



Research Article

PREVALENCE OF ROTAVIRUS, ADENOVIRUS, ASTROVIRUS AND ENTEROVIRUSES INVOLVED IN ACUTE INFANTILE AND CHILDHOOD GASTROENTERITIS IN BAGHDAD: A STOOL-BASED ANTIGENIC STUDY

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ARTICLE INFO

Article History:

Received 24th March 2016

Received in revised form

19th April 2016

Accepted 31st May 2016

Published online 30th June 2016

Keywords:

Pediatric Gastroenteritis,
Single and Mixed infection,
Rotavirus,
Adenovirus,
Astrovirus,
Enterovirus.

ABSTRACT

Background: Diarrhea in children less than five years of age is a leading cause of morbidity and mortality. The etiology of diarrhea in more than one third of the cases is unknown. Viral agents are now the most common identifiable causes of childhood gastroenteritis.

Objectives: The aims of the study were to determine the prevalence of Rotavirus, Adenovirus, Astro virus and Enterovirus in pediatric patients up to five years of age with acute gastroenteritis.

Methods: An outpatient-hospital based prospective study was carried out at AL-Elweya Pediatric Hospital and Children Welfare Teaching Hospital (Baghdad) during the period from February to October 2013.

Rotavirus, Adenovirus, Astrovirus and Enterovirus in fecal samples of 465 symptomatic pediatric patients with acute gastroenteritis were detected by immunochromatographic assay (ICA). Their age ranged from less than one month to 60 months with a median age of seven months.

Results: Out of the 465 samples analyzed, detection of one virus- alone was found in (22.8%), (10.1), (7.3%) and (5.6%) of the diarrheal cases regarding Rota virus, Astro virus, Enterovirus, and Adenovirus, respectively, yet co-infection with all four studied viral types was found in only one case (0.2%), two viruses in 65 cases (14%), and three viruses in 8 cases (1.7%). Rota- and Astro- viral diarrheas tend to occur significantly in younger- aged pediatric patients than their negative counterparts.

Conclusions: A significant prevalence of gastroenteritis that associated with Rotavirus, Adenovirus, Astrovirus and Enterovirus was observed while mixed viral infections constituted a respective bulk among Iraqi pediatric patients with gastroenteritis less than five years in Baghdad.

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INTRODUCTION

Worldwide, acute gastroenteritis is now one of the most common diseases among humankind (Wilhelmi de Cal *et al.*, 2008). Its greatest morbidity and mortality are among those at the extremes of age, children and the elderly. Gastroenteritis is a common cause of death in children < 5 years in developing countries, which could be linked to a wide variety of pathogens. While deaths from diarrhea are less common in developed countries, this illness leads to many hospitalizations or medical visits (Glass *et al.*, 2001).

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In the past decade, the availability and application of more sensitive immunoassays and molecular techniques for detection and characterization of the infectious agents of have opened up new avenues both in understanding these pathogens themselves, particularly their molecular epidemiology, their disease burden and their prevention and control, as well as have led to advances and new developments in these diagnostic generations (Glass *et al.*, 2001; Olives *et al.*, 2007; Wilhelmi, 2003). Since the era of identification of Norwalk virus as a cause of gastroenteritis, the numbers of viruses that associated human diarrheal diseases have progressively increased and viral agents are now the most common identifiable causes of childhood gastroenteritis (Wilhelmi de Cal *et al.*, 2008). Worldwide, two epidemiologic patterns of viral diarrhea were

recognized; endemic-pattern in children and outbreaks-pattern affecting people of all ages. These viral gastroenteritis are affecting all children in their first few years of life regardless their level of hygiene, quality of water, food or sanitation, or type of behavior (Glass *et al.*, 2001). Much of the gastroenteritis in children is caused by viruses belonging to four distinct families: Rotaviruses (RV), Caliciviruses (CV), Astroviruses (AsV), and Adenoviruses (AD). Although Rotaviruses (RV), are the main causative agents of viral gastroenteritis in infant and young children (Glass *et al.*, 2001; Ozdemir *et al.*, 2010), however, the development of a safe and highly effective vaccine against Rotavirus could prevent cases of severe diarrhea and reduce the mortality attributable to this disease (Wilhelmi *et al.*, 2008). Viral gastroenteritis due to Rotavirus (RV), Adenovirus (AD) and Astrovirus (AsV), are common pediatric pathogens accounting for many physician visits, hospital admissions, and nosocomial infections⁶. Enteroviruses are now important pathogens known to contribute significantly to the etiology of diarrheal diseases in both developing and developed countries (Nagabushana *et al.*, 2014). In addition, other viruses such as Noroviruses (NV), Toroviruses (TV), Coronaviruses (CoV), Picobirnaviruses (PBV), Aichi virus (AiV) and human bocavirus (BV) are also increasingly being identified as causative agents of diarrhea (Wilhelmi *et al.*, 2008).

While RV is the most common cause of severe diarrhea in children under 5 years of age (1), however, the average age of children infected with these agents was less than one year (Basu *et al.*, 2003). In a three-year passive surveillance of gastroenteritis, (AsV) and (RV) infections peaked simultaneously in winter months (Rodriguez-Baez *et al.*, 2002)⁶. In another study, an increase of RV was noted in the autumn-winter season but no seasonal pattern or prevalence was neither observed in AD nor in AsV shedding could not be determined, too (Basu *et al.*, 2003). Emphasis on the familiarity with molecular epidemiology of these viruses and studying their antigenic diversity would elaborate application of an adequate vaccines as well as prophylactic procedures to them (Oldak *et al.*, 2006). Since viral gastroenteritis diseases are gradually increasing in the developed countries and are among the leading causes of illness in young children in developing countries (10), this study aimed to determine the prevalence of non-microbial [bacterial fungal, parasitical] diarrheal diseases associated with acute gastroenteritis caused by Rotavirus, Adenovirus, Astrovirus and Enterovirus infections in children from Baghdad up to five years of age.

MATERIALS AND METHODS

Study population

From February to October 2013, a prospective study was conducted on a fresh stool samples from a total number of 465 infants and young children less than five years old with acute diarrhea at two pediatric hospitals in Baghdad; AL-Elweya Pediatric Hospital and Children Welfare Teaching Hospital (CWTH). Macroscopic and microscopic laboratory examinations of stool samples was done. The inclusion criteria was to include in this study a watery stool samples (at macroscopic examination) & a parasite-free stool samples at microscopic examination (using saline & iodine preparations) from diarrheal cases that were not lasting more than seven days after the onset of illness.

The exclusion criteria was neither to include a reported hemorrhagic fresh stool samples nor containing parasitic agents (*Giardia lamblia* or *Entamoeba histolytica*) in their stools. Cer Test immunochromatographic assay was used for this research. Stool samples were collected in a labeled screw-cap clean container and were tested by immunochromatographic assay (purchased from CerTest, Spain) for antigenic detection of Rotavirus, Adenovirus, Astrovirus, and Enterovirus and were done according to instructions of the manufacturers. The Cer Test Rotavirus, Adenovirus, Astrovirus, and Enterovirus equalitative immunochromatographic assays for determination of rotavirus, adenovirus, astrovirus, and enterovirus in fecal samples. The membrane is pre-coated with mouse monoclonal antibodies, on the test band regions, against viral antigens. During testing, the sample is allowed to react with the colored conjugate (anti-Rotavirus mouse monoclonal antibodies-red microspheres, anti-Astrovirus mouse monoclonal antibodies-red microspheres, and anti-Enterovirus mouse monoclonal antibodies-red microspheres and anti-adenovirus mouse monoclonal antibodies-blue microspheres) which was pre-dried on the test.

The mixture then moves upward on the membrane by capillary action. As the sample flows through the test membrane, the colored particles migrate. In the case of positive result the specific antibodies present on the membrane will capture the colored particles. Different colored lines will be visible, depending upon the virus content of the sample. The mixture continues to move across the membrane to the immobilized antibody placed in the control band region, a green-colored band always appear. Regarding the Rotavirus, Adenovirus, Astrovirus and Enteroviruses, the same procedural steps were applied for CerTest Rotavirus, Adenovirus CerTest, Astrovirus CerTest and Enteroviruses CerTest card device tests were used.

However, to detect Adeno& Rotaviruses, approximately 100 mg or 100 microliter (while for Astroviruses and Enteroviruses, 200-300 mg or 200-300 microliter and 150 mg or 150 microliter of stool sample was put and shaken in collection tube containing the diluents. Four drops or 100 microliter was dispensed in S circular window of card and the results (appearance of the colored bands) were read after 10 minutes. Negative results indicated by the appearance of only one green band (control line) while positive results, in addition to the green control band, a red band (for positive Rotavirus, Astrovirus and Enterovirus), and a blue band (for positive Adenovirus) also appeared in the site of result line. A total absence of the control colored band (green) regardless the appearance or not of the results lines (red or blue) was evaluated as an invalid results. In our research, T test, ANOVA test, and Chi square were applied for statistical analysis of the obtained results and were done by using the SPSS program (version-10) and Excel application.

RESULTS

From February to October 2013 a total of 465 (261 males, 204 females) children with acute diarrhea were studied; 386 cases attending AL Elweya Pediatric Hospital (215 males, 171 females) and 79 attending Children Welfare Teaching Hospital (CWTH) (46 males, 33 females), male : female ratio was 1.3:1 (Figure 1). Their age ranged from less than one month to 60 months with a median age of seven months.

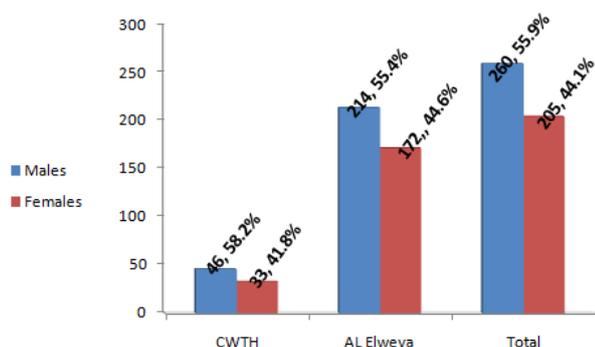


Figure 1. Distribution of pediatric patients with acute diarrhea according to their genders

On assessing the results of immunochromatographic assay (ICA) for viral antigens detection it was found that (61.7%) of the total stool samples from the studied diarrheal cases have showed positive results for one or more viruses, while (38.3%) of total cases showed negative results for these viruses. Out of the total diarrheal cases, co-infection with all four studied viral types was found in only one case (0.2%), while two viruses were found in 65 cases (14%), three viruses in 8 cases (1.7%), yet detection of one virus alone was found in (22.8%), (10.1%), (7.3%) and (5.6%) of the diarrheal cases regarding Rota virus, Astro virus, Entero virus, and Adeno virus, respectively (Figure 2), and table (1) shows the co- infection combinations of the two as well as three viruses.

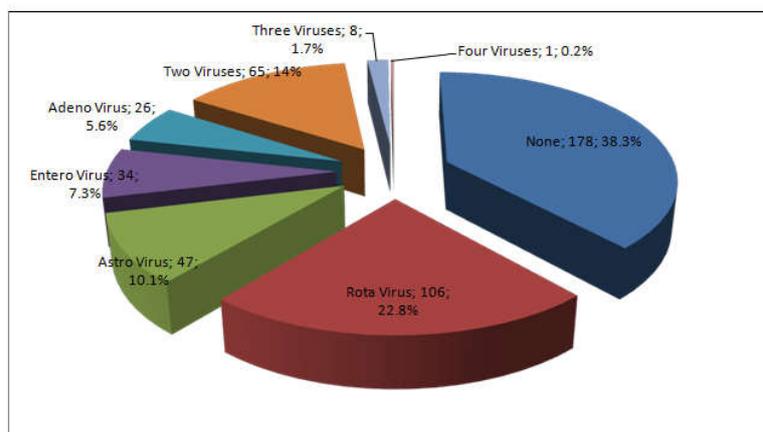


Figure 2. Distribution of pediatric patients with acute diarrhea according to their single or multiple viral infections

Table 1. Distribution of children with acute diarrhea infected with two or more viruses

Immunochromatographic Assay Results	Viral combinations	No.	%
Positive for two viruses	Rota V.& Adeno V.	31	47.8
	Rota V. & Astro V.	14	21.5
	Rota V. & Entero V.	12	18.5
	Adeno V. & Entero V.	4	6.1
	Astro V. & Entero V.	4	6.1
Total		65	100.0
Positive for three viruses	Rota V., Adeno V.& Astro V.	2	25
	Adeno V., Astro V.& Entero V.	2	25
	Rota V., Astro V.& Entero V.	3	37.5
	Rota V., Adeno V.& Entero V.	1	12.5
Total		8	100.0

The age pediatric patients have ranged from less than one month to 60 months with a median age of seven months. Rotavirus antigen was the most frequently detected agent in children as 64.1% in (0-12) months group, 30.2% in (13-24) months group and 5.7% detected in children with the age of >24 months group. The rates of adenovirus positivity were 65.4%, 19.2 and 15.4% for in (0-12) months, (13-24) months, and more than 24 months respectively. Of children who have Astrovirus- antigen positive, 80.8% and 19.1%, for (0-12) months and (13-24) months groups, respectively, while No Astrovirus positivity was detected in children with the age of >24 months. At a time, these rates were found as 76.5%, 17.6%, and 5.9%, respectively for (0-12) months, (13-24) months, and more than 24 months for Enteroviral infections.

Regarding age distribution among cases in relation to the types of viral infection, table 2 showed that viral diarrhea tend to occur among younger age groups. However, no significant differences were found in all age stratifications between those with positive and negative results of all viral infections, except for Rota- and Astro- viral infections where those cases with positive viral infections were significantly younger than their negative- Rota- and Astro virus counterparts (Table 2). Although rates of males exceeded females among both viral and non-viral diarrhea yet the association between gender and cause of diarrhea was statistically not significant association ($\chi^2=6.6$, $df=5$, $P=0.2555$) (Table 3).

Table 2. Age of diarrheal pediatric patients in association with types of their viral infection

Diarrhea associated with	Age in months								P Value
	≤12		13-24		>24		Total (465)		
	No.	%	No.	%	No.	%	No.	%	
Rota virus	68	64.1	32	30.2	6	5.7	106	22.8	0.009*
Adeno virus	17	65.4	5	19.2	4	15.4	26	5.6	0.66
Astro virus	38	80.9	9	19.1	0	0.0	47	10.1	0.05*
Entero virus	26	76.5	6	17.6	2	5.9	34	7.3	0.68
≥Two viruses	66	89.2	6	8.1	2	2.7	74	15.9	0.01*
Non- viral	131	73.6	28	15.7	19	10.7	178	38.3	

*Statistically significant association (χ^2 test with Yeat's correction)**Table 3. Distribution of children with acute diarrhea according to gender and viral cause of diarrhea**

Diarrhea associated with	Gender						P Value
	Males		Females		Total (465)		
	No.	%	No.	%	No.	%	
Rota virus	60	56.6	46	43.4	106	22.8	0.2555*
Adeno virus	16	61.5	10	38.5	26	5.6	
Astro virus	30	63.8	17	36.2	47	10.1	
Entero virus	17	50.0	17	50.0	34	7.3	
≥Two viruses	33	44.6	41	55.4	74	15.9	
Non- viral	105	59.0	73	41.0	178	38.3	
Total	261	56.1	204	43.9	465	100.0	

*Statistically not significant association ($\chi^2 = 6.6$, $df=5$, $P=0.2555$)

DISCUSSION

Diarrheal diseases are causes of major public health problems worldwide and among these, infectious diarrheal diseases are the third leading cause of death while viral gastroenteritis are gradually increased in young children in the developed as well as developing countries (Basu *et al.*, 2003; Khoury *et al.*, 2011; Ifeanyi *et al.*, 2009). As such, understanding the contribution of viral gastroenteritis is essential for implementation of appropriate public health measures in controlling these diseases. Since the clinical features of viral gastroenteritis do not differ from those of gastroenteritis caused by other pathogens, the purpose of our study was to determine the prevalence of acute gastroenteritis associated with Rotavirus, Adenovirus, Astrovirus and Enteroviruses. Diarrheal diseases caused by Rotavirus and other viral agents are shed in high concentration in the stool of children with gastroenteritis and feces as a specimen is the preferred for diagnosis (Nakawesi *et al.*, 2010). In this study and for this reason, stool samples were examined using antigen- based Immunochromatographic assay (ICA) for Rotavirus, Adenovirus, Astrovirus, and common Entero viruses. Clinical data was abstracted from patient's family and recorded in a special forma for this purpose. Although Rotaviruses and enteric adenovirus types 40 and 41 were found to be the most prevalent etiological viruses of childhood gastroenteritis in developed countries (Topkaya *et al.*, 2006). In this study (Table 2), rotaviruses has also ranked the top of list of the studied viruses (22.8%) but are followed immediately by Astrovirus infection (10.1%), then enteroviral gastroenteritis (7.3%) and the least was the prevalence of enteric adenovirus types 40 & 41 gastroenteritis (5.6%).

Previous studies in neighboring countries have similarly shown that Rotavirus is an important pathogen, responsible for 14.1%-62.2% in Saudi Arabia (Wilhelmi *et al.*, 2003; Ozdemir *et al.*, 2010; Rodriguez-Baez *et al.*, 2002; Nagabushana *et al.*, 2014; Basu *et al.*, 2003; Oldak *et al.*, 2006; Khoury *et al.*, 2011; Ifeanyi *et al.*, 2009; Nakawesi *et al.*, 2010; Topkaya *et al.*, 2006; Akhter *et al.*, 1999; Hani *et al.*, 2005), 32.5% in Northern Jordan (16) 16.7% - 32.2% in Turkey (17,18,), and 42% and 62% in south and north of Iran, respectively (19-20). However, in a study in Tahrán conducted by Zamani *et al.* Rotavirus antigen has been detected in (15.3%) of patients (Zamani *et al.*, 2004). Among Egyptian population, one of the enteric viruses was detected in 50% of fecal samples, of which 57.4% were positive for rotavirus (Kamel *et al.*, 2009). In another African studies, Shedding of RV was detected in from 6% to 56% (Basu *et al.*, 2003; Oldak *et al.*, 2006; Khoury *et al.*, 2011; Ifeanyi *et al.*, 2009; Nakawesi *et al.*, 2010).

In Egypt, 60% and 86% of Rotavirus -positive samples were related to patients younger than 1 and 5 years of age, respectively (Kamel *et al.*, 2009). In Korea a study conducted by Hong Koh and associates (2008) on children with acute watery diarrhea revealed that the detection rate of viral agents was most common in children between the ages of 1 and 3 yr. Rotavirus was detected in (41.3%) (Koh *et al.*, 2008). In Europe countries (France, Germany, Italy, Spain and United Kingdom), European surveillance data (24 & 25) found that most rotavirus gastroenteritis occurred in children aged 6 to 23 months (56.7% to 74.2% and even up to 80.9%) and 15.9% to 18.1% and up to 31.9% in those children aged <6 months and also cases were

observed among infants (<2 months) of age (Forster *et al.*, 2009; Van Damme *et al.*, 2007). Studies in the United States (26 & 27), confirm a burden of rotavirus infections in 55% to 71% of hospitalizations for AGE in children aged \leq 5 years. Enteric adenovirus are the most prevalent viruses in the etiology of pediatric gastroenteritis in developed as well as developing countries (Topkaya *et al.*, 2006; Modarres *et al.*, 2006). Our study is in good agreement with many studies in Iran and Turkey, where Adenoviruses (40 and 41) have been detected in 2.3%-15% and 10.5% of diarrheal patients, respectively. However, this study is in disagreement with other Turkish study (0.8% rate of infection) (Modarres *et al.*, 2006; Ozdemir *et al.*, 2010; Hamkar *et al.*, 2010; Motamedifar *et al.*, 2013). These data draw our attention and thought that these rates of infection detection are being dependent on the level of economic status of each country or related to the geographical regions or areas of these studies where each of them done. Age of children in relation to the rates of enteric viral infections: In an attempt to find a relationship between age distribution among cases in relation to the rate of each type of enteric viral infections, Table (2) shows that all the studied viral diarrhea tend to occur among younger age groups in a sequence from (0-12) months, (13-24) months and the least in (13-24) months group.

In comparison, the present results are in disagreement with two studies conducted in Turkey, where Rotavirus was detected as 23.7% and 14% in children aged less than 14 months, but this study in agreement with these studies where viral rates were decreasing with advancing the children age (13, 18). However, our study was also in good agreement with other reports from Bahrain, south as well as Northern Iran, Southern Korea, Indonesia, Nigeria and Central Africa, United States, and UK, which had showed that maximum rates of rotaviruses were in those children aged less than 1-year (Ozdemir *et al.*, 2010; Hamkar *et al.*, 2010; Motamedifar *et al.*, 2013; Kamel *et al.*, 2009; Subekti *et al.*, 2002; Odimayo *et al.*, 2008; Cortese and Parashar, 2006; Dutta *et al.*, 1990; Huh *et al.*, 2009; Grace *et al.*, 2010; Georges *et al.*, 1984). Regarding the rate of infection by Enteric adenovirus types 40 and 41, which are considered to be the second major causative agent of viral gastroenteritis after Rotaviruses among infants and young children less than two years of age in both industrialized and developing countries in most parts of the world, our adenovirus rates (5.6%) are in a good agreement with most of the findings of the others.

Herein, from north to south Iran (2.3 -15%), in Turkey (0.8-10.5%), while studies in developing countries such as in China, Saudi Arabia, Egypt, Gaborone Botswana, Singapore, Korea, Indonesia and Brazil showed that enteric adenovirus was found in 2.5%, 5.3%, 10.4%, 7.8%, 3%, 7.1%, 4% and 3.6% of diarrheal patients, respectively (28,19,20,18,6,13, 37,4,29,38, 39, 40,22,41, 42). Differences in rates of detection in the present as compared to other studies might be related to the level of economic status or geographical region of the study area (18, 28, 29, and 43). Astrovirus infections have been increasingly identified as important agents of acute gastroenteritis in children (30-33). Community studies identified rates between 5 to 17%, while 2 to 16% among the hospitalized children (Hamkar *et al.*, 2010; Walter *et al.*, 2000; Dalton *et al.*, 2002; Schnagl *et al.*, 2002). Astrovirus have been associated with 3-12% of diarrhea episodes in developed countries (19, 47, 48,49), whereas various developing countries have their specified detection rates; 2.7% in East Africa and 3.1% in Brazil

(Basu *et al.*, 2003; de Paula *et al.*, 2008; Cardoso *et al.*, 2002). However, other Brazilian authors have reported higher detection rates (Cardoso *et al.*, 2002; Victoria *et al.*, 2007; Silva *et al.*, 2001). Our results (Table 2) have recognized a higher rate of Adenoviral infection than a study conducted in Turkey among those aged (0-12) months (65.4% Vs 7.2%) while are consistent with that study regarding (13-24) months, and more than 24 months which revealed rates 19.2 % Vs 18.8%, and 15.4% Vs 8.1%, respectively. (18).

The present results are higher than those in a study conducted in Iran (1-11% in different age groups) (19, 20). However, the current results are consistent with them regarding more frequent rates in children under 14 months old as well as those findings reported by Kheyami *et al* and Akhter *et al* in Saudi Arabia (Kheyami *et al.*, 2006; Akhter *et al.*, 1999) and Barnes (1993) from Australia and Modarres *et al* from Iran (Modarres *et al.*, 2006). This present data are compatible with Carraturo in Italy (41.2%) (59.) and Lin *et al.* in Taipei (76.6%) (60). which were also more frequent among children younger than 24-36 months. After reviewing all these variations in the results of current and previous studies of Adenoviral infection in relation to age could be related to the fact that enteric adenoviruses produced milder symptoms than in patients with rotavirus infection so as to result in under-reporting as well as leading that fewer numbers of children families with adenovirus infections seeking hospital care (Hamkar *et al.*, 2010). Astroviral infections have been increasingly recognized as important agents in children with acute gastroenteritis (Hamkar *et al.*, 2010; Dalton *et al.*, 2002; Schnagl *et al.*, 2002).

Community studies had found a prevalence of Astroviral gastroenteritis in 5%-17% while in hospitalized children was 2 to 16%. (61,44). Globally, and depending on the geographical area as well socio-economic situation of each population studied, the detection rates varied from 1.8%-13.9% (Kheyami *et al.*, 2006; Akhter *et al.*, 1999; Barnes *et al.*, 1993; Kheyami *et al.*, 2006; Caracciolo *et al.*, 2007; Phan *et al.*, 2005). In developed countries prevalence of 3% -12% was reported (19, 47-48- 43) while in developing countries, such as East Africa was 2.7%, in Iran 3% and in Brazil was 3.1% (19, 47-48, 50-52).

However, other Brazilian authors have reported higher detection rates (Cardoso *et al.* 2002, Resque *et al.* 2007, Silva *et al.* 2001, and Victoria *et al.* 2007) (Cardoso *et al.*, 2002; Victoria *et al.*, 2007; Resque *et al.*, 2007; Silva *et al.*, 2001). These could be related to the socio-economic differences among Brazilian population. Although Astroviral infections have been detected among all studied ages, it is noteworthy that most surveys had concentrated on children as well as immunocompromised patients because they were at greater risk (Hamkar *et al.*, 2010; Bhattacharya *et al.*, 2006; Belliot *et al.*, 2010). In Iran as well as in Turkey, a maximum rates were observed among 2-5 years children (Ozdemir *et al.*, 2010; Hamkar *et al.*, 2010; Rodriguez-Baez *et al.*, 2002). However no Astrovirus was detected in children older than 3 years. in another study conducted in Turkey (Ozdemir *et al.*, 2010). In Korea, the Astroviral infection rate among children less than 5 years was 15.7%, however, among those aged of 1 and 3 years was (0.6%) (Hong Koh *et al.*, 2008). Noteworthy, Ozdemir S *et al* (2010) have concluded that no significant differences between adenovirus and astrovirus rates according to age were observed (5).

In our study, Astrovirus infections rate according to age were 80.8% and 19.1% for (0-12) months and, (13-24) months groups respectively. , while no Astrovirus detection in children with the age of >24 months. In comparison, a study in Iran Astrovirus infection rates were 16.7% in children <1 yr of age; 16.7% in those between 1 and 2 yr old while in those between 2 and 5 yr was 58.3% (Hamkar *et al.*, 2010). However, in a Turkish study reverse proportional distributions of Astrovirus infections were noticed, respectively (Ozdemir *et al.*, 2010). In Brazil, the rate of distribution of the astrovirus and adenovirus positive samples according to age group <1,1-6,7-12,13-24,25-36,37-48 months were zero%,3.5%,3.5%,2.2%, zero %, zero % of Astrovirus and zero %,3.0%,3.0%,5.7%, zero %, zero % for Adenovirus infections, respectively . These data mean that the maximum rate of infection for Astrovirus is restricted between 1-12 months age group⁵¹. Diarrheal etiology in about 40% of the cases is unknown although is a leading cause of morbidity and mortality in children less than five years of age. Enterovirus as a cause of diarrhea is well known, but there are few studies till date focusing on the epidemiological and clinical variables .associate this viral gastroenteritis. In fact, the 3 studied viruses constitute the major bulk of recent studies regarding acute gastroenteritis, however, this is a trial to shade light and adding some facts about Enteroviral gastroenteritis entity in children less than 5years age (Nagabushana *et al.*, 2014).

In the present study, 7.3% (34/ 465) of cases have positive- fecal Enterovirus antigen; in those aged ≤12 months, 13-24 months, and >24 months the rates of detection were 76.5%,17.6%, and 5.9%,respectively. These results are consistent with two Indian studies (Nagabushana, 2014 & Borade *et al.*, 2011) who reported a total rate of 11.5% and 14.50%, respectively, and was most commonly seen in 0-12 months infants (Nagabushana *et al.*, 2014; Aherkar *et al.*, 2009). Reviewing a preceding studies conducted in some regions of the world, it was specifically reported in Vietnam the predominance of Coxsackie viruses (51.9%) and echoviruses (29.6%) in feces from infants and children with acute gastroenteritis where these enteroviruses were identified throughout the year while the highest number was in December (Phan *et al.*, 2005). In Greece, EV 71 was found in 6.9%, EV 96 in 3.4% where as enteroviruses in Thailand were detected in (2.5%) of children with acute gastroenteritis. In Spain, EV 75 implicated in less severe cases, particularly in younger children (Kokkinos *et al.*, 2013; Chaimongkol *et al.*, 2012; Reina-Gonzalez *et al.*, 2007). In a study conducted in India from April 2009 to May 2011 and among the 282 studied diarrhea cases, 41 cases (14.5 %) were positive for Enteroviral infection.

These authors revealed a seasonal variations in enterovirus infection rates where highest rates have been documented in the months of June to August. In addition, and despite clean hygienic conditions, enterovirus is still an important cause of infantile diarrhoea in urban area like Pune (A Borade *et al.*, 2009). Finally, a study that was done by Nagabushana in India has confirmed the positivity of enterovirus antigens in 14 (11.5%) among 122 diarrheic children stool samples and noticed a maximal rate (30.8%) of Enterovirus infections in the months of monsoon (June to August) (Nagabushana *et al.*, 2014). Co-existence of more than one agent is not rare in gastroenteritis cases. Regarding distribution of rate of infections of pediatric patients with acute diarrhea according to their single or multiple viral infections (Table1), it was found that co-

infection of Rota V. with Adeno V. has occurred in (47.8 %); Rota V. with Astro in (21.5 %); Rota V. with Entero V. in (18.5 %); Adeno V. with Entero V. in (6.1 %); and lastly Astro V. & Entero V. in (6.1 %). Compared with previous studies, a study conducted in Tikrit / Iraq, and out of 30 tested stool samples, mixed infections of Rota-Adeno viruses were observed in 10(33.3%) cases. Koh and associates in Korea found co- infection of rotavirus with adenovirus in (12.9%) (Hong Koh *et al.*, 2008; Zaman *et al.*, 2012; Parashar *et al.*, 2003). Triple viral antigen positivity of (Rota V., Adeno V. & Astro V.) and (Adeno V., Astro V. & Entero V.) were each detected in 25%, whereas (Rota V., Astro and Entero V.) and (Rota V., Adeno V.& Entero V.) were detected in 37.5% and 12.5%,respectively. Different rates of such co-infection in other countries were reported. Compared with previous study conducted in Turkey revealed that (0.6%) of children with viral gastroenteritis yielded triple (Rota, Adeno, Astroviruses) infections while (9.6%) of them yielded two at a time.

Rotavirus & adenovirus association was (7.2%) the most frequently detected co-infections (Ozdemir *et al.*, 2010). In Iranian studies, Rotavirus-adenovirus co-infection rates were ranged from 4% to 64% of co-infected cases. (20, 37, 73). Mixed infections in Egypt constituted 10% while in Italy (1.3%), in Turkey (8%) and in Korea, co-infection of Rotavirus and Noro virus was most common (12.9%) including detection of co-infections with Adenovirus. Marcia *et al.*, (2008) in Brazil observed 50% co- infection of Rotavirus and Astrovirus, 40% for Rotavirus and Adenovirus and 10% for Astrovirus and Calici virus (Topkaya *et al.*, 2006; Kamel *et al.*, 2009; de Paula *et al.*, 2008; Lin *et al.*, 2000). A study conducted in Thailand revealed Rotavirus- mono infection prevalence of 27.5%, Enterovirus (2.5%), and Astrovirus as well as Adenovirus (each 0.6%). In addition, A rates of Norovirus (11.9%), Sapovirus (3.1%), and Human Parechovirus (1.9%), were observed, too. Bi- and triple-infections were observed in (4.4%) of patients. However, monthly distribution analysis revealed that all those viruses were detected continually throughout the year at a low level of infection (Chaimongkol *et al.*, 2012). The differences in the rates of diarrheal lesions associated with Rotavirus, Adenovirus, Astrovirus and Enterovirus as well as their rates of mixed infection could critically correlated with differences in respect to the variations in the standards of health, both at public services as well as individual levels, the tested patients sample size enrolled, seasonal variation at time of collection of these samples, and the types of techniques assigned and performed in each research work.

Conclusion

- Diarrhea associated with Rotavirus, Adenovirus, Astrovirus and Enterovirus has a significant prevalence among children less than five years with gastroenteritis in Baghdad, yet other types of viruses could played a role in those who have negative results for these 4 viral antigens.
- Rota virus-related gastroenteritis in Iraqi pediatric patients has showed similar popular trend to that global accepted one. However, Astroviral-related gastroenteritis showed a rising trend of infection followed by Adenoviral gastroenteritis.

- Mixed viral-gastroenteritis has constituted a respective bulk in Iraqi pediatric patients with gastroenteritis under 5 years of age.
- It is worthy to emphasize on the use of other molecular procedures such as RT-PCR for specifying the rates of common serotypes of rotavirus and other viral agents incriminated in those children with gastroenteritis.

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