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ORIGINAL ARTICLE

Parasitic and Viral Gastroenteritis among Pediatric Egyptian Patients less than Five Years

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ABSTRACT

Key words:
Gastroenteritis, Children, Parasites, Rotavirus, Adenovirus

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Background: *Acute gastroenteritis is one of the most common human infectious diseases. In low-income countries, diarrhea is the second principal cause of mortality in children less than the age of sixty months.* ***Objectives:*** *This cross-sectional research aimed to detect intestinal parasites, rotavirus, and adenovirus and their coinfection occurrence in 100 children not more than 5 years with acute gastroenteritis.* ***Methodology:*** *Part of each stool sample collected was examined microscopically to detect enteric parasites using a direct wet mount, sedimentation techniques, and modified Ziehl-Nelseen stain. The other part was used in the identification of rotavirus and adenovirus antigens by immunochromatographic assay.* ***Results:*** *The percentages of the infective causative agents of diarrhea were as follows: rotavirus (80%), adenovirus (19%), Blastocystis spp. (4%) and finally, Cryptosporidium spp. (1%). Co-infection percentages of rotavirus with adenovirus, was 15%, rotavirus with Blastocystis spp. (3%), and rotavirus with Cryptosporidium spp. (1%). Seasonal distribution of rotavirus infection recorded a high occurrence in autumn, winter, and spring (92.6%, 92%, and 87%, respectively) (P-value <0.001). The seasonal peak of adenovirus was in winter (60.9%) which was significant statistically P-value < 0.001. According to the seasonal pattern of intestinal parasites, Blastocystis spp. infection occurrence was 8% in autumn, 4% in summer, and 3.7% in spring. Only one case of Cryptosporidium spp. was detected in summer (4%).* ***Conclusion:*** *Rotavirus and adenovirus constitute a significant portion of acute gastroenteritis causative agents in children, while Blastocystis spp. and Cryptosporidium spp. appeared to be the most likely encountered parasitic infections in children with acute gastroenteritis.*

INTRODUCTION

Microbial gastrointestinal diseases affect people of all ages, but they specifically increase morbidity and mortality in infants and children[1]. Although viruses, bacteria, and parasites are the causes incriminated in infectious gastroenteritis, viruses became a leading cause with recent progress in viral infections diagnosis, particularly rotavirus and adenovirus. According to the Global Enterics Multicenter Study, adenovirus was the second most common viral agent of gastroenteritis following rotavirus in infants[2]. Other viruses involved in childhood diarrhea include norovirus, sapovirus, and astrovirus[3]. Both rotavirus and adenoviruses spread feco-orally leading to diarrhea and other symptoms[4].

The World Health Organization (WHO) reported that acute gastroenteritis caused above hundred thousand child deaths in 2015 worldwide, and 9% of deaths in children under five years[5]. Rotavirus vaccines have been recommended for widespread use for infant in 2009 and were used in many countries causing a decrease in rotavirus-specific gastroenteritis frequency and death. Due to their impact, rotavirus vaccines might be anticipated to change the epidemiology of incriminated enteropathogens[6].

Using quick, simple, low-cost procedures to diagnose viral acute gastroenteritis is preferable. Immunochromatography tests (ICT) used to detect copro antigens of rotavirus and adenovirus show high level of sensitivity and specificity. ICT is simple, rapid, and equipment-independent test[7].

Consumption of raw vegetables, animal keeping, education level, and water supply resources play a great role in the spread of intestinal parasites that cause gastroenteritis[8]. *Cryptosporidium* spp., *Giardia intestinalis*, and *Entamoeba histolytica* are the most common causative parasitic agents as regards childhood diarrhea[9]. Parasitic infections may manifest as plentiful mucus or bloody stools, abdominal colic, tenesmus (*E. histolytica*), or as a malabsorption disorder (*Giardia*)[10].

In the current study, we aimed to detect intestinal parasitic infections versus rotavirus as well as adenovirus infections in addition to their co-infection occurrence in children whose ages range from one month to 60 months suffering from diarrhea and to investigate possible factors correlated to their occurrence particularly as coinfections and possible associations with certain risk factors, seasonal variation, and the different inducers of infection could be revealed.

METHODOLOGY

Study design:

This cross-sectional study enrolled 100 stool samples taken from 100 infants and young children, whose age was less than five years who were suffering from acute gastroenteritis and attending the gastroenteritis emergency unit of Abu El-Reesh Pediatric Teaching Cairo University Hospital from December 2023 to November 2024 to investigate parasitic and viral causes of gastroenteritis. The study was approved by the Clinical Research Ethics Committee of the Faculty of Medicine, Cairo University **(Approval number: N-427-2023)**. The study adhered to the principles of Helsinki Declaration. Written informed parental or guardian consent was obtained for each participant.

Study population:

Participants of both genders, aged from 1 month to 60 months, suffering from gastrointestinal complaints were enrolled in this study. Gastrointestinal manifestations included abdominal pain, diarrhea, nausea, and vomiting. Exclusion criteria were patients above 5 years of age receiving anti-diarrheal or anti-parasitic treatment. Using a designed questionnaire, patients' age, gender, breastfeeding duration, animal contact history, season of admittance, and vaccination history were recorded.

Parasitological diagnosis:

Stool samples from each participant were collected in labeled sterile cups and were examined in the Diagnostic and Research Unit of Parasitology (DRUP) at Medical Parasitology Department, Faculty of Medicine, Cairo University. Samples were inspected macroscopically for consistency, mucus, and/or blood, then examined microscopically by low-power and high-power magnifications. After adding a drop of iodine, a wet mount examination was done, followed by sedimentation technique[11] for the detection of parasitic infections, including trophozoites, cysts, oocysts, or helminths' eggs. A modified Ziehl-Neelsen (MZN) stain[12] was done from concentrated fecal samples and examined for oocysts of *Cryptosporidium*.

Detection of adenovirus and rotavirus antigens in stool:

Adenovirus and rotavirus antigens in the stool were screened in all patients' stool samples using the Rotavirus and Adenovirus Combo Rapid Test Cassette {feces} (RightSign, Biotest, China) following the manufacturer's instructions. It is a rapid (providing results within 10 minutes), chromatographic, lateral flow immunoassay for selectively specific detection of rotavirus and adenovirus in human feces qualitatively **(Figure 1)**.

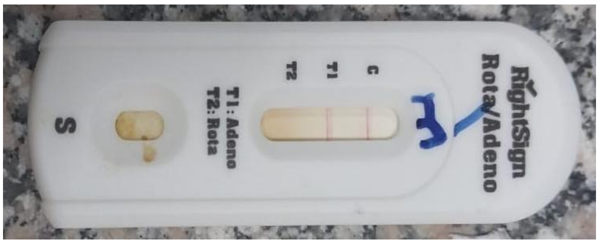

Fig. 1: Rotavirus and Adenovirus Combo Rapid Test Cassette (RightSign). It harbored two apertures; smaller one on the left side (S) slot for prepared stool sample administration and a longer one to the right for reading reaction bands results. Result interperatation was as follows; (C) control band (must appear indicating cassette integrity as in image above), (T_1) adenovirus detection band and (T_2) rotavirus detection band. One or both viral detection bands could be positive; in this cassette sample showed positive result for adenovirus (T_1).

Procedure steps according to the manufacturer's instructions were done as follows:

PPE was worn, and the infection control measures were applied, and all the used items were discarded according to the local regulations.

The sealed pouches of the test were stored at room temperature (15-30℃) till usage. Samples were collected at the onset of diarrheal symptoms as stated by the manufacturer. Sufficient feces specimens were put (1-2 ml or 1-2 g) in a clean, dry, waterproof container. The specimens were neither refrigerated nor stored. The assay was performed in the cassette within minutes after collection of fresh specimens to obtain the best results.

For solid specimens: The specimen collection tube cap was unscrewed, and the specimen was randomly stabbed in at least three different sites by the specimen collection applicator to collect 50 mg of feces. The sample was not scooped (None of the specimens collected was solid as all cases complained of watery diarrhea).

For liquid specimen: The fecal specimens were aspirated with the dropper held vertically, and two drops (~ 50 µL) were transferred into collection tube with the extraction buffer, cap was tightened and shaken for mixing the specimen with the extraction buffer.

The collection tube was held upright. The tip of it was unscrewed then inverted, and 2 drops of extracted specimens (~ 80 µL) were transferred to the specimen well in cassette. Air bubbles were avoided.

The results were read within 10 minutes and interpreted as follows:

- **Positive for rotavirus:** if red lines appeared in control region (C), and T2 region.
- **Positive for adenovirus:** if red lines were seen in C region, and T1 region.

- **Positive for both:** if three red lines were seen in C, T1 and T2 regions, respectively.
- **Negative:** if one red line appeared only in C region and **invalid** if no lines were seen.

Data statistical analysis:

Data were coded and entered using the statistical package for the Social Sciences (SPSS) version 28 (IBM Corp., Armonk, NY, USA). Data were summarized using mean, standard deviation, median, minimum, and maximum in quantitative data and using frequency (count) and relative frequency (percentage) for categorical data. The non-parametric Kruskal-Wallis and Mann-Whitney tests were used to compare between quantitative variables[13]. Chi-square ($\chi 2$) test was performed to compare categorical data. The exact test was used instead when the expected frequency was less than 5[14]. Logistic regression was done to detect independent predictors of rotavirus and adenovirus infection[15]. *P*-values less than 0.05 were considered statistically significant.

RESULTS

The age of participant's mean± standard deviation (SD) was 11.25±10.38 months.

The ratio of males: females was 2:1. None of the participants were vaccinated by any of the rotavirus vaccines. Most patients were complicated with dehydration (92%). Fewer patients suffered from vomiting and fever. The majority of the participants were breastfed for more than 6 months. History of animal contact was reported in only 9% of the cases. Data regarding the demographic and clinical characterization of participants has been displayed in Table 1.

Table 1: Demographic and clinical characterization of the participants

	Mean	Standard Deviation	Median	Minimum	Maximum
Age (months)	11.25	10.38	8.00	1.00	60.00
				Count	**%**
Sex			**Male**	66	66.0%
			Female	34	34.0%
Animal contact			**Positive**	9	9.0%
			Negative	91	91.0%
Breastfeeding			**Positive**	63	63.0%
			Negative	37	37.0%
Dehydration			**Positive**	92	92.0%
			Negative	8	8.0%
Vomiting			**Positive**	83	83.0%
			Negative	17	17.0%
Fever			**Positive**	40	40.0%
			Negative	60	60.0%

For the effect of seasonal variation on demographic and clinical factors affecting diarrhea among the involved children, a significant occurrence change was recorded for clinical symptoms namely vomiting, with highest percentage in spring and lowest during the summer (P- value = 0.003), to be followed by fever, with highest frequency during autumn and least in the winter season (P-value < 0.001). Dehydration seemed to be a constant finding in all seasons with no significant change. Also, animal contact increased during autumn and summer, while breastfeeding was reported more during winter and spring, but both with no significant relation detected **Table 2.**

By analysis of the causative agents of diarrhea among the participants, the percentages of the enteric pathogens were as follows: rotavirus (80%), adenovirus (19%), *Blastocystis* spp. (4%) **(Figure 2)** and finally, *Cryptosporidium* spp. (1%) **(Figure 3)** [**Table 3].**

Table 2: Factors affecting diarrhea with seasonal variation among involved children

		Spring (N=27)		Summer (N=25)		Autumn (N=25)		Winter (N=23)		*P* value
		Count	%	Count	%	Count	%	Count	%	
Sex	**Male**	19	70.4%	15	60.0%	17	68.0%	15	65.2%	0.877
	Female	8	29.6%	10	40.0%	8	32.0%	8	34.8%	
Animal contact	**Positive**	1	3.7%	3	12.0%	4	16.0%	1	4.3%	0.387
	Negative	26	96.3%	22	88.0%	21	84.0%	22	95.7%	
Breastfeeding	**Positive**	19	70.4%	12	48.0%	12	48.0%	20	87.0%	0.011
	Negative	8	29.6%	13	52.0%	13	52.0%	3	13.0%	
Dehydration	**Positive**	27	100.0%	23	92.0%	21	84.0%	21	91.3%	0.198
	Negative	0	0.0%	2	8.0%	4	16.0%	2	8.7%	
Vomiting	**Positive**	27	100.0%	16	64.0%	20	80.0%	20	87.0%	0.003
	Negative	0	0.0%	9	36.0%	5	20.0%	3	13.0%	
Fever	**Positive**	15	55.6%	6	24.0%	16	64.0%	3	13.0%	<0.001
	Negative	12	44.4%	19	76.0%	9	36.0%	20	87.0%	

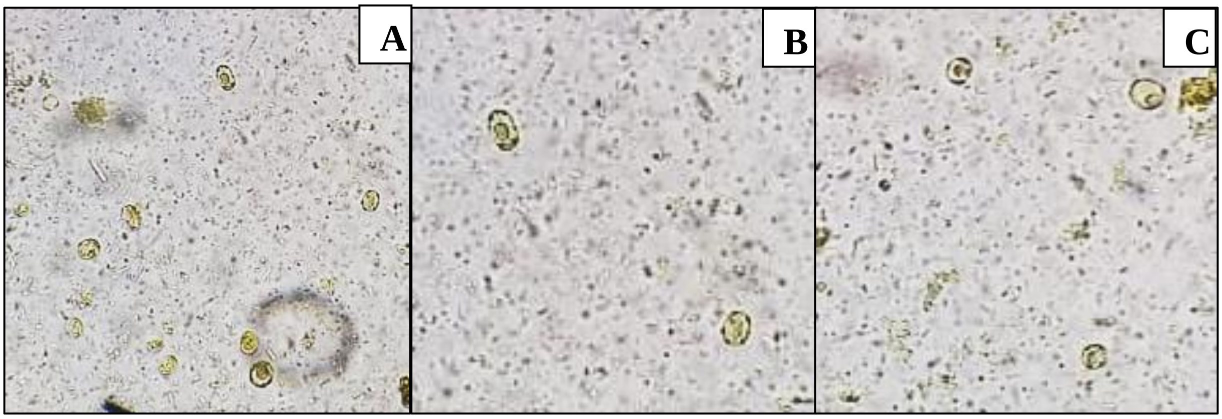

Fig. 2: Light microscopic high power images of *Blastocystis* spp. wet mount stained with Lugol's iodine. *Blastocystis* appeared numerous, of variable sizes within average range, with an outer rim of cytoplasm lodging more than one nucleus (vacuolar forms) (A). Many *Blastocystis* showed stained granular inclusions in central vacuole (granular forms) (zoomed images B and C) (mag. x400).

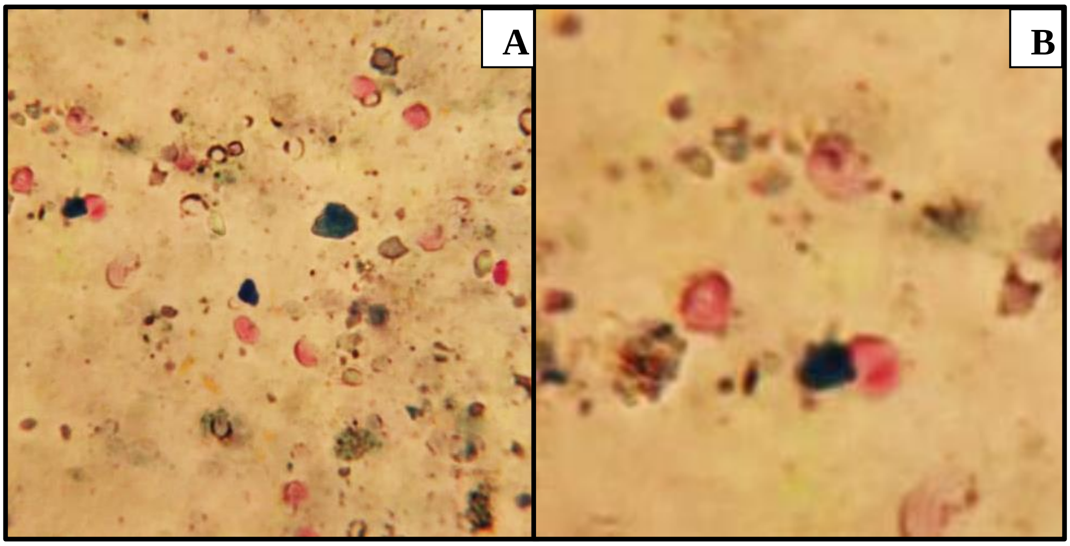

Fig. 3: An oil immersion lens - light microscopic image of *Cryptosporidium* spp. oocysts stained with Modified Ziehl Nelseen stain. Oocysts appear as spherical pale heterogeneous red bodies against a molted greyish blue background. Heavy infection with multiple oocysts (A) could be detected, with some incompletely stained oocysts. Heterogeneous staining representing internal sporozoites could be seen in zoomed image (B) (mag. x1000).

Table 3: Distribution of pathogens detected in children with gastroenteritis

		Count	%
Rotavirus	**Positive**	80	80.0%
	Negative	20	20.0%
Adenovirus	**Positive**	19	19.0%
	Negative	81	81.0%
***Blastocystis* spp.**	**Positive**	4	4.0%
	Negative	96	96.0%
***Cryptosporidium* spp.**	**Positive**	1	1.0%
	Negative	99	99.0%

As regards coinfection of rotavirus with adenovirus, 15 patients (15%) were positive for both infections, while coinfection of rotavirus with *Blastocystis* spp. was found in 3 patients (3%). In addition, rotavirus coinfection with *Cryptosporidium* spp. existed in 1 patient only (1%). Therefore, 80% of detected intestinal parasites appeared as co-infections with rotavirus [**Table 4].**

Table 4: Coinfection occurrence of rotavirus with adenovirus, *Blastocystis* spp., and *Cryptosporidium* spp.

Coinfection		Count	%
Rotavirus+ adeno virus	**Positive**	15	15.0%
	Negative	85	85.0%
Rotavirus+ *Blastocystis* spp.	**Positive**	3	3.0%
	Negative	97	97.0%
Rotavirus+*Cryptosporidium* spp.	**Positive**	1	1.0%
	Negative	99	99.0%
Rotavirus+ all intestinal parasites	**Positive**	4	4.0%
	Negative	96	96.0%

The results showed the prevalence of rotavirus-associated diarrhea across different seasons, with a significant *P*-value of <0.001, showing a strong statistical relationship between season and presence of rotavirus infection.

Rotavirus infection had a high prevalence in spring, autumn, and winter (92.6%, 92%, and 87%, respectively) (*P*-value <0.001). Summer season had a significantly lower prevalence of rotavirus infection (48%), with a nearly equal distribution of positive and negative cases.

The highest prevalence of adenovirus-associated diarrhea was noticed in winter (60.9%), which was significant statistically (*P*-value < 0.001), with no occurrence of adenovirus infections in autumn.

According to the seasonal pattern of intestinal parasites, none was significant as *Blastocystis* spp. infection prevalence was 8% in autumn, 4% in summer, and 3.7% in spring, with no occurrence of *Blastocystis spp*. infection in winter. In addition, only one case of *Cryptosporidium* spp. was detected in the summer (4%), shown in **Table 5**.

Table 5: Seasonal distribution of infectious agents of gastroenteritis

		Spring		Summer		Autumn		Winter		*P* value
		Count	%	Count	%	Count	%	Count	%	
Rotavirus	**Positive**	25	92.6	12	48.0	23	92.0	20	87.0	<0.001
	Negative	2	7.4	13	52.0	2	8.0	3	13.0	
Adenovirus	**Positive**	1	3.7	4	16.0	0	0.0	14	60.9	<0.001
	Negative	26	96.3	21	84.0	25	100.0	9	39.1	
***Blastocystis* spp.**	***Positive***	1	3.7	1	4.0	2	8.0	0	0.0	0.845
	Negative	26	96.3	24	96.0	23	92.0	23	100.0	
***Cryptosporidiu m* spp.**	**Positive**	0	0.0	1	4.0	0	0.0	0	0.0	0.730
	Negative	27	100.0	24	96.0	25	100.0	23	100.0	

The logistic regression analysis for rotavirus, the rate of positivity in all seasons except summer was 10.524 times which meant that individuals in all seasons except summer were approximately 10.5 times more common to be infected with rotavirus in comparison with those in summer (OR=10.524 with lower and upper 95% CI of 3.486 and 31.772 respectively) (*P*-value <0.001)

The logistic regression analysis for adenovirus infection highlighted a significant finding (*P*-value <0.001), suggesting that the winter season is a strong predictor of adenovirus infection. The odds ratio was 22.4 with upper and lower 95% CI of 76.946 and 6.521 respectively, indicating that individuals in winter were approximately 22.4 times more common to be infected with adenovirus in comparison with individuals in the other seasons.

DISCUSSION

Enteropathogenic agents causing diarrhea in children include viruses, bacteria, helminths, and protozoa conveyed via ingestion of contaminated food and water, a consequence of poor hygiene[16].

In our study, the prevalence of viral and intestinal parasitic coinfection by enteric rotavirus, adenovirus, and intestinal parasites was assessed in 100 children (below 5 years); admitted to Abu El-Reesh hospital complaining of acute gastroenteritis, from December 2023 to November 2024.

In the current study, the age of participants ranged from 1 to 60 months and the mean±SD was 11.25±10.38 months in agreement with Li *et al.*[17] who reported that the highest rate of acute gastroenteritis in children aged between 12 and 23 months. On the contrary, Khan[18] stated that gastroenteritis was higher in children <6 months.

In this study, the ratio of males to females was 2:1, which is consistent with a previous study[17].

The history of animal contact in this study was (9%). According to Pielok *et al.*[19], a case of a young veterinary student with diarrhea whose stool contained *Cryptosporidium* spp. oocysts and *Blastocystis* spp. were reported. In addition, khan[8] stated that animal breeding is a risk factor in parasitic disease transmission in children.

According to our results, a history of breastfeeding was (63%) in children with acute gastroenteritis. Santos *et al.*[20] stated that breastfeeding has a role in infant protection from diarrhea caused by parasitic agents such as protozoa.

In this study, the clinical manifestations were diarrhea with dehydration (92%), and vomiting (83%) followed by fever (40%). Similarly, Zhang & Yang[21] reported that fever, vomiting, dehydration, and severe diarrhea were common symptoms among rotavirus acute gastroenteritis. This could be attributed to that rotavirus infection activates vagal nerves that are associated with nausea and vomiting. An American study revealed that children experiencing rotavirus gastroenteritis, show elevated levels of IL-6 in blood serum and experience fever. Moreover, children having diarrhea and fever also show elevated levels of TNF-α[22].

In the current study, different clinical data such as fever and vomiting showed a statistically significant correlation with seasonal patterns of participants while dehydration was statistically non-significant. Aman[23] reported that all acute gastroenteritis studies in Indonesia registered diarrhea in nearly all cases, the second most frequently reported symptom was vomiting, and the third most frequent symptom was fever. In addition, Özmen *et al.*[24] declared that vomiting was more common in the viral acute gastroenteritis group than in the parasitic group.

In this study, viral pathogens were reported to cause 99 % of GE in children under 5 years of age with rotavirus and adenovirus accounting for 80% and 19% of cases respectively. Similarly, in Germany, viral pathogens accounted for 93% of GE in children below five years[25].

In this study, the percentages of enteric pathogens were as follows: rotavirus (80%), adenovirus (19%), *Blastocystis* spp. (4%) and *Cryptosporidium* spp. (1%).

According to Saha *et al.*[26], rotavirus was most prevalent (25.9 %) than adenovirus-F (10.6 %) in children with diarrhea using multiplex PCR.

In the current study, the human adenovirus rate was (19%) which agreed with another research in Egypt (20%)[27].

In this study, *Cryptosporidium* spp. rate was (1%) while the prevalence of *Cryptosporidium* in children with diarrhea in South Africa was 6%[28]. In addition, Calderaro *et al.*[29] reported that prevalence rate of *Cryptosporidium* was 3% in children suffering from gastroenteritis.

In this study, *Blastocystis* spp. rate was (4%) in a study conducted in El Salvador, Yellanthoor[30] had a case report of acute gastroenteritis in a 13-year-old boy due to blastocystosis.

In the current study, the lower prevalence rate of parasitic agents in comparison with viral agents may be due to the enhancement in sanitation that has considerably decreased gastroenteritis cases caused by parasites and bacteria, yet it has a slight effect on viral gastroenteritis[31].

As regards coinfection, 15% of our patients were positive for both rotavirus and adenovirus infections. Khalife *et al.*[32], reported that rotavirus was the most common viral agent (28%), followed by adenovirus (12.3%) and mixed infections (5.5%). Montasser *et al.*[33] stated that the coinfection of rotavirus with adenovirus was 8% among Egyptian hospitalized children complaining of gastroenteritis.

Our current study revealed that rotavirus infection had a prevalence rate in spring, autumn, and winter (92.6%, 92%, and 87% respectively), while the summer season showed the lowest prevalence rate, with a statistical significance ($P < 0.001$). While the peak season for viral gastroenteritis caused by human adenovirus infection was winter (highest prevalence 60.9%) with a statistical significance ($P < 0.001$) and no occurrence of adenovirus infections in autumn.

Calderaro *et al*.[29] found that adenovirus cases were more detectable in the autumn-winter season, while rotavirus cases were more frequent in the winter-spring season. Another study highlighted the high incidence of viral infections throughout the cold climate, with adenovirus infections peak in November[34]. Khan[35] stated that acute gastroenteritis cases increased between winter and spring.

On the contrary, De Francesco *et al.*[36] detected adenovirus in 7.1% of children <2 years with a peak in April and June. Furthermore, in Shanghai, Lu *et al.*[37] found that the adenovirus positivity rate was about 3.5%, and most common in children <3 years during the summer months.

In the current study, gastroenteritis caused by *Blastocystis* spp. occurred more in autumn (8%), followed by summer (4%), with the lowest rate in spring (3.7%). In concordance, a study reported that autumn months were where most of the parasitic agents observed[38]. Ozmen *et al.*[24] reported that the rate of parasites was common during the summer months. Hakkoymaz *et al.*[39] reported that gastroenteritis due to parasites was more observed in summer with the lowest rate in spring. In this study, the only positive case of *Cryptosporidium* was detected in the summer (4%). This agrees with Calderaro *et al.*[29] who observed that the prevalence of *Cryptosporidium* cases was more frequent from July to October.

In our study, the coinfection of rotavirus with *Blastocystis* spp. was (3%). In addition, rotavirus coinfection with *Cryptosporidium* spp. existed in 1 patient only (1%). All these rates were statistically insignificant. Furthermore, 80 % of all intestinal parasites detected in this study were coinfected with rotavirus. This agrees with a previous study which reported coinfection of rotavirus with *Cryptosporidium* among children with gastroenteritis in Iraq[40].

CONCLUSIONS

In our work, rotavirus causes gastroenteritis more than adenovirus and intestinal parasitic infection with *Blastocystis* and *Cryptosporidium* spp. in children below 5 years. It is necessary to make an accurate and rapid diagnosis in children with gastroenteritis to treat early. ICT could be very useful to detect rotavirus and adenovirus quickly in clinical practice. Hygiene practices, food and water safety, appropriate waste disposal may help to decrease the number of parasitic and viral cases of gastroenteritis. Rotavirus vaccines administration can decrease mortality and morbidity rates in children with acute gastroenteritis.

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The research contained in this manuscript has not been previously published, and the manuscript is not under consideration elsewhere.

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