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Seroprevalence of human sapovirus IGM and IGG among children (0-5) years in some selected hospitals in Birni Kebbi, Kebbi state, Nigeria

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Abstract

The studies investigate the seroprevalence of human sapovirus IgG/IgM among children 0-5 years attending some selected hospitals in Kebbi State, Nigeria. Two hundred venous blood samples were collected from Argungu (35.42%), Dakingari (34.90%) and Sir Yahaya memorial hospital (29.69). Sapovirus IgG/IgM was detected using ELISA techniques and confirmed by molecular techniques using universal and specific primers set to detect the sapovirus genogroup among children. Male (51.04%) children are predominance of the respondent with high sapovirus infection 9.18%. Children within the age group 49-60 month have the highest sapovirus infection 12.50%. Children parents/guidance with secondary school level of education have 12.50% of sapovirus infection, children residing in the rural areas has 12.90% of sapovirus infection Family size from 4 and above has the highest sapovirus infection 12.90%. Parents/ guidance engaged in business activity their children are more prone to sapovirus infection (11.91%). The result showed an association among children presented with vomit 11.40%, Diarrhoea 8.00% and crèches 14.61% with sapovirus infection. Children depend on River and well water as source of drinking water have 22.73% and 7.90% of sapovirus infection respectively, Result further show that children with inclusive and exclusive mode of breast feeding had 10.26 and 17.39% of sapovirus infection. The PCR using universal primer sets detect 3 samples are sapovirus positive. The amplified sera positive were sequence and phylogenetic tree was constructed using neighbouring joining method based on the relationship with members from GenBank of NCBI. Genogroup II and Genogroup IV were identifying. The study demonstrated a high seroprevalence 7.81% and GII and GIV are detected in the study area.

Keywords: Elisa, genogrouped, infection, prevalence and sapovirus

1. Introduction

Human sapoviruses belong to the *Caliciviridae* family within the order Picornavirales (genus Sapovirus, species Sapporo virus) (Vinje *et al.*, 2019) [21]. Sapovirus is an enteric virus and is recognized 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) [12], it has been associated with persistence vomiting suggested to possibly cause gastroenteritis in humans (Yan *et al.*, 2013) [23].

Viral particles spread from person to person through faecal-oral route by consuming contaminated food and drinking water, and/or handling sapovirus-positive faeces (Kobayashi *et al.*, 2012) [7]. The increase of acute gastroenteritis associated with sapovirus (SV) has been reported and recognized as a major public health problem particularly in developing countries (Liu *et al.*, 2016) [9]. It is documented that after the successful deployment of the Rotavirus vaccine, SVs have emerged as the second most commonly etiological virus behind Norovirus in children with acute diarrhoea (Liu *et al.*, 2016) [9].

A large number of microorganisms, including viruses, have been identified as the causative agents of gastroenteritis. Throughout the world and among all age groups, human sapovirus infections are associated with acute gastroenteritis, in both sporadic cases and outbreak settings (Iritani *et al.*, 2016) [24].

The incubation period of sapovirus infections varies from less than 1 to 4 days and generally causes a mild disease consisting of diarrhoea, vomiting and sometimes co-infections (Thongprachun *et al.*, 2016) [20]. Major clinical symptoms include diarrhoea and vomiting; however, additional constitutional symptoms (i.e., nausea, stomach/abdominal cramps, chills, headache, myalgia, or malaise) are also frequently reported. Similar to the case for norovirus illness, fever is a rare clinical symptom. Diarrhoea usually resolves within 1 week;

however, individuals showing symptoms for a longer time (i.e., from over a week to up to 20 days) were also reported (Sakai *et al.*, 2001) ^[17]. In general, the severity of sapovirus gastroenteritis is milder than that for rotavirus and norovirus (Rock *et al.*, 2002) ^[15].

Gastroenteritis symptoms are usually self-limiting, and patients usually recover within a couple of days; however, the symptoms, severity and duration of disease are dependent on the individual and sapovirus infection sometimes leads to hospitalization (Vinje *et al.*, 2019) ^[21]. Mortality is rare, but it was reported from outbreaks that occurred in a long-term-care facility for the elderly (Lee *et al.*, 2012) ^[8].

Throughout the world and among all age groups, human sapovirus infections are associated with acute gastroenteritis, in both sporadic cases and outbreak settings. Among patients with sporadic gastroenteritis sapoviruses were shown to rank second to fourth as the major viral pathogens (Oka *et al.*, 2015) ^[12]. In the post-rotavirus vaccine era, their role further increased (Pitkanen *et al.*, 2019) ^[14]. Though tending to be somewhat milder, the clinical symptoms of sapovirus gastroenteritis are indistinguishable from those caused by noroviruses (Sakai *et al.*, 2001) ^[17]. There are information available about human sapovirus infection in different countries such as Peru (Sanchez *et al.*, 2018) ^[19] Iran (Romani *et al.*, 2012) ^[16], Ethiopia (Galaw *et al.*, 2019) ^[4] and Egypt (Lee *et al.*, 2012) ^[8]. Human sapovirus infection is not routinely diagnosed in most Nigerian hospitals probably due to the cost of the diagnosis and the clinical spectrum of signs and symptoms are similar to other gastroenteritis caused by viruses: Rotavirus, astrovirus, and norovirus. The clinical symptoms of sapovirus gastroenteritis are indistinguishable from those caused by noroviruses. Therefore, laboratory diagnosis is essential to identify the pathogen and risk factors associated with sapovirus infection. There is no information available about the human Sapovirus circulating among children attending some selected hospitals in Kebbi State, Nigeria. This prone the research to investigate the seroprevalence of human Sapovirus Among children attending some selected hospitals in Kebbi State, Nigeria

2. Materials and Methods

2.1 Sampling Techniques

The study adopted a purposive sampling technique among the children attending general hospital Argungu, Dankigari and sir Yahaya memorial hospital Birnin Kebbi

2.2 Sample size Determination

The formula described by Petronella (2012) ^[13] with the prevalence of 6%. Prevalence of 6% was adopted, considered the prevalence of Human sapovirus in children aged 0-5 years is not known in Kebbi State.

$$N = \frac{Z^2 P(1-P)}{D^2}$$

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

Two hundred (200) samples were collected among children 0-5 years attending some selected hospitals in Kebbi State. To make a sample size that was giving a fair representation of the study area.

2.3 Ethical approval

Ethical approval for the study was collected from the ethical Research committees of various hospitals

2.3.1 Inclusion criteria

- Children within 0-5years as inn and outpatient observed in a short stayed unit or prolong stayed in the hospitals were include in the study,
- Children with diarrhoea, nausea, vomiting and fever were included in the study
- Children parents or legal guidance that accept consent to participate in the research were included in this study

2.3.2 Exclusion criteria

- Children above 0-5years as inn and outpatient observed in a short stayed unit or prolong stayed in the hospitals will be excluded in the study
- Children with a vomiting, no diarrhoea with respiratory illness that parents/guidance could not be able to explain the cause of vomiting were excluded in the study
- Children parents or legal guidance that refuses consent were excluded in the study.

2.4 Determination of socioeconomic, Risk and clinical factors

A structural questionnaire was design to capture the socio-demographic, risk and clinical information of the respondents these include residences, age group, sex, inclusive or exclusive breast feeding, educational status, source of drinking water and occupation of the parent.

2.5 Sample collection

One hundred (200) blood and stool samples were collected from children age 0-5years attending the selected hospitals in Kebbi state. The blood samples were collected in an EDTA bottle for serological screening for sapovirus and the stools sample were collected in a sterile plastic wide mouth container for sapovirus shedding detection and stored at +4°C until processing.

2.6 Serological Detection of IgG and IgM antibodies of human sapovirus

The detection of Human sapovirus-specific antibodies (IgG and IgM) was performed by ELISA. Humam sapovirus IgM and IgG were detected using commercial ELISA kits (Melson Medical Co limited: Kuancheng Distric Changohun Jillin province china) according to the manufacturer's instructions and as previously described by Oka *et al.* (2012) ^[12]

The Kit uses enzyme linked immunosorbent assay double antigen sandwich principle to analyze 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. Following washed to remove unbound substance. Finally chromogen solution A and chromogen solution B were

added, a blue color developed. The reaction is then stopped and color turns to yellow when stooping solution (acidic) was 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

The serum samples were transferred into sterile Eppendorf test tube. All reagents were brought to room temperature before used. Two positive and two negative controls well were set on pre coated microplate respectively. Separately 50ul was added to positive and negative well. Add tested sample 10ul, then add sample diluents 40ul to test sample well. Blank well doesn't add anything. 100ul of HRP-conjugate reagent to each well, cover with an adhesive strip and incubate for 60minutes at 37°C. Each well was aspirated and washed for four times for a total of five washed. Washed by filling each well with washed solution (400ul) using a squirt bottle. Chromogen solution A 50ul and chromogen B 50ul were added to each well respectively. Gently mixed and incubated for 15minutes at 37°C. And stop solution was added to each well. The optical density was measured at 450nm using a microtiter 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 negative control well ≤ 0.15

CO = the absorbance value for 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 \geq Calculated critical (cut off), the result is positive

2.7 Extraction of RNA and complementary DNA preparation

The stool samples were subjected to the extraction of RNA of sapovirus using QIAamp Viral RNA kit (Qiagen). The extraction was performed according to the instructions supplied by the manufacturer. Complementary DNA (cDNA) was generated by adding 100 nanograms of the extracted RNA to 32.5 μ l prepared mixture composed of 1 μ l hexamers primers (Fermentas, Latvia), 4 μ l of 5 x buffer, 0.5 μ l of Ribolock RNase inhibitor, 2 μ l of dNTP mixture composed of 10 mM each of dNTP, 200 units of reverse transcriptase enzyme and 19 μ l of RNase free water (Fermentas, Latvia). The RT assay was performed at 42 °C for one hour (Gelaw *et al.*, 2019)^[4]

2.8 Polymerase Chain Reaction for Human sapovirus

The amplification process was carried out using a set of primers with nucleotide sequences of primers. The amplification Master Mix Kit mixture was used with 5 μ l of cDNA added to a 20 μ l PCR mix. The amplification procedures were performed using the following conditions: denaturation at 94 °C for 5 minutes, then 35 cycles composed of 94 °C for 45 seconds- 55 °C for 45 seconds and 72 °C for 1 minute, then final extension of 7 minutes at 72 °C. PCR products were visualised under UV illumination after electrophoresis on a 1% agarose gel stained with ethidium bromide. The estimated amplified fragment size for sapovirus was 434 bp (Gelaw *et al.*, 2019)^[4]

Table 2.1 Primers for PCR amplification of Human Sapovirus

HSV Universal Primer	Polarity	Sequence (5' - 3')	Nucleotide Position	Amplicon size
SLV5317	+	CTCGCCACCTACRAWGCBTGGTT	5083-5105	434
SLV3749	-	CGGRCYTCAA VSTACCBCCCCA -	5494-5516	
Specific primer				
SaV4579a	+	CCATCTGGGATGCCATTYAC	4525-4544	
SaV1245R	-	CCCTCCATYTCAAACACTA	5159-5177	

Source: Yan *et al.* (2003)^[22]

Key: *IUB codes: B = C, G or T; H = A, C or T; N = any base; R = A or G; S = G or C; V = A, C or G; W = A or T; Y = C or T. + Forward Primer - Reverse Primer

2.9 Statistical Analysis

Demographic data were analysed using the Statistical Package for the Social Sciences (SPSS) software version 16. Categorical variables were compared using chi-square; P - values < 0.05 were regarded as statistically significant

3. Results

3.1 Seroprevalence of Human sapovirus

The seroprevalence of specific antibodies to Human

sapovirus was determined in a total of 192 human serum samples randomly collected from Birnin Kebbi using an indirect enzyme-linked immunosorbent assay (ELISA). Our study showed a seroprevalence of human sapovirus in the population aged from 0- years among children. Among the 192 analyzed sera, 9 (4.67%) and 6 (3.12%) were positive for IgG and IgM antibodies respectively to human sapovirus.

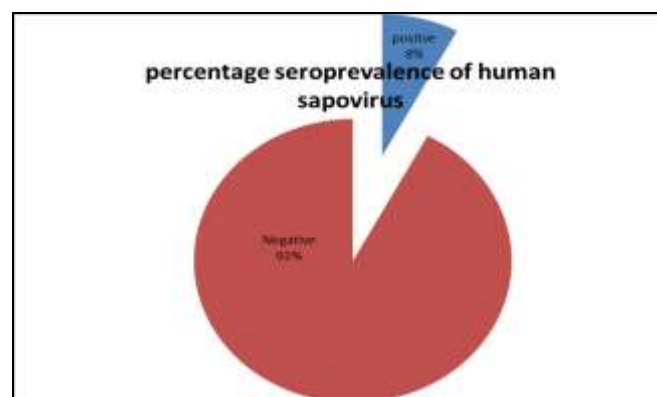


Fig 1: Percentage SAPOVIRUS antibodies detection using ELISA kit

Total of 192 samples were analysed for human SAPOVIRUS infection in different sampling sites, samples from Argungu (35.42%), Dakingari (34.90%) and Sir Yahaya memorial hospital (29.69) Birnin Kebbi, Kebbi State. Human SAPOVIRUS were more detected among samples collected from Argungu 11.77% there was an association of SAPOVIRUS infection among the different sampling sites.

Table 3.1: Distribution of human SAPOVIRUS according to sample sites

Location	Samples	Percentage (%)		P-Value
		Positive (%)	Negative (%)	
Argungu	68 (35.42)	8 (11.77)	60 (88.23)	0.74
Dakingari	67 (34.90)	6 (3.13)	61 (96.87)	
Sir- Yahaya	57 (29.69)	1 (1.75)	56 (98.25)	
Total	192 (100.00)	15 (7.81)	177 (92.19)	

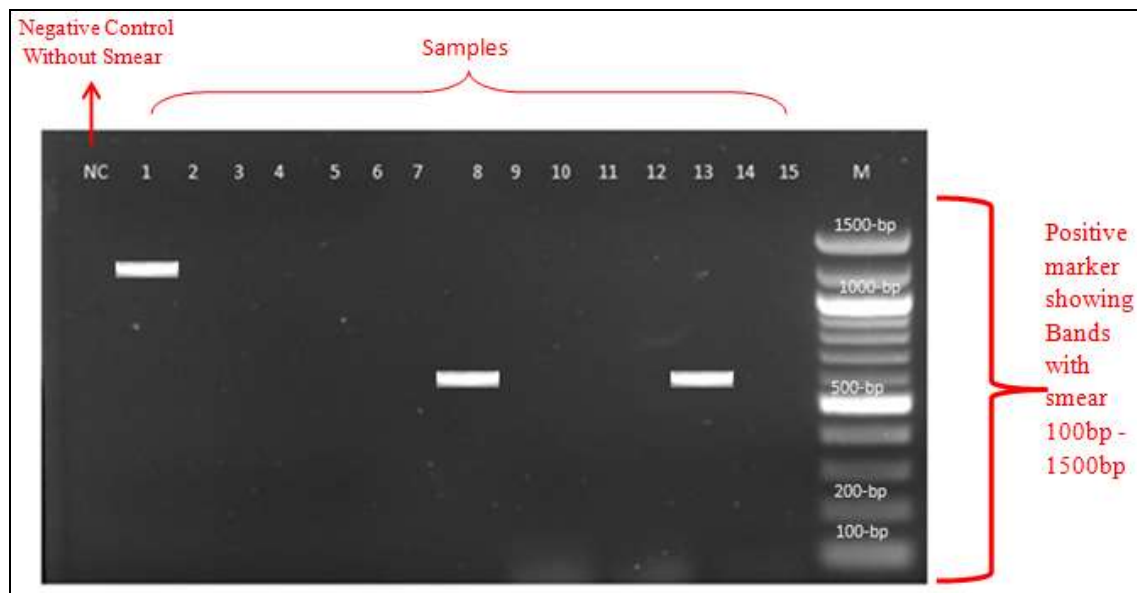
The study include 192 children 0-5 years attending some selected hospital in Kebbi State, Male 51.04% are predominance of the respondent with high SAPOVIRUS

infection 9.18%. Sapovirus infection are widely distributed among the striated of age groups. Children within the age group 49-60 month had the highest sapovirus infection 12.50%. Children parents/guidance with secondary school level of education had 12.50% of sapovirus infection, children residing in the rural areas had 12.90% of sapovirus infection. The presents study showed that sapovirus infection increases as the number of family size increases. Family size from 4 and above had the highest sapovirus infection 12.90%. Parents/ guidance engaged in business activities their children are more prone to sapovirus infection. The present result showed that business men and women had the highest prevalence of sapovirus infection 11.91%. The result showed an association among children presented with vomit 11.40%, Diarrhoea 8.00% and crèches 14.61% with sapovirus infection. Children depend on River and well water had 22.73% and 7.90% of sapovirus infection respectively, Result further show that children with inclusive and exclusive mode of breast feeding had 10.26 and 17.39% of sapovirus infection

Table 3.2 Distribution of socioeconomic, clinical factors associated with human sapovirus among children

Gender	Sample examined	Percentage (%)		p- value
		Positive (%)	Negative (%)	
Male	98 (51.04)	9 (9.18)	89 (90.82)	0.62
Female	94 (49.96)	6 (6.33)	88 (93.620)	
Total	192 (100.00)	15 (15.51)	177 (92.13)	
Age				
0-12	25 (13.02)	2 (8.00)	23 (92.00)	0.60
13-24	38 (19.79)	4 (10.53)	34 (89.47)	
25-36	46 (23.96)	3 (6.52)	43 (93.48)	
37-48	43 (22.39)	1 (2.33)	42 (97.67)	
49-60	40 (20.83)	5 (12.50)	35 (87.50)	
Total	192 (100.00)	15 (7.81)	177 (92.19)	
Educational level				
Primary	67 (34.89)	4 (5.97)	63 (94.03)	0.03
Secondary	56 (29.17)	7 (12.50)	49 (87.50)	
Tertiary	45 (23.44)	3 (1.57)	42 (93.33)	
Others	24 (12.50)	1 (4.17)	23 (95.83)	
Total	192 (100.00)	15 (7.81)	177 (92.20)	
Residences				
Rural	93 (48.44)	12 (12.90)	81 (87.10)	0.09
Urban	99 (51.56)	3 (3.03)	96 (96.97)	
Total	192 (100.00)	15 (7.81)	177 (92.19)	
Family size				
1-3	81 (42.19)	1 (1.24)	80 (98.77)	0.55
4-6	63 (32.81)	6 (3.13)	57 (90.48)	
7 and above	48 (25.00)	8 (16.67)	40 (83.33)	
Total	192 (100.00)	15 (7.81)	177 (92.19)	
Occupation				
Farmer	97 (50.52)	9 (9.29)	88 (90.71)	0.89
Civil savant	53 (27.60)	1 (1.89)	52 (98.11)	
Business	42 (21.86)	5 (11.91)	37 (88.09)	
Total	192 (100.00)	15 (7.81)	177 (92.19)	
Vomiting				
YES	114 (59.48)	13 (11.40)	101 (88.60)	0.70
No	78 (40.63)	2 (2.56)	76 (97.44)	
Total	192 (100.00)	15 (7.81)	177 (92.19)	
Fever				
Yes	167 (86.98)	11 (6.59)	156 (93.41)	0.46
No	25 (13.02)	4 (16.00)	21 (84.00)	
Total	192 (100.00)	15 (7.81)	177 (92.19)	
Cough				
YES	183 (95.31)	13 (7.10)	170 (92.90)	0.47
No	9 (4.69)	2 (22.22)	7 (77.77)	

Total	192 (100.00)	15 (7.81)	177 (92.19)	
Diarrhoea				
YES	175 (91.15)	14 (8.00)	166 (94.86)	0.87
No	17 (8.86)	1 (5.88)	16 (94.12)	
Total	192 (100.00)	15 (7.81)	177 (92.19)	
Abdominal pain				
YES	163 (84.90)	9 (5.56)	149 (94.44)	0.65
No	29 (15.10)	6 (20.69)	23 (79.31)	
Total	192 (100.00)	15 (7.81)	177 (92.19)	
Dehydration				
YES	154 (80.21)	11 (7.14)	143 (92.86)	0.43
No	38 (19.79)	4 (10.53)	34 (89.47)	
Total	192 (100.00)	15 (7.81)	177 (92.19)	
Crèche				
YES	89 (46.35)	13 (14.61)	75 (85.39)	0.85
No	103 (53.65)	2 (1.94)	101 (98.06)	
Total	192 (100.00)	15 (7.81)	177 (92.19)	
Source of Drinking water				
Tap	54 (28.13)	3 (5.56)	51 (94.44)	0.67
Borehole	28 (14.58)	1 (3.57)	27 (96.43)	
Well	76 (39.58)	6 (7.90)	70 (92.10)	
River	22 (11.46)	5 (22.73)	17 (77.27)	
Sachet	12 (6.25)	0 (0.00)	12 (100.00)	
Total	192 (100.00)	15 (7.81)	177 (92.19)	
Breast feeding				
Exclusive	78 (40.63)	8 (10.26)	70 (89.74)	0.03
Inclusive	23 (11.98)	4 (17.39)	19 (82.61)	
Bottle feeding	79 (41.15)	3 (3.80)	76 (96.20)	
Complementary	12 (6.25)	0 (0.00)	12 (100.00)	
Total	192 (100.00)	15 (7.81)	177 (92.19)	



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

Fig 2 A view of a gel electrophoresis run captured using a Android phone Techno Spark 10.

Each gel run contained samples (1- 15) loaded in rows. The row contained both the negative and positive controls for each gel run. A 100bp ladder was loaded on the flanking end wells of each gel rows as seen in the picture above. A clear band without smears was considered as negative

amplified DNA

The sapovirus strains clustered into two genogroups of GII and GV. Genogroup GII and GV constituted most of the infections at 100% and 99%, respectively.

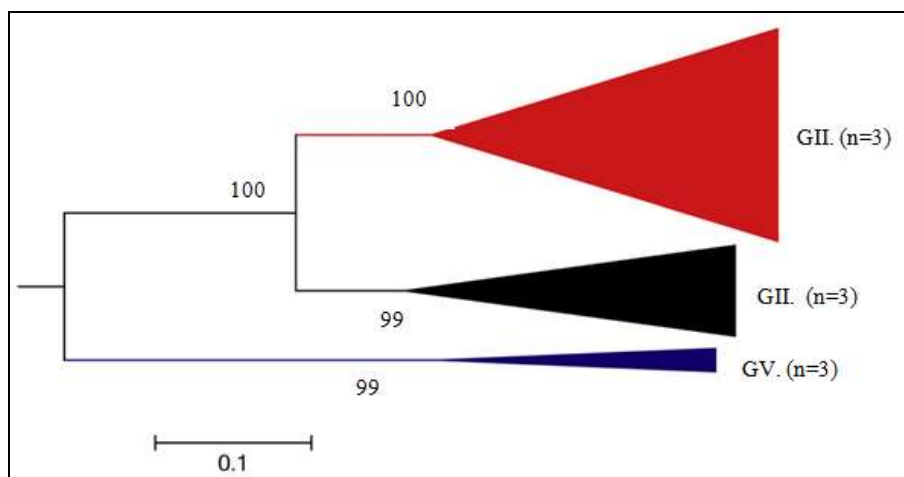


Fig 3 The phylogenetic tree of sapovirus identified using neighbour joining method based on the relationship with members from GenBank of NCBI

4. Discussion

Viral pathogens represent a significant aetiology for acute gastroenteritis. These infections are usually self-limited in developed countries while it may lead to mortality in underdeveloped countries, especially in children (Shame *et al.*, 2017) [18]. Out of 192 samples examined results revealed a seroprevalence of 7.81% among children 0-5 years within the selected studied areas. The seroprevalence in this study was higher than the prevalence 0.0% reported by Japhet *et al.*, (2019) [6] in Nigeria, 3.9% reported by Petronella (2012) [13]. The detection of IgM among sample examined implies that there is a recent exposure to HSV particles while the presence of IgG implies a prior exposure or chronic exposure to the HSV. Exposure to sapovirus is higher among male 9.18% than female 6.33% this result is in agreement with the work of Mariam *et al.* (2020) [10] that male had 10.5% of sapovirus infection than female 0.00%. This implies that male children are more active, stayed longer time outdoor and have become more in contact with environmental than females this make them more likely to be infected with sapovirus than females (Al-Shuwaikh, 2016) [1]. There is a strong association of sapovirus infection among gender as p-value 0.62. There was an association sapovirus infection among age group of respondent. Children within 49-60 months are more exposed to sapovirus infection 12.50% compared to other age group. The present study revealed that sapovirus are more frequently exposed among children that parents/ guidance had secondary level of educational (12.50%) and resided on rural areas (12.90%) but there is no association of sapovirus infection among parents/guidance educational level and their residential areas as p-value = 0.03 respectively. There is an association among children with 7 and above family size 16.67% as p-value = 0.55. This result is in disagreement with the work of Peni *et al.* (2025) [25] that children living in homes with four- six members (4-6) were at greater risk of sapovirus infection and agreed with the work of Dey *et al.*, (2007) [2] in their studies reported that children living in homes with more than seven children were at greater risk of sapovirus infections. This implies that children living in over crowded homes are exposed to sapovirus infections. The present study showed an association of sapovirus infection among children that had vomit (11.40%) p = 0.70, diarrhoea (8.00%) p = 0.87 and abdominal pain (5.56%) p = 0.65 this work is in line with the work of Peni *et al.*, (2025) [25] in

there studied reported that vomiting, diarrhoea, abdominal and fever are the common clinical sign of sapovirus infection among children 0-5 years. There is no association of sapovirus infection among children presented with cough (22.22%) p = 0.47 re Dehydration (7.14%) = 0.43. This present work is in line with that of Dove *et al.* (2005). There is an association among children that had access to crèches (14.61%) p = 0.85 and their source of drinking water is river (22.73%) p = 0.67

The 7.81% IgG/IgM positive sera detected using ELISA kit were further screened with more sensitive and effective techniques (PCR) than the ELISA. The antibody tests also yielded high sensitivity and specificity for the detection of individual IgG/IgM antibodies. The results indicate that these tests could play a very important role in diagnosis of HSV infection when used in conjunction with the molecular tests. The detection of the antibodies collectively at the time when the PCR becomes negative implied that they may have a role in the clearance of the virus. The PCR techniques revealed that 3 (20.00%) samples are positive 12 (80.00%) are negative for human sapovirus genogroup using universal primer set (SLV5317 forward SLV3749 backward). This result is contrary with the work of Peni *et al.* (2025) [25] in their study reported 0 (0.00%) of sapovirus among children with universal primer set. Similar Japhet *et al.* (2019) [6] also reports 0.00% of human sapovirus using PCR. The prevention of sapovirus infection depends mainly upon efficient hand hygiene practice, environmental disinfection, proper sewage disposal, and limited contact with ill individuals. There is an argument about the role of improvement of water sanitation in the prevention of sapovirus as it is a common pathogen in both high and low-income countries. However, as it is transmitted by contaminated water and food (Rockx *et al.*, 2002) [15] increasing food and water sanitation will reduce the burden of the infection. The differences observed in sapovirus prevalence rates may also be due to the sensitivity of the detection methods used in these studies.

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