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Seroprevalence and Socioeconomic Characteristics of Human Sapovirus Infection among Children Attending Specialist Hospital, Sokoto, Sokoto State, Nigeria

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Abstract

The study investigates the seroprevalence and socioeconomic characteristics of human sapovirus infection among children aged 0-5 years attending a specialist hospital in Sokoto, Sokoto State, Nigeria. A total of 100 blood samples were collected for human sapovirus detection and Sapovirus IgG/IgM. A questionnaire was used to obtain the socioeconomic characteristics associated with human sapovirus infection. Sapovirus IgG/IgM was detected using ELISA kit. The results showed a prevalence of 7.70% sapovirus infection among children. There was an association of sapovirus among males (4.40%) and females (3.30%). Children within 49-60 months had the highest sapovirus infection of 17.60% while Parents/Guidance with secondary level of education had the highest sapovirus infection of 3.30%. Parents/Guidance residing in the rural areas had a higher sapovirus infection rate of 8.20%. Children living in families of 4-6 had the highest sapovirus infection rate of 4.0%. There was an association between the occupational status of the children's parents/guidance and the prevalence of sapovirus infection; farmers had the highest prevalence of 3.30%. The serological assays (ELISA) provide information about the prevalence of HSaV infection among children in the study area. Routine testing for all enteric viruses, especially Human Sapovirus, is needed.

Key Words: ELISA, IgG/IgM, Prevalence and Sapovirus

INTRODUCTION

Sapovirus is an enteric virus and is recognised as a public health problem causing acute gastroenteritis in people of all age groups globally and it also causes outbreaks in semi-closed settings, like orphanages and elderly care facilities (Oka *et al.*, 2015), The increase of acute gastroenteritis associated with sapovirus (SV) has been reported and recognised as a major public health problem particularly in developing countries (Liu *et al.*, 2016). It is documented that after the successful deployment of the Rotavirus vaccine, SVs have emerged as the second most common etiological agent behind Norovirus in children with acute diarrhoea (Liu *et al.*, 2016).

In children with acute diarrhoea IgG antibodies are not detectable in peripheral blood in immune-mediated diseases in particular, IgM serology can be the test of choice since the patient presents when IgM levels are rising and virus titers have declined (Khira *et al.*, 2010) the acquisition of antibodies to Sapovirus begins early in life and antibody prevalence rates for adults. The antibody prevalence studies showed that virtually all children are infected with Sapoviruses by the time they are 5 years of age, indicating that Sapovirus infection is widespread, although the illness most likely is sporadic with a high rate of asymptomatic infections (Khira *et al.*, 2010)

Sapoviruses can be detected by ELISA, EM and/or RT-PCR in faeces of infected humans. The ELISA, among other techniques, allows testing of numerous samples in a short time, without any specialised equipment. ELISAs have been developed and used for large-scale epidemiologic studies of human sapoviruses (Yomashita *et al.*, 2010). Moreover, a major weakness of ELISAs is the high specificity that each antiserum has shown for the homologous strain used to produce it, but not for heterologous strains (Yomashita *et al.*, 2010). This is a disadvantage for screening samples in epidemiologic studies because the circulating strains are often highly diverse.

Human sapovirus infection was detected in most of Africa, such as Peru (Sanchez *et al.*, 2018), Iran (Romani *et al.*, 2012), Ethiopia (Galaw *et al.*, 2019) and Egypt (Maysaa *et al.*, 2022). In Nigeria, especially in Sokoto, Human sapovirus infection is not routinely diagnosed, probably due to the cost of the diagnosis, and the clinical spectrum of signs and symptoms is similar to other gastroenteritis caused by viruses: Rotavirus, Astrovirus, and Norovirus. These previous data suggest that sapovirus plays an important role in pediatric gastroenteritis. Pursuing our research on seroprevalence of Sapovirus, we report the first seroprevalence of antibodies (IgG/IgM) to Sapovirus in Sokoto, Sokoto State. Nigeria

Therefore, the present study aimed to investigate the seroprevalence of Human Sapovirus among Children attending the Specialist Hospital, Sokoto, Sokoto State, Nigeria. The results of the study will provide information on human sapovirus circulation in Sokoto, including other agents of gastroenteritis in children, and the association between human sapovirus and socio-economic factors associated with human sapovirus infection. Additionally, the study will serve as reference material for further research work in the study area.

METHODOLOGY

Study Area

The specialist hospital is located at latitude 13.06269^{0}N and longitude 5.24322^{0}E in Sokoto South Local Government Area, situated in the State Capital. It was established by the colonial master in 1937. It is one of the major referral hospitals within and outside the state capital (Alhassan *et al.*, 2012). Sokoto State lie between longitude 3° and 7° E and latitude 10° and 14° N of the Equator. It has 23 LGAs, and is bordered to the North by the Republic of Niger, Zamfara State to the East, and Kebbi State to the South and West. Sokoto State covers an area of 28,232 km^2 and has a population of 3,702,676 (NPC, 2006) census. The predominant tribes are Hausa, Fulani, Gobirawa, and Zabarmawa, while Islam is the main religion. Agriculture, petty trading, craftsmanship, and civil service are the main occupations of the people (NPC, 2006).

Sampling size

The study adopted a purposive sampling technique among the children attending the Specialist Hospital, Sokoto, Sokoto State, Nigeria. The study population consisted of children aged 0-5 years with mild to severe symptoms of diarrhoea attending a special Hospital in Sokoto, Sokoto State, Nigeria. A Total of one hundred (100) blood samples were collected.

Sample size was determined using the formula described by Petronella (2012) formula with a prevalence of 6%. A prevalence of 6% was considered since the prevalence of Human sapovirus in children aged 0-5 years is not known in Sokoto, North Western Nigeria.

$N = [Z^2 P (1-P)]/ D^2$

Where:

N = Minimum sample required

Z = 1.96 standard error

P = Prevalence 7% = 0.007

D = the desired degree of accuracy at 5% confidence level= 0.05

$N= [(1.96)^2 \times 0.07 (1- 0.08)]/(0.05)2$

N = 100.04

Serological Detection of IgG and IgM antibodies of human sapovirus

The detection of Human sapovirus-specific antibodies (IgG and IgM) was performed by ELISA. Human sapovirus IgM and IgG were detected using commercial ELISA kits (Melson Medical Co., Ltd., Kuancheng District, Changohun Jillin province China) according to the manufacturer's instructions and as previously described by Becker *et al* (2019).

The Kit uses an enzyme-linked immunosorbent assay double antigen sandwich principle to analyse the existence or not of Sapovirus IgM (SAV-IgM/IgG) in the samples. The micro-ELISA strip plate provided in this kit was coated with antigen. Add samples to wells with sapovirus IgM (SAV-IgM/IgG) conjugate HRP. Any antibodies specific for the antigen present will bind to the pre-coated antigen. Followed by washing to remove unbound substance. Finally, chromogen solution A and chromogen solution B were added, and a blue colour developed. The reaction is then stopped, and the colour turns to yellow when the stooping solution (acidic) is added. The existence or not of sapovirus IgM (SAV-IgM/IgG) in the samples is then determined by comparing the O.D. of the samples to the CUT OFF

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The serum samples were transferred into sterile Eppendorf test tubes. All reagents were brought to room temperature before used. Two positive and two negative controls were set on a pre-coated microplate. Separately, 50ul was added to the positive and negative wells. Add 10ul of the tested sample, then add 40ul of sample diluents to the test sample well. Blank well doesn't add anything. Add 100 µL of HRP-conjugate reagent to each well, cover with an adhesive strip, and incubate for 60 minutes at 37 °C. Each well was aspirated and washed four times for a total of five washed. Washed by filling each well with washed solution (400 µL) using a squirt bottle. Chromogen solution A (50 µL) and chromogen B (50ul) were added to each well, respectively. Gently mixed and incubated for 15minutes at 37^0c. And a stop solution was added to each well. The optical density was measured at 450nm using a microtitter plate reader (BioTek, Elx800, US) within 15 minutes. These results were then interpreted against specific negative and positive controls included in each plate.

The average positive control well ≥ 1.00 the average of the negative control well ≤ 0.15

CO = the absorbance value for the negative control well + 0.15

Negative control: Sapovirus IgM (SAV-IgM) O.D. < Calculated critical (cut off), the result is negative

Positive control: sample IgM (SAV-IgM) O.D ≥ Calculated critical (cut off), the result is positive

Ethical approval

Ethical approval for the study was collected from the ethical Research committee of the Specialist Hospital in Sokoto, Sokoto State, Nigeria.

Inclusion criteria

i Children within 0-5years, as in or outpatient,s observed in a short-stay unit or prolonged stayed in the hospital were included in the study.

ii Children with diarrhoea, nausea, vomiting and fever were included in the study

iii Children's parents or legal guardians that gave consent to participate in the research were included in this study

Exclusion criteria

i Children above 0-5years, as in and outpatient observed in a short-stay unit or prolong stayed in the hospital were excluded from the study

ii Children with vomiting, or diarrhoea, with respiratory illness for which the parents/guidance could not explain the cause of vomiting, were excluded from the study

iii Children's parents or legal guardians that refused consent were excluded from the study.

Determination of Socioeconomic characteristics

The socioeconomic information of the parents/guidance and children was generated using a structured questionnaire (Residence, age group, sex, exclusive or inclusive breastfeeding, educational status, and occupation of the parents/guidance).

Statistical Analysis

Questionnaire data generated from patients were analysed using the Statistical Package for the Social Sciences (SPSS) software version 16. Categorical variables were compared using chi-square; P values of > 0.05 were regarded as statistically significant.

RESULTS

A total of one hundred (100) blood samples were randomly collected from children 0-5 years attending the specialist hospital in Sokoto, Sokoto State. Figure 1. 24% and 41.76% are male and female children aged 0-5years involved in the studied. Males have the highest Sapovirus infection rate, 4.4%, compared to females, 3.3%. There was no association with gender (P-value = 1.000) is significant at 0.05 Table 1. The highest sapovirus infection was observed among children within 49-60 months (17.60%), compared to other age groups, but there was no association (P-value = 0.064 < 0.05). There is an association among the educational levels of parents/guidance: Primary 23.08, secondary 27.47, Tertiary 19.78, and others 29.67. Sapovirus infection was recorded in 3.3% of parents/guidance with a secondary level of education out of the total examined (53.85%) from rural areas and (46.15%) from urban areas. Rural areas constitute the highest number of

sapovirus infection (8.20%), while urban areas have 7.10% of Sapovirus infection. There is an association between the rural and urban areas, as the P-value of 1.000 is greater than $x^{2.}$ Value 0.03. Family size: Out of 91 samples examined (37.36%) have a family size of 1-3, (26.37%) have a family size of 4-6 and (36.26%) have a family size of 7 and above. Family size of 4-6 has the highest Sapovirus infection (4.4%) showing that there is no association as the x^2 = 3.697 > p Occupation status: Out of 91 samples examined, 39.56 % are farmers, 24.18% are civil servants, and 33 (36.26%) are businessmen and women. Sapovirus infection was higher among children whose guidance/parents are farmers (3.3%), Table 1. There is an association among the occupation of the guidance /parent as p-value> 0.05 0.760 at 0.

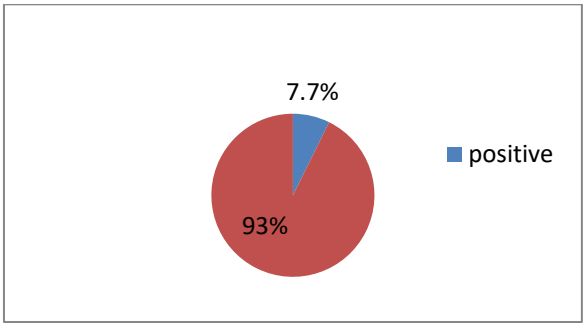

Figure 1: Seroprevalence of Human Sapovirus (HSaV) Among Children (0-5) Year

Table 1: Distribution of Human Sapovirus Infection According to Socioeconomic Characteristics

Gender	Number of samples	Percentage		P value	
		Positive (%)	Negative (%)		X^2 value
Male	53(58.24)	4(4.4)	49 (53.8)	0.004	1.000
Female	38 (41.76)	3 (3.3)	35 (38.5)		
Age					
0-12	16	1(6.67)	15 (93.80)	**8.902**	**0.064**
13-24	20	0(0.00)	20(100)		
25-36	22	1(4.50)	21(95.50)		
37-48	16	2(12.50)	14(87.50)		
49-60	17	3(17.60)	14(82.40)		
Educational level					
Primary	21(23.08)	2 (2.2)	19 (20.90)	0.129	0.660
Secondary	25(27.47)	3 (3.3)	22 (24.20)	0.901	0.343
Tertiary	18(19.78)	1 (1.1)	17 (18.70)	0.144	1.000
Others	27(29.67)	1 (1.1)	26 (28.60)	0.123	0.245
Residential					
Rural	49 (53.85)	4 (8.20)	45 (91.80)	0.033	1.000
Urban	42 (46.15)	3 (7.10)	39(92.90)		
Family size					
1-3	34 (37.36)	2 (2.6)	32 (31.4)	0.250	0.708
4-6	24 (26.37)	4 (4.4)	20 (22.0)	3.697	0.76
7 and above	33 (36.26)	1 (1.1)	32 (35.2)	1.585	0.202
Occupation					
Farmer	36 (39.56)	3 (3.3)	33 (36.3)	0.019	0.853
Civil savant	22 (24.18)	2 (2.2)	20 (22.0)	0.030	0.777
Business	33 (36.26)	2 (2.2)	31 (34.1)	0.046	0.659

P - Value > 5% (0.05) are statistically significant

DISCUSION

Sapovirus is one of the main enteric viruses that cause acute gastroenteritis in children, often causing outbreaks in the population and public health problems. Sapovirus mainly infects people with lower immunity, such as children and the elderly, especially children under 5 years of age (Magwalivha *et al.*, 2018). HSaV is dominant in infants under 24 months old (Becker *et al.*, 2019).

Ninety-one (91) blood samples were examined for Sapovirus using ELISA for detection of human sapovirus IgG and IgM. Sapovirus was detected in 7.7% of the samples examined. The IgG and IgM are among the different types of antibodies presented during immunological responses to pathogens (Hasman *et al.*, 2007). The detection of IgM in the examined sample implies that there is a recent exposure to HSV particles, while the presence of IgG implies a prior exposure or chronic exposure to HSV in the area. The

prevalence in this study was higher than the prevalence of 0.0 % reported by Japhet *et al.* (2019) in Nigeria, and 3.9% reported by Petronella (2012).

The prevalence of 7.7% in this study was lower than the prevalence reported in the United Kingdom, 11.1% (Pang *et al.*, 2001). In Nicaragua, 16.6% were reported by Becker et al. (2014). In the Netherlands, 7.8% were reported by Svraka et al. (2010). Furthermore, 2.4% were reported by Amar et al. (2007). In Nicaragua, 1.9% were also reported by Becker et al. (2014). The prevalence of 7.7% in the present study, obtained from different regions and countries, was due to variation in sample size, duration, and method of detection (Japhet et al., 2019; Kgomotso *et al.*, 2020).

Gender: The results showed that males had the highest occurrence rate (4.4%) and females had 3 (3.3%) of HSV positive cases. The result showed a significant difference between genders with sapovirus infection. The result is in line with the work of Mariam *et al.* (2020), who reported that males had the highest rate of Sapovirus infection (6, 10.5%), followed by females (0, 0.00%). However, several studies reported that there are no significant differences related to gender in HSV infections (Fazeli *et al.*, 2016; Biscaro *et al.*, 2018). Al-Shuwaikh (2016) reported that male predominance may be due to social factors rather than a higher rate of infection. This high prevalence associated with males can be due to the fact that male children are more active than females (Al-Shuwaikh, 2016).

Hussein (2016) reported in their studies that male children spend more time outdoors and are more exposed to environmental factors than females. This makes them more likely to be infected with sapovirus than females.

The results of the study showed that HSV revealed a higher percentage in the age group (49-60) months compared to other age groups. However, there was a significant association between sapovirus infection and age group. This result is in line with the work of Magwalivha et al. (2018), who reported that human sapovirus mainly infects people with lower immunity, such as children under 0 to 5 years of age. The results showed lower detection rates of sapovirus in children aged 0-12 months. This may possibly reflect a protective effect of breastfeeding and/or transferred maternal antibodies (Saito *et al.*, 2014 and Nakata *et al.*, 2007). Becker *et al.* (2019) reported a contrary result that HSaV was dominant in infants under 24 months old. This can be due to the fact that maternal antibodies are only protective in the first few months of infant life because of their short half-life. As a result, this protection is missing when infants reach six (6) months, when the antibodies decrease (Hussein *et al.*, 2016). There are many factors that contribute to the transmission of Sapovirus among these ages, and important among these factors are the low health awareness and attention to personal hygiene for people with children, contamination of food and drinking water and contamination of toys (Ahmed *et al.*, 2004)

The results showed that parents/guidance with 3.3% and 2.2% of the level of education for both primary and secondary school, respectively, are more infected than those with tertiary and other levels of education (1.1%), respectively. Although the current study shows that the rate of infection was significantly associated with the level of education of parents/guidance, Hussein (2016) reported that children from parents/guidance who had no education or primary education only have a higher risk of contracting sapovirus infections. This may be due to the fact that children spend more time with their parents/guardians, whose educational level may dictate the quality of care and other social and environmental factors that a child may be exposed to various infections.

The results showed that the majority of children examined are from rural areas, accounting for 4 (8.20%) of Sapovirus infections. This result is in line with the work of Maysaa *et al.* (2022), who reported that children with Sapovirus were all from rural regions. The variation in prevalence rates may be due to socioeconomic factors and cultural practices.

This result indicated that children living in homes with four- six members (4-6) were at greater risk of Sapovirus infection. The result is contrary to the work of Dey *et al.* (2007), who reported that children living in homes with more than seven children were at greater risk of Sapovirus infections. This implies that children living in overcrowded homes are at risk of contracting sapovirus infections.

CONCLUSIONS

The results of the study indicated that children aged 0-5 years are infected with sapovirus, with a 7.7% sereoprevalence. Male children had 4.4% of sapovirus infection, which is more infected than female children, 3.3%. Children within 38-

48 months are more infected with sapovirus. Parents with a secondary level of education have children with a 3.3% seroprevalence rate. Children whose parents/guardians reside in rural areas had the highest sapovirus infection rate of 8.20%, and children within families of 4-6 are more infected (4.4%) than those with smaller families. The results further revealed that parents/ guardians whose occupation is family are more infected with 3.3% of sapovirus infection than children whose parents/guardians are not farmers

RECOMMENDATION

Based on the findings of this research, the following recommendations were drawn

1. Routine testing for all enteric viruses should be warranted, especially Sapovirus, which is the second most frequent viral causative agent. The other enteric viruses should also be included in the various surveillance studies being carried out among children. The presence of dual infections also warrants further epidemiological and pathogenic research, especially using the more advanced molecular techniques for detection.

2. Routine surveillance of all enteric viruses will eventually give a clear picture on the seasonality. This study involved only randomly selected serum samples from children.

3. Attitudes on disease severity scores should be conducted on enteric viruses to give a clearer picture of children in both rural and urban areas.

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