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Abstract

Background: Gastroenteritis is considered as one of the leading causes of morbidity and mortality in children less than 5 years of age, especially in developing countries. Rotavirus and Adenovirus are recognized as common etiologies of this condition. Objectives: In Erbil province, little information are reported about these viral infections among children under 5 years old, so we investigated two of the most important agents, Rotavirus and Adenovirus to provide epidemiological evidence for a better understanding of their role among children with diarrhea. Methods: A total of 300 stool samples from pediatric patients (less than 5 years old) with gastroenteritis who were admitted to Raparin pediatric hospital, Erbil, Iraq, from May to October 2016 were collected and screened for presence of Rotavirus Group A (RovA) and Enteric Adenovirus type 40/41 (EAd40/41) antigens by using rapid immunochromatographic assay (Plasmatic Laboratory Products, DT6 5BU United Kingdom). Results: Out of the 300 samples examined, Rotavirus was detected in 65 samples (21.67%), Adenovirus was identified in 19 (6.33%) of patients and 12 (4%) of specimens had Rotavirus-Adenovirus co-infection. The findings showed that Rotavirus is significantly higher than Adenovirus. The Infections were high predominant among children <5 years>old, with a maximum incidence among infants 1-12 months. The frequency of viral infection was higher in male 33.33% patients compared to females 30.3%. The viral infection in patients from rural areas (34.64%) was more frequent than those from urban areas (30.06%). The result revealed that the highly incidence were in infant using bottle-feed (36.55%) in comparing with infants who are breast-feed (28.20%) and mixed-feed (30.43%). No significant associations were observed with gender, residency and feeding types.

Keywords

children gastroenteritis, Rotavirus, Adenovirus, Immunochromatographic assay.

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ABSTRACT

Background: *Gastroenteritis is considered as one of the leading causes of morbidity and mortality in children less than 5 years of age, especially in developing countries. Rotavirus and Adenovirus are recognized as common etiologies of this condition.*

Objectives: *In Erbil province, little information are reported about these viral infections among children under 5 years old, so we investigated two of the most important agents, Rotavirus and Adenovirus to provide epidemiological evidence for a better understanding of their role among children with diarrhea.*

Methods: *A total of 300 stool samples from pediatric patients (less than 5 years old) with gastroenteritis who were admitted to Raparin pediatric hospital, Erbil, Iraq, from May to October 2016 were collected and screened for presence of Rotavirus Group A (RovA) and Enteric Adenovirus type 40/41 (EAd40/41) antigens by using rapid immunochromatographic assay (Plasmatic Laboratory Products, DT6 5BU United Kingdom).*

Results: *Out of the 300 samples examined, Rotavirus was detected in 65 samples (21.67%), Adenovirus was identified in 19 (6.33%) of patients and 12 (4%) of specimens had Rotavirus-Adenovirus co-infection. The findings showed that Rotavirus is significantly higher than Adenovirus. The Infections were high predominant among children <5 years old, with a maximum incidence among infants 1-12 months. The frequency of viral infection was higher in male 33.33% patients compared to females 30.3%. The viral infection in patients from rural areas (34.64%) was more frequent than those from urban areas (30.06%). The result revealed that the highly incidence were in infant using bottle-feed (36.55%) in comparing with infants who are breast-feed (28.20%) and mixed-feed (30.43%). No significant associations were observed with gender, residency and feeding types.*

Conclusions: *The present results revealed that Rotaviruses and Adenoviruses have an important role in diarrhea among children especially those less than 5 years old and viral pathogens should be investigated routinely in diarrhea stool specimens.*

Keywords: ***children gastroenteritis, Rotavirus, Adenovirus, Immunochromatographic assay.***

INTRODUCTION

Gastroenteritis is recognized as one of the most important causes of illness and death in children under the age of 5 years throughout the world [1]. Gastroenteritis is transmitted by direct person to person contact, fecal-oral route, or respiratory droplets [2, 3]. Approximately more than 700 million diarrheal cases occurred in children less than five years of age each year, with a mortality rate estimated to be 3-5 million cases, the majority of these conditions appear in developing countries [4, 5, 6].

Gastroenteritis is often caused by a variety of enteropathogens, such as; bacteria, parasites and viruses [7]. Enteric viruses have been recognized as the most significant etiological agents of the gastroenteritis. More than 20 different types of viruses have been identified as infectious agents for gastroenteritis, and four categories of viruses are being considered clinically relevant: Rotavirus, Adenovirus, astrovirus, and calicivirus [7, 8]. Rotavirus and Adenoviruses have proven to be important causes of diarrhea in children within the age group of 0 to 5 years [9].

Rotaviruses are non-enveloped, icosahedral, double-stranded RNA and they are belonging to the family Reoviridae [46]. Seven groups of rotavirus (A-G) have been described, with group A rotaviruses being the leading cause of severe dehydrating gastroenteritis in children less 5 years of age worldwide [5].

Rotavirus is the most common cause of viral gastroenteritis among infants and young children. Rotavirus infection is associated with high rates of morbidity worldwide and high rates of mortality in developing countries, it has been estimated that they result in 800000 cases of death a year due to malnutrition and lack of health care and that up to 85% of these deaths occur in poorest countries [10, 11, 12]. For this reason, many researchers have gone into developing a vaccine to protect children from this virus [8]. Fever, abdominal pain, nausea, diarrhea and vomiting are the major clinical manifestations of Rotavirus gastroenteritis that may lead to hypovolemic shock, dehydration and death [13, 14]. Infection can also be symptomless [5].

Adenoviruses are non-enveloped viruses with an icosahedral nucleocapsid containing a linear double stranded DNA and they are belonging to the family Adenoviridae [5, 43]. There were six groups of Adenoviruses symbolized by the letters (A-F) and 51 serotypes [7]. The serotypes 40 and 41 enteric adenoviruses are associated with gastroenteritis in pediatrics [14].

Adenoviruses are second only to Rotavirus as the most important causative agents of acute infantile gastroenteritis [15]. They are considered as the primary cause of epidemic diarrhea and onset of diarrheal disease in hospitals and day-care centers. The rate of enteric Adenoviruses varies from 1-8% in developed countries to 2-31% in developing countries [16]. When compared to Rotavirus infections, high fever and dehydration are less frequently observed and the infection duration is longer. It may be accompanied by vomiting and fever [17, 18].

Epidemiological data on the prevalence of viral gastroenteritis appear to be very dispersed in most developing countries including Iraq. The prevalence of viral pathogens in children with diarrhea in the neighboring countries was found to be 48.8%, 20% and 6% for Rotavirus, Adenovirus and co-infection respectively in Iran [19], 17.0%, 2.4% and 0.3% for Rotavirus, Adenovirus and co-

infection respectively in Turkey [20], it were 18.7% and 3.9% for both Rotavirus and Adenovirus respectively in Saudi Arabia [21], 39.9% and 44.8% for Rotavirus in Jordan and Bahrain, respectively [22, 23].

Regarding the prevalence of viral gastroenteritis in Iraq, 33.3%, 6.6% and 33.3% for Rotavirus, Adenovirus and co-infection respectively in Kirkuk city [24], %43.3% for Rotavirus in Basrah city [25], 51.23%, 27.6% and 4.21%, 34.4% for Rotavirus and Adenovirus in Najaf-AL-Ashraf city respectively [26, 27], 50.5% for Rotavirus and 20.4% for Adenovirus in Babylon city [28], 18.55%, 33.33% and 39.26% for Rotavirus in Tikrit, Mosul and Ramadi cities respectively [29, 30, 31], 91.3%, 2.6% and 6.09% for Rotavirus, Adenovirus and co-infection in Baghdad city respectively [15], 22% for Rotavirus, 3% for Adenovirus and 2% for co-infection in Sulaimani city [18], 10.92%, 69.35% and 2.30%, 30.64% for Rotavirus and Adenovirus in Duhok and Zakho cities respectively [3, 32], finally in Erbil was 37% for Rotavirus [33].

Although diarrhea is a major cause of illness and death in Iraqi children under 5 years, the epidemiology of the Adenovirus and Rotavirus is not well-known in Erbil province, therefore, the aim of this study was to investigate the frequencies of both viruses and their role in viral diarrhea.

MATERIALS AND METHODS

Sample collection:

This cross sectional study was carried out on 300 children less than five years of age were suffering from diarrhea hospitalized Raparin pediatric hospital over a period of 6 months, started from May to the end of October 2016 in Erbil city.

Diarrhea Stool samples were collected from each child in sterile disposable screw-cap containers. These were labeled with number, date and name of each subject. A questionnaire containing demographic, clinical and environmental data was obtained from each case. The presence of Rotavirus and Adenovirus in fresh stool samples was investigated at the microbiology laboratory of the same hospital by using an immunochromatographic test.

Detection of Rotavirus Group A and Enteric Adenovirus type 40/41:

The diarrhea stool specimens were tested as soon as possible after collection; they were directly tested with immunochromatographic assay (purchased from Plasmatic Laboratory Products, United Kingdom) for antigenic detection of Rotavirus and Adenovirus in human stool, and were done according to instructions of the manufacturers.

Test Principle:

The principle of the test based on the use of a homogenous nitrocellulose membrane system which pre-coated with mouse monoclonal anti-Rotavirus and anti-Adenovirus antibodies against specific viral antigens on the test band regions.

Test Methods:

However, to detect Rotaviruses and Adenoviruses, approximately 100 mg or 100 microliter of stool specimen was transferred by a stick into the diarrhea stool collection tube with diluents samples, and then the tube was shacked in order to assure good sample dispersion. After that, 3-4 drops of the solution were added into the circular window marked with an arrow; finally the results were read at 5-10 minutes by observing the coloring bands.

Interpretation of results:

- *Rotavirus positive:* Two distinct red lines appear in the left kit. One line should be in the control region (C) and the other line should be in the test region (T).
- *Adenovirus positive:* Two distinct red lines appear in the right kit. One line should be in the control region (C) and the other line should be in the test region (T).
- *Rota-Adenovirus positive:* All the lines will appear in both sides. Two lines should be in the control region (C) and the other two lines should be in the test region (T).
- *Negative Rotavirus or Adenovirus:* One red line appears in the control region (C) left or right. No apparent red or pink line appears in the test region (T), as shown in figure (1).

Invalid: No red lines appear or control line fails to appear, indicating that the operator error or reagent failure

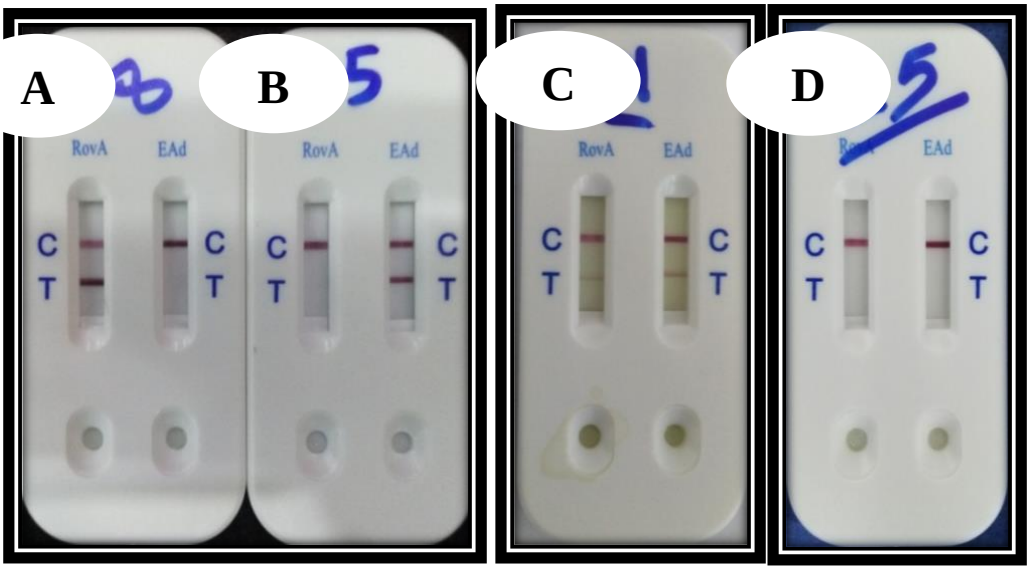

Figure 1: A- Rotavirus Positive B- Adenovirus Positive C- Rota-Adenovirus Positive D- Negative

STATISTICAL ANALYSIS

The data were analyzed using Statistical Package for the Social Sciences (*SPSS*), version 21.0. The proportion and their frequencies were checked by applying chi-square (x2) test to estimate the relative risk of Rotaviruses and Adenoviruses to diarrheal infection. P-value < 0.05 was considered significant.

RESULTS

Rotavirus and Adenovirus detection rates

From the 300 examined diarrhea stool specimens from children under 5 years with gastroenteritis using immunochromatographic assay, the proportion of viral infection was 32%. This average involves the following rates 21.67%, 6.33% and 4% for Rotavirus Group A (RovA), enteric Adenovirus type 40/41 (EAd40/41) and Rota-Adenovirus co-infection respectively. Statistically significant differences were noticed ($p<0.05$) as appeared in figure (2).

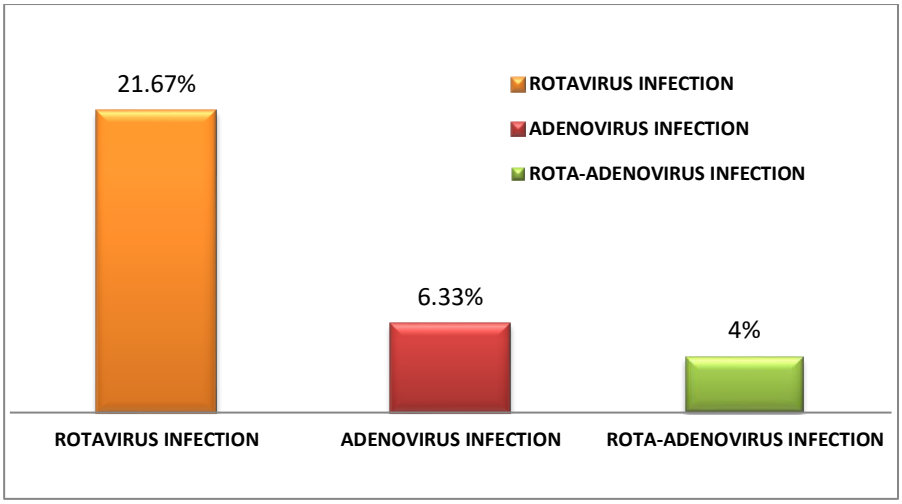

Figure 2: Distribution of pediatric patients with gastroenteritis according to their viral infections (P=0.001)

Rotavirus and Adenovirus detection rates according to gender

As shown in table (1), out of 300 study samples, 168 were males and 126 were females. Males showed a higher rate of viral infection (33.33%) than females recorded (30.3%). But statistically was non-significant (p>0.05).

Table 1: Distribution of pediatric patients with gastroenteritis according to their genders

Genders	No. Tested	Virus type			Total
		Rotavirus (%)	Adenovirus (%)	Co-infection (%)	
Male	168	38 (22.61)	11 (6.55)	7 (4.17)	56 (33.33%)
Female	132	27 (20.45)	8 (6.06)	5 (3.79)	40 (30.3 %)
Total	300	65 (21.67)	19 (6.33)	12 (4)	96 (32%)
P = 0.5765					

Rotavirus and Adenovirus detection rates by age groups

Table (2) shows the distribution of viruses causing gastroenteritis by age groups. The higher infection rates (47.95%) was appeared in age group 1-12 months, while the lower infection (20.45%) reported in age group 49-<60 months, the differences among different age groups were statistically significant (p<0.05). Rotaviruses were the most common cause of acute gastroenteritis and occurred most frequently with 30.14% in the age group 1-12 months, while the least common rate of Rotavirus infection was 13.63% among 49-<60 months age group. The majority of Adenovirus detection occurred in the 1-12 months age group while only a single case was positive for Adenovirus in the 37-48 months age group.

Table 2: Distribution of pediatric patients with gastroenteritis according to their age groups

age groups (months)	No. Tested	Virus type			Total
		Rotavirus (%)	Adenovirus (%)	Co-infection (%)	
1-12	73	22(30.14)	8(10.96)	5(6.85)	35(47.95)

13-24	61	17(27.87)	6(9.84)	3(4.91)	26(42.62)
25-36	70	12(17.14)	2(2.86)	1(1.42)	15(21.42)
37-48	52	8(15.38)	1(1.92)	2(3.85)	11(21.15)
49-<60	44	6(13.63)	2(4.55)	1(2.27)	9(20.45)
Total	300	65	19	12	96
P = 0.0003					

Rotavirus and Adenovirus detection rates according to residency

Table (3) showed that most cases of viral diarrhea that were caused by Rotaviruses (23.62%) and adenoviruses (7.09%) belonged to rural areas. Overall there were 30.06% and 34.64% viral infections from urban and rural areas, respectively, with no-statistically differences between them ($p<0.05$).

Table 3: Distribution of pediatric patients with gastroenteritis according to their residency

Residency	**No. Tested**	**Virus type**			**Total**
		Rotavirus (%)	Adenovirus (%)	Co-infection (%)	
Urban	173	35(20.23)	10(5.78)	7(4.05)	52(30.06)
Rural	127	30(23.62)	9(7.09)	5(3.93)	44(34.64)
Total	300	65	19	12	96
P= 0.4000					

Rotavirus and Adenovirus detection rates according to feeding type

Regarding the relationship between viral diarrheal infections with feeding methods, the results showed that viral infections among bottle feeding, mixed feeding and breast-feeding children were 36.44, 30.43% and 28.20% respectively (Table 4). The higher rate of infection was observed in bottle fed children and lower rate was in breast fed. There was no significant difference in occurrence of infection among types of feeding $p>0.05$.

Table 4: Distribution of pediatric patients with gastroenteritis according to their type of feeding

Feeding type	**No. Tested**	**Virus type**			**Total**
		Rotavirus (%)	Adenovirus (%)	Co-infection (%)	
Breast	78	16(20.51)	4(5.12)	2(2.56)	22(28.20)
Bottle	107	27(25.23)	6(5.60)	6(5.60)	39(36.44)
Mixed	115	22(19.13)	9(7.82)	4(3.48)	35(30.43)
Total	300	65	19	12	96
P= 0.4451					

DISCUSSION

Diarrheal diseases are causes of major public health problems in developed and developing countries [1]. Viral gastroenteritis is the most common cause of hospitalization for infants and older children with severe dehydration resulting from diarrhea; it is also a cause of infant mortality [9].

The present study showed that the overall rate of infection with viruses causing gastroenteritis was 96/300 (32%) as illustrated in figure (1) (P=0.001). This result is comparable to others studies where the incidence of enteric viruses was reported as: 32% in both Turkey and Baghdad-Iraq [17, 35] and 33% in Saudi-Arabia [36]. While, higher rates than our results were recorded in Iran, Jordan and Najaf-Iraq (55%) (39.9%) and (55.4%), respectively [14, 22, 26]. Furthermore, in, Portugal, Sudan and Brazil lower rates (13.2%, 13.9% and 10.7%) of viral prevalence among children has been recorded, respectively [12, 14, 37]. These variations could be explained by the duration of the study, patients age, number of samples, social habits of the population, socioeconomical status, seasonal variations, endemicity of enteropathogens, diagnostic techniques, Geographical distribution, breast feeding of premature infants and weak medical information's [5, 2, 38, 10].

Our finding shows that Rotavirus is most commonly detected and responsible for 65/300 (21.67%) of gastroenteritis (Figure 1); this observation was a good agreement with (22%) reported in Sulaimani, Saudi Arabia and Beijing, respectively [18, 36, 39], and with (21%) in Baghdad and France [35, 40]. In contrast, our rate was higher than the prevalence of Rotavirus diarrhea estimated in Duhok (10.91%), Baghdad (14.36%), Basrah (10.34%), Saudi Arabia (15.7%), Turkey (17%), Tikrit (18.5%) and Korea (20.3%), respectively [3, 5, 6, 11, 20, 29, 41]. However, the results of the present study are lower than those observed in other countries Yemen, Sudan, Jordan, Bahrain, Iran and Saudi Arabia, respectively [10, 14, 22, 23, 43, 44]. This variation in the prevalence of Rotavirus infection among different areas is probably due to the social habits of the population, e.g. personal hygiene, and/or environmental variations that may be related to growth of Rotavirus pathogens particularly in contaminated water [22].

On the other hand, Adenovirus were detected in 19/300 (6.33%) of the samples (Figure 1). This result is comparable to other studies where the prevalence of enteric adenoviruses was reported as: 5.6% and 6% in Baghdad, respectively [1, 34], 6.7% in Iran [47] and 6.5% in Korea [48]. These figures are high in comparison to other reports from Duhok at 2.3% [3], 3% in Sulaimani [18] and 2.4% in Turkey [20]. While studies in other countries such as in Turkey, Sudan and Iran showed that Adenovirus was found 26.2%, 16.2% and 20.30% of diarrheal patients, respectively [9, 14, 43]. Differences in rates of detection in the present study as compared to other studies might be related to the level of economic status or geographical region of the study area or epidemiological condition of the virus in regions with different climates or study design or difference of the age groups or laboratory methods used [1, 2, 16].

In general Rota-Adenovirus co-infection is less frequent than mono-infections.co-infection were founded in 12 cases presented 4% of all samples. This rate was very similar to that observed in Iran (4%) [14], and supported by other studies in Turkey 4.4% [17] and 4.5% [42]. But differ from those founded in Baghdad [15], Iran [19] and Kirkuk [24] in which a higher prevalence of co-infection was recorded. While it is lower than other studies done in Beijing [39] and in Sulaimani [18].

According to table (1), the frequency of viral infection in male children 33.33% higher than in female children (30.3%), but no significant relationship between gender and virus type was observed. In some studies it was reported that the higher rate of viral gastroenteritis was seen in males and similarly this difference was usually insignificant [20, 22, 27]. This is in contrast to the findings in Baghdad and Basrah, where the majority of viral infections significantly occurred in males [15, 25]. These results may be explained by the fact that the males are more active, mobile and integrated to the environment especially among agricultural communities, more ever; it may involve gender specific immunological differences [6, 38].

According to the age groups table (2), the highest frequency of viral infection showed in infants less than one year (47.95%), whereas the lowest rate (20.45%) were happened in children with 49-<60 months of age. The significant viral infection was shown in all age groups (P=0.0003). The significant differences between viral infection and different age groups are agreed with other reports from Baghdad and Jordan [5, 22]. The rate of viral infection in infants aged less than 1 year was 47.95%. This finding is higher than those recorded in Baghdad 36.6% [4], Saudi Arabia 23.3% [11], Tikrit 36.5% [29], and Al-Ramadi 32% [31], but it is lower than those reported in Saudi Arabia 57.1% [21], Babylon 51.5% [28] and Congo 55.9% [45]. However, in some studies viral infection was more common in children less than 2 years of age and in children aged 5 to 12 months [9, 32]. The differences between the presented results and results of the other studies may be due to the age, immunity, nutrition status of the patients, season, inadequate sanitation and geographic region [5, 35]. Our study indicated that there was a tendency of decreasing rates of viral infection in older children. This might be explained by the fact that older children may have acquired protective immunity as a result of previous exposures to virus and become more resistant to infection with this agent [2, 18, 30].

As regards to geographical region distribution (Table 3), the higher rate of infected children with viral infection was found in rural area 34.64% than in urban area 30.06%, with no significant deference (P>0.05). This comes in accordance with others in Sulaimani [18], Basrah [25] Babylon [28], and Al-Ramadi [31]. These differences between patients from rural and urban areas might be due to poor water supply, poor sewage disposal, social habits of the population and low education [6, 38].

In our study, the relationship between the prevalence of viral infection and the type of feeding in children showed in table (4). The rate of virus infection associated with breast feeding was 28.20% less than the proportion of bottle and mixed feeding which was registered 36.44% and 30.43% respectively, with no significant difference (P > 0.05). These results are similar to studies in Al-Najaf [7], Iran [16], Sulaimani [18], Babylon [28], Mosul [30], Kirkuk [38], and Burkina Faso [49]. But the results are not consistent with another study performed in Basrah [25]. The lowest infection with breast-fed children indicates that antibodies produced by mothers play a protection role against viral infection [7]. Also the high rate of viral infection among those with bottle-fed or mixed-fed may can be explained by poor hygienic condition of the child and contamination during milk preparation, in addition to this, the immunity of the child decreased in age <1 year depending on solid food [38].

CONCLUSION

Viral pathogens play an important role in childhood viral gastroenteritis. In this study, we concluded that gastroenteritis in Erbil province is associated with Rotavirus and Adenovirus which has a significant prevalence among children less than five years of age suffering from diarrhea. The majority of diarrheal cases were below 1 year. Ages of children are significant predisposing factors for virus infections. Also it was confirmed that the prevalence of this viral infection was higher in males and rural area than in females and urban area. It can be concluded that using immune-chromatography assay reveal high efficacy in diagnosis of virus infections in stool specimens.

RECOMMENDATION

Accordingly, we recommend routine detection of rotavirus and Adenovirus by immunochromatographic assay for all patients with diarrhea to identify the viral causative agent involved.

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