

Original Article

Etiology, epidemiology and clinical characteristics of acute diarrhea in hospitalized children in rural Peru

Angela Cornejo-Tapia¹, Fiorella Orellana-Peralta¹, Pablo Weigl¹, Jorge Bazán-Mayra^{2,3}, Hernán Cornejo-Pacherres³, Gabriella Ulloa-Urizar¹, Miguel Angel Aguilar-Luis^{1,4}, María Jesus Pons^{1,4}, Juana del Valle-Mendoza^{1,4}

¹ School of Medicine, Research Center and Innovation of the Health Sciences Faculty, Universidad Peruana de Ciencias Aplicadas, Lima, Peru

² Hospital Regional Docente de Cajamarca, Cajamarca, Peru

³ Regional Laboratory of Cajamarca, Dirección Regional de Salud de Cajamarca (DIRESA), Cajamarca, Peru

⁴ Instituto de Investigación Nutricional, Lima, Peru

Abstract

Introduction: Diarrhea remains one of the main causes of morbidity and mortality in children under five years of age especially in low-income countries. In Peru, epidemiological reports about enteropathogens related to acute diarrhea are scarce in rural areas. The aim of this study was to describe the etiology, epidemiology, and clinical characteristics of the principal causes of acute infectious diarrhea in a northern rural region of Peru.

Methodology: A prospective study was conducted from January 2011 to December 2012 to describe the main pathogens causing acute diarrhea using PCR assay.

Results: A total of 117 children diagnosed with acute diarrhea were included in the study. A single etiological agent was identified in 41.03% of samples, being rotavirus followed by norovirus and *Shigella*. Co-infections containing virus and bacteria were found in 22.22% of samples. Vomiting was most commonly found symptoms in 58.97% cases followed by fever (54.70%). Malnutrition was detected in 14.53% of the children.

Conclusions: High prevalence of rotavirus, as well as adenovirus and norovirus, was observed in the present study. *Shigella* was the most common bacteria found in acute diarrhea in the area. The implementation of a better surveillance system is mandatory in order to identify the principal etiologies of gastroenteritis in the rural areas of Peru and to develop of better prevention strategies and reduce diarrhea-associated mortalities.

Key words: diarrhea; rural area; children; bacteria; virus; low-income countries.

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Introduction

Although in recent years deaths caused by diarrhea are declining worldwide, mainly as a consequence of improved socioeconomic conditions, pediatric diarrheal diseases still account for more than 800,000 annual deaths globally, representing 11% of the 7.6 million estimated child deaths per year around the world [1]. Similarly in Peru, according to the 2007 National Surveillance of Health Ministry (MINSA-DGE) the incidence of acute diarrhea in children less than 5 years of age was of 2,722 cases per 10,000 inhabitants, with around 750,000 episodes of acute diarrhea annually (<http://www.dge.gob.pe/boletines/2007/52.pdf>).

Diarrhea is a consequence of intestinal infection that spreads through food, the consumption of contaminated water or person-to-person transmission as

a result of poor hygiene [2]. Multiple etiological agents are known to be responsible, including viruses, bacteria and parasites. The most important agent that causes severe gastroenteritis accompanied by acute diarrhea in young children worldwide is rotavirus [3]. In Peru, human rotaviruses are responsible for approximately 810 deaths annually in children under 5 years of age [4]. Among bacteria, the most common pathogens are *Escherichia coli*, followed by *Campylobacter jejuni*, *Shigella* spp. and *Salmonella* spp. [5]. Diarrheagenic *E. coli* (DEC) are frequently reported as a cause of diarrhea in children in developing countries, however, these pathogens are not included in the routine diagnosis in microbiology clinical laboratories [5]. The clinical relevance of each specific enteropathogen depends on the geographical area and greater local

knowledge is the key to design specific strategies for appropriate interventions [5].

In most rural areas of low and middle-income countries, etiological diagnosis of diarrhea is not determined due to limited access to healthcare facilities, or due to the absence of adequate diagnostic tools. Nonetheless, the possibility to transfer samples to reference centers may be an option in order to achieve accurate diagnosis by direct molecular tools [6]. Therefore the aim of this study was to describe the etiology, epidemiology, and clinical characteristics of acute infectious diarrhea in children under 5 years in a northern rural region of Peru.

Methodology

Samples Management and Study Area

A total of 117 stool samples were obtained from children less than 5 years of age who were hospitalized with acute gastroenteritis from January 2011 to December 2012 in the “Hospital Regional de Cajamarca” located in Cajamarca Department, in rural Northern Peru. The size of this department, with great climatic diversities, is greater than 35,000 Km² with a total population of approximately 1,500,000 inhabitants. This study was approved by the Ethics Committee of the Universidad Peruana de Ciencias Aplicadas (Lima, Peru).

The inclusion criteria were: children under 5 years of age, hospitalized with acute gastroenteritis. The exclusion criteria were considered when the informed consent was not correctly obtained. Parents or guardians of children collected the stool sample, and they gave it to our training personnel in sterile containers, between 1–3 days after the onset of disease. Physicians in the hospital registered the clinical data and all information was obtained by review of hospital records. Acute gastroenteritis was defined as the occurrence of diarrhea lasting 14 days along with symptoms such as vomiting, fever, dehydration and abdominal pain as described by the European Society for Pediatric Infectious Diseases [7]. Fecal samples were transported at 4°C to the laboratory of the Microbiology Department of Dirección Regional de Salud de Cajamarca (Cajamarca, Peru). Samples were stored at -20°C until processed in the laboratory of the Research Center for Health Sciences, Universidad Peruana de Ciencias Aplicadas (Lima, Peru).

The Ethics Committee of the “Universidad Peruana de Ciencias Aplicadas” approved the study protocol and informed consent was signed by the parents or legal tutors before the sample collection was performed.

Nucleic acid extraction

Genetic material from fecal suspensions was extracted using a QIAamp Viral RNA Kit (QIAGEN GmbH, Hilden, Germany) in accordance with the manufacturer’s instructions. The extraction was stored at -20°C until use.

Pathogen detection

The following pathogens were evaluated in this study: gene amplification of rotavirus [4], norovirus and adenovirus [8,9] was performed by RT-PCR as described previously. Previous virus amplification, dsRNA was reverse transcribed using one Step RT-PCR Kit (Merck Biosciences, Darmstadt, Germany). Following denaturation of RNA at 80°C for 5 minutes, RT-PCR comprised a reverse transcription step at 50°C for 30 minutes.

Campylobacter jejuni, *Campylobacter coli*, *Shigella* spp. and *Salmonella* spp. were identified by PCR as previously described [10-12]. DEC, in particular enteroaggregative (EAEC), enterotoxigenic (ETEC) and enteropathogenic *E. coli* (EPEC) were identified by multiplex PCR as previously described [13].

Data analysis

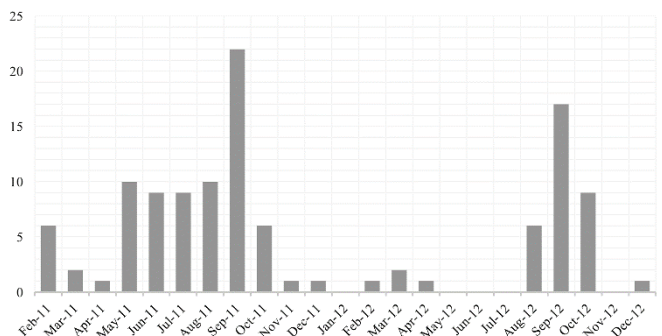
Statistical significance was established using the Fisher’s exact test. Differences were considered significant at p-value ≤ 0.05. Statistical analyses were performed with SPSS version software 21.0 (SPSS Inc., Chicago, IL, USA).

Results

Seasonal distribution of diarrheal cases

We observed a peak of diarrheal cases in September, thus our results indicate a seasonal component in the distribution of cases (Figure 1).

Figure 1. Episodes of diarrhea according to seasonality during the studied period.



Clinical characteristics of hospitalized infants

The characteristics of the hospitalized infants with diarrhea are shown in Table 1. Most of the samples were from children less than 17 months of age (60.7%). Regarding breastfeeding habits, 39.3% received a combination of breastfeeding and formula, and only 28.2% of children were exclusively breastfed. The most common clinical symptoms found in this cohort were vomiting (53%) and fever (52.2%). In relation to the number of depositions, 57 children (48.7%) presented between 1 to 6 depositions. No dehydration symptoms were presented in 56 (47.9%) of children.

Vomiting was more prevalent in children with viral infection (34,8%). Of these, 30.4% were children with viral monoinfection and 4.35% were associated with viral coinfections. Vomiting due to bacterial infection was found in only 14.5%, of them 10.2% had bacterial monoinfection and 4.4% had bacterial coinfection). Malnutrition was more frequently observed in viral

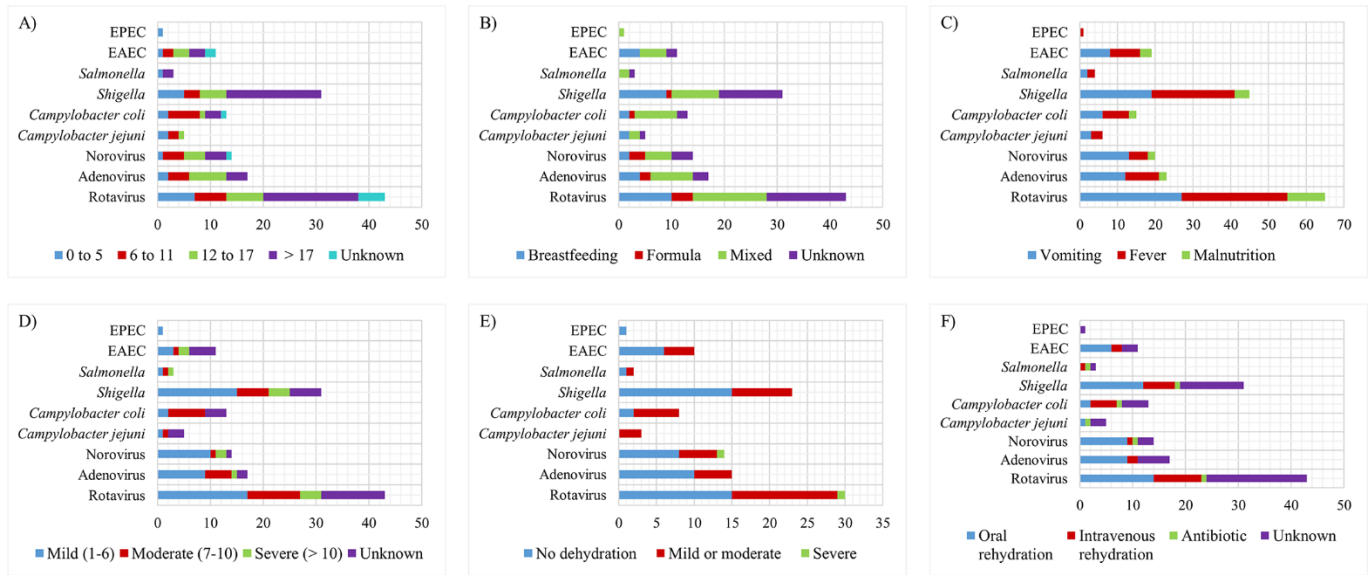
infection (41.2%) (Table1). No differences were reported in the degree of dehydration and number of depositions during the diarrheal episode due to viral or bacterial infection (Table 1).

The most frequently administrated treatment was oral rehydration (40.2%), with 17 cases receiving intravenous rehydration. In the hospital, antibiotic treatment was only administrated in 9 (7.7%) cases; of them 2 with viral and 4 with bacterial infection (Table 1). It is important to highlight that 26 (22.2%) children received antibiotic treatment prior to hospital admission. Among these, 12 cases received cotrimoxazole, 3 azithromycin, 2 amoxicillin, 2 chloramphenicol, 1 cephalosporin, 1 tetracycline, 1 amoxicillin plus clavulanic acid and 4 were given unknown antibiotics. Sixty-six patients (56.4%) did not receive any previous antibiotic treatment. In addition, in 17 (64.4%) cases receiving antibiotics, no bacterial pathogen was detected. No statistical association of

Table 1. General characteristics of the studied children.

| | Total cases (N = 117) | Only one virus (N=30) | Only one bacteria (N=18) | Coinfection (38/117 = 32.48%) | | | No infectious agent identified |
|------------------------------|--------------------------|--------------------------|-----------------------------|----------------------------------|----------------------------------|----------------------------|-----------------------------------|
| | | (30/117 = 25.64%) | (18/117 = 15.38%) | virus-virus (N = 6) | bacteria- bacteria (N = 6) | virus-bacteria (N = 26) | N = 31 (31/117 = 26.5%) |
| Age(months) | | | | | | | |
| 0 to 5 | 21 (17.95%) | 5 (23.8%) | 2 (9.5%) | 0 | 2 (9.5%) | 4 (19.04%) | 8 (38.09%) |
| 6 to 11 | 22 (18.80%) | 7(31.8%) | 5 (22.7%) | 2 (9.09%) | 2 (9.09%) | 3 (13.63%) | 3 (13.63%) |
| 12 to 17 | 28 (23.93%) | 3(10.71%) | 5 (17.85%) | 4 (14.28) | 1 (3.57) | 4 (14.28) | 11 (39.28) |
| > 17 | 38 (32.48%) | 11 (28.95%) | 6 (15.78%) | 0 | 1 (2.63) | 13 (34.21) | 7 (18.42) |
| Unknown | 8 (6.84%) | 4 (50%) | 0 | 0 | 0 | 2 (25) | 2 (25) |
| Lactation | | | | | | | |
| Breastfeeding | 34 (29.06%) | 9 (26.47) | 7 (20.59) | 1 (2.94) | 2 (5.88) | 5 (14.7) | 10 (29.41%) |
| Formula | 10 (8.55%) | 4 (40) | 1 (10%) | 2 (20) | 0 | 1 (10) | 3 (30%) |
| Mixed | 46 (39.32%) | 10 (21.74) | 7 (15.22) | 1 (2.17) | 3 (6.52) | 10 (21.73) | 15 (32.61%) |
| Unknown | 27 (23.08%) | 7 (25.93) | 3 (9) | 2 (7.41) | 1 (3.7) | 10 (37.04) | 4 (14.81%) |
| Clinical symptoms | | | | | | | |
| Vomiting | 69 (58.97%) | 21 (30.43) | 7 (10.15) | 3 (4.35) | 3 (4.35) | 19 (27.54) | 16 (23.19) |
| Fever | 64 (54.70%) | 17 (26.56) | 10 (15.63) | 2 (3.13) | 4 (6.25) | 17 (26.56) | 14 (21.88) |
| Malnutrition | 17 (14.53%) | 6 (35.29) | 1 (5.88) | 1 (5.88) | 1 (5.88) | 5 (29.41) | 3 (17.65) |
| Diarrhea | | | | | | | |
| Mild (1-6) | 57 (48.72%) | 13 (22.81) | 10 (17.54) | 4 (7.02) | 1 (1.75) | 10 (17.5) | 19 (33.33) |
| Moderate (7-10) | 23 (19.66%) | 7 (30.44) | 4 (17.39) | 1 (4.35) | 1 (4.35) | 7 (30.43) | 3 (13.04) |
| Severe (> 10) | 13 (11.11%) | 3 (23.08) | 0 | 1 (7.69) | 2 (15.38) | 2 (15.38) | 5 (38.46) |
| Unknown | 24 (20.51%) | 7 (29.16) | 4 (16.66) | 0 | 2 (8.33) | 7 (29.16) | 4 (16.66) |
| Degree of dehydration | | | | | | | |
| No dehydration | 56 (47.86%) | 12 (21.43) | 8 (14.28) | 4 (7.14) | 2 (3.57) | 10 (17.86) | 20 (35.71) |
| Mild or moderate | 38 (32.48%) | 11 (28.95) | 8 (21.05) | 2 (5.26) | 3 (7.89) | 7 (18.42) | 7 (18.42) |
| Severe | 2 (1.71%) | 2 (100) | 0 | 0 | 0 | 0 | 0 |
| No information | 21 (17.95%) | 5 (23.81) | 2 (9.54) | 0 | 1 (4.76) | 9 (42.85) | 4 (19.05) |
| Treatment | | | | | | | |
| Oral rehydration | 47 (40.17%) | 13 (27.66) | 6 (12.76) | 4 (8.5) | 2 (4.26) | 9 (19.15) | 13 (27.66) |
| Intravenous rehydration | 17 (14.53%) | 6 (35.29) | 0 | 0 | 3 (17.65) | 6 (35.29) | 2 (11.76) |
| Antibiotic | 9 (7.69%) | 2 (22.2) | 4 (44.44) | 0 | 0 | 0 | 3 (33.3) |
| Unknown | 44 (37.61%) | 9 (20.45) | 8 (18.18) | 2 (4.54) | 1 (2.27) | 11 (25) | 13 (29.54) |

Figure 2. Distribution of each pathogen according A) age group, B) lactation of children C) clinical symptoms, D) diarrhea intensity, E) degree of dehydration and F) treatment received.



specified pathogen with demographic and clinical characteristics and treatment was observed (Figure 2).

Pathogen detection

Co-infections were frequent (38 cases, 32.5%), with virus-bacteria co-infection being reported in 26 cases (22.2%). While infections caused by either a virus or bacterial pathogen occurred in 30 (25.6%) and 18 cases (15.4%) respectively. No pathogenic microorganism was detected in 31 samples (26.5%).

Analyses of distribution for virus and bacteria monoinfection or coinfection according to the age revealed that 40.9% of children of 6-11 months of age presented viral infection (virus monoinfection or virus-virus coinfection). In relation to breastfeeding habits, “formula” feeding was significantly related to the presence of viral infection ($p < 0.05$).

At least one pathogen was isolated in 86 out of the 117 fecal samples (73.5%). Rotavirus was most frequently isolated, followed by *Shigella*, adenovirus, norovirus, *C.coli* and DEC. (Table 2). Other pathogens showed a frequency less than 10. Monoinfection was found in 41% of the episodes. There were no significant associations between the pathogen and age groups as shown in Figure 2.

The presence of rotavirus and *Shigella* was found to be significantly associated with an age of more than 17 months, no special pathogen distribution was observed in other age groups (Figure 2).

Virus-bacteria co-infections were the most frequent, followed by virus-virus co-infections and bacterial-bacterial co-infections. In virus-bacterial

Table 2. Frequency of pathogens found (Absolute number) in diarrhea samples.

| Microorganisms | Prevalence N = 117 (%) |
|-----------------------------|------------------------|
| Rotavirus | 42 (35.9) |
| <i>Shigella</i> spp. | 30 (25.6) |
| Adenovirus | 17 (14.5) |
| Norovirus | 14 (11.9) |
| <i>Campylobacter coli</i> | 12 (10.3) |
| EAEC | 11 (9.4) |
| <i>Campylobacter jejuni</i> | 5 (4.3) |
| <i>Salmonella</i> spp. | 3 (2.6%) |
| EPEC | 1 (0.5) |
| ETEC | 0 (0) |

infections, rotavirus and *Shigella* was the most frequently found in 26 (22.22%) cases, followed by adenovirus with *Shigella* in 3 cases. Three samples contained at least 3 microorganisms in each sample. Viral-viral coinfection of rotavirus and adenovirus was found in 3 cases, followed by rotavirus and norovirus coinfection in 2 cases. Co-infections between bacteria were also observed. Notably *Shigella* and *Salmonella* coinfection and *Shigella* and *Campylobacter* coinfection were frequently observed (Table 3).

Discussion

The etiology and clinical course of acute gastroenteritis may vary depending of children characteristics and geographical regions. In the present study, we performed hospital surveillance using PCR methodology to determine diarrheagenic pathogens circulating in rural areas of Northern Peru in hospitalized children. In other studies, the isolation rate

Table 3. Etiological agent and coinfections found in diarrhea samples.

| Pathogen N = 48 (41.03%) | Isolates number(%) |
|---|---------------------------|
| Rotavirus | 18 |
| Norovirus | 9 |
| <i>Shigella</i> | 9 |
| Adenovirus | 3 |
| <i>Campylobacter jejuni</i> | 3 |
| <i>Campylobacter coli</i> | 3 |
| Diarrheagenic <i>E. coli</i> | 2* |
| <i>Salmonella</i> | 1 |
| Virus-Virus N = 6 (5.13%) | |
| Rotavirus+Adenovirus | 3 |
| Rotavirus+Norovirus | 2 |
| Adenovirus+Norovirus | 1 |
| Bacteria-bacteria N = 6 (5.13%) | |
| <i>Shigella-Salmonella</i> | 2 |
| <i>Campylobacter coli-Shigella</i> | 2 |
| <i>Shigella- EAEC</i> | 1 |
| <i>Campylobacter coli-EAEC</i> | 1 |
| Virus-bacteria N = 26 (22.22%) | |
| Rotavirus- <i>Shigella</i> | 7 |
| Adenovirus- <i>Shigella</i> | 3 |
| Rotavirus- <i>Campylobacter coli</i> | 2 |
| Rotavirus- <i>Campylobacter coli-Shigella</i> | 2 |
| Rotavirus- <i>Shigella-EAEC</i> | 2 |
| Adenovirus- <i>Campylobacter coli</i> | 2 |
| Rotavirus-Adenovirus- <i>Shigella</i> | 2 |
| Adenovirus-EAEC | 1 |
| Rotavirus-EAEC | 1 |
| Rotavirus-Norovirus-EAEC | 1 |
| Rotavirus- <i>Campylobacter coli-Campylobacter jejuni</i> | 1 |
| Rotavirus-Adenovirus- <i>Shigella-Campylobacter jejuni-EAEC</i> | 1 |
| Rotavirus-Norovirus-Adenovirus-EAEC | 1 |
| No infectious agent N = 31 (26.5%) | 31 |

*1 EAEC and 1 EPEC.

and prevalence of each pathogen also varies with the age of the patient and the type of study, i.e. community versus hospital, passive community surveillance versus active surveillance [14].

Although, the rotavirus vaccine was introduced by the Peruvian National Immunization Program in 2009, national reports from 2015 have shown that there is still a low vaccination coverage of 80.9% in children under 12 months [15]. Moreover, despite the increase in rotavirus immunizations by 17.1% in Cajamarca in the last 5 years, vaccination coverage was only 83.8% in 2015, with even lower values in the rural areas [16]. This low coverage couple with the high prevalence of rotavirus makes Cajamarca a very vulnerable population for severe cases of gastroenteritis [4]. In addition, the high prevalence of rotavirus observed in our study population can be explained due to the presence of new circulating genotypes not covered by the available vaccine (e.g. G12/P[6]) which have been previously reported by our investigation group [4]. In a

recent study performed in a peri-urban area of Lima, rotavirus was reported as a cause of diarrhea in only 4.2% cases, which is smaller than that found in our study [17]. This could potentially be due to differences in rotavirus vaccine coverage, which may be slightly lower in rural areas than in peri-urban, nutritional status, the origin of samples (hospital vs. community), or diagnostic techniques used.

We report a total of 14 (11.9%) cases of norovirus, 9 of which were found as the single causative agent. This contrasts with higher values previously reported in Lima, where norovirus was detected in 17.4% of 224 diarrhoeal samples from children younger than 24 months of age [18]. However, this difference may be related to the fact that this study was performed in samples which were already negative for other pathogens. In addition, norovirus was identified more frequently in children older than 12 months of age compared to younger children [18]. It is also reported that in previous years, following the introduction of

rotavirus vaccine, norovirus has been described as an emerging etiological agent of diarrhea among children in tropical areas [19].

The frequency of bacterial pathogens in this study such as *Salmonella* (2.6%) and *Shigella* (25.6%) was similar to previous reports [20]. Surprisingly, in the present study, diarrheagenic *E. coli* was less frequent than what has previously been reported in similar aged children in this country (9.2% STEC) [21]. Since previous reports in rural areas have described a high presence of diarrheagenic *E. coli*, we expected our results related to pathogenic *E. coli* to be higher [22].

Although exclusive breastfeeding is recommended, especially in the first 6 months of life, only 12 out of 24 (50%) infants under 6 months of age were exclusively breastfed in our study. Adherence to breastfeeding practice should be reinforced, since it is considered to be the most cost effective intervention against diarrhea in children, due to the protective compounds in mother's milk [23]. High rates (14.5%) of malnutrition were found in the studied children. This is consistent with national figures of 19.3% chronic malnutrition reported in the same age group in Peru. Although our rates are higher than the 1.4% reported in children up to 3 years of age in the same region [24], it is clearly apparent that malnutrition is an important challenge in some rural Peruvian regions [25].

Molecular tools such as PCR are rapid and sensitive, thus justify the increase in their use for diagnosis and epidemiology of enteric pathogens in stool samples. However, it may detect traces of asymptomatic carriage that does not cause a real infection, which is considered as limitation of this technique especially for studies performed in endemic areas. [26] This fact limits the use of conventional PCR for establishing the enteric pathogens detected from stool samples as the actual case of an infectious gastrointestinal disease. [27]

One of the study limitations is that detection of parasites was not performed. It has been previously reported that the prevalence of some pathogenic enteroparasites such as *Giardia lamblia*, is up to 28.1% in diarrheic children [28].

Conclusions

A high prevalence of rotavirus, as well as adenovirus and norovirus, was observed in this study. *Shigella* was the most common bacteria found in acute diarrhea. These results represent an important addition to the understanding of etiological agents implicated in diarrheal diseases in rural areas of Northern Peru and may contribute to the development of guidelines for

detection, better surveillance and prevention of diarrhea. Implementation of a better surveillance system is mandatory to identify the principal etiologies of gastroenteritis and to develop better prevention strategies and to reduce diarrhea associated mortalities.

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Authors' contributions

AC, PWE, FOP and JdVM designed the study protocol; JdVM was responsible for obtaining funding and laboratory work supervision; PWE, FOP, MJP, MA, GU carried out the laboratorial experiments. JB, HC responsible for the clinical assessment, AC responsible of sample collection and database completion; MJP drafted the manuscript; All authors read and approved the final manuscript. JdVM is guarantor of the paper.

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Corresponding author

Juana del Valle Mendoza, PhD
 Facultad de Ciencias de la Salud, Universidad Peruana de Ciencias Aplicadas,
 Avda. San Marcos cuadra 2,
 Lima 9, Chorrillos, Lima (Perú).
 Phone: 00-51-1-3133333 extension: 2701
 Fax: (0051) 313-3334
 E-mail: jdelvall@upc.edu.pe

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