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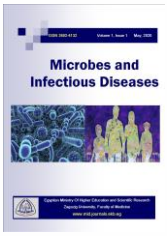

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Original article

Screening for Rotavirus Group A and *Campylobacter* co-infection among Egyptian children with acute gastroenteritis in a tertiary care hospital

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ABSTRACT

Background: This cross-sectional screening study aims to investigate the frequency, epidemiologic, and clinical features of Rotavirus group A (RVA) and *Campylobacter* co-infection among children diagnosed with acute gastroenteritis (AGE). **Methods:** Ninety-two stool samples were collected from children under 5 years of age with AGE. Stool samples were screened for RVA and *Campylobacter* using enzyme immunoassays. **Results:** RVA VP6 antigen was detected in 22 samples, representing 23.9%. Four samples tested positive for *Campylobacter*, accounting for 4.3% of the total samples analyzed. All samples tested negative for *Campylobacter* mono-infection. *Campylobacter* and RVA co-infection was more common in older children (17.5 months vs. 10 months, p=0.01) and was prevalent in the summer (p=0.019). Compared to RVA mono-infection, *Campylobacter* and RVA co-infection was associated with elevated fever (p=0.0001), increased total leukocyte count (p=0.012), higher CRP levels (p=0.007), and a positive response to anti-infective medications (p=0.047). **Conclusion:** RVA infection is a common etiology of acute diarrhea among children in Egypt. Despite the infrequency of RVA and *Campylobacter* co-infections, laboratory analyses can aid in predicting these conditions and should be incorporated into the design of individualized treatment plans.

Introduction

Diarrhea remains a significant contributor to child mortality, particularly in developing countries, making precise pathogen diagnosis essential for effective surveillance, prevention, and control strategies**[1].** Among the etiological agents, Rotavirus group A (RVA) represents a significant contributor to diarrhea in infants worldwide, accounting for nearly 20% of diarrhea-related mortalities in children under five years of age. Low-income countries that lack RVA vaccination programs are particularly affected by RVA diarrhea **[2, 3].** Despite substantial global reductions in the burden of RVA over the past three decades, it continues to be high in regions such as Africa, Oceania, and South Asia **[4].**

The rapid and accurate diagnosis of RVA continues to pose significant challenges, especially in middle- and low-income countries. RVA infection is diagnosed through the detection of its viral antigens or the RNA genome. Reverse transcription-polymerase chain reaction (RT-PCR)

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demonstrates greater sensitivity. However, its high cost renders it often impractical for surveillance studies, especially in resource-limited settings. Conversely, latex agglutination tests and immunochromatographic assays are cost-effective but have limitations in sensitivity and specificity, which restricts their widespread application. Enzyme immunoassay (EIA) serves as an effective and cost-efficient diagnostic tool for RVA surveillance studies **[5].**

Campylobacteriosis, a well-known gastrointestinal infection, is primarily caused by C. jejuni and C. coli. These bacteria can infect humans via multiple pathways, including the ingestion of undercooked meat, especially poultry, and the consumption of contaminated water and milk. Furthermore, direct interaction with farm animals, including poultry and livestock, may facilitate the transmission of these bacteria. Campylobacteriosis in humans presents with symptoms including watery and/or bloody diarrhea, abdominal pain, cramps, fever, malaise, and vomiting **[6]**. It poses a particular risk to young children, who are more susceptible to dehydration and nutrient loss, including sodium and protein, due to diarrheal illness **[7].**

Stool culture has traditionally been regarded as the most reliable method for diagnosing *Campylobacter* infection. The challenges of culturing *Campylobacter*, which can be time-consuming and may require several days for conclusive results, restrict its effectiveness relative to rapid molecular techniques. Molecular testing may be impractical in resource-limited regions. Considering the potential severity of *Campylobacter* infections and the risk of complications, EIA represents a rapid and dependable diagnostic method, particularly in resource-constrained areas **[8]**. Moreover, EIA has been shown to exhibit greater sensitivity than culture methods, which may significantly under-detect *Campylobacter*. In contrast, EIA effectively detects a wider range of *Campylobacter* species, including non-C. jejuni/ coli species **[9]**.

Previous studies have demonstrated the occurrence of co-infections between enteric bacteria and viruses, highlighting the important influence of co-infection by bacterial and viral pathogens on disease progression. Infections caused by RVA and *Campylobacter* exhibit a spectrum of severity, from mild to severe, and may result in complications **[10-14]**.

This study adopted a cross-sectional screening design examining the frequency, epidemiologic, and clinical features of RVA and *Campylobacter* co-infection among children with AGE.

Methods

Study design and Ethical approval:

This cross-sectional study was conducted at the Medical Microbiology and Immunology Department and the Pediatrics Department of the Faculty of Medicine, Zagazig University, during the period from December 2021 to December 2023 in compliance with the Declaration of Helsinki. Approval was obtained from the Institutional Review Board (IRB) no. 9272. Written informed consent was obtained from the guardians of all pediatric patients participating in this study. Assuming a prevalence of 10% for Rotavirus-associated diarrhea among Egyptian children **[15]**, and with a total of 272 children meeting the inclusion criteria during the study period at our hospital, the sample size was estimated to be 92 children at a 95% confidence level, calculated using Epi Info version 7.2.5.0.

Sample collection:

Ninety-two stool samples were collected from children with AGE who were admitted to Zagazig University Pediatric Hospital. The inclusion criteria were children aged <5 years who had been diagnosed with acute diarrhea and had been hospitalized within less than 48 hours. Acute diarrhea was defined as the passage of 3 or more loose or watery stools a day for 14 days. The exclusion criteria included children with chronic diarrhea persisting for more than 2 weeks and children with other gastrointestinal disorders eg. malabsorption, chronic diseases, or immune disorders. Data regarding medical history, clinical examination, and laboratory investigations were recorded. Samples were collected in clean, sterile, leak-proof containers, transported to the laboratory within 1 hour **[16],** and stored at -20°C for EIA tests.

Screening for RVA in stool samples:

Stool samples were screened for RVA using the RIDASCREEN® Rotavirus EIA kit (R-Biopharm AG, Germany) to detect the RVA-specific VP6 antigen following the sandwich ELISA technique according to the manufacturer's instructions **(Fig 1a)**.

Screening for *Campylobacter* in stool samples:

Screening for *Campylobacter* in stool samples was conducted using the RIDASCREEN® *Campylobacter* EIA kit (R-Biopharm AG, Germany) to detect *Campylobacter* antigen utilizing the sandwich ELISA technique according to the manufacturer's instructions **(Fig 1b)**.

Statistical analysis:

Data were collected, tabulated, and statistically analyzed using IBM Corp., released in 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp. Quantitative data were expressed as Mean ± Standard deviation or Median (Interquartile Range), while qualitative data were expressed as numbers and percentages. The Mann-Whitney U test was used to compare two groups exhibiting non-normal distribution. Percentages of categorical variables were compared using the Chi-square test or Fisher's exact test when appropriate. All tests were two-sided. A p-value < 0.05 was considered statistically significant, whereas a p-value ≥ 0.05 was considered statistically insignificant.

Results

The demographic, clinical, and laboratory data of the study population are presented in **Table 1**. RVA was detected in 22 of 92 samples, representing 23.9% of the entire study population. Of the 22 samples examined, 18 were positive only for RVA mono-infection (19.6% of the total study population), while 4 of the 22 samples were positive for both RVA and *Campylobacter* co-infection (4.3% of the entire study population), as demonstrated in **Fig 2**.

RVA and *Campylobacter* co-infection and RVA mono-infection demonstrated no statistically significant differences in terms of gender or feeding pattern. However, RVA and *Campylobacter* co-infection was more common in older children (17.5 months vs. 10 months, $p < 0.01$) and was prevalent in summer ($p = 0.001$), as presented in **Table 2**.

The clinical and laboratory findings of the studied children indicated that RVA and *Campylobacter* co-infection was associated with higher fever ($p = 0.0001$), elevated total leukocyte count ($p = 0.012$), higher CRP levels ($p = 0.007$), and positive response to anti-infective medications ($p = 0.047$) compared to RVA mono-infection, as presented in **Table 3**

Table 1. Demographic, clinical, and laboratory characteristics of the total study population (n = 92).

Variables		**N.**	**%**
Sex	**Females**	40	43.5
	Males	52	56.5
Age per months	**Median (IQR)**	13(9-18)	
Feeding pattern	**Breast feeding**	30	32.6
	Other feeding	62	67.4
Duration of diarrhea in days	**Median (IQR)**	5(3-6)	
Frequency of diarrhea time/day	**Median (IQR)**	8(7-11)	
Body temperature°C	**Mean ±SD**	39.06±1	
Duration of Vomiting in days	**Median (IQR)**	3(1-4)	
Frequency of vomit time per day	**Median (IQR)**	6(5-6)	
Total leucocyte count *10^3/mm^3	**Median (IQR)**	15*10^3(11.5-19)	
C reactive protein mg/l	**Median (IQR)**	10.5(7.3-14)	
Medications	**Antidiarrheal drugs + Zinc supplement**	40 (43.5)	
	Anti-infective drugs	52 (56.5)	

IQR: interquartile range, SD: standard deviation

Table 2. Demographic data and seasonal distribution of cases of RVA and Campylobacter co-infection compared to RVA mono-infection.

Variable		RVA and Campylobacter co-infection N. 4	RVA mono-infection N. 18	x^2	p value
Age in months	**Median (IQR)**	17.5(16.25-24)	10(7.75-12)	**2.56**	**0.01***
Gender	**Male**	1(8.3%)	11(91.7%)	f	0293
	Female	3(30.0%)	7(70.0%)		
Feeding pattern	**Breast feeding**	1(33.3%)	2(66.7%)	f	0.94
	Other feeding	3(15.8%)	16(84.2%)		
Season	**Winter season**	1(9.1%)	10(90.9)	x^2 10.01	**0.019 ***
	Spring	1(12.5%)	7(87.5%)		
	Summer season	2(100.0%)	0(0.0)		
	Autumn season	0(0.0)	1(100.0%)		

x^2: chi-square test, f: fisher's exact test, p: ≥ 0.05 non-significant, p: < 0.05 significant, IQR: interquartile range.

Table 3. Clinical and laboratory findings of children with RVA and Campylobacter co-infection compared to RVA mono-infection.

Variable		RVA and *Campylobacter* co-infection N. 4	RVA mono-infection N.18	*u*	p value
Duration of diarrhea/days	**Median (IQR)**	4(3-8)	5(3-5)	0.044	0.97
Frequency of diarrhea time/day	**Median (IQR)**	10(7-14)	8(6-13)	0.699	0.49
Body temperature	**Mean±SD**	40 ±0.88	38.4±0.5	5.22	**0.0001***
Duration of Vomiting/days	**Median (IQR)**	2(1-6)	3(1.75-3)	0.22	0.825
Frequency of vomit time/day	**Median (IQR)**	8(5-12)	7(4-11)	0.70	0.480
Total leucocyte count *103/mm3	**Median (IQR)**	21(12.75-25)	8.6(7.3-10.08)	2.51	**0.012***
C reactive protein mg/l	**Median (IQR)**	21.5(11.5-25.65)	7.5(5.75-9)	2.69	**0.007***
Medications	**Antidiarrheal drugs + Zinc supplement**	1(25.0)	16(88.9)	f	**0.047***
	Anti-infective drugs	3(75.5)	2(11.1)		

x^2: chi-square test, f: fisher's exact test, u: mann-whitney test, IQR: interquartile range, SD: standard deviation, p: ≥ 0.05 not significant, p: < 0.05

Figure 1. ELISA test for (a) Rotavirus A VP6 antigen and (b) for *Campylobacter* antigen.

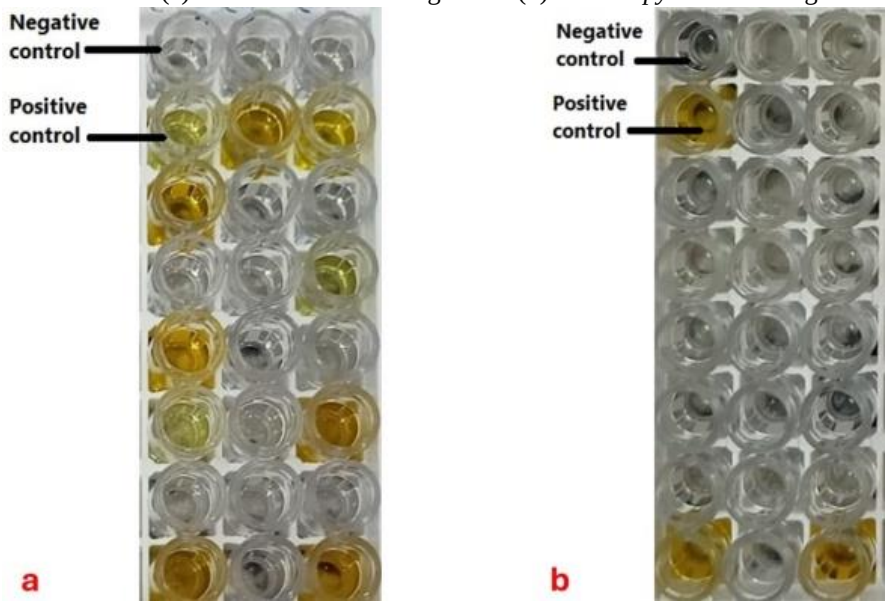
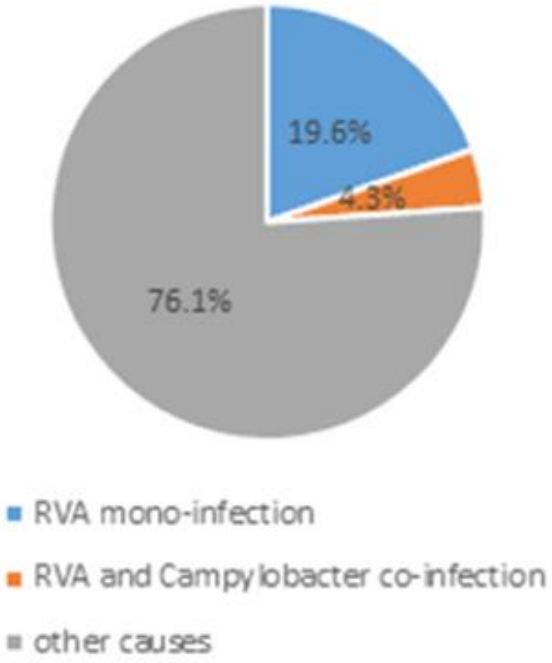


Figure 2. Frequency of Rotavirus A and *Campylobacter* infections in the study population.



Discussion

Rotavirus and *Campylobacter* are prevalent etiological agents of gastroenteritis in infants and children**.** Traditional laboratory diagnostic tests commonly used in less developed countries exhibit limitations in detecting slow-growing bacteria and non-bacterial pathogens that cause diarrhea in children under five years of age, resulting in significant delays in treatment **[17]**. The current study aims to investigate the frequency, epidemiologic, and clinical features of RVA and *Campylobacter* co-infection among children with AGE.

In the study, RVA was identified in 22 out of 92 stool samples, accounting for 23.9% of the total. Previous studies have demonstrated variations in the prevalence of RVA infections among children in Egypt. According to Matson et al **[18]**, approximately 25.2% of samples collected from hospitalized children with gastroenteritis tested positive for RVA. Similarly, another study examining hospitalized children in Egypt under five years of age revealed that 31% of the samples tested positive for RVA **[19].** In contrast, a surveillance study conducted in two different hospitals, which primarily observed clinic-based cases of diarrhea in Egyptian children, revealed that 23% and 10% of the cases, respectively, were associated with RVA **[15]**. Additionally, a study conducted in a primary health care center involving Egyptian children under five years of age with acute diarrhea indicated that RVA was the most frequently detected organism in 10.7% of cases **[20].** Another investigation involving both inpatient and outpatient children found that 39.1% of the children tested positive for RVA, with a higher detection rate among inpatients (43.9%) compared to outpatients (29.9%) **[21]**. The discrepancies in findings may be due to variations in the diagnostic techniques utilized in these studies **[22]**.

Four samples positive for RVA were also positive for *Campylobacter*, accounting for 4.3%, with no samples exhibiting *Campylobacter* mono-infection. Previous investigations conducted in Egypt have exhibited significant variations in their findings concerning the prevalence of *Campylobacter* infections. For instance, a study carried out in Assiut found that 27.5% of the 80 human stool samples collected from both children and adults tested positive for *Campylobacter* using culture and molecular techniques **[23]**. A prospective study conducted in Abu Homos from 1995 to 2003 utilized culture-based methods to isolate *Campylobacter* from 9.37% of 6,562 fecal samples collected from 1,057 children aged up to 36 months **[24]**. A culture-based analysis conducted in the Gharbia Governorate involving 106 children aged 7 to 15 years revealed a prevalence rate of 12.3% for C. jejuni and 2.8% for C. coli **[25]**. These disparities could be attributed to variations in the age

groups included in the studies and the diverse detection techniques utilized.

According to a study conducted in Pakistan, the prevalence of RVA and *Campylobacter* infections among children with diarrhea was investigated. The study found that the detection rate of RVA was 26.4%, while *Campylobacter* was detected in 52% of the samples. Furthermore, co-infection with both pathogens was identified in 21.8% of the cases **[12]**. Similarly, a study from Nepal reported that 30.0% of stool samples from children under five years of age with AGE were positive for co-infection with RVA and *Campylobacter*. Additionally, RVA mono-infection was detected in 20.1% of the samples, and *Campylobacter* mono-infection was found in 26.7% of the samples **[13]**. A cross-sectional study conducted in Vietnam evaluated the prevalence of RVA infections and associated co-infections by analyzing 171 rectal swabs from children with acute diarrhea. The findings revealed that RVA was the most commonly identified pathogen, present in 42.7% of samples, whereas *Campylobacter* was detected in 7.6% of cases. The co-infection rate with other bacterial or viral pathogens alongside RVA was 45.2%. Co-infection with both RVA and *Campylobacter* accounted for 21.2% of the co-infection cases **[14].**

The present study indicates that co-infection with RVA and *Campylobacter* was more common in older children compared to RVA mono-infection (17.5 months vs. 10 months, $p < 0.01$). This may be associated with an increased risk of recurrent *Campylobacter* infection, which is foodborne and zoonotic, in children over one year of age due to the consumption of contaminated water or food and contact with animals. However, the rate of *Campylobacter* infection usually declines with age due to acquired immunity **[26].**

In this study, co-infection with *Campylobacter* was more prevalent in the summer ($p = 0.019$). A previous cohort study conducted in Egypt over three years reported a peak in *Campylobacter* enteritis during the summer **[27]**. Developed countries generally exhibit elevated rates of *Campylobacter* infections during summer and autumn. Conversely, developing countries usually show no seasonal preference regarding *Campylobacter* infections, and the peaks in the isolation of *Campylobacter* vary geographically, not only between countries but also within the same country. This variance can be attributed to inadequate surveillance of epidemics in specific regions **[28]**.

This study revealed a significant association between co-infection and various clinical and laboratory findings in the study population. Compared to RVA mono-infection, co-infection was associated with a higher fever ($p = 0.0001$), an elevated total leukocyte count ($p = 0.012$), higher CRP levels ($p = 0.007$), and a positive response to anti-infective medications. A previous study found that patients co-infected with both a virus and a bacterium had significantly higher levels of C-reactive protein (a marker of inflammation) compared to those infected with multiple viruses or with RVA alone **[29]**.

There was no significant association between the severity of diarrhea and vomiting among children with co-infections compared to RVA mono-infections. This finding contradicts some studies that proposed co-infections from multiple pathogens could enhance each other's pathogenic strategies, leading to severe diarrhea **[30; 13]**.

In conclusion, this study highlights the significant impact of RVA infection as a common etiological factor of acute diarrhea in Egyptian children. Despite the lower prevalence of RVA and *Campylobacter* co-infection observed in this study, laboratory investigations could predict such conditions and should be considered when designing individual treatment plans. Prompt identification of the causative pathogen among various potential infections facilitates improved management of acute diarrhea in children.

Statements and Declarations

Financial disclosure

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Conflict of interests

The authors have no relevant financial or non-financial interests to disclose.

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by **DAA**, **HAA** and **GMA.** The first draft of the manuscript was written by **EAA** and **DAA** and all authors

commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Institutional Review Board of Zagazig University (IRB) no. 9272

Consent to participate

Written informed consent was obtained from the parents or legal gardians of all pediatric patients participating in this study

Consent to publish

Consent to publish has been received from the parents or legal gardians of all pediatric patients participating in this study

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Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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