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Original Article

Etiology of diarrhea among children under five years in Thai Binh, Vietnam: a prospective study

Xuan Duong Tran[1,2,3 #], Thi Loi Dao[3 #], Van Thuan Hoang[3], Ndiaw Goumballa[1,2], Thi Thom Vu[4], Trong Kiem Tran[4], Hong Ha Pham[5], Duy Cuong Nguyen[3], Thanh Binh Nguyen[3], Philippe Parola[1,2], Pierre Marty[6,7], Philippe Gautret[1,2,3]

[1] *Aix Marseille Univ, AP-HM, SSA, RITMES, Marseille, France*
[2] *IHU-Méditerranée Infection, Marseille, France*
[3] *Thai Binh University of Medicine and Pharmacy, Hung Yen, Vietnam*
[4] *Thai Binh Pediatric Hospital, Hung Yen, Vietnam*
[5] *Department of Health, Hung Yen, Vietnam*
[6] *Université Côte D'Azur, Inserm, C3M, Nice Cedex 3, France*
[7] *Parasitologie-Mycologie, Centre Hospitalier Universitaire L'Archet, Nice Cedex 3, France*

# Authors contributed equally to this work.

Abstract

Introduction: In developing countries like Vietnam, childhood diarrhea remains frequent and is often treated empirically without an etiological diagnosis.

Methodology: Patients aged under five years, hospitalized at a gastroenterology department with acute diarrhea, were recruited at one hospital. Enteric pathogens were tested by real-time PCR.

Results: 451 children with diarrhea were included, 65.2% were male. 56.3% were aged under 12 months. Upon inclusion, 49.7% (224/451) had nausea and vomiting, and 17.5% had bloody diarrhea. 27.1% of children had a fever, and 37.7% and 4.2% had moderate and severe dehydration, respectively. Almost all patients (437/451, 96.9%) received empirical antimicrobial treatment. 76.5% of children were positive for at least one pathogen, with 37.9% positive for two to four pathogens. Adenovirus, norovirus, and enterovirus were the most frequent viruses detected, with a proportion of 35.7%, 25.7%, and 20.6%, respectively, while *Campylobacter jejuni* was the most frequent bacterium detected (14.2%), followed by *Salmonella* spp. and *Escherichia coli* pathotypes. Male gender, patients positive for at least one virus, and rotavirus were associated with an increased risk of severe disease (OR = 1.55, $p = 0.04$, OR = 2.23, $p < 0.001$, and OR = 1.86, $p = 0.03$, respectively).

Conclusions: These findings underscore the complex interplay of viral and bacterial pathogens in pediatric diarrheal illness and highlight the need for targeted interventions focusing on appropriate diagnostic strategies, antimicrobial stewardship, and gender-specific considerations to mitigate the burden of childhood diarrhea in resource-limited settings like Vietnam.

Key words: Diarrhea; children; Vietnam; adenovirus; enterovirus; *C. jejuni*.

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Introduction

Diarrhea, characterized by the passage of loose or watery stools, represents a leading cause of morbidity and mortality among children globally [1]. It is estimated that nearly 1.7 billion cases of childhood diarrhea occur annually, resulting in approximately 500,000 deaths, primarily in resource-constrained settings [2]. In low- and middle-income countries, where healthcare infrastructure, access to clean water, sanitation, and proper healthcare services may be limited, diarrhea poses a particularly severe threat to child health, often exacerbating the cycle of poverty and inequity [3]. While significant progress has been achieved in reducing diarrhea-related mortality, the burden of childhood diarrhea remains substantial, warranting continued research and intervention efforts [4]. Among the most vulnerable populations, children under five years of age bear the brunt of this preventable and treatable condition, which continues to exert a heavy toll on their health and development [1].

Vietnam, a country with a rich cultural heritage and a rapidly evolving economy, is no exception to this global health challenge. Vietnam's socio-economic transformation over the past few decades has been remarkable, leading to substantial improvements in child health outcomes [5]. From a country grappling with communicable diseases and undernutrition, Vietnam has made substantial strides in improving child health indicators, including reductions in under-five mortality, improved vaccination coverage, and

improvements in nutritional status [5,6]. The government's commitment to healthcare expansion and equitable access to services has contributed to these successes. However, the persistence of childhood diarrhea underscores the complexity of health challenges that continue to affect Vietnamese children [7–9]. As Vietnam strives for sustainable development and improved child health, a nuanced understanding of the etiology of diarrhea among these young lives and the factors contributing to childhood diarrhea becomes indispensable [10]. In addition, the overuse of antibiotics in children with diarrhea is also a notable health problem in Vietnam [11], it not only damages the economy but also leads to an increase in the rate of resistant bacteria.

Diarrhea is a major cause of morbidity and mortality among children globally [12]. Despite progress in reducing diarrheal mortality, it remains a significant concern, especially in low- and middle-income countries like Vietnam. Inappropriate use of antibiotics further complicates the situation, leading to antimicrobial resistance [13]. Given the limited microbiological diagnostic facilities in resource-limited settings, this study aimed to determine the etiological agents of acute diarrhea in children under five years at a pediatric hospital in Thai Binh, Vietnam.

Methodology

Study design and criteria for selection

This was a one-year prospective study, conducted at Thai Binh Pediatric Hospital (TBPH) from July 2020 to July 2021. TBPH is a provincial hospital of Thai Binh that receives approximately 2000 monthly hospitalized patients, but the facility for microbiological investigations is limited.

The criteria for inclusion in this study were: patients aged under five years, hospitalized at the gastroenterology department with acute diarrhea (passage of loose or watery or bloody stools at least three times in 24 hours).

Clinical data collection

Children's characteristics (age, gender, chronic disease, exclusive breastfeeding for the first 6 months of life, vaccination status against rotavirus, number of family members, age of mother, occupation of mother), and clinical data were collected using standardized forms.

Routine laboratory investigations were performed in the TBPH, including microbiological testing at the discretion of the receiving clinician, and were based on hospital guidelines. Blood specimens were collected from all patients for white blood cell (WBC) counts and C-reactive protein (CRP) measurement. WBC and hemoglobin levels were classified as decreased, normal, and increased according to the age of patients [14]. In patients with suspected sepsis, blood cultures were performed. Stool cultures were performed according to the doctor's decision when bacterial diarrhea was suspected due to the presence of fever, bloody stools, or severe dehydration. In addition, a rotavirus antigen rapid test (Atlas Medical GmbH, Blankenfelde-Mahlow, Germany) was performed if children presented with abundant diarrhea.

Specimen collection and real-time PCR procedure

Stool samples were taken immediately after the children had a bowel movement and transferred to Sigma-Virocult® medium. Then, samples were transported within 48 hours of collection to the laboratory of TBPH for storage at -80 °C. The samples were transferred to the Institut Hospitalo-Universitaire, Méditerranée Infection, Marseille, France, on dry ice before processing.

Identification methods of enteric pathogens by PCR assay are detailed elsewhere [15]. The Multiplex RNA Virus Master Kit (Roche Diagnosis, France) was used for detecting hepatitis A and E virus, adenovirus, rotavirus, norovirus, astrovirus, and internal controls MS2 phage using one-step simplex real-time quantitative RT-PCR amplifications. Real-time PCR amplifications were performed using the LightCycler® 480 Probes Master kit (Roche Diagnostics, France) according to the manufacturer's recommendations. The mapA gene of *Campylobacter jejuni*, the ipaH gene of *Shigella spp.*/EIEC, the invA gene of *Salmonella spp*., the stx1 and stx2 genes of enterohemorrhagic *E. coli*, the EAF and eae genes of enteropathogenic *E. coli* (EPEC), and the pCVD432 gene of enteroaggregative *E. coli* (EAEC) were amplified with internal DNA extraction controls. The 18S gene was used to detect *Entamoeba histolytica* and *Giardia lamblia*, while the Hsp70 gene was used to test *Cryptosporidium spp*. All quantitative real-time PCR to detect respiratory and gastrointestinal pathogens was performed using a C1000 Touch™ Thermal Cycler (Bio-Rad, Hercules, CA, USA). Negative controls (PCR mix) and positive controls (DNA from a bacterial strain or RNA from a viral strain) were included in each run. A cycle threshold (CT) value ≤ 35 was used to assess positive results of bacteria or virus amplification.

Data analysis

Data were double entered using Microsoft Access

and were then cleaned and exported to STATA software version 17.0 (Copyright 1985–2021 StataCorp 4905 Lakeway Drive College Station, Texas 77845, USA) for analysis. Continuous variables were analyzed and expressed as median and range. Categorical variables were presented as numbers and proportions. To analyze the risk factor for the severity of GIIs, the main outcome measured was the presence of moderate or severe dehydration. The association between the main outcome and independent factors such as age and occupation of mother, age, sex of ill children, chronic comorbidities, exclusive breastfeeding for the first 6 months, vaccination against rotavirus, nutrition status, and pathogens identified by qPCR was firstly evaluated using univariable analysis. A Chi-square test was used to evaluate the difference in proportions. Variables with a $p < 0.2$ in the univariable analysis were imported into the multivariable analysis using logistic regression. The results were presented as odds ratio (OR) and 95% confidence interval (95%CI). A $p < 0.05$ was considered statistically significant.

Ethical approval

The protocol was approved by the Thai Binh University of Medicine and Pharmacy institutional review board (No. 498/HDDD, project "Molecular epidemiology of infectious diseases among children under five years in Thai Binh, Vietnam"). The study was performed according to the good clinical practices recommended by the Declaration of Helsinki and its amendments. All parents or legal guardians of participants provided their written informed consent.

Results

Sociodemographic characteristics of the studied population

Among 451 children with diarrhea, 56.3% (252/451) of children were aged under 12 months, and 294 (65.2%) were male. Four patients (0.9%) had chronic comorbidities. A proportion of 72.5% (327/451) of children were exclusively breastfeeding for the first 6 months of life, and 38.4% (173/451) received the vaccination against rotavirus. The majority of patients (408/451, 90.5%) had a normal status of nutrition (Table 1).

Table 1. Characteristics of the patients.

Characteristic	n	%
Age (months)		
Mean	12.8 ± 9.6	
< 12	252	55.9
12 - 36	183	40.6
37 - 59	16	3.6
Gender		
Male	294	65.2
Female	157	34.8
Chronic comorbidities		
Yes*	4	0.9
No	447	99.1
Exclusive breastfeeding for the first 6 months		
Yes	327	72.5
No	124	27.5
Vaccination against rotavirus		
Yes	173	38.4
No	278	61.6
Malnutrition		
No	408	90.5
Moderate malnutrition	23	5.1
Severe malnutrition	0	0
Overweight	20	4.4
Number of members in family		
Mean	4.5 ± 1.3	
≤ 5	377	83.6
> 5	74	16.4
Age of mother (years)		
≤ 35	317	70.3
> 35	134	29.7
Occupation of mother		
Housewife, farmer, small business	88	18.5
Worker	125	27.7
State officials	59	13.1
Private officer	179	39.7

*Three patients with congenital heart diseases and one patient with G6PD deficiency

Table 2. Clinical features at admission and routine laboratory findings at Thai Binh Pediatric Hospital.

Clinical symptoms and routine laboratory findings	n	%
Fever	122	27.1
Nausea, vomiting	224	49.7
Bloody diarrhea	79	17.5
Dehydration		
No	262	58.1
Moderate	170	37.7
Severe	19	4.2
Acute associated diseases		
Yes*	29	6.4
No	422	93.6
White blood cell count (× 10^9/L)		
Decreased	30	6.6
Normal	359	79.6
Increased	62	13.8
CRP levels (mg/dL)		
< 50	347	76.9
≥ 50	104	23.1
Hemoglobin levels		
Decreased	195	43.2
Normal	253	56.1
Increased	3	0.7
Positive blood culture (N = 11)		
Acinetobacter baumannii	1	9.1
Positive stool culture (N = 13)		
Salmonella spp	1	7.7
Proteus mirabilis	1	7.7
Rotavirus antigen rapid test (N = 297)		
Negative	242	81.5
Positive	55	18.5

13 patients beneficed parasitological examen of stool, but the results were negative; *29 patients with upper respiratory tract infections.

Clinical features at admission and routine laboratory findings at Thai Binh Pediatric Hospital

Upon inclusion, 27.1% (122/451) of children had a fever, 49.7% (224/451) had nausea and/or vomiting, and 17.5% (79/451) had bloody diarrhea. A proportion of 37.7% (170/451) and 4.2% (19/451) had moderate and severe dehydration, respectively (Table 2).

Upon admission, 17.1% (77/451) and 13.8% (62/451) of diarrheal children had increased white blood cell count and elevated CRP levels, respectively. 43.2% (195/451) of children had anemia (Table 2).

Among 11 patients with blood cultures, Acinetobacter baumannii was identified in one patient. Stool cultures detected *Salmonella spp.* and *Proteus mirabilis* in one case each. The rotavirus antigen test was positive in 55 of 297 cases (18.5%).

PCR findings

PCR detected at least one pathogen in 76.5% of cases. Viruses were more frequent than bacteria, with adenovirus (35.7%), norovirus (25.7%), and enterovirus (20.6%) being the most common. Among bacteria, *C. jejuni* (14.2%) was most prevalent (Table 3).

Table 3. Results of real-time PCR performing in stool swabs.

Pathogens	n	%
Pathogens identified among 451 patients		
Adenovirus	161	35.7
Enterovirus	93	20.6
Norovirus	116	25.7
Rotavirus	67	14.9
Hepatitis A virus	0	0.0
Hepatitis E virus	0	0.0
Astrovirus	0	0.0
At least one virus	294	65.2
Campylobacter jejuni	64	14.2
Salmonella spp.	30	6.7
Enteroaggregative *Escherichia coli*	38	8.4
Enterohemorrhagic *Escherichia coli*	1	0.2
Enteropathogenic *Escherichia coli*	13	2.9
Shigella spp. / Enteroinvasive *Escherichia coli*	1	0.2
At least one bacterium	132	29.3
Entamoeba histolytica	0	0.0
Giardia lamblia	0	0.0
Cryptosporidium spp.	1	0.2
At least one pathogen	345	76.5
Pathogens identified among 79 bloody diarrheal patients		
Adenovirus	12	15.2
Enterovirus	13	16.5
Norovirus	11	13.9
Rotavirus	10	12.7
Hepatitis A virus	0	0
Hepatitis E virus	0	0
Astrovirus	0	0
At least one virus	34	43.0
Campylobacter jejuni	12	15.2
Salmonella spp.	12	15.2
Enteroaggregative *Escherichia coli*	8	10.2
Enterohemorrhagic *Escherichia coli*	1	1.3
Enteropathogenic *Escherichia coli*	1	1.3
Shigella spp. / Enteroinvasive *Escherichia coli*	1	1.3
At least one bacterium	31	39.2
Entamoeba histolytica	0	0
Giardia lamblia	0	0
Cryptosporidium spp.	1	1.3
At least one pathogen	52	65.8

Among 55 patients with positive rotavirus antigen rapid test, 30 (54.6%) were positive by PCR test, while 12/242 (5.0%) were positive with rotavirus by PCR, but antigen rapid test was negative (Supplementary Table 1). Notably, vaccination against rotavirus decreased the risk for rotavirus carriage with OR = 0.54, 95% CI = [0.30 – 0.97], $p = 0.04$.

Among 79 bloody diarrheal patients, 31 (39.2%) were positive with at least one bacterium, and 34 (43.0%) were positive with at least one virus (Table 3).

Treatment and outcome of the patients

Almost all patients (437/451, 96.9%) received empirical antimicrobial treatment with cephalosporins, the most frequently used antibiotic (366/451, 81.2%), from 5 to 21 days (median = 5 days). About 20% of children received multiple antibiotics (Table 4). The median duration of hospitalization was five days (2 to 39 days).

A total of 92.0% (415/451) of cases were discharged, while 8.0% (36/451) left against medical advice. No deaths were reported.

Risk factor for severity of disease

Table 5 shows the results of risk factors for moderate and severe dehydration. Age (aOR = 1.02, $p = 0.03$), male gender (aOR = 1.53, $p = 0.04$), and rotavirus infection (aOR = 2.62, $p < 0.001$) were associated with an increased risk of dehydration. Children whose mothers were workers and private officers had a higher likelihood of severe disease (aOR = 2.64, $p = 0.002$ and aOR = 2.15, $p = 0.01$,

Table 4. Treatment and outcome of the patients.

	n	%
Empirical antimicrobial treatment	437	96.9
Betalactam	12	2.7
Cephalosporins	366	81.2
Aminoglycosides	19	4.2
Macrolides	9	2.0
Carbapenems	4	0.9
Glycopeptide antibiotics	1	0.2
Imidazole	101	22.4
Multiple antibiotic	89	19.7
Duration of antibiotic treatment in days, median (range)	5 (2 - 21)	
Length of stay in days, median (range)	5 (2 - 39)	
Length of stay ≥ 7 days	161	35.7
Outcome		
Discharge	415	92.0
Discharge against medical advice*	36	8.0
Death	0	0

*Four patients were transferred to the National Hospital for Pediatrics at the request of the guardian.

Table 5. Risk factors for moderate and severe dehydration.

Variables	n (%)	Univariate analysis		Multivariate analysis	
		OR [95%CI]	*p*	Adjusted OR [95%CI]	*p*
Age (months)	14.0 ± 9.6	1.02 [1.01- 1.04]	0.02	1.02 [1.01 – 1.04]	0.03
Gender					
Female	55 (29.1)	reference	0.03	reference	
Male	134 (70.9)	1.55 [1.04 – 2.32]		1.53 [1.01 – 2.32]	0.04
Exclusive breastfeeding for first 6 months[1]					
No	56 (29.6)	reference	0.39	reference	
Yes	133 (70.4)	0.83 [0.55 – 1.26]		0.78 [0.49 – 1.26]	0.31
Vaccination against rotavirus					
Yes	77 (40.7)	reference	0.38		
No	112 (59.3)	1.19 [0.81 – 1.75]			
Malnutrition					
No	176 (93.1)	reference		reference	
Malnutrition	6 (3.2)	0.46 [0.18 – 1.20]	0.12	0.50 [0.17 – 1.26]	0.13
Overweight	7 (3.7)	0.71 [0.28 – 1.82]	0.48	0.78 [0.30 – 2.06]	0.62
Number of members in family	4.5 ± 1.2	0.97 [0.83 – 1.13]	0.70		
Age of mother					
≤ 35	129 (68.2)	reference	0.42		
> 35	60 (31.8)	1.18 [0.79 – 1.78]			
Occupation of mother					
Housewife, farmer, small business	29 (15.3)	reference		reference	
Worker	59 (31.2)	1.81 [1.03 – 3.20]	0.04	2.64 [1.42 – 4.94]	0.002
State officials	23 (12.2)	1.30 [0.65 – 2.58]	0.45	1.86 [0.89 – 3.89]	0.10
Private officer	78 (41.3)	1.57 [0.92 – 2.68]	0.10	2.15 [1.18 – 3.92]	0.01
Microbiological findings by PCR					
Adenovirus	74 (39.2)	1.29 [0.88 – 1.91]	0.19	1.32 [0.88 – 1.98]	0.18
Enterovirus	41 (21.7)	1.12 [0.71 – 1.77]	0.63		
Norovirus	54 (58.6)	1.29 [0.84 – 1.97]	0.24		
Rotavirus	41 (21.7)	2.51 [1.48 – 4.28]	0.001	2.62 [1.49 – 4.61]	0.001
Campylobacter jejuni	26 (13.8)	0.95 [0.55 – 1.61]	0.82		
Salmonella spp.	10 (5.3)	0.68 [0.31 – 1.48]	0.33		
Enteroaggregative *Escherichia coli*	13 (6.9)	0.70 [0.35 – 1.41]	0.32		

[1]Because exclusive breastfeeding for the first 6 months of life is widely recognized as a protective factor against infectious diseases, including diarrhea, it was included in the logistic regression analysis, despite it was not statistically associated with moderate and severe dehydration in univariate analysis.

respectively) (Table 5).

Table 6 shows the associated factors with bloody diarrhea. In multivariate analysis, adenovirus and norovirus infections were negatively associated with bloody diarrhea with aOR = 0.29, $p < 0.001$, and aOR = 0.47, $p = 0.03$, respectively. However, salmonella infection increased 3 times the risk of bloody diarrhea with aOR = 3.10, $p = 0.006$.

Discussion

Our study provides comprehensive insights into the etiology, clinical manifestations, and treatment outcomes of diarrhea among children under five years in Vietnam. Our findings underscore the high prevalence of diarrheal pathogens among this vulnerable population, with a substantial proportion testing positive for at least one pathogen. The use of real-time PCR testing allowed for the detection of a wide range of pathogens, revealing a complex microbial landscape associated with childhood diarrhea.

Consistent with previous studies, viral pathogens, particularly adenovirus, norovirus, and enterovirus, were prominently identified among the positive cases [16–21]. This highlights the significant burden of viral etiologies in pediatric diarrheal illnesses in Vietnam, aligning with global trends reported in similar settings [20]. Furthermore, patients with rotavirus infection were significantly more at risk for dehydration, underlining the potential severity of this viral infection. Moreover, our study identified a discrepancy between PCR and antigen rapid tests for rotavirus detection. While the antigen rapid test identified 18.5% of cases, PCR detected additional positive cases, including among those testing negative by the rapid test. This suggests that antigen tests may underestimate rotavirus prevalence due to lower sensitivity. Given these limitations, PCR-based methods should be considered, where feasible, to enhance diagnostic accuracy and guide management strategies.

Interestingly, bacterial pathogens, including *C. jejuni, Salmonella spp*., and various pathotypes of *E. coli*, were also prevalent, indicating the coexistence of viral and bacterial etiologies contributing to diarrheal morbidity. The high frequency of *C. jejuni* underscores its importance as a leading bacterial cause of childhood diarrhea in Vietnam, consistent with findings from other regions [22–26].

Our study found no cases of *G. intestinalis*, which contrasts with existing data on its prevalence in Vietnamese children [27,28]. This discrepancy may

Table 6. Risk factors for bloody diarrhea.

Variables	n (%)	Univariate analysis		Multivariate analysis	
		OR [95%CI]	*p*	Adjusted OR [95%CI]	*p*
Age	11.8 ± 9.5	0.98 [0.96 – 1.01]	0.30		
Gender					
Female	27 (34.2)	reference	0.90		
Male	52 (65.8)	1.03 [0.62 – 1.72]			
Exclusive breastfeeding for the first 6 months					
No	19 (24.0)	reference	0.45	reference	0.57
Yes	60 (76.0)	1.24 [0.71 – 2.18]		1.18 [0.66 – 2.12]	
Vaccination against rotavirus					
Yes	29 (36.7)	reference	0.74		
No	50 (63.3)	1.09 [0.66 – 1.78]			
Malnutrition					
No	68 (86.1)	reference		reference	
Malnutrition	7 (8.8)	2.19 [0.87 – 5.52]	0.10	2.41 [0.89 – 6.54]	0.08
Overweight	4 (5.1)	1.25 [0.40 – 3.85]	0.70	1.21 [0.37 – 3.93]	0.75
Number of members in family	4.4 ± 1.2	0.94 [0.77 – 1.16]	0.58		
Age of mother					
≤ 35	60 (76.0)	reference	0.23		
> 35	19 (24.0)	0.71 [0.40 – 1.24]			
Occupation of mother					
Housewife, farmer, small business	18 (22.8)	reference		reference	
Worker	15 (19.0)	0.53 [0.25 – 1.12]	0.10	0.52 [0.23 – 1.18]	0.12
State officials	14 (17.7)	1.21 [0.55 – 2.67]	0.64	1.20 [0.49 – 2.91]	0.69
Private officer	32 (40.5)	0.85 [0.44 – 1.61]	0.61	0.94 [0.44 – 1.98]	0.87
Microbiological findings by PCR					
Adenovirus	12 (15.2)	0.27 [0.14 – 0.51]	< 0.001	0.29 [0.15 – 0.56]	< 0.001
Enterovirus	13 (16.5)	0.72 [0.38 – 1.37]	0.32		
Norovirus	11 (13.9)	0.41 [0.21 – 0.81]	0.01	0.47 [0.23 – 0.93]	0.03
Rotavirus	10 (12.7)	0.80 [0.39 – 1.65]	**0.55**		
Campylobacter jejuni	12 (15.2)	1.10 [0.56 – 2.18]	0.78		
Salmonella spp.	12 (15.2)	3.52 [1.62 – 7.65]	0.001	3.10 [1.39 – 6.90]	0.006
Enteroaggregative *Escherichia coli*	8 (10.1)	1.28 [0.56 – 2.92]	0.55		

stem from differences in diagnostic methods, as PCR may have lower sensitivity for *Giardia* detection, compared to microscopy or antigen-based tests. Additionally, environmental and regional factors, including hygiene practices and socioeconomic conditions in different regions of Vietnam, could contribute to differences in *Giardia* prevalence. Future studies using multiple diagnostic methods and broader sampling are needed for a more accurate assessment.

This study also identified key risk factors associated with severe diarrheal disease in children, notably the role of viral and bacterial pathogens, as well as patient-specific characteristics. A significant finding was the association between age and male gender with an increased risk of moderate to severe dehydration. This trend has been documented in previous studies and may be attributed to several biological and behavioral factors [29–31]. Exclusive breastfeeding for the first six months of life is widely recognized as a protective factor against infectious diseases, including diarrhea [32]. In our study, this was not significantly associated with diarrhea in the analysis. However, the absence of statistical significance does not necessarily negate its protective effect. The lack of association in our findings could be attributed to several factors, including potential recall bias in breastfeeding history, variations in feeding practices, or other confounding factors that were not fully accounted for in the analysis. Given the well-established benefits of exclusive breastfeeding in reducing diarrhea risk, it remains a key public health recommendation.

The association between the occupation of the mother and the severity of diarrhea among children might be attributed to reduced maternal availability for childcare, limited time for breastfeeding or meal preparation, and potential reliance on external caregivers, which could influence hygiene practices and dietary habits. The increased exposure to group childcare settings or suboptimal feeding and sanitation conditions in working households may also contribute to the observed higher severity. These findings underscore the importance of strengthening public health policies to support working mothers, such as improved parental leave and enhanced childcare hygiene standards.

Our study also demonstrated the protective effect of rotavirus vaccination, with vaccinated children showing a significantly reduced risk of rotavirus carriage. This finding supports the effectiveness of rotavirus immunization in reducing the burden of rotavirus-associated diarrhea, in alignment with global evidence. However, the relatively low vaccination rate observed

in our cohort underscores the need for improved vaccine coverage in Vietnam. Strengthening vaccination efforts through increased awareness campaigns, integration into national immunization programs, and addressing vaccine accessibility barriers could further mitigate the impact of rotavirus-related diarrheal disease in young children.

Despite the considerable burden of diarrheal illness observed in our study cohort, the overall clinical outcomes were favorable, with no reported deaths and the majority of children being discharged following treatment. However, a notable proportion of children were discharged against medical advice, highlighting potential challenges in adherence to treatment recommendations or healthcare access issues. Most children experienced relatively short hospitalizations (5 days), but some had prolonged stays of up to 39 days. The longer hospitalizations could be explained by acute associated diseases, which were present in 6.4% of children. Future studies should explore the impact of comorbid conditions on diarrheal disease outcomes to better inform treatment and discharge planning. Identifying children at risk of prolonged hospitalization could help optimize resource allocation and improve patient outcomes.

Our study also revealed a concerning pattern of antimicrobial use, with almost all children receiving empirical antibiotic treatment, predominantly cephalosporins. This highlights a potential overuse of antibiotics in pediatric diarrhea management, raising concerns about antimicrobial resistance and the need for improved antibiotic stewardship. The widespread use of antibiotics may be attributed to several factors, including diagnostic uncertainty, lack of rapid point-of-care testing, and clinical concerns about bacterial co-infections, particularly in cases presenting with severe symptoms such as fever, dehydration, or bloody diarrhea. While bacterial pathogens were identified in a subset of cases (29.3%), our findings suggest that a significant proportion of antibiotic prescriptions may not have been warranted based on microbiological evidence. This emphasizes the necessity of implementing stricter guidelines for empirical antibiotic use, promoting evidence-based prescribing practices, and expanding access to rapid diagnostic tools to differentiate viral from bacterial infections [33,34]. Further research and policy interventions should focus on optimizing antibiotic use to prevent unnecessary prescriptions and mitigate the risk of antimicrobial resistance in pediatric populations.

In resource-limited settings like Vietnam, the routine use of CBC and CRP testing for every diarrheal patient should be critically evaluated. While these tests can help identify infections and inflammation, their routine implementation may not be cost-effective, especially when clinical symptoms alone can guide management in most cases [35]. In our study, 17.1% of patients had elevated white blood cell counts, and 13.8% had increased CRP levels. However, their direct impact on treatment decisions remains unclear. Prioritizing targeted testing based on clinical severity or specific risk factors may optimize resource utilization without compromising patient care.

Our study has several limitations, including its single-center nature and the potential for selection bias inherent to the study design. Additionally, the reliance on caregiver-reported data for certain variables may introduce information bias. The method of identifying pathogens using real-time PCR cannot distinguish between living and dead microorganisms. Furthermore, we cannot distinguish between carriage and infection. Future multicenter studies incorporating larger sample sizes and longitudinal follow-up would provide further insights into the epidemiology and clinical course of pediatric diarrhea in Vietnam.

Conclusions

In conclusion, this study highlights the distinct clinical impacts of viral and bacterial infections in pediatric diarrhea cases. Viral infections, particularly rotavirus, norovirus, and adenovirus, were significantly associated with an increased risk of dehydration, while bacterial pathogens, such as *Salmonella* spp., were linked to a higher likelihood of bloody diarrhea. These findings emphasize the need for targeted management strategies, including rehydration-focused care for viral infections and appropriate antimicrobial use for bacterial cases. Further research is warranted to validate these findings and inform evidence-based interventions for improving pediatric diarrheal care and outcomes.

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Corresponding author

Professor Philippe Gautret, MD, PhD
Institut Hospitalo-Universitaire Méditerranée Infection
19-21 Boulevard Jean Moulin, 13385
Marseille Cedex 05, France
Email: philippe.gautret@ap-hm.fr

Conflict of interest

No conflict of interest is declared.

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Annex – Supplementary Items

Supplementary Table 1. Correlation between rotavirus carriage detected by qPCR and rotavirus vaccination and rotavirus antigen rapid test.

	Rotavirus carriage detected by qPCR		*p*
	Negative, n (%)	**Positive, n (%)**	
Rotavirus vaccination			
No	229 (59.6)	49 (73.1)	0.04
Yes	155 (40.4)	18 (26.9)	
Rotavirus antigen rapid test (N = 297)			
Negative	230 (90.2)	12 (28.6)	< 0.001
Positive	25 (9.8)	30 (71.4)	