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MAJOR ARTICLE

Correlation Between Prevalence of Selected Enteropathogens and Diarrhea in Children: A Case–Control Study in China

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Background. The application of nucleic acid detection methods improves the ability of laboratories to detect diarrheal pathogens, but it also poses new challenges for the interpretation of results. It is often difficult to attribute a diarrhea episode to the detected pathogens. Here we investigated the prevalence of 19 enteropathogens among diarrheal and nondiarrheal children and provided support for understanding the clinical significance of the pathogens.

Methods. A total of 710 fecal samples were collected from children under 5 years old in 2 different regions of China from May 2017 to March 2018, comprising 383 mild to moderate diarrheal cases and 327 nondiarrheal controls. The enteropathogens were detected using real-time polymerase chain reaction (PCR) or real-time reverse transcription PCR (RT-PCR).

Results. Enteropathogens were detected in 68.9% of cases and 41.3% of controls. Rotavirus A (adjusted OR [aOR], 9.91; 95% CI, 4.99–19.67), norovirus GI and GII (aOR, 3.82; 95% CI, 2.12–6.89), and *Campylobacter jejuni* (aOR, 20.12; 95% CI, 2.57–157.38) were significantly associated with diarrhea ($P < .05$). Adenovirus, norovirus GII, rotavirus A, and enteroaggregative *Escherichia coli* (pCVD432) gave lower cycle threshold (Ct) values in cases than in controls ($P < .05$). Rotavirus A and norovirus GII were associated with diarrhea when the Ct values were ≤30 and ≤25, respectively.

Conclusions. The types and loads of enteropathogens are likely to influence the interpretation of the clinical significance of positive results.

Keywords. case–control; children; diarrhea; enteropathogen.

Diarrheal disease is one of the leading causes of morbidity and mortality worldwide. The GBD2019 project reported 6.58 billion cases of diarrheal disease globally, resulting in 1.53 million deaths and 80.9 million disability-adjusted life-years (DALYs) [1]. The highest DALYs were among children younger than age 5 years [1]. Diarrhea can be caused by a wide range of etiological agents, including viruses, bacteria, and parasites. Accurate identification of gastroenteritis pathogens is, therefore, crucial for surveillance purposes and outbreak investigations. Molecular diagnostic technology offers high sensitivity and wide coverage for the identification of diarrheal pathogens. Positive samples may come from infected patients, asymptomatic infection, or convalescent patients [2, 3]. It is difficult to determine whether diarrhea is caused by the detected pathogens and which pathogen plays a major role in a mixed infection.

Quantitative methods such as real-time polymerase chain reaction (PCR) or real-time reverse transcription PCR (RT-PCR) can be used to determine or evaluate the correlation between pathogen and diarrhea, including the severity of diarrhea [4–6]. Recent studies have provided important data for better understanding the clinical significance of pathogens and interpreting the positive results. However, the prevalence of pathogens is usually region-specific [7]; thus, research conclusions cannot be directly applied to other areas or populations. Here, we conducted a study to investigate the prevalence of 19 different diarrheal pathogens among diarrheal and nondiarrheal children under 5 years old in 2 regions of China. Either real-time PCR or RT-PCR was used to detect fecal specimens to improve the etiology estimation.

METHODS

Study Sites and Enrollment

A total of 710 fecal samples were collected in Hunan Province and Changning District in Shanghai Municipality from May

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2017 to March 2018, comprising 383 outpatient diarrheal cases and 327 nondiarrheal controls.

Inclusion criteria for the case group were ≤5 years of age, ≥3 episodes of diarrhea within a 24-hour period, residing in the area where the sampling hospital is located, and duration of diarrhea was <4 weeks, with priority given to those who did not use antibiotics before enrollment. Symptoms or signs such as diarrhea, vomiting, fever, dehydration level, and hospitalization were used to calculate a severity score using the Vesikari 20-point scale. Cases were divided into 3 categories: mild (scores ≤10), moderate (scores of 11–15), and severe (scores ≥16) [8, 9].

Asymptomatic controls were children without diarrhea who had no history of diarrhea within 4 weeks before recruitment. Controls were (1) enrolled in the community (n = 143, 43.7%) or (2) obtained from the same hospitals as the cases (n = 184, 56.3%) hospitalized for reasons other than gastroenteritis and did not receive antibiotics before enrollment.

Process of Laboratory Testing

Each stool specimen was collected and divided into 2 tubes, 1 containing Cary-Blair transport medium and another empty without any liquid. The specimens, preserved in Cary-Blair transport medium, were used for *Salmonella* and *Vibrio* culture. The liquid-free specimens were frozen at −80°C before nucleic acid extraction. The target pathogens were detected using either real-time PCR or real-time RT-PCR. All positive results were verified by another round of real-time PCR/RT-PCR or by sequence analysis of the amplified products.

Culture of *Salmonella* and *Vibrio*

Vibrio in the specimens was enriched with alkaline peptone water (APW; Luqiao, Beijing, China) and selected on thiosulfate-citrate-bile-sucrose (TCBS) agar (Oxoid, Basingstoke, UK). *Salmonella* was enriched with selenite brilliant green (SBG) broth (Luqiao) and selected on *Salmonella* Chromogenic Medium (Kemajia, Shanghai, China). Suspicious colonies were identified using serum agglutination tests and biochemical reactions with VITEK 2 Gram-Negative Identification (GN) cards (bioMérieux, France). One milliliter of an overnight culture of APW and SBG was stored at −80°C for nucleic acid extraction.

Real-time PCR and Real-time RT-PCR Assays

The frozen specimens and overnight cultures of enrichment media (SBG and APW) were thawed at room temperature. Nucleic acids were extracted using QIAcube HT with the *cador* Pathogen 96 QIAcube HT kit (Qiagen, Hilden, Germany) at Changning CDC or using the automatic nucleic acid purification system NP968 with the EX-DNA/RNA virus extraction kit (Tianlong, Xi'an, China) at Hunan CDC.

We tested for 19 enteric pathogens in fecal specimens, including (1) 3 viruses: adenovirus (AdV), norovirus GI and GII (NoV GI/GII), and rotavirus A (RVA); (2) 13 bacterial pathogens: *Campylobacter coli*, *C. jejuni*, *Clostridium difficile* (toxin A/B), 5 pathotypes of diarrheagenic *Escherichia coli* (DEC; enteroaggregative *E. coli* [EAEC], enteropathogenic *E. coli* [EPEC], enterotoxigenic *E. coli* [ETEC], Shiga toxin–producing *E. coli* [STEC], and enteroinvasive *E. coli* [EIEC]), *Shigella* spp., *Salmonella* spp., *Vibrio cholerae*, *V. parahaemolyticus*, and *Yersinia enterocolitica*; (3) 3 parasites: *Cryptosporidium*, *Entamoeba histolytica*, and *Giardia lamblia.* The target genes were screened by real-time PCR or real-time RT-PCR, with primers and probes listed in Supplementary Table 1. Nucleic acids extracted from specimens were used as templates to detect all pathogens, except for *Salmonella* and *Vibrio,* for which nucleic acids from the enrichment cultures were used.

All the reaction mixtures of real-time PCR/RT-PCR were 20 μL in volume, containing 1 μL of the template. The concentration of each primer (and probe if applicable) was 200 nM. Viral pathogens were screened with a One-Step PrimerScript RT-PCR Kit (TaKaRa, Dalian, China) and validated with a One-Step TB Green PrimeScript RT-PCR kit II (TaKaRa) under the following conditions: 42°C for 5 minutes, 95°C for 10 seconds, and 40 cycles of 95°C for 5 seconds and 60°C for 40 seconds. *V. cholerae* and *V. parahaemolyticus* were screened with Premix Ex Taq (Probe qPCR; TaKaRa). The remaining targets were detected with TB Green Premix Ex Taq II (Tli RNaseH Plus; TaKaRa). The cycling conditions were 95°C for 30 seconds, followed by 40 cycles of 95°C for 5 seconds and 60°C for 40 seconds. Melting curves were analyzed using TB Green real-time PCR/RT-PCR. Real-time PCR/RT-PCR detections were performed using the LightCycler 96 system (Roche, Indianapolis, IN, USA). Samples were recorded as positive when the cycle threshold (Ct) was ≤35.

Two target genes, aggR and pCVD432, were used in EAEC screening. When both genes were positive, EAEC was considered positive; if only 1 gene was positive, the amplified products were sequenced to verify the results. For the 3 parasitic pathogens, the amplified products of the screening test were sequenced to verify the results. For other pathogens, if the screening test was positive, another real-time PCR/RT-PCR was used for verification. When the verification test was positive, or when the verification test was negative but the sequence of the amplified product of the screening test was correct, the result was considered a true positive. If *Salmonella* or *Vibrio* strains were isolated using the culture method, the samples were considered positive regardless of whether the nucleic acid test was positive.

Statistical Analysis

The Fisher exact test or chi-square (χ^2) test was used to compare categorical variables. The difference in Ct values between the case and control groups was analyzed with the Mann-Whitney test. Logistic regression models were constructed for each of

the pathogens of interest. Odds ratios (ORs) and their 95% CIs were estimated and adjusted for age, gender, enrollment site, and month of recruitment. The results were considered significant at an α level ≤.05. All analyses were conducted using SAS 9.4 (SAS Institute, Cary, NC, USA) or GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA).

RESULTS

Basic Characteristics of Cases and Controls

The basic characteristics of 383 cases and 327 controls are shown in Table 1. The median age (interquartile range [IQR]) was 15 (12–24) months and 21 (14–33) months for cases and controls, respectively. The age of the case group was younger, and the number of infants <1 year old was greater than that of the control group. All cases were defined as nonsevere by the Vesikari 20-point scale with a mean score (±SD) of 5.06 ± 1.63, which included 379 (99.0%) mild (score ≤10) and 4 (1.0%) moderate (score 11–15) cases.

Frequency of Enteropathogens

Enteropathogens were detected in 264 samples (68.9%) from diarrheal cases and 135 (41.3%) from controls (χ^2 = 54.8; P < .001). More than 1 pathogen was identified in 84 samples (21.9%) from cases and 40 (12.2%) from controls (χ^2 = 11.5; P < .001). The prevalence (cases vs controls) of pathogens that were more frequently (P < .05) identified in cases than in controls was RVA (21.9% vs 3.4%), NoV (GI and GII; 16.4% vs 4.9%), *Salmonella* (13.8% vs 8.6%), *C. jejuni* (4.2% vs 0.3%), and EIEC/*Shigella* (1.6% vs 0.0%) (Table 2).

The detection rates of ST-ETEC (including STh and STp) in cases and controls were 1.04% and 1.53%, respectively, and the detection rates of LT-ETEC were 1.04% in cases and 2.45% in controls. For the 2 pathogens, there was no significant difference in detection rates between the 2 groups.

RVA was the least likely pathogen to be detected in a mixed infection in both cases and controls, as only 26 of 84 RVA-positive specimens (31.0%) in the case group were mixed infection, compared with 27.3% (3/11) in the control group.

The prevalence of pathogens stratified by age group and sampling site is presented in Supplementary Figure 1 and Supplementary Table 2. Some pathogens had seasonal characteristics (Supplementary Figure 2) in both cases and controls, especially RVA, NoV GI/GII, and DEC. For cases who were positive for a single pathogen, the frequency of various symptoms was analyzed and is shown in Supplementary Table 3.

Correlation Between Pathogens and Diarrhea

Multivariate logistic regression analysis showed that RVA (adjusted OR [aOR], 9.91; 95% CI, 4.99–19.67), NoV GI/GII (aOR, 3.82; 95% CI, 2.12–6.89), and *C. jejuni* (aOR, 20.12; 95% CI, 2.57–157.38) were significantly associated with diarrhea (P < .05). The pathogenic significance of EIEC/*Shigella*, STEC, *V. cholerae*, *V. parahaemolyticus*, *Y. enterocolitica*, *Cryptosporidium*, and *E. histolytica* was not evaluated because of the small number of positive detections. Of the 79 NoV-positive samples, 78 (98.7%) were NoV GII and 1 (1.3%) was NoV GI, which was detected in the control group. Therefore, the data in this study mainly reflected the prevalence of NoV GII.

The pathogen loads in the specimens can be reflected by the Ct values of real-time PCR/RT-PCR (Figure 1). The Ct values of AdV, NoV GII, RVA, and EAEC (pCVD432) in the case group were significantly lower than those in the control group

Table 1. Basic Characteristics of Children With and Without Diarrhea

	Cases (n = 383), No. (%)	Controls (n = 327), No. (%)	χ^2 Value	*P* Value
Age, mo				
0–12	127 (33.2)	65 (19.9)	32.308	<.0001
13–36	229 (59.8)	199 (60.9)		
37–60	27 (7.0)	63 (19.3)		
Gender				
Male	229 (59.8)	199 (60.9)	0.084	.773
Female	154 (40.2)	128 (39.1)		
Site				
Hunan	196 (51.2)	197 (60.2)	5.871	.015
Shanghai	187 (48.8)	130 (39.8)		
Seasons				
Summer (May 2017–Jul 2017)	62 (16.2)	51 (15.6)	7.969	.047
Autumn (Aug 2017–Oct 2017)	124 (32.4)	91 (27.8)		
Winter (Nov 2017–Jan 2018)	149 (38.9)	119 (36.4)		
Spring (Feb 2018–Apr 2018)	48 (12.5)	66 (20.2)		
Disease severity by the Vesikari 20-point scale				
Mild (scores ≤10)	379 (99.0)			
Moderate (scores of 11–15)	4 (1.0)			
Severe (scores ≥16)	0			

Table 2. The Prevalence of Pathogens in the Case and Control Groups and the Correlation Between Pathogens and Diarrhea

Pathogens	Cases (n = 383), No. (%)	Controls (n = 327), No. (%)	*P* Value[b]	Multivariate Analysis[a] aOR (95% CI)	*P* Value
Viruses					
Adenovirus	21 (5.5)	13 (4.0)	.382	1.32 (0.63–2.77)	.456
Norovirus GI and GII	63 (16.4)	16 (4.9)	<.001	3.82 (2.12–6.89)	.000
Rotavirus A	84 (21.9)	11 (3.4)	<.001	9.91 (4.99–19.67)	.000
Bacteria					
Campylobacter coli	5 (1.3)	2 (0.6)	.461	1.92 (0.36–10.13)	.442
Campylobacter jejuni	16 (4.2)	1 (0.3)	<.001	20.12 (2.57–157.38)	.004
Clostridium difficile (toxin A/B)	30 (7.8)	28 (8.6)	.784	0.81 (0.47–1.42)	.462
Diarrheagenic *Escherichia coli* (DEC)/*Shigella*	75 (19.6)	65 (19.9)	.925	0.99 (0.65–1.50)	.955
EAEC	40 (10.4)	28 (8.6)	.444	1.16 (0.67–2.00)	.591
EPEC	35 (9.1)	38 (11.6)	.322	0.77 (0.45–1.31)	.332
ETEC	6 (1.6)	13 (4.0)	.061	0.40 (0.14–1.12)	.082
EIEC/*Shigella*	6 (1.6)	0 (0.0)	.034	NA	.976
STEC	4 (1.0)	0 (0.0)	.129	NA	.981
Salmonella	53 (13.8)	28 (8.6)	.033	1.53 (0.93–2.53)	.094
Vibrio cholerae	1 (0.3)	1 (0.3)	>.999	0.97 (0.06–16.53)	.982
Vibrio parahaemolyticus	1 (0.3)	0 (0.0)	>.999	NA	.986
Yersinia enterocolitica	1 (0.3)	0 (0.0)	>.999	NA	.986
Parasites					
Cryptosporidium	1 (0.3)	0 (0.0)	>.999	NA	.987
Giardia lamblia	1 (0.3)	5 (1.5)	.100	0.22 (0.02–1.94)	.172

Abbreviations: aOR, adjusted odds ratio; EAEC, enteroaggregative *E. coli*; EIEC, enteroinvasive *E. coli*; EPEC, enteropathogenic *E. coli*; ETEC, enterotoxigenic *E. coli*; NA, not applicable; OR, odds ratio; STEC, Shiga toxin–producing *E. coli*.

[a]Pooled analysis controlling for age, gender, enrollment site, and month of recruitment.

[b]*P* value was calculated by Fisher exact test.

($P < .05$). There were no significant differences in the Ct values of *C. coli*, *C. difficile* toxin A/B, EAEC (*aggR*), EPEC, ETEC (LT), and ETEC (STh) between the 2 groups.

The Ct values of positive results were divided into 4 levels, namely "≤20," ">20 to ≤25," ">25 to ≤30," and ">30 to ≤35." As shown in Figure 2, RVA and NoV GII were associated with diarrhea when the Ct value was low (≤30 for RVA and ≤25 for NoV GII); positive results with a higher Ct value had no significant correlation with diarrhea. *C. jejuni* was also correlated with diarrhea when Ct ≤30, but the correlation between the Ct value ranging from 30 to 35 and diarrhea was not analyzed due to lack of positive samples. There was no significant difference in

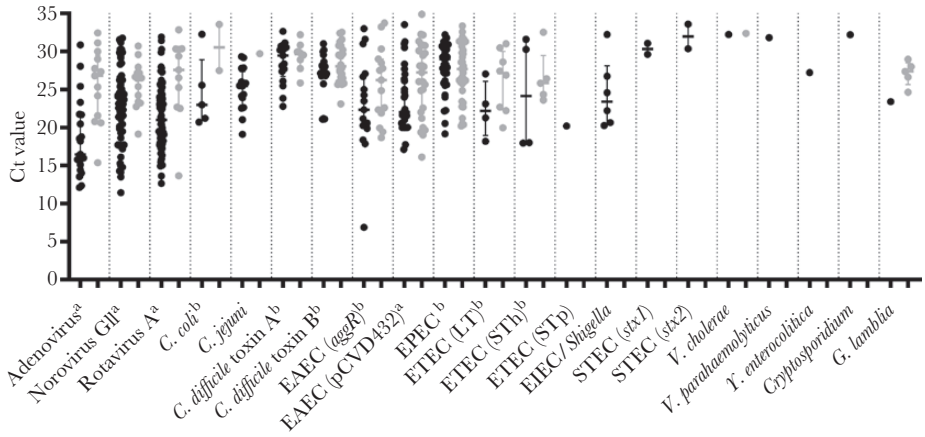

Figure 1. Ct values of pathogens in positive samples. Solid black circle (●), positive detections in case group; solid gray circle (●), positive detections in the control group; short bar and line (┼), median with interquartile range. [a]Significant difference in Ct values between the 2 groups analyzed by Mann-Whitney test. [b]No significant difference in Ct values between the 2 groups. The difference in Ct values was not analyzed when the number of positives in either of the groups was <2. The Ct value of *Salmonella* was not analyzed as the enrichment culture was used as the template. Abbreviations: Ct, cycle threshold; EAEC, enteroaggregative *E. coli*; EIEC, enteroinvasive *E. coli*; EPEC, enteropathogenic *E. coli*; ETEC, enterotoxigenic *E. coli*; STEC, Shiga toxin–producing *E. coli*.

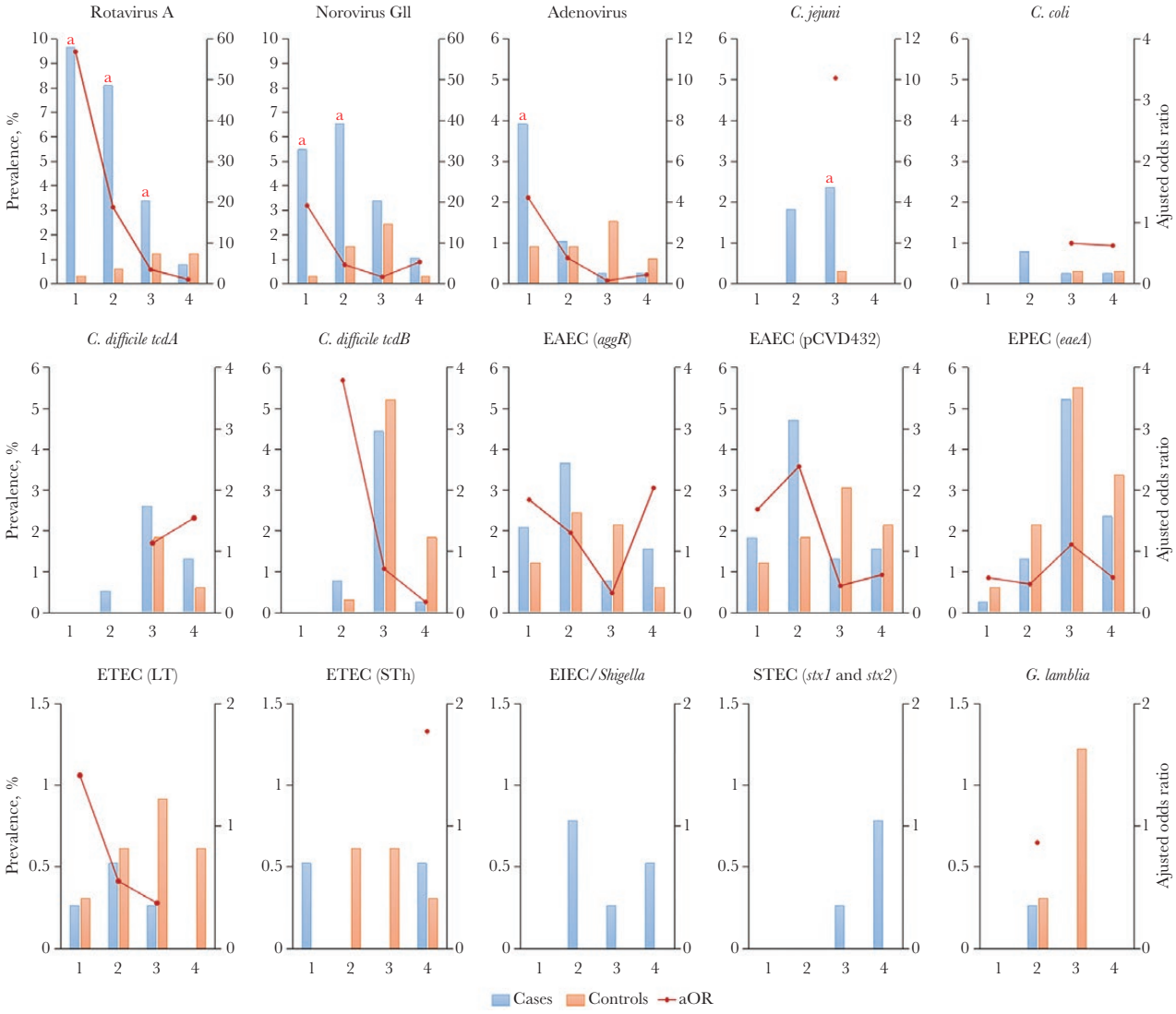

Figure 2. Variation in pathogen prevalence and adjusted odds ratio with Ct level of pathogens. X-axis: 1–4 correspond to 4 Ct value levels, ie, 0 < Ct ≤ 20, 20 < Ct ≤ 25, 25 < Ct ≤ 30, and 30 < Ct ≤ 35. Left y-axis, the prevalence of pathogens in cases and controls. Right y-axis, adjusted odds ratio by multivariate logistic regression analysis controlling for age, gender, enrollment site, month of recruitment, and Ct level. [a]The pathogen was significantly associated with diarrhea at the Ct-labeled level ($P < .05$). Abbreviations: aOR, adjusted odds ratio; Ct, cycle threshold; EAEC, enteroaggregative *E. coli*; EIEC, enteroinvasive *E. coli*; EPEC, enteropathogenic *E. coli*; ETEC, enterotoxigenic *E. coli*; STEC, Shiga toxin–producing *E. coli*.

the detection rate of AdV between the case and control groups (5.5% vs 4.0%). However, positive results of AdV with Ct ≤20 were correlated with diarrhea (aOR, 4.21; 95% CI, 1.16–15.29; $P < .05$). When the Ct value was >20, the detection rate of AdV in the case group was close to or lower than that in the control group, but the difference was not significant.

DISCUSSION

With the widespread use of nucleic acid detection technology, the detection rate of diarrheal pathogens has increased, but at the same time, interpretation of the test results has become a great challenge. It is difficult to ascribe unequivocally 1 episode of diarrhea to the pathogens detected. In this study, we investigated the prevalence of 19 pathogens in outpatient diarrheal and nondiarrheal children in China. We analyzed the correlation between pathogens and diarrhea, which helps to elucidate the clinical significance of pathogens to diarrhea and the meaning of the detection results.

The prevalence of AdV 40/41 in cases and asymptomatic children suggested a significant association of this pathogen with diarrhea or severe diarrhea [10–12]. In this study, the prevalence of AdV in cases and controls was 5.5% and 4.0%, respectively. Unfortunately, we did not identify the serotypes of AdV and could not analyze whether there were differences between F40/41 serotypes and non-F40/41 AdV species. The duration of shedding of AdV ranged from 1 day to 10 days [13] after

infection. When the children in the control group were positive for AdV, they may have been infected with non-F40/41 species or have been excreting virus after the last infection.

RVA and NoV are the leading causes of childhood diarrhea in many regions of China [7] and were the 2 pathogens with the highest detection rates in the case group. The detection rates of RVA and NoV GI/GII in the control group were 3.4% and 4.9%, respectively, which were lower than the data of other studies [13, 14]. It was reported that NoV shedding could be detected within 3–14 hours before the onset of symptoms and lasted for 13–56 days after inoculation [2]. Duration of RV excretion ranged from 4 to 57 days after the onset of diarrhea [3]. The positive specimens in the control group were likely from asymptomatic infected individuals, patients before the onset of symptoms, or children with viral shedding after a previous infection.

RVA and NoV GII were significantly associated with diarrhea, while AdV was not. The Ct values of the 3 viruses in the case group were significantly lower than those in the control group. The correlation between virus and diarrhea was significant only when the Ct value was ≤30 for RVA, ≤25 for NoV GII, and ≤20 for AdV. Therefore, when real-time RT-PCR is used to detect clinical diarrhea samples, we suggest using the above Ct values as the cutoff to reduce nonclinical significance reports. It should be noted that the Ct value of infected samples is related to many factors, such as the stage of infection, sampling location, and specimen quality. A high Ct value cannot be used as the only criterion to exclude this pathogen as the possible cause of the diarrhea episode.

Differences in fecal viral load between symptomatic and asymptomatic infection have been observed in several studies [11, 14]. In the detection of RVA in children under 5 years of age, a Ct cutoff value of 24 in RT-PCR has been proposed to be equivalent to the detection limit of enzyme-linked immunosorbent assay and to be clinically significant [15]. Similarly, in one study, a Ct cutoff value of 30 was clinically significant in detecting NoV in children under 5 years of age [14]. There have also been studies with differing results. For example, Qiu's study found no significant differences in AdV load between the diarrhea and control groups [10]. Different real-time RT-PCR assays and detection platforms have different detection efficiencies, so the cutoff values mentioned above may not be directly applied to other assays. The prevalence of pathogens has regional characteristics, so it may be necessary to establish a specific cutoff value in different areas and populations.

Whether *Campylobacter* is associated with diarrhea is not consistent in different studies. Platts-Mills et al. suggested that *Campylobacter* spp. contributed substantially to the burden of diarrhea in children [16], and some studies have shown that *C. jejuni* or *C. coli* is moderately associated with diarrhea even at the highest quantities [5]. However, some studies did not observe the association between *Campylobacter* and diarrhea [12]. In this study, *C. jejuni* with Ct ≤30 was significantly associated with diarrhea, while *C. coli* was not. As there was no positive specimen of *C. jejuni* with Ct >30, we did not analyze whether *C. jejuni* was associated with diarrhea at a low load.

Typical EPEC possess a virulence plasmid pEAF encoding the bundle-forming pilus (BFP), which is strongly associated with diarrhea in infants and young children [4, 12]. However, the proportion of typical EPEC is very low in China, and atypical EPEC without BFP are now the dominant EPEC [17]. Therefore, although this study did not distinguish between typical and atypical EPEC, we speculated that the data obtained were mainly from atypical EPEC. The roles of atypical EPEC and EAEC in diarrhea are not clear as they have often been detected at similar rates in both diarrheal and nondiarrheal patients and have been frequently identified in coinfections among both groups [12, 18]. In our study, the prevalence of EPEC (9.1% vs 11.6%) and EAEC (10.4% vs 8.6%) was similar in the case and control groups. There was no significant difference in the Ct values of EPEC (*eaeA*) and EAEC (*aggR*) between the case and control groups. Still, the Ct value of EAEC (pCVD432) in the case group was significantly lower than that in the control group. The multivariate analysis results suggested no significant correlation between EPEC/EAEC and diarrhea at all Ct levels. The pathogenic role of EAEC and EPEC in mild to moderate diarrhea in children remains unclear.

ETEC is an important pathogen of diarrhea in developing countries, and epidemiologic studies suggest a significant correlation between ST-producing ETEC and diarrhea [5, 12]. However, in this study, the detection rate of ETEC in the case group was lower than that in the control group (1.6% vs 4.0%, no significant difference). More specifically, there was no significant difference in the prevalence or Ct value of LT and STh between the case and control groups.

C. difficile has often been detected in diarrheal stool specimens [7, 19]. The detection rate of *C. difficile* in cases was close to that in controls (7.8% vs 8.6%), and most of the positive samples were from children aged 0–3 years. The pathogen was reported to colonize about 60%–70% of healthy newborns and infants, with gradual reduction between 12 and 24 months [20]. Therefore, *C. difficile* in young children may be a state of asymptomatic colonization and does not necessarily mean a disease-related infection.

Salmonella was identified as a diarrhea-associated pathogen [5, 12], and the strength of the association was higher with increasing pathogen loads [4, 5]. However, in this study, no correlation between *Salmonella* and diarrhea was found. In our real-time PCR detection of *Salmonella*, the nucleic acid of enrichment culture was used as the template, which was more sensitive for analyzing low-load samples than using fecal nucleic acid. There might be some asymptomatic individuals carrying *Salmonella* in both the case group and the control group.

The detection of these low-load specimens might have weakened the difference in the prevalence of *Salmonella* between the 2 groups.

In this study, we compared the prevalence of selected enteropathogens in children with and without diarrhea, evaluated the association of pathogens with diarrhea, and attempted to give clinical Ct cutoffs for some of the pathogens. It should be noted that a high Ct value cannot be used as the only criterion to exclude this pathogen as the possible cause of the diarrhea episode. Limitations: (1) The primers and probes used in this study were mainly from the literature, rather than methods recognized by different laboratories. The absence of "gold standard methods" and the insufficient sample size led to some uncertainties in the results, which need to be further validated; (2) 56.3% of nondiarrheal controls came from patients hospitalized for reasons other than gastroenteritis, which may have confounded the results.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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Potential conflicts of interest. All authors: no reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Data were not publically available.

Patient consent. Written informed consent was obtained from the legal guardians of all diarrheal children and all nondiarrheal children from community. Stool samples of nondiarrheal hospitalized children in the control group were selected from the preserved feces left by the patients when they were admitted to the hospital according to the inclusion criteria. Therefore, informed consent was not signed.

Author contributions. H.Z., H.Z.X., and W.Z.Q.: local data collection and analysis. L.Z.P.: statistical analysis. W.W., X.X., Q.D., Z.L., and G.J.Y.: bacterial culture and nucleic acid extraction. L.J., D.B.W., and Z.Z.F.: verification of results. Z.J.Y.: study design, data analysis, and paper writing. K.B. and Z.M.: study design and paper review.

Ethical approval. The design of the work was approved by the ethics committee of the National Institute for Communicable Disease Control and Prevention, China CDC.

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