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RESEARCH ARTICLE

Prevalence and Clinical Manifestations of Rotavirus and Adenovirus Infections in Children Under Five Years Old in Katsina State, Northwestern Nigeria.

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Abstract

Gastroenteritis is a leading cause of childhood morbidity and mortality in developing countries. Our aim was to determine the prevalence of rotavirus and adenovirus infection and their co-infection in children aged < 5 years in Katsina State, Northwestern Nigeria. A total of 400 (322 diarrhoiec and 78 non-diarrhoiec) stool specimens were collected from children attending six hospitals located across the three senatorial zones of the state from June 2013 to April 2014. Their socio-demographic information and clinical presentations were noted with the aid of questionnaire. Viral antigens were detected by enzyme linked immunosorbent assay (ELISA). Rotavirus was detected in 5.3% of the diarrhoiec and none in the non-diarrhoiec specimens while adenovirus was detected in 12.4% of the diarrhoiec and 5.1% of the non-diarrhoiec specimens. Co-infection of rotavirus and adenovirus was observed in 0.6% of the diarrhoiec children. Generally, children < 2 years old were more vulnerable to rotavirus and adenovirus infection. There was a significant association between dehydration and rotavirus and adenovirus infections ($p<0.05$).

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Introduction:-

Acute infective gastroenteritis is a major global health problem which manifests as three or more watery or loose bowel evacuations in a 24 hour period that may last several days, with fever and vomiting. Children under 5 years of age are particularly susceptible, and global estimates indicated a mean of between 3.5 and 7 episodes of severe diarrhoea during the first 2 years of life, and the greatest burden is in the developing countries because of poor sanitation, lack of safe drinking water, and bad sanitary habits (WHO, 2009).

Rotavirus infection is the single most important cause of infectious, severe, dehydrating diarrhoea and death globally in children aged 5 years and below (Ahmed *et al.*, 2009; Dhama *et al.*, 2009), and continues to have a great impact on childhood morbidity and mortality (Dennehy, 2008).

Adenoviruses, particularly enteric adenoviruses (EAds) type 40 (Ad40) and type 41(Ad41), can cause acute and severe diarrhoea in young children worldwide (Seranti *et al*., 2004). Adenoviruses are often recovered from the stools of young children and are considered to be the second most important viral agent associated with infantile gastroenteritis (Grimwood *et al.*, 1995; Audu *et al.*, 2002; Basu *et al.*, 2003). Further studies indicate that adenoviruses are associated with 4-15% of all hospitalized cases of viral gastroenteritis (Grimwood *et al*., 1995).

In Nigeria, few available studies conducted in the Southern and North central regions have associated adenoviruses with 3.8%and 6.7% of pediatric diarrhoea. In Northwestern Nigeria, there is paucity of data regarding the epidemiology of enteric adenoviruses, which some studies have implicated as the second most important viral agent

associated with gastroenteritis in children (Nimzing *et al.*, 2000). Therefore, this study was undertaken in order to establish the role of rotavirus and adenovirus in gastroenteritis in Nigerian children and in Katsina State in particular.

Methodology:-

Study design

The study was a cross-sectional hospital survey of children between 0 to 5 years of age with or without diarrhoea. Those without diarrhoea served as control. Informed consent was obtained from the parent/guardian of each child.

Study Area

The study was carried out in Katsina State between July 2013 and April 2014. The state is located at the extreme northern margin of Nigeria, covers a total area of about 23,938sqkm (3,370sq) with a total population of 5,801,584 people, going by 2006 census (FGN 2007). The state is bounded by Niger Republic to the north, by Jigawa and Kano States to the east, by Kaduna State to the South and by Zamfara State to the West. Katsina state has predominantly Hausa-Fulani indigenes. About 75% of the people are farmers and others are traders and livestock owners.

Inclusion criteria and Exclusion criteria

The inclusion criterion was any child between the ages of 0-5 years of both sexes who were presented or admitted for diarrhoea illness in the selected hospitals.
The exclusion criterion was children above 5 years of both sexes.
A diarrhoea case in the study was defined as a child passing loose, watery or a bloody loose stool three or more times in a 24-hour period as reported by the parents. The control was considered as any child that was presented for an illness other than diarrhoea and with no history of it on the day of, or in three weeks preceding sampling.

Approval

Ethical approval was obtained from the ethical committee of Katsina State Ministry of Health.

Sample collection

320 faecal specimens were collected from children between the ages of 0-5 years that were presented or admitted at clinics or hospitals for acute diarrhoeal illness, and 80 from non diarrhoeic patients as controls. The specimens were collected in a clean, labeled screw capped tubes and transported immediately in ice-cooler boxes to Postgraduate Microbiology Laboratory Ahmadu Bello University, Zaria and stored at 4^{o}C.

Sample processing and laboratory analysis

A 10% faecal suspension was prepared by adding 0.1g of solid stool or 100µl of liquid stool to 900µl distilled water in a screw cap test tube, vortex 30-60 seconds to mix thoroughly.

Viral Antigen Detection using ELISA

Each of the 10% faecal suspension was screened for the presence of rotavirus and adenovirus antigens using commercially available enzyme immunoassay (Diagnostic Automation, Inc, US) kit.
The assay was done strictly according to the manufacturer`s instruction in order to determine enteric virus positive specimens.

Data analysis and presentation

Analysis of rotavirus and adenovirus infection in children according to age, sex and dehydration was done using SPSS version 20.0. P values < 0.05 were considered statistically significant.

Result:-

A total of 400 stool samples were screened for rotavirus and adenovirus antigen. Of these, 322 and 78 were diarrhoeic and non-diarrhoiec samples respectively. Among the 322 children that were presented with diarrhoea, 5.3% (17/322) were positive for rotavirus and no viral antigen was detected in the non-diarrhoeic samples. Rotavirus was significantly associated with diarrhoea in this study (χ^2= 4.3008, df= 1, p= 0.038095). Adenovirus was detected in 12.4% (40/322) of the children with diarrhoea and in 5.1% (4/78) of the non-diarrhoeic children. Even though, there was no statistically significance difference of adenovirus infection between the diarrhoeic and non-diarrhoeic

children (χ^2= 3.4124, df= 1, p= 0.064707), those with diarrhoea were about three times more likely to be infected with adenovirus than those without diarrhoea (OR = 2.6241, 95% CI= 0.9098-7.5685) (Table 1).

Co-infection of rotavirus with adenovirus was observed in 0.6% (2/322) of the diarrhoiec children (Figure 1). Analysis by sex and age showed that co-infection was found in one male and one female with 24 and 36 months old respectively. All the co-infected children were found in the diarrhoeic population. Examination of their stool samples showed that they both had watery stool and which was passed atleast1-3 times in 24 hour period.

82.4% of positive cases of rotavirus gastroenteritis were under 2 years of age with highest prevalence in children 7-12 months of age, while adenovirus was most prevalent among children aged 0-6 months (Table 2).

There was no significant difference in the prevalence of rotavirus (χ^2= 0.005, df= 1, p= 0.944) and adenovirus (χ^2= 3.1942, df= 1, p= 0.073901) infection among male and female children in this study, although rotavirus shedding was slightly higher in male (5.5%: 10/182) than in female children (5.0%: 7/140). Conversely, adenovirus infection was observed to be slightly higher in female (13.2%) than in male children (11.0%) (Table 3).

In this study, rotavirus and adenovirus infections were significantly associated with dehydration (P<0.05). Among the diarrhoeic children positive for rotavirus, the degree of dehydration among the children was found to be mild, moderate, severe and absent in 13.5% (5/37), 2.8% (4/145), 8.3% (7/84) and 1.8% (1/56) of cases respectively while in adenovirus infected children, it was severe, mild and absent in 40.9% (18/44), 45.5% (20/44), and 13.6% (6/44) of children respectively.

The major clinical features presented by the rotavirus positive children included a combination of diarrhoea, fever and vomiting (35.2%: 6/17), diarrhoea and fever (29.4%: 5/17), dairrhoea and vomiting (23.5%: 4/17) and diarrhoea only (11.8%: 2/17). In adenovirus infected children, a combination of diarrhoea, fever and vomiting occurred in 52.5% (21/40) of the children, diarrhoea and fever in 17.5% (7/40), diarrhoea and vomiting in 20.0% (8/40) and diarrhoea only in 10.0% (4/40).

Analysis of stool specimen in rotavirus infected children showed watery stool in 70.6% (12/17), stool with mucus in 23.5% (4/17), stool with mucus and blood in 5.9% (1/17). Analysis of stool specimen in adenovirus infected children showed watery stool in 54.5% (24/44), stool with mucus in 22.7% (10/44), stool with mucus and blood in 13.6% (6/44) and normal stool 9.1% (4/44) (Figure 2).

The frequency of stool passed per day varied among the children, about 52.9% (9/17) of the children infected with rotavirus passed stool 4-6 times a day, 35.3% (6/17) passed stool 1-3 times a day and 11.8% (2/17) 7-10 times a day respectively. Of the 44 adenovirus positive diarrhoeic children, 60% (24/40) passed stool 4-6 times a day, 32.5% (13/40) passed stool 1-3 times day and 7.5% (3/40) passed stool 7-10 times a day (Figure 3).

Table 1: Prevalence of Rotavirus and Adenovirus Infection in Diarrhoeic and Non-diarrhoeic Children 0-5 Years Old in Katsina State, Nigeria

	Rotavirus		**Adenovirus**		
Population	**Positive (%)**	**Negative (%)**	**Positive (%)**	**Negative (%)**	**Total**
Diarrhoeic	17 (5.3)	305 (94.7)	44 (12.4)	282 (80.5)	322
Non-Diarrhoeic	0 (0.0)	78 (100)	4 (5.1)	74 (19.5)	78
Total	**17 (5.3)**	**383 (95.7)**	**44 (11.0)**	**356 (89)**	**400**

Rotavirus: χ^2 = 4.3008, df= 1, p= 0.03
Adenovirus: χ^2= 3.4124, df= 1, p= 0.06; (OR = 2.6241, 95% CI= 0.9098-7.5685)

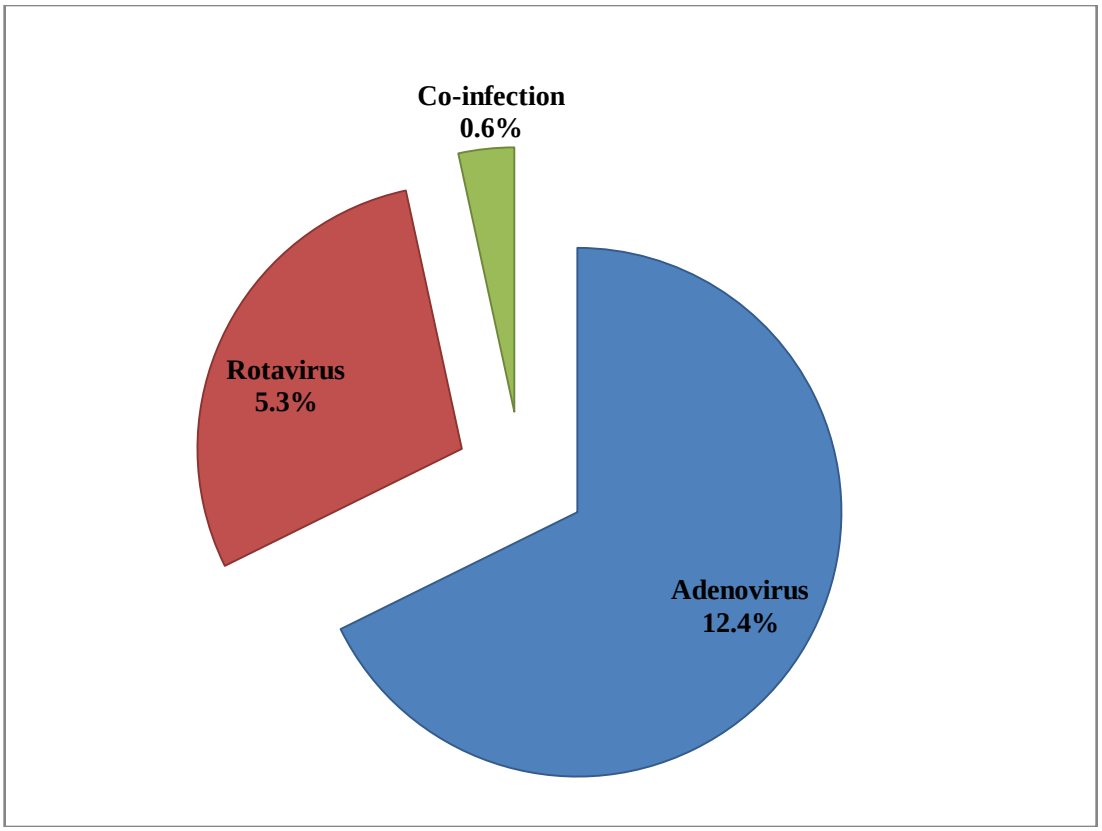

Figure 1: Co-infection of Rotavirus with Adenovirus in Diarrhoiec Children 0-5 Years Old in Katsina State, Nigeria.

Table 2: Distribution of Rotavirus and Adenovirus Infection in Relation to Sex in Diarrhoiec Children 0-5 Years Old in Katsina State, Nigeria

	Rotavirus		Adenovirus		
Sex	**Positive (%)**	**Negative (%)**	**Positive (%)**	**Negative (%)**	**Total**
Male	10 (5.5)	172 (94.5)	20 (11.0)	162 (89.0)	182
Female	7 (5.0)	133(95.0)	24 (13.2)	116 (63.7)	140
Total	**17 (5.3)**	**305 (94.7)**	**44 (12.4)**	**278 (87.6)**	**322**

Rotavirus: χ^2= 0.005, df= 1, p= 0.9444
Adenovirus: χ^2= 3.1942, df= 1, p= 0.073901

Tarble 4.3: Age Distribution of Rotavirus and Adenovirus Infection among the Under 5 Children with Diarrhoea in Katsina State, Nigeria

	Rotavirus		Adenovirus		
Age	**Positive (%)**	**Negative (%)**	**Positive (%)**	**Negative (%)**	**Total**
0-6	0 (0.0)	25	5 (20.0)	20	25
7-12	5 (7.7)	60	8 (12.3)	57	65
13-18	1(3.0)	32	6 (18.1)	27	33
19-24	8(7.0)	107	11 (9.6)	104	115
25-36	2(4.0)	48	8 (16.0)	42	50
37-60	1 (3.0)	33	2 (5.9)	32	34
Total	**17 (5.3)**	**305**	**40 (12.4)**	**282**	**322**

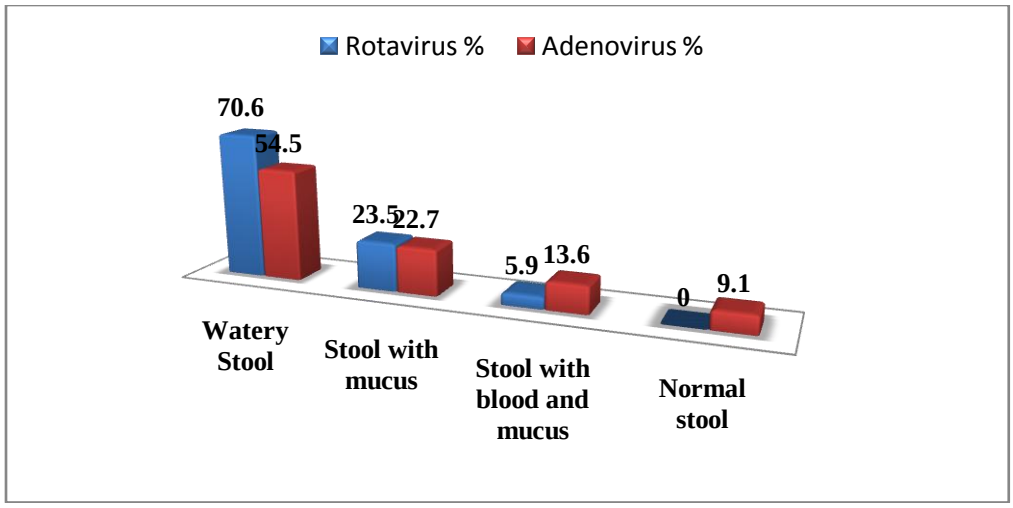

Figure 2: Stool type in Rotavirus and Adenovirus Infected Children 0-5 Years old in Katsina State, Nigeria

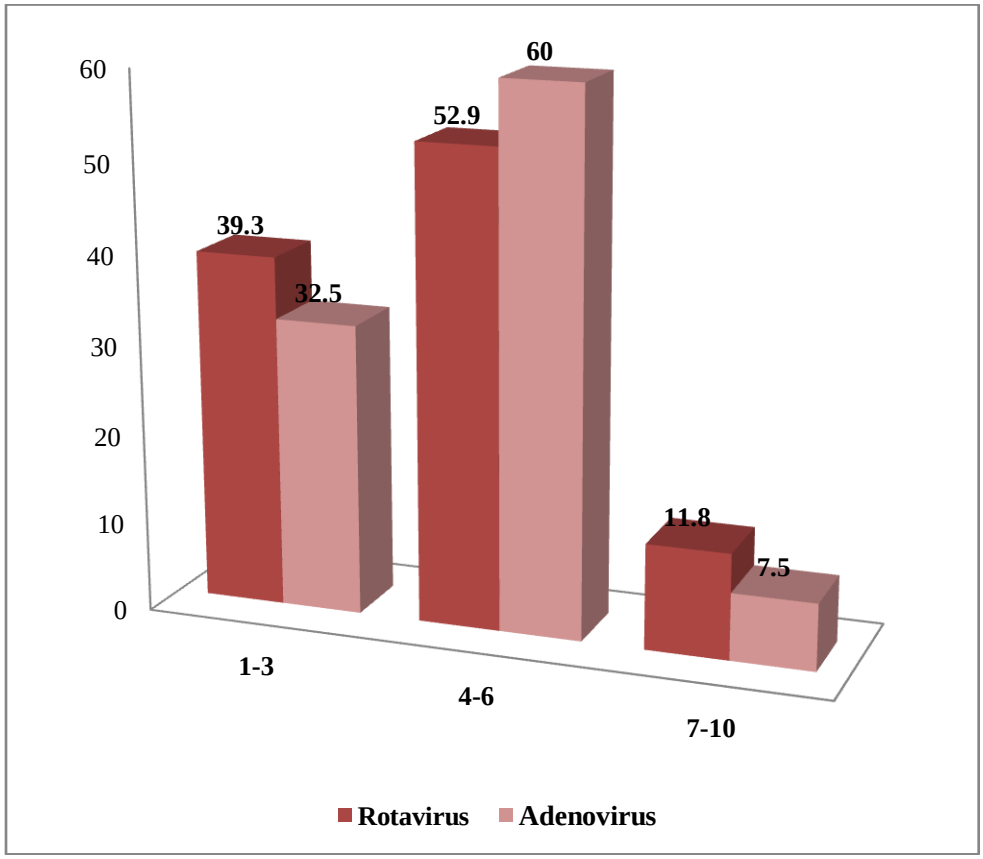

Figure 3: Frequency of Stool Passed in 24 hour period (per day) Among the Rotavirus and Adenovirus Infected Children 0-5 Years Old in Katsina State, Nigeria.

Discussion:-

In this study, rotavirus infection occurred in 5.3% of children 0-5 years old that presented with diarrhoea and did not occur in the control group. This implies that rotavirus is significantly associated with diarrhoea. Rotavirus has been shown to be the most important cause of viral diarrhoea in children less than five years of age in Nigeria (Pennap *et al.*, 2010; Aminu *et al.*, 2008; Kuta *et al.*, 2014), Ghana (Armar *et al.*, 2003) and Cameroon (Esona *et al.*, 2003).

The prevalence of rotavirus infection in this study is comparable to the report of Kuta *et al,* (2014) where a prevalence of 4.5% was reported in their study in three North Central States and Federal Capital Territory Abuja, Nigeria. However, the result is lower than previous reported studies in other parts of the country such as 13.0% in Ibadan, 11.0% in Jos, 11.9% in Maiduguri, 18.0% in Northwestern Nigeria, 16.3% in Zaria and 13.8% in Jos, (Ojeh et al., 1995; Nimzing *et al.*, 2000; Adah *et al.*, 2001; Aminu *et al.*, 2008; Pennap and Umoh 2010; Junaid *et al.*, 2011).

On the other hand, the prevalence of adenovirus infection (12.4%) in this study is comparable to the results of other studies were a prevalence of 14% was reported in Bangladesh (Kim *et al.*, 1990),13% in Mexico (Maldonado *et al.*, 1998), 16.7% in Lagos (Audu et al., 2002), 16.2% in Khartoum (Elhag et al., 2013). In Africa and other parts of the world, lower prevalence of 3.8% in Jos, Nigeria (Nimzing *et al.*, 2000), 7.8% in Gaborone (Basu *et al*., 2003), 5% in Northern France (Tran *et al.*, 2010), 3.3% in South Western Iran (Kajbaf *et al.*, 2013), 3.7% in Najra region, Saudi Arabia (AlAyed *et al*., 2013) have been reported. However, a higher prevalence of 22.3% in Nigeria has been reported (Aminu *et al*., 2007).The presence of adenoviruses in non-diarrhoeic children may probably reflect the facts that adenoviruses other than enteric adenoviruses (EAds) are often excreted in stool. Similar studies by Audu *et al*, (2002) and Aminu *et al,* (2007) have previously reported presence of adenoviruses in non-diarrhoeic children.

The observed differences in prevalence might be due to the differences in geographical location or alternatively it might reflect changing trends (CDC, 2008). The differences could also be due to difference in climatic conditions, environmental and socio-economic factors or cultural practices. Overcrowding, poverty and poor general sanitation, which are all factors enhancing the transmission of human rotavirus infection (Aminu *et al.*, 2008) might account for high level of rotavirus infection seen in other parts of the country.

The lower prevalence of rotavirus observed in this study might also be due to seasonal factor or long period of specimen storage, which might have led to disintegration of the viral particles, hence insufficient antigen in the specimen. In addition, the patients might be shedding rotavirus antigen lower than the detection limits of the test assay.

Co-infection of rotavirus with adenovirus in this study was observed in 0.6% of the diarrhoeic children. This result is comparable with the results of Nimzing *et al,* (2000) where a prevalence of 1.1% was reported. However, our prevalence is lower than that previously reported by Aminu *et al,* (2008) and Tran *et al,* (2010) where a prevalence of 3.2% was obtained. The dual infection observed in this study may be attributable to either a single virus is responsible for the diarrhoea or the two viruses act in synergy. More so, the multiple infections observed may be attributable to poor hygienic conditions and overcrowding peculiar to the study area. Poor hygienic condition has been suggested to contribute to multiple infections in developing countries (Guix *et al*., 2002).

Rotavirus infection was observed to be slightly higher in male (5.5%) than in female (5.0%) children, hence there was no statistically significance difference in rotavirus infection between male and female children with diarrhoea in this study ($P>0.05$). This is similar to other studies in Nigeria where rotavirus was detected in slightly higher rates in male than in females (Aminu *et al.*, 2008, Pennap and Umoh 2010; Junaid *et al.*, 2011). Similar studies in other countries such as in South Western Iran (Kajbaf *et al*., 2013), and in Sudan (Magzoub *et al.*, 2013) have reported higher detection rates of rotavirus infection in males than in females. However, studies from Cameroon (Ndze *et al*., 2012) reported higher detection rates in females (45.3%) than in males (40.8%). Conversely, adenovirus infection was observed to be slightly higher in females (13.2%) than in males (11.0%), hence there was no statistically significance difference in adenovirus infection between male and female children with diarrhoea in this study ($P>0.05$). This is similar to the findings of many studies (Audu *et al.*, 2002; Samarbaf-Zadeh *et al.*, 2010) where they found no any significant difference in infection between both sexes. The reason for the lack of statistically significance difference in detection rate between male and female children may be explained by the fact that at younger age, both sexes have little or no major differences in their life style. Alternatively, the slight differences observed might be due to sex susceptibility or by chance, whether this difference is due to sex susceptibility or by chance is however questionable and needs further investigation.

In this study, 82.4% of the children positive for rotavirus were under two years of age, emphasizing the fact that rotavirus infection occurs early in life. This is in consistent with a number of similar studies in Nigeria (Audu *et al.*, 2002; Aminu *et al*., 2008; Junaid *et al.,* 2011; Anochie *et al.,* 2013) and most parts of the world (Basu *et al.,* 2003; Kargar *et al.,* 2012; Almusawi *et al.,* 2013; Magzoub *et al.,* 2013; Karakus *et al.,* 2014).

The highest prevalence of rotavirus infection occurred in the age-group 7-12 months (7.7%). This age-group distribution is comparable to previous reports by Junaid *et al*, (2011) and Kajbaf *et al*, (2013) where they reported higher prevalence in children 7-12 months old. The least prevalence of rotavirus infection was observed in the age-group 0-6 months. The low rate of infection in infants may be attributed to a higher rate of breast feeding thereby providing partial protection due to present of maternal antibodies in breast milk.

The detection of rotavirus mostly in children under 2 years in this study is in accordance with the assumption that in under-developed areas the early peak of rotavirus diarrhoea may result from early exposure to contaminated sources as well as over-crowded homes, more so, since almost all humans experience at least one rotavirus infection by 3 years of age and circulating rotavirus antibodies remain detectable indefinitely (Bernstein and Ward 2004). This may lead to protection against rotavirus infection and disease or at least milder forms of disease, which result in lower rate of rotavirus gastroenteritis in older children.

Further analysis of adenovirus infection by age, showed that (70.5%) of positive cases were in children under 2 years of age. This result is consistent with the findings of most studies conducted worldwide where children less than 2 years of age are more vulnerable to adenovirus infection (Basu *et al.,* 2003; Filho *et al.,* 2007; Samarbaf-Zadeh *et al.,* 2010). However, this result is in contrast to the findings of Aminu *et al*, (2007) where the detection of adenovirus infection was mostly in children older than 2 years of age.

The highest prevalence of adenovirus infection occurred in the age-group 0-6 months (20.0%). Our observation of a higher prevalence in children within the age brackets below 24 months of age and a lower prevalence in the age groups of 24 to 60 months of age may mean that the children are infected much earlier in life but go on to develop resistance to infection. In other words, this could be explained by the fact that older children acquire protective immunity during repeated exposures to the virus and therefore subsequent infections are mild or asymptomatic (White *et al.,* 2008).

In this study, rotavirus and adenovirus was significantly associated with dehydration. Viral infection rate was higher among children with mild dehydration. Previous studies by Aminu, (2006), Parashar *et al*, (2006) and Pennap and Umoh, (2010) have made similar observations.

Rotavirus and adenovirus shedding was highest when a combination of all the three symptoms (diarrhoea, fever and vomiting) occurred together and lower when two occurred together and lowest when diarrhoea occurred alone. These clinical features observed are the major symptoms that accompanied rotavirus diarrhoea. This is similar to the observations made by Aminu (2008), Parashar *et al*, (2006), Pennap and Umoh (2010) and Junaid *et al*, (2011). Similar observations of these clinical features among adenovirus infected children have been made Audu *et al*, (2002) and Aminu *et al*, (2007). These clinical features observed are suggestive of the sign and symptoms cause by adenovirus associated diarrhoea.

Conclusion:-

In this study, rotaviruses and adenoviruses were found to be an important cause of diarrhoea in children 0-5 years old in Katsina State, Nigeria. However, the prevalence of rotavirus infection among the children appears to be relatively low while adenoviruses were shown to circulate at a higher frequency in association with gastroenteritis in children less than five years old. Our study provides evidence that adenoviruses can be a leading cause of viral gastroenteritis infection in children less than five years of age.

Rotavirus infection was more prevalent children 7-12 months old while adenovirus virus was more prevalent in children 0-6 months old. Rotavirus and adenovirus detection was greatest when diarrhoea, vomiting and fever occurred together and lowest when diarrhoea occurred alone.

Recommendations:-

Extensive research on adenoviruses should be carried out because their role and position in viral gastroenteritis as shown by this study and many others, is becoming increasingly higher as compared to other viral agents of gastroenteritis.

Since the detection of rotavirus in this study was mostly in children 2 years and below, rotavirus vaccine should be included into the Expanded Program on Immunization (EPI) as this will greatly reduce rotavirus associated morbidity and mortality within this group.

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