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Detection of *Cryptosporidium spp*. Co-infection with Rotavirus in Children Under Five Years with Diarrhea

Faten K. Shayyal[1] *MSc*, **Huda D. Al-Marsomy**[2] *PhD*, **Shatha Hussain Ali**[3] *FIBMS*

[1]Al-Imamein Al-Kadhimein Medical City, Baghdad, Iraq, [2]Dept. of Microbiology, College of Medicine, Al-Nahrain University, Baghdad, Iraq, [3]Dept. of Pediatrics, College of Medicine, Al-Nahrain University, Baghdad, Iraq

Abstract

Background *Cryptosporidium spp*. and Rotavirus are recognized as important causes of acute gastroenteritis in children worldwide.

Objective To determine the frequency of *Cryptosporidium spp*. and Rotavirus in children under five years with diarrhea.

Methods This cross-sectional study included 100 stool samples collected from children complaining from diarrhea with age ranged between 1-60 months. Samples were collected from Al-Imamein Al-Kadhimein Medical City, Al-Kadhimiya Children Hospital and Central Child Teaching Hospital in Baghdad during the period from December 2021 to May 2021. Conventional polymerase chain reaction was used to detect *Cryptosporidium spp.* while enzyme-linked immunosorbent assay was used to detect Rotavirus.

Results The rate of *Cryptosporidium* infection was 39% and the rate of Rotavirus infection was 20.43%. Co-infection *Cryptosporidium* and Rotavirus found in seven patients.

Conclusion Infection with *Cryptosporidium* may facilitate with infection with Rotavirus.

Keywords Gastroenteritis, Cryptosporidium, Rotavirus, co-infection

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List of abbreviations: ELISA = Enzyme-linked immunosorbent assay, PCR = Polymerase chain reaction, RV = Rotavirus, SSU rRNA = Small subunit ribosomal 18S rRNA

Introduction

Acute gastroenteritis considered as the major cause of morbidity and mortality worldwide. About 63% of all diarrhea cases globally occur in children below five years of age. The mortality rate has been reached to 3-5 million cases per year and the majority of this rate occurs in developing countries [1,2].

Cryptosporidiosis is a worldwide infection caused by the protozoan of parasite of the Apicomplexan genus *Cryptosporidium*, usually present as gastroenteritis-like syndrome. The range of disease in seriousness from mild to severe and the site of infection, nutritional and immune status of the host are responsible of sign and symptoms. There is variation in the clinical picture with infecting species that emerging [3].

Cryptosporidium is the second major cause of moderate to severe diarrhea in children under five years, and an important cause of mortality worldwide after Rotavirus [4].

Rotavirus is the most common viral cause of acute gastroenteritis in infants and young

children throughout the world and a leading cause of high rates of morbidity and mortality. Approximately 702,000 children die per year by Rotavirus infection [5]. Rotavirus is a member of the genus Rotavirus, family Reoviridae; there are five species of this virus, referred to as A, B, C, D, and E. Rotavirus A, the most common species, causes more than 90% of infections in humans [6]. Nearly all children are infected by Rotavirus at least once by the age five years [7].
This study aimed to determine the frequency of *Cryptosporidium spp*. and Rotavirus as co-infection among children under five years with diarrhea that it helps to guide treatment decisions, which improves patient outcomes

Methods

One hundred patients with an age range from 1-60 months suffering from diarrhea were included in this cross-sectional study. Samples were taken from Al-Kadhimyia Hospital for children, Al-Imamain Al-Kadhimein Medical City and Central Child Teaching Hospital in Baghdad, Iraq. The study was conducted through the period from December 2021 to May 2021. Stool specimens were collected from each child included in this study in clean dry containers. Stool samples were divided into two portions. The first portion was for molecular and the second portion was for enzyme-linked immunosorbent assay (ELISA) that stored at -20 C°.
Verbal consent was taken from families of the children involved in the study. The study was approved by the Institutional Review Board (IRB) in 4-1-2022 approval No. (20211166)
Conventional polymerase chain reaction (PCR) was performed to detect *Cryptosporidium spp*. based on SSU rRNA gene from children with diarrhea stool sample. This method was carried out according to method described by Mead (2020) [8]. DNA extraction from stool sample by using EasyPure® Stool Genomic DNA Extraction Kit and according the manufacturer's instruction. The primer used for SSU rRNA gene is shown in table 1 [8].

Table 1. Primer used in this study

Primer	Sequence (5`→3`)
Cryptosporidium (830 bp)	
F	GGAAGGGTTGTATTTATTAGATAAAG
R	CTCATAAGGTGCTGAAGGAGTA

PCR products were examined electrophoretically in 1% agarose gels and visualized with advanced DNA stain after staining. Detection of Human Rotavirus antigen was done by ELISA Kit (Human Rotavirus RV-Ag ELISA Kit, SunLong, Biotech Cat.No.SL1544Hu) according to manufacturing instructions.

Statistical analysis

Microsoft Excel 2016 and the statistical package for social sciences (SPSS) 20.0 were used to conduct the statistical analysis for this prospective research. Using the independent sample t-test to compare numerical data. The lower acceptable threshold for statistical significance is below or equal to (0.05).

Results

According to the result of conventional PCR of this study, (39 out of 100) samples give positive result for *Cryptosporidium spp.* among children with diarrhea as shown in the table (2). Samples were subjected to molecular analysis by using specific primers sequences of small subunit ribosomal 18S rRNA gene specific primers (830bp), (Figure 1).

Table 2. Rate of *Cryptosporidium spp.* infection in patient samples according to the conventional PCR

Conventional PCR for *Cryptosporidium spp.*	Frequency	Percent
Negative	61	61.0
Positive	39	39.0
Total	100	100

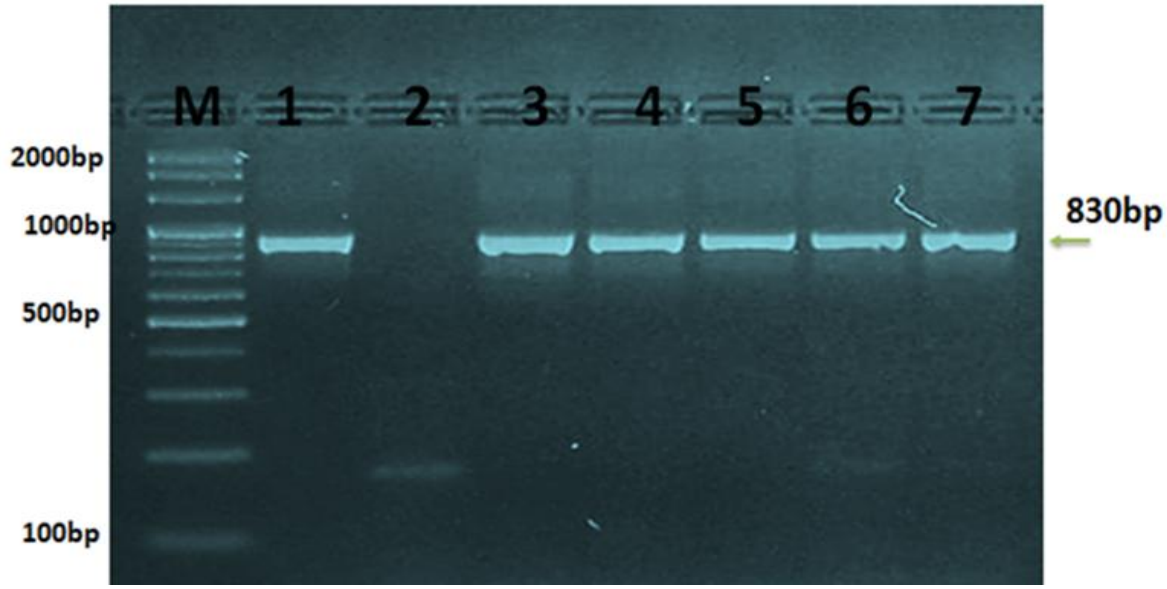

Figure 1. Gel electrophoresis of PCR product of 18S rRNA gene. Amplicon size shows 830bp for *Cryptosporidium spp.* (1,3,4,5,6,7) was positive while (2) was negative. Where M: marker (100-2000 bp)

In this study, the detection of Rotavirus antigen in 93 stool samples was screened by ELISA, the result show that 19 (20.43%) out of 93 patient samples were positive for Rotavirus antigen as shown in the table (3).

This study shows that 7 of the *Cryptosporidium spp.* infected children are co-infected with Rotavirus as shows in the table (4).

Table 3. Number of positive and negative Rotavirus antigen in patient

Rotavirus Ag	Frequency	Percent
Negative	74	79.57
Positive	19	20.43
Total	93	100

Table 4. Rate *of Cryptosporidium spp.* co-infected with Rotavirus

Cryptosporidium spp.* Rotavirus Crosstabulation		Rotavirus		Total	P value by Chi square test
		Positive	Negative		
Cryptosporidium spp.	Positive	7	32	39	0.8
	Negative	12	42	54	
Total		19	74	93	

Discussion

The rate of infection of *Cryptosporidium spp.* in current study was 39% using conventional PCR technique. Compared to previous studies from Iraq, the infection rate in humans by using the molecular technique in Baghdad was 47.33% and Thi-qar 45% [9,10]; the current study rate was nearly similar with them. Much lower infection rate by using PCR was recorded from neighboring countries such as Iran the infection rate was 4.94% [11], and in Jordon the frequency of *Cryptosporidium* infection by using molecular examination was 8.3% [12]. The disparity in infection prevalence of *Cryptosporidium spp.* among different studies may be attributed to the difference in local climatic situations, which determine the survival of oocysts and favors the dissemination of oocysts, samples size, sampling method, large or small population, diagnostic methods, the status of public health and sanitary services, season, sources of drinking water, living conditions, nutritional status, immune status and personal hygiene co-infection and levels of close contact with domestic animals, the infection rate reported being influenced by education level, socioeconomic status and age groups [13].

This study showed that 20.43% infection rate with Rotavirus antigen after testing 93 stool samples of children under 5 years of age suffering from diarrhea from Baghdad by using ELISA, compared to the previous studies performed locally like 17% in Baghdad city in 2015 [14], also, in Baghdad 79.6% in 2020 [15], Thi-Qar 45% in 2019 [16], and 42.5% in Tikrit city in 2022 [17]. Globally performed researches for Rotavirus also shows the different in range like 23% in Poland in 2021 [18] and 58% in Egypt in 2022 [19].

This study showed that 7 cases of *Cryptosporidium spp.* infected children are co-infected with Rotavirus. These results differ from other studies in Zakho city that showed the mixed infection of Rotavirus and *Cryptosporidium* was 1 (0.55%) [20].

Each diarrhea pathogen is capable of causing disease alone, but diarrhea also occurs in the presence of two or more pathogens, referred to as co-infections. Several studies have described diarrhea caused by co-infections in both developed [21-23] and developing countries [24-26].

The association of clinical severity and co-infections is controversial. Some studies have found that co-infections may cause more severe diarrhea than infection with either pathogen alone [22,24]. Other studies reported no differences in clinical severity between mono-infection and co-infections [27,28].

In conclusion, from 39 patients infected with *Cryptosporidium spp.*, seven cases were rotavirus positive, thus, the prolonged infection with *Cryptosporidium spp.* may facilitate the infection with the virus.

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Author contribution

Shayal: did the laboratory work and write the article. Dr. Al-Marsomy: Supervision of the study and final editing of the manuscript. Dr. Ali: consultant.

Conflict of interest

The authors declared that they have no conflict of interest.

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Correspondence to Dr. Huda D. Al-Marsomy
E-mail: hudadhaher@nahrainuniv.edu.iq
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