

RESEARCH ARTICLE

DETECTION OF POLIO VIRUS ANTIBODIES LEVEL AMONG CHILDREN IN KANO STATE, NIGERIA.

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Abstract

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Key words:-

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The study was conducted to detect poliovirus antibody and its level in children aged \leq 5 years in three local government areas (LGAs) of Kano state, Nigeria. The study was a hospital-based study and purposive sampling was used in selecting one health institution each from the three LGAs. Random sampling was employed in selecting 423 children whose blood samples were collected and analyzed for the presence of poliovirus antibody and its level using polyclonal Elisa detection kits. The study revealed that 94.1% of the children were seropositive for poliovirus with mean titer value of 1.6938, and age was significantly associated with seroprevalence rates with 33.4% and 37.4% of the seropositive children in the age groups 0-12 and 13-24 months respectively (p<0.05). Also, no significant difference existed in the seropositivity rates of the children regarding their sex as well as their mother's educational level (p>0.05), although the father's educational level significantly affected it (p<0.05). The Poliovirus antibody mean titer level significantly increases in routine and campaign oral poliovirus (OPV) immunization as the number of the vaccine doses increases (p < 0.05). The study detected a high seroprevalence rates to poliovirus among children in Kano state and recommends that fathers should be considered as a target group in the course of enlightenment campaign as well as the need for more efforts to improve literacy level of women and to target the unimmunized children whose lack of immunity poses a risk of re-emergence of the poliovirus infection in Nigeria.

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Introduction:-

Poliomyelitis is a disease caused by poliovirus which according to Mark (2006) and Tapani (2006) have been known to cause paralysis especially in unimmunized or sub-optimally immunized children. The World Health Organization (WHO) recorded that there were an estimated 350,000 case of polio worldwide in 1988 and this number reduced to 291 in 2012, representing a 99% reduction, although in 2013 and 2014 there was a bounce back in some countries towards more cases (WHO, 2014). In 2015 only 2 countries remained where the disease is endemic-Afghanistan and Pakistan, whereas Nigeria was declared wild polio free in 2015 and was reported as the first time that Nigeria has interrupted transmission of wild poliovirus, bringing the country and the African region closer than ever to being certified polio free (WHO, 2015).

Poliomyelitis is a highly contagious disease caused by three serotypes (types 1, 2 & 3) of poliovirus which are spread by faecal-to-oral transmission (Shibuya and Murray, 2004; WHO, 2014). Children are thought to play a dominant role in the transmission of Polioviruses within populations (Fine and Ritchie, 2006), and age of children, number of doses the children had taken and educational level of the children's fathers have been identified as significant risk factors on the prevalence of poliovirus antibodies (Mawashi *et al.*, 2015).

In 1988 the World Health Assembly launched a campaign to eradicate polio by 2000 and CDC (2014) revealed that the Polio eradication programs most important step is the interruption of endemic transmission of polio virus which according to Maigari *et al.* (2015) was mainly based on successful administration of polio vaccine through the massive immunization programs targeted towards preventing poliomyelitis.

Earlier studies revealed that reduction in poliomyelitis cases were due to extensive use of two vaccines, the live attenuated oral Poliovirus vaccine (OPV) or the Sabin vaccine, and the inactivated Poliovirus vaccine (IPV), or the Salk vaccine (Friedrich, 2000). Thus, the Global Polio Eradication Initiative aimed at immunization of children with multiple doses of OPV, via both routine immunization (RI) and supplementary immunization activities (SIAs) (CDC, 2010).

Pasca *et al.* (1994) earlier revealed that it is customary in any country where a comprehensive program to vaccinate children against Polioviruses was undertaken to investigate the immune status of the eligible children or even the entire population to Polioviruses from time to time. The WHO (2015) further expounded that Polio sero-prevalence surveys (SPS) revealed a positive correlation between number of OPV doses received and immunity levels. Specifically children receiving >7 OPV doses have higher antibody titres of 90% and above, and this also addresses the unfounded concerns on 'too many OPV doses' often expressed by sceptical health professionals and outright non-compliant parents.

In Nigeria, Polio Sero-Prevalence Surveys started in 2011 following an Expert Review Committee (ERC) recommendation (WHO, 2015). The National Primary Health Care Development Agency (NPHCDA) with the support of WHO immediately initiated SPS in Kano State (WHO, 2015), however most of the surveys on polio virus antibodies in Kano state were conducted in urban areas (Iliyasu *et al.* 2014; Mawashi *et al.*, 2015). Thus, this study was aimed at detecting polio virus antibodies and its level among children five years and below attending health facilities in some rural areas of Kano state.

Materials and Methods:-

Study Design:-

The study was a cross-sectional health facility based study conducted at three local government areas (LGAs) which were selected from three senetorial zones of Kano state. Purposive sampling method was used in selecting the health facility while random sampling was used in selecting the children that participated in the study. Thus, at each LGA the most popular Community hospitals which provide free medical services, have a large turnover in pediatric OPD and which is patronized by patients from all the area were selected. The hospitals selected were; Kura General Hospital, Dambatta General Hospital and Wudil General Hospital.

Study Area:-

The study was conducted in Kano State Nigeria located at latitude $9^0 55$ 'N and $8^0 53$ 'E at a latitude of 1300 meters, 4100ft above sea level. The climate condition is characterized by breeze harmattan between October and March and a rainy period between April and September with anannual rainfall of about 1300mm. According to Bureau of statistics (2008) the state has an estimated land mass of about 130,913 square kilometers, and a population of 9,383,682 people with population density of 403 per square kilometers, based on the 2006 national population census. The main occupation is farming with most of the urban populations being civil servants and traders. The adult literacy rate was estimated at 29.1% and 16.5% for male and female respectively (Bureau of statistics, 2008).

Dambatta LGA is located at the latitude 12.43306 0 N and 8.5152 0 E. It has an area of 732 km² and a population of, 268,985 with10759 being children as at 2015 (NIPOST, 2010). It has a total of 32 health facilities. Kura LGA is located at the latitude 11.77139⁰ N and 8.43028 0 E, with an area of 206 km² and a population of 181,896, with 7,276 being children \leq 5 (NIPOST, 2010). The town in particular was notoriously known for the rejection of polio vaccine and has a reported case in the year 2014 (WHO, 2014). Wudil is a LGA which has an area of 362 km² and a population of 239524 with 9581being children \leq 5 years of age (NIPOST, 2009).

Ethical Clearance and Consent:-

Ethical approval and permission to conduct the study was received from the Ethics Committee of Kano State Ministry of Health, Hospital management Board, and the selected LGAs hospitals of the study (appendices I). Provision of Helsinki declaration concerning research on human subjects were also strictly adhered to and the parent of the children consented prior to sample collection

Study Population:-

The subjects for the study were children ≤ 5 years of age, who were residents of the selected LGAs, and attending the health facilities from June to October 2015.

Inclusion and Exclusion Criteria:-

Any child in the specified age group of ≤ 5 years with a history of polio immunization brought to the General Outpatient Department (OPD) of the selected hospital and residing in the LGA for at least six months preceding the study and whose Parent/Guardian gave consent for participation was included in the study. All infants or children outside the specified age group or who reside outside the specified LGAs, and those whose Parent/Guardian do not provide consent for their participation in the study were excluded. In addition, children with contraindication for venipuncture, very sick requiring hospitalization and those diagnosed or suspected of having congenital immunodeficiency disorder were also excluded.

Sample Size Estimation:-

The sample for the study was calculated based on 51% sero-prevalence rates of polio type 3 among children aged 6-9 months in Kano reported by Iliyasu *et al.* (2014), and using the formula for sample estimation by Araoye (2003) as follows:

 $n = z^2 pq/d^2$; thus number of samples (n) = $(1.96)^2 x 0.51 x 0.49 / (0.05)^2 = 383.8$.

The calculated sample size was 383.8. However, to minimize error, 10% of the calculated sample size (38.4) was added to the number (Iliyasu *et al.*, 2014) giving a total of 423 which was the sample size used for the study.

Sample Collection and Processing:-

A total of 423 blood samples were collected from the children upon the consent of their parents from June to October 2015. Standard antiseptic procedures were strictly adhered to before and after the study. Exactly 2mls of blood was aseptically collected by venipuncture in a plain vacutainer tube as described by Jawetz *et al.* (2010). Immediately after collection, the blood samples were allowed to clot at room temperature for 30 minutes and kept in the refrigerator at 4-8°C for up to 6 hours at the LGA hospital. Thereafter they were transported to Aminu Kano Teaching Hospital (AKTH) Laboratory in cold cryoboxes in which ice-packs were earlier placed so as to preserve the samples as described by WHO (2000). The tubes containing the samples were centrifuged at 3000 revolutions per minute for five minutes. Exactly 2ml of serum from each sample was pipetted into a cryovial, labeled with respective ID number of the study subject. Cryovials were put in cryoboxes and stored at $-60^{\circ}C$ at AKTH until all samples are collected for processing (Iliyasu *et al.*, 2014).

Prior to sample collection, an interviewer administered questionnaire adapted from Iliyasu *et al.* (2014) was used in generating data for the study. Some of the aspects reflected in the questionnaire include physical examination of the children including weight and length, their mothers and fathers educational level and immunization history.

Detection of Antibodies to Polio Virus:-

The poliovirus antibody and its level was detected from the processed blood samples using polyclonal Enzyme linked immunosorbent assay (ELISA) detection test kits (Demeditec Diagnostics GmbH, Germany) which detects antibodies against the three types of polio simultaneously, due to a past illness or to immunity by vaccination (http://www.demeditec.com). The Polio IgG antibody test kit is based on the principle of the enzyme immunoassay (EIA) in which Polio antigen bounded on the surface of the microtiter strips reacts and bind with IgG antibodies present in diluted patient serum or ready-to-use standards upon addition into the wells. A ready-to-use anti-human-IgG peroxidase conjugate is added followed by the addition of substrate (TMB) solution which induces the development of a blue dye in the wells. The color development is terminated by the addition of a stop solution, which changes the color from blue to yellow and the resulting absorption of the dye is measured at the wavelength

of 450 nm and the concentration of the IgG antibodies is directly proportional to the intensity of the color (http://www.demeditec.com).

The assay was carried out according to manufacturer's instructions (http://www.demeditec.com). Ninety six (96) wells that contained an immobilized polio antigen were labeled 1-12 vertically and A-G horizontally. The wells A1-A5 were labeled as substrate blank, negative control, cut-off standard, weak positive control, and positive control respectively. Both the samples the standards and controls were diluted 1:101. Then for each diluted sample 100µl of it was added to the respective labeled well, similarly the ready-to-use standards and controls were pipetted respectively into the wells. One well was left empty for the substrate blank. The plate was covered with the re-usable plate cover and incubated at room temperature for 60 minutes and the wells were then emptied by dumping. Then 300µl of diluted washing solution was added and procedure was repeated totally three times, and rests of the washing buffer were afterwards removed by gentle tapping of the microtiter plate on a tissue cloth. Then 100µl each of ready-to-use conjugate was pipette into all of the wells excluding the substrate blank well and then the plate was covered with the re-usable plate cover and incubated at room temperature for 30 minutes. The wells of the plates were then emptied by dumping, and 300µl of diluted washing solution was added. This procedure was repeated three times and rests of the washing buffer were afterwards removed by gentle tapping of the microtiter plate on a tissue cloth. Finally 100ul each of the ready-to-use substrate was pipetted into the wells including the substrate blank well. The plate was covered with the re-usable plate cover and incubated at room temperature for 20 minutes in the dark (drawer). Then 100µl each of the ready-to-use stop solution was pipetted into all the wells to terminate the substrate reaction. This was thoroughly mixed and the bottom of the plate was wiped. The reading was performed and measured spectrophotometrically at the wavelength of 450 nm. The concentration of the IgG antibodies is directly proportional to the intensity of the color and the results were read and interpreted according to the manufacturer's instructions by comparing the optical density (OD) of the cut-off standard values to that of the sample OD values as indicated below:

1. Cut-off standard (OD) value = 0.391.

- 2. Sample OD value greater than (>) cut-off standard OD value (0.391) = Positive result.
- 3. Sample OD value less than (<) cut-off standard OD value (0.391) = Negative result.

Data Analysis:-

Data generated from the study was analysed using SPSS version 17 Software. Results generated were presented as percentages, while chi-squire and student T-test were used in determining any significant association with regards to demographic and other associated risk factors among the studied children. A p-value of 0.05 or less was considered significant at 95% confidence interval.

Results:-

The results of the study revealed that, of the 423 studied children, 398 (94.1%) had antibodies to polio virus with a mean titer value of 1.6938. The mean weight of the studied children was 10.93kg and their mean height was 11.143cm.

Results on Table 1 revealed that of the 398 children seropositive to poliovirus, 205 (51.5%) were males and 193 (48.5%) were females (P=0.734). Results shown on Table 2 further revealed that children in the age groups 0-12 and 13-24 months had higher significant seroprevalence rates of 33.8% (133/398) and 37.4% (149/398) respectively compared with children in the age groups 37-48 and 49 – 60 years who both had prevalence rates of 1(4.0%) (P=0.044). The results also revealed that polio virus antibody was uniformly distributed among the children in the three LGAs (p>0.05), with 34.2%, 33.4% and 32.5% in Dambatta, Kura and Wudil respectively (Table 3).

There was no significant difference in the prevalence of polio virus antibody among the studied children regarding their mothers' educational status, although 317 (79.6%) of the seropositive children had mothers whose educational level was identified as primary, and 81(20.4%) having mothers with educational status identified as secondary or tertiary level (P=0.310) (Table 4). However, there was a significant relationships between the prevalence of polio virus antibody among the studied children with regards to their fathers' educational status (P=0.001) with 160 (40.2%) of them having fathers whose educational was identified as secondary or tertiary level (Table 5).

The results further revealed that children that received at least four doses of routine OPV had a significantly higher mean antibody titer value of 2.0913 compared to those with 1-3 doses who had mean titer values of at least 0.889 (P

= 0.001) (Table 6). Similarly, with regards to the number of campaign OPV, children that received \geq 7 doses of the OPV had significantly higher titer values of 1.8929 compared to those with 1-3 doses who had at least a titer value of 1.4929 (P = 0.001) (Table 7).

Tuble 1. Distribution of pono virus antibodies antong enhance according to sex in Rano State.			
Sex	No. Positive (%)	No. Negative (%)	Total
Male	205 (51.5)	12 (48.0)	217
Female	193 (48.5)	13 (52.0)	206
Total	398 (94.1)	25 (5.9)	423

Table 1:- Distribution of polio virus antibodies among children according to sex in Kano State.

P=0.734

 Table 2: Age distribution of children with polio virus antibody in Kano State.

Age group	Number studied	No. Positive (%)
0-12	153	133 (33.4)
13-24	150	149 (37.4)
25-36	55	53 (13.3)
37-48	28	27 (6.8)
49-60	37	36 (9.0)
Total	423	398 (94.1)

P=0.044

Table 3:- Distribution of Polio Virus Antibodies among the Studied Children With Regard to their Local Government Area.

LGA	Number studied	No. Positive (%)
Dambatta	141	136 (34.2)
Kura	141	133 (33.4)
Wudil	141	129 (32.5)
Total	423	398 (94.1)

P=0.086

Table 4:- Distribution of polio virus antibodies in studied children based on their mothers' educational level

Educational level	Number studied	No positive (%)
Primary	339	317 (79.6)
Secondary or Above	84	81 (20.4)
Total	423	398

P=0.310

Table 5:- Distribution of polio virus antibodies in studied children based on their fathers' educational level

Educational level	Number studied	No Positive (%)
Primary	262	238 (59.8)
Secondary/tertiary	161	160 (40.2)
Total	423	398

P=0.001

Table 6:- Mean polio virus antibody titer value of studied children with regards to the number of routine OPV doses received.

No of routine OPV	Mean titer value
0	0.8890
1	0.9156
2	1.3984
3	1.5887
4	2.0913

P = 0.001

No of Campaign OPV	Mean titer value
0	1.3197
1-3	1.4925
4-6	1.7064
>7	1.8930

Table7:- Mean polio virus antibody titer value of studied children with regards to the number of campaign OPV doses received.

P = 0.001

Discussion:-

This study revealed that 94.1% of the blood samples collected from children \leq 5 years of age from the three senatorial zones of Kano state were sero-positive to poliovirus antibody (IgG). These finding were similar to earlier studies which reported that greater than 90% of school age children, adolescent and young adults had detectable antibodies to Poliovirus (Kelley *et al.*, 1991; Orenstein *et al.*, 1988). Recently, Dashe *et al.* (2010) in Jos reported 97.8% sero-positivity among 183 studied population. Yusouf *et al.* (2015) also reported a seroprevalence rate of 98.8% (168) among 170 children between the ages of 10 to 59 months in Gadap Town Karachi, Pakistan. The relatively high sero-prevalence of the poliovirus antibody in this study may not be unconnected with the consistent polio immunization campaign which improves and increases population turnout to receive the vaccination. As observed in this study, the level of awareness on the need to get children immunized against the virus has now increases day by day as almost all the respondents know about routine and campaign OPV. Yusuf *et al.* (2015) earlier reiterated that high seroprevalence rates may be as a result of improved access to immunization across the country by successive governments through campaign immunizations. This study further indicates that children have effectively responded to the vaccine being used in the ongoing polio eradication initiative.

Seroprevalence reports of other regions in Nigeria revealed lower rates when compared to the results of this study. For example Adewunmi *et al.* (2005) and Baba *et al.* (2012) reported prevalence rates of 59.6% and 53% among children in Ibadan and Maiduguri respectively. Giwa *et al.* (2012) explained that seroprevalence to poliovirus serotypes, although higher than values found in previous studies done in Nigeria, was lower than in the developed world. This was corroborated by Iliyasu *et al.* (2014) who revealed that seroprevalence levels found in their survey, specific to Kano, were much lower than in corresponding serosurveys in India and Egypt (Estívariz *et al.*, 2012; El-Sayed *et al.*, 2013).

The study further revealed that 5.9% of the studied children were seronegative to poliovirus serotypes indicating that these children constituted the unimmunized and unprotected children who could serve as a source of re-emergence of poliovirus infection which could obstruct the success of polio immunization programme. The study further revealed that although the seronegative children have been documented with a history of poliovirus immunization, their seronegative status indicated that they did not either respond properly to the vaccine administered to them or the vaccine might have lost its immunogenicity at the time of administration or it may even be that their immunization history was incorrectly documented. Earlier, a study in Delta state revealed only 4% (8) of the children had no detectable antibody (Danboraye *et al.*, 2011). In another study, 7.9% seronegativity to poliovirus antibodies was reported by Adeniji *et al.* (2015) in a study conducted in North-central and South-western Nigeria among 229 Children between 10 months and 13years. Also, in a study conducted in Zaria, North Western Nigeria between 2008 and 2009, 1.9% of 264 studied children aged 1-10 years had no antibodies to all the three poliovirus serotypes (Giwa *et al.*, 2012).

The findings of this study revealed that prevalence of poliovirus antibodies was significantly associated with younger children compared to the older ones. This indicates that parents and care givers are more likely to expose their children especially those in the earlier months of life to receive both routine and campaign OPV, hence the need to put more effort to reach children in this category as they are the most vulnerable to receive the immunization. In a similar study Mawashi *et al.* (2015) earlier explained that the high seroprevalence of poliovirus antibodies among children in lower age groups such as 12-23 months might be as a result of antibody boosting through routine and campaign OPV and probable exposure to wild poliovirus (WPV) through unsanitary habits.

Contrary to the results of this study, Yusuf *et al.* (2015) observed a higher prevalence of poliovirus antibodies among children aged 48-59 months. This observation as explained by Danboraye *et al.* (2012) could be as a result

of antibody boosting resulting from contagious to the virus in an endemic area as such seropositivity will not decrease with increasing age of the study participants. In similar studies in 2013, Iliyasu *et al.* (2014) also reported that the seroprevalence was unexpectedly low among infants aged 6–9 months, and remained high among children aged 36–47 months. They further explained that the marked drop in seroprevalence for all 3 serotypes in the group aged 6–9 months between 2011 and 2013 brought into focus the quality of IPD operations and the need to devise new strategies to reach the younger children, especially newborn babies and infants, most of whom would be with their mothers and therefore be in the category of "child absent" during IPDs. Thus, the evidence provided by the study yielded an increase in resources for demand-creation activities targeting mothers and caregivers of children in this age group. As a result an improvement was recorded in 2014, compared with 2013 (Iliyasu *et al.*, 2014). In this regard, the baseline seroprevalence among infants aged 6–9 months in 2014 was reported to be better than that in 2013 by Craig *et al.* (2016).

The findings of this study confirms earlier observations by other studies that gender was insignificantly associated with seroprevalence of poliovirus antibodies among children (P=0.734). Although studies have shown that more females were immune than males, yet no significant association exist in relation to polio specific IgG. In contrast Iliyasu *et al.* reported that male gender tended to have higher seroprevalence for the three serotypes, although not statistically significant (Iliyasu *et al.*, 2014). Baba *et al.* (2012) uphold the same view that no correlation existed between gender and antibody to the poliovirus serotypes.

In this study the difference in seropositivity among the three LGAs was not statistically significant (P=0.086). This study is in line with the study by Yusuf *et al.* (2015) who reported that there was no association among seroprevalence of poliovirus antibody and location of child's place of residence. Although, a study by Mawashi *et al.* (2015) stated that Children in the urban areas had more poliovirus antibody prevalence than those from their rural counterparts.

The level of mother's education does not significantly affect sero-prevalence to polio virus among studied children in this study (P=0.310), with only 81 (20.4%) of the children having mothers with secondary level of education and above. However, the study revealed that the father's educational level significantly affects seroprevalence rates. Children whose fathers received education up to secondary level and above had the highest poliovirus antibody prevalence of 40.2% (P=0.001). Yusuf et al. (2015) reported that children whose fathers were educated up to tertiary level were all seropositive in their study. This study had reaffirmed what was already known, that Father's educational level positively affects child's antibody prevalence. Thus, results from the study emphasize Father's educational level as an important factor in the course of a successful immunization programmes. This observation may not be unconnected to the fact that in the area where this study was carried out men predominate in the affairs of the family. Earlier, Vincelik et al. (2007) stated that educational and cultural differences in communities were found to play a significant role in transforming attitudes and behaviors towards vaccination. Mohammed et al. (2014) further reported that noncompliant (to immunization) heads of households had low level of education compared to compliant heads of households in Sokoto State, Nigeria. Similar report revealed that low level of parents' education was found to be associated with non-vaccination during mass campaign in India (Singh et al., 1997). However, according to Omer et al. (2009) only in few instances in developed countries, had it been seen that advanced educational status was a major factor in vaccine refusals.

The number of routine OPV doses received by a child was shown in this study to be a very significant factor to the development of polio virus antibody as revealed by the antibody titre mean value. Children who did not receive even a single dose of OPV had a significantly lower mean antibody titer value of 0.8890 compared to those who had received four (4) doses of routine OPV recording a mean titre value of 2.0913 (P=0.001). Likewise, the mean titer value increases significantly with increase in the number of campaign OPV doses (P=0.001). This study therefore revealed that the mean antibody titer increases with increase in the number of both routine and campaign OPV doses received by the study participants with the highest mean titer value reported in children that received complete doses of OPV. Similar reports by Ilyasu *et al.* (2014) indicated the existence of a significant relationship between the number of routine OPV doses taken and the presence of antibody to the virus. Thus, this study supports previous findings that Vaccine doses taken by children affect poliovirus antibody prevalence in the population (Chen *et al.*, 1996; Habib *et al.* 2013; Iliyasu *et al.* 2014; Mawashi *et al.*, 2015).

Conclusion:-

The study detected a high sero-prevalence rate of poliovirus antibody of 94.1% among children aged \leq 5 years in three local government areas of Kano state indicating that majority of the children in the studied area have been immunized. The study also revealed that age was significantly associated with seroprevalence rates with younger children having higher prevalence rates. However, the seroprevalence rates were not significantly associated with sex of the studied children. Mothers educational levels did not significantly affect the prevalence rates among the studied children, however, the father's educational level, significantly affects the seroprevalence rates. Poliovirus antibodies mean titer level significantly increased in children who had received more number of OPV doses in both the routine and campaign OPV immunization.

It is recommended therefore that; (i) fathers should be considered as a target group in the course of enlightenment campaign as they can encourage and fully engage mothers about immunization programmes and its benefit to the children and (ii) more efforts should also be put by relevant authorities to improve the literacy level of women and to target the unimmunized children whose lack of immunity poses a risk of re-emergence of the poliovirus infection in Nigeria

Aknowlegdement:-

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Statement Of Conflict Of Interest:-

None of the authors had any financial relationship or otherwise with the commercial entities whose products have been used for the study as well as the respective hospitals from where samples of the study where generated, thus they had no interest with regards to subjects of the study as well as outcome of the study.

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Appendix 1:- Ethical approval from ethics committee of Kano state hospital management board.



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