaxime (CTX), and cefuroxime (CXM) were active against the *E. coli* pathogens, while high frequencies of resistance to ampicillin (AMP), chloramphenicol (CHL), and trimethoprim/sulfamethoxazole (SXT) were shown, with resistance prevalences of 86.4%, 77.2%, and 88.3%, respectively. Nearly 89% of the *Shigella* strains were resistant to trimethoprim/sulfamethoxazole, 75% were resistant to ampicillin, and 53.6% were resistant to chloramphenicol. More than 85% of the strains were susceptible to cefuroxime, cefotaxime, nalidixic acid, ciprofloxacin, and imipenem. Multi-antibiotic resistance was detected in 145/162 (89.5%) of the diarrheagenic *E. coli* and 22/28 (78.6%) of the *Shigella* strains.

The most prevalent multiresistance patterns (the resistance to at least two antibiotics) for all *E. coli* and *Shigella* strains were AMP^r CHL^r CXM^s CTX^s NAL^s CIP^s IPM^s SXT^r and AMP^r CHL^s CXM^s CTX^s NAL^s CIP^s IPM^s SXT^r in 89.5% of all *E. coli* and in 35% of *Shigella* strains, respectively.

In the diarrhea group the reasons for visiting the hospital were: (i) diarrhea only; (ii) diarrhea and vomiting; (iii) diarrhea and fever; and (iv) diarrhea together with vomiting and fever. The different categories accounted for 54.5, 19.3, 16.7, and 9.5%, respectively. Overall, when being examined, 43.6% of children with diarrhea were febrile, 53.8% had vomiting, and 82.6% had dehydration. Types of diarrheal

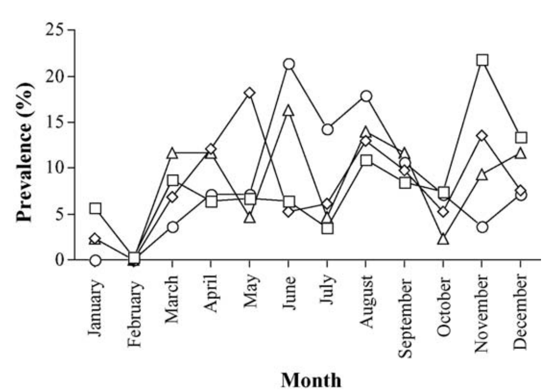

Figure 1 Seasonal prevalence of the identified enteric pathogens in children less than five years of age in Hanoi, Vietnam. Rotavirus (□); *Escherichia coli* (◇); *Shigella spp* (○) and enterotoxigenic *Bacteroides fragilis* (△).

stool were noted. Watery stool was predominant with a prevalence of 66.4% followed by mucous stool (21%). There was one bloody stool (0.2%). Other types of stool accounted for 12.4%. The mean episodes of stools per day was seven, ranging from 2 to 23. Before attending the hospital, 162/587 (27.6%) of children had been given antibiotics and 523/587 (89.1%) of the children received oral rehydration fluid.

Epidemiology of diarrhea

During the period from March 2001 to April 2002, we studied 836 subjects living in Hanoi including 587 children with diarrhea and 249 controls. The male/female ratio was 1.64 for the diarrhea group and 1.18 for the control group showing a significant difference (Table 4). All were less than five years of age. The age distribution of all subjects is shown in Table 5. Of those with diarrhea, 40.9% were less than one year old and 71.0% were less than two years old. Table 4 shows some characteristics of the children in both groups. The children without diarrhea had a current average weight significantly higher than those with diarrhea both in children ≤2 years old and in those >2 years old. Of patients less than six months of age, 22.3% from the diarrhea group were fully breast-fed as compared to 36.4% of the controls. The difference is not statistically significant. However, the corresponding figures in children up to three months of age were 36.8% and 71.4%, respectively ($p = 0.026$).

Table 4 Epidemiological factors related to the risk for diarrhea

Characteristic	Group of children		*p* Value
	Diarrhea (*n* = 587)	Control (*n* = 249)	
Child characteristics			
Gender male/female:	365/222	135/114	<0.05
Weight			
Mean weight at birth (kg)			
Children ≤2 years old	3.06	3.16	<0.05
Children >2 years old	3.10	3.16	>0.05
Mean of current weight (kg)			
Children ≤2 years old	8.50	9.61	<0.01
Children >2 years old	13.66	14.92	<0.01
Only breast-fed (children ≤6 months)	23 (22.3%)[a]	12 (36.4%)[a]	>0.05
Clinical symptoms (diarrhea only)			
Fever	256 (43.6%)		
Vomiting	316 (53.8%)		
Dehydration	485 (82.6%)		
Kinds of stool			
Watery	390 (66.4%)		
Bloody	1 (0.2%)		
Mucous	123 (21.0%)		
Other	73 (12.4%)		
Stools per day[b] (mean)	7		
Mother characteristics			
Age <25	102 (17.4%)	14 (5.6%)	<0.0001
High education level	206 (35.1%)	128 (51.4%)	<0.0001
Hygiene conditions			
Hand washing[c]	250 (42.6%)	211 (84.7%)	<0.001
Water resource (piped water)	408 (69.5%)	231 (92.8%)	<0.0001
Convenience (hygienic latrine)	469 (79.9%)	247 (99.2%)	<0.0001
Living standard			
Very poor	18 (3.1%)	1 (0.4%)	Chi-square
Poor	152 (25.9%)	58 (23.3%)	for
Middle	204 (34.7%)	90 (36.1%)	trend
Fair	121 (20.6%)	53 (21.3%)	=3.403
Rich	92 (15.7%)	47 (18.9%)	*p* = 0.065
Information on health and sanitation			
Daily, weekly	112 (19.1%)	65 (26.1%)	<0.05

[a] Percentage in the defined group.
[b] Episodes per day when children were examined.
[c] Mother's hand washing before feeding children.

Table 5 Distribution of children by age group

Age group (months)	No. (%) of children		Total
	Diarrhea	Control	
0–12	240 (40.9)	48 (19.3)	288 (34.4)
13–24	177 (30.1)	71 (28.5)	248 (29.7)
25–36	95 (16.2)	48 (19.3)	143 (17.1)
37–48	41 (7.0)	49 (19.7)	90 (10.8)
49–60	34 (5.8)	33 (13.2)	67 (8.0)

Table 4 shows data concerning other epidemiological factors related to demography and the socio-economic situation of the children's families. A significantly higher prevalence of mothers aged under 25 was seen in children with diarrhea compared to the controls. With regard to living standards, there was a decreasing trend in children with diarrhea with an increasing level of family income ($p = 0.065$). Overall, the prevalence of children with diarrhea was significantly higher in families where hygienic water and latrines were lacking, where mothers less often washed their hands before feeding the children, where mothers had a low level of education, and where information on health and sanitation reached the family less often.

Discussion

Etiology and clinical properties of diarrhea

Diarrhea in developing countries is caused by an increasingly long list of viral, bacterial, and parasitic pathogens with rotavirus, diarrheagenic *E. coli*, *Shigella spp*, *Salmonella spp*, and *V. cholerae* heading the list.[22] In the present study, no *Salmonella* or *V. cholerae* strains were found. The reason for this could be the low prevalence of these pathogens as shown in previous studies in Vietnam.[23,24] However, in other investigations in developing countries,[16,25–27] these agents have been identified more often in fecal samples from children with diarrhea. The other pathogens: group A rotavirus, DEC, *Shigella spp*, and ETBF were identified with prevalences of 46.7, 22.5, 4.8, and 7.3%, respectively. A recognized pathogen was identified in 67.3% patients. This is a rather high prevalence as compared to other studies, even in adult patients,[12,16,28,29] but still lower than the data of Albert et al.[26] with a potential enteric pathogen in 74.8%.

Rotavirus is the most important cause of diarrhea in children in developing countries, causing around one million deaths per year.[30] Several studies in Vietnam have shown that group A rotavirus plays an important role in diarrheal disease in children.[31–34] In this study we have confirmed the high prevalence of rotavirus infection in Vietnamese children with diarrhea, showing a significant difference compared to controls. Moreover, information concerning clinical properties and co-infections of rotavirus has been investigated and this has contributed to the knowledge of diarrhea caused by rotavirus in children in Vietnam. A limitation with respect to rotavirus was that we could not identify the serotypes of this pathogen.

Diarrheagenic *E. coli* are recognized as an emerging etiology causing diarrhea in children, especially in developing countries. The epidemiological significance of each *E. coli* category in childhood diarrhea varies with geographical area.

ETEC causes a significant number of cases of childhood diarrhea and gastroenteritis among travelers. In our study, we did not see any strong association of ETEC with diarrhea as compared to controls ($p = 0.076$). However, the prevalence of ETEC was significantly higher in children over two years of age as compared to those less than two years of age in the diarrhea group (Figure 2) ($p < 0.005$). LT^+ ETEC, ST^+ ETEC, and LT^+ ST^+ ETEC strains were isolated with a lower prevalence, 2.2% in patients, as compared to the study by Wolk et al.[35] but the same prevalence as in the study by Nishi et al.[36] As described in previous studies, the prevalence of ETEC is different in different geographical areas. According to Mayatepek et al., the figures in the diarrhea group and in the control group were 28% and 16%, respectively;[37] Wolk et al. showed the prevalence of ETEC to be 20.7% in patients.[35]

EHEC is an important food-borne pathogen, especially in developed countries. Clinical manifestations of EHEC infection range from asymptomatic carriage to diarrhea to hemorrhagic colitis. Hemolytic uremic syndrome (HUS) is a common complication in children.[38] No EHEC was isolated in this study. This is not uncommon and similar results have been obtained by others.[27,39,40]

EPEC continues to be an important cause of diarrhea in children up to two years of age.[41] Our study showed a slightly higher prevalence of EPEC in patients compared to controls. It seems to be associated with children under 2 years of age ($p = 0.053$) as compared to the older ones. All of the isolated EPEC were atypical strains having only the *eae*A gene but no *bfp*A, which is the structural gene encoding BFP (bundle-forming pilus). Since *bfp*A is encoded on the EAF plasmid[42] it could be lost during culture and isolation, resulting in the failure to identify them in our study.

In other studies, children colonized with EPEC were rarely infected with other enteric pathogens.[10,43–45] In this study, 20 of the EPEC-positive children (19 from the patients and one from the controls) were also positive for group A

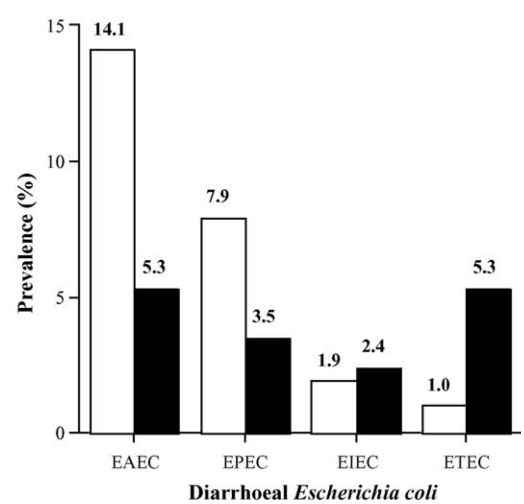

Figure 2 Comparison of the prevalence of diarrheagenic *Escherichia coli* in the diarrhea group in children younger (□) and older (■) than two years of age. Significant differences are seen in EAEC ($p < 0.005$) and ETEC ($p < 0.005$).

rotavirus, and one harbored group A rotavirus, EPEC, and ETBF, simultaneously (Table 2). The co-infection of EPEC with other pathogens has also been described in another study.[26]

Regarding EIEC infection, several previous studies[27,46,47] have found no or very few EIEC from fecal samples in children with diarrhea. Only 2% of EIEC were isolated from patients in our study. It has been shown that in some developing countries, EIEC strains can be responsible for 1 to 7% of cases of diarrhea or dysentery.[12,48–50] EIEC and *Shigella spp* are both responsible for dysentery diarrhea. Although *Shigella spp* were isolated with a higher prevalence, it was still lower than that of other studies.[26,49,51]

EAEC was the most predominant bacterial pathogen in this study for both children with and without diarrhea. It was strongly associated with diarrhea in children less than two years of age. Many surveillance studies have demonstrated the importance of EAEC in pediatric diarrhea.[15,42,52,53] However, there have been conflicting reports on the association of EAEC with acute and persistent diarrhea.[15,25,27,54,55] EAEC was not associated with persistent diarrhea in this study. It could be due to the largely unknown or different epidemiological characteristics of EAEC (e.g., likely sources, reservoirs of infection, routes of transmission, seasonality, and age-distribution).

Very few studies on DEC have been carried out in Vietnam.[23,24] The findings concerning this emerging pathogen will be helpful for pediatricians and microbiologists in diagnosis and treatment of children with diarrhea.

ETBF has been studied intensively for the last 20 years since it was recognized as an important anaerobic bacterial pathogen causing diarrhea in small children.[56–58] This is the first study of the role of ETBF in children's diarrhea in Vietnam and it is concluded that this pathogen is an important causative agent of diarrhea in children in Hanoi, Vietnam.

There were no striking differences regarding vomiting, dehydration, and episode/day diarrhea in children infected with different categories of DEC and Shigella. Children infected with Shigella had fever with a temperature average significantly higher than that of those infected with rotavirus, DEC, and ETBF. In addition, the average age of these children was also significantly higher. Nevertheless, vomiting and watery diarrhea were strongly associated with rotavirus infection. Children infected with rotavirus alone had a significantly higher prevalence of vomiting and watery diarrhea as compared to those infected with other pathogens ($p < 0.05$ and $p < 0.05$, respectively). These can be useful symptoms in clinical diagnosis and examination of rotavirus diarrhea as mentioned in previous studies.[59–62]

The peak of rotavirus infection occurred during the winter months, and the bacterial infections were often predominant during the summer or warm months. These trends agree with the findings of other studies.[10,63–65]

There is an increasing concern for the possible development of resistance to antimicrobial agents in the *Enterobacteriaceae*, especially in DEC when this pathogen has been emerging, and the resistance is a result of inappropriate use of antibiotics in hospitals and communities.[66–68] Our data show that ampicillin, chloramphenicol and trimethoprim/sulfamethoxazole should not be considered as appropriate for empirical therapy of children with diarrhea in Vietnam. Moreover, the use of quinolones (nalidixic acid and ciprofloxacin) in the treatment of diarrhea caused by DEC and *Shigella spp* should also be carefully considered since many strains have developed resistance to these agents as shown in previous studies.[69–72] To our knowledge, there have not been any published studies on antibiotic resistance of all five different categories of DEC in Vietnam, so far.

Epidemiology of diarrhea

The epidemiology of enteric pathogens that cause diarrhea suggests that most infections are acquired from food, water, and hand contact and many diarrheal diseases can be prevented by simple rules of personal hygiene and safer food preparation.[73,74] Among the contributors to diarrhea in children, the household health environment, living standards, and mothers' knowledge play important roles. Women play a major role in making or breaking the 'chain of contamination' within the household sphere.[75] Their knowledge of caregiving for their children with diarrhea is very important. In the present study, 89.1% of children with diarrhea had been given oral rehydration fluid before hospitalization. Seventy percent of them had received oral rehydration solutions (ORS). In an investigation in Vietnam,[76] 60% of mothers knew about ORS and 40% of children with diarrhea were given ORS. However, the awareness is different in different age groups, the lowest level of awareness being in the under 25s. Education level also plays an important role in the knowledge of mothers. It has been shown that the more education they have, the more they know about the way to take care of children with diarrhea, and the lower prevalence of diarrhea their children may have. This study, along with others, has shown that diarrhea is more common among children whose mothers are in the younger age group, and have a low level of education.[8,77,78]

Diarrheal diseases are water-, hygiene-, food-, and sanitation-related and have multiple oral–fecal transmission routes.[79–81] In this study, significantly higher prevalences of families who had piped water, a hygienic latrine, and where the mothers more often washed their hands before feeding their children were seen in the group of healthy children as compared to the group of children with diarrhea.

Hand washing can interrupt some transmission routes of enteric pathogens to the host. There are a number of epidemiological studies on hand washing which claim substantial reduction in diarrheal morbidity.[82–85] However, water availability is likely to have an impact on the frequency of hand washing.

It is not easy for every family to get a hygienic water supply for domestic use. It depends on the geographical area, supply infrastructure, and the supply capacity of the water plant. In Hanoi, Vietnam, people in some areas still do not have access to sufficient piped water. During the summer, when water consumption rises dramatically, some families face a shortage of hygienic water. They may have to find additional sources or try to store water in different ways. These facilitate microbial contamination resulting in diarrhea, especially in children. This could partially explain the high prevalence of diarrhea in children during the summer months.

As mentioned above, there is an association between stool disposal and child diarrhea. We have seen that diarrhea could be prevented in about 20% of the children by having access to hygienic latrines in the households. In fact, not all the households in Hanoi have their own hygienic latrines. In some cases they have to share with others. Sometimes these types of conveniences are not hygienic. People in some areas use an indigenous composting latrine or defecation pit. The problem of safe stool disposal has also been investigated in many other studies.[86–89] Safe stool disposal is one of the key barriers to the transmission of enteric pathogens.

Diarrhea prevention is an important public health issue and it is necessary to disseminate information and teach skills, and appropriate activities to the public. However, the availability of this type of information and delivery to the households, plays an important role in the anti-diarrhea campaign. In our study, to the question: "How often does your family receive information about health and sanitation?", 26.1% of households in the healthy group claimed that they received the information daily or weekly, compared to 19.1% in the diarrhea group, showing a significant difference. This shows that, although many diarrheal diseases can be prevented by following the simple rules of personal hygiene and safe food preparation, public healthcare workers should pay more attention to effectively inform the population about health and sanitation through mass media to the households.

In conclusion, many aspects of diarrhea need to be further investigated in order to decrease the prevalence of diarrhea in Vietnamese children under five years of age.

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