MOLECULAR DETECTION OF POLIOVIRUSES AND ANTIBODIES FROM CHILDREN IN KANO, KEBBI AND ZAMFARA STATES, NIGERIA.

BY

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BY

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DECLARATION

I declare that the Work in this dissertation entitled '**MOLECULAR DETECTION OF POLIOVIRUSES AND ANTIBODIES FROM CHILDREN IN KANO, KEBBI AND ZAMFARA STATES, NIGERIA.**' was carried out by me in the Department of Microbiology, under the supervision of Prof. E. D. Jatau, Prof. O. S. Olonitola and Prof. S. E. Yakubu of the Department of Microbiology Ahmadu Bello University, Zaria.

The information derived from the literature has been duly acknowledged in the text and a list of references provided and no part of this dissertation was presented for another degree, diploma or certificate at this or any other institution.

Kabir Mawashi YUSUF ---------- ----------

Signature Date

CERTIFICATION

This project dissertation entitled "**MOLECULAR DETECTION OF POLIOVIRUSES AND ANTIBODIES FROM CHILDREN IN KANO, KEBBI AND ZAMFARA STATES, NIGERIA.**" by YUSUF Kabir Mawashi meets the regulations governing the award of the degree of Doctor of Philosophy in Microbiology of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to all those who aspire to self-help and strong determination to succeed.

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ABSTRACT

A high proportion of poliovirus antibodies amongst susceptible children, must at all times be sustained in a community if poliomyelitis infection is to be curtailed and its final eradication achieved. For some time now, children have been vaccinated against poliomyelitis through various strategies (Routine Immunization (RI) and Supplemental Immunization Activities (SIAs) in Northern Nigeria without commensurate measures to systematically evaluate the effectiveness or otherwise of such activities. This research was embarked upon to determine the poliovirus antibody prevalence to serotypes 1, 2 and 3 in the three study areas of Kano, Zamfara and Kebbi States, to determine the effect of some probable risk factors of poliovirus antibody amongst the children and also detect if present, the poliovirus in the stool sample of children positive to all the three serotypes. This will give room to assess the overall performance of the Immunization programme through a serosurvey. A cross-sectional survey was designed to enroll children whose mothers had access to Health Facilities across the study area. Two hundred and fourty blood and stool samples each were collected and tested for the presence of poliovirus antibodies and poliovirus particles respectively. Indirect ELISA was used to detect the presence of poliovirus antibodies, while Reverse Transcription Polymerase Chain Reaction (RT-PCR) was used for poliovirus detection. Nine variables were evaluated for their role/contribution or otherwise to overall poliovirus antibody in children in the selected areas in the States. Out of the samples collected, 189 (78.8%) had antibodies to all the three poliovirus serotypes. While 223 (92.9%), 205 (85.4%) and 221 (92.1%) had antibodies to poliovirus serotypes 1, 2 and 3 respectively. About 21.2% of the children sampled had no detectable antibody to any of the poliovirus serotypes. Children numbering 196 (81.7%), 214 (89.2%) and 196 (81.7%) had poliovirus

antibodies to poliovirus types 1 and 2, 1 and 3 and 2 and 3 respectively. About 56.3% of the children sampled in Kano State had antibodies to all the poliovirus serotypes. The percentages of children who had antibodies to poliovirus serotypes 1, 2 and 3 were 65.2%, 86.5% and 68.2% respectively. Seropositivity prevalence to all the polioviruses in Kebbi State was 83.3%. Children who had antibodies to poliovirus serotypes 1, 2 and 3 were about 89.3%, 89.3% and 88.2% of the entire children sampled respectively. Zamfara State children sampled had 96.3% antibody prevalence to all the three poliovirus serotypes. Overall, the children had antibody prevalence to poliovirus serotypes 1, 2 and 3 as 97.5%, 98.7% and 97.5% respectively. Older children 48 – 59 months had a higher poliovirus antibody prevalence (93.5%) than the 0 – 11 months old (66.0%). Female children had higher poliovirus antibody prevalence (86.0%) than Male children (72.2%). Poliovirus antibody prevalence showed increase with Polio Vaccine taken by the children sampled. Children who were not administered any dose had the lowest poliovirus antibody prevalence (66.7%), those that received more than four doses had the highest poliovirus antibody prevalence with (85.7%). Urban children had higher poliovirus antibody prevalence (75.2%) than their rural counterpart (48.7%). Children whose father's educational level was up to tertiary level had highest poliovirus antibody prevalence (100%) than those with either primary (56.0%) or secondary school (87.8%) educational levels. Children of the civil servants had the highest poliovirus antibody prevalence (80.2%) while children whose fathers were traders had the lowest prevalence (76.2%). Children of other tribes resident in the study areas had higher poliovirus antibody prevalence (83.1%) than the indigenous tribes (Hausa 72.8% and Fulani 81.7%). Children whose household's major water source was pipe borne water had the highest poliovirus antibody prevalence (83.6%) while those with well water as their major water source had the lowest antibody prevalence (75.0%). This study found out that age of the children, sex, number of

vaccine doses taken, State of residence and educational level of the fathers had a significant effect on the prevalence of poliovirus antibodies ($p < 0.05$). However, place of residence (urban/rural), father's occupation, father's tribe and household's water source had no statistically significant effect on poliovirus antibody prevalence ($p \square 0.05$). Detecting the poliovirus in one of the study areas was an indication that the virus was still circulating in the area. Therefore more interventional efforts need to be put in place to boast stronger herd immunity which will enable achieving eradication in the three states.

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ABBREVIATIONS

Abbreviation	Meaning
AFP	Acute Flaccid Paralysis
bOPV	Bivalent Oral Polio Vaccine
CDC	Center for Disease Control
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
ELISA	Enzyme Linked Immunosorbent Assay
GPEI	Global Polio Eradication Initiative
IPV	Inactivated Polio Vaccine
mOPV	Monovalent Oral Polio Vaccine
NC	Non coding
NIDs	National Immunization Days
nm	Nano meter
NPEVs	Non Polio Enteroviruses
NPHCDA	National Primary Health Care Development Agency
OPV	Oral Polio Vaccine
PCR	Polymerase Chain Reaction
RI	Routine Immunization
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
tOPV	Trivalent Oral Polio Vaccine
UNICEF	United Nations Children Fund
VDPV	Vaccine Derived Polio Virus
VP	Virion proteins
VPg	Genomic virion protein
WHA	World Health Assembly
WHO	World Health Organization
WPV	Wild Polio Virus
µl	Micro liter

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

The Poliovirus belongs to the genus *Enterovirus* within the family *Picornaviridae*. With a diameter of 27-30nm they are amongst the smallest viruses known, and contain a single-stranded ribonucleic acid (RNA) molecule of positive polarity linked to a small protein at the 5' region of the genome designated as the genomic virion protein (VPg). The entire nucleotide sequence has been determined and the total genome consists of 7440, 7440 and 7435 nucleotides for serotypes 1, 2 and 3 respectively (White and Fenner, 1994). The viral capsid consists of 20 copies of each of the four structural virion proteins (VP1, VP2, VP3 and VP4) (Lodish *et al.,* 2000). These viral capsid proteins protect the encapsidated nucleic acids from degradation and interact with a specific cellular receptor on susceptible host cells (Roos *et al.,* 2007). These viruses have been classified into three distinct serotypes based on their reaction to reference panels of antisera (CDC, 2015). Antibodies produced against one of the three serotypes do not protect against the other types, although some cross-reactivity has been described (Bryce *et al.,* 2004). However, antibodies produced against serotype 1 can confer significant protection against Type 2 (Davies *et al.,* 1975; Masaud *et al.,* 2011).

The epitopes responsible for inducing poliovirus neutralizing antibodies are located at the end of the loops on the three structural proteins: VP1, VP2, and VP3. The VP1 epitope is the most exposed surface protein and plays a major role in the induction of neutralizing

antibodies for all the three serotypes (Tineke *et al.*, 2000). All the three serotypes 1, 2, and 3 infect cells via a specific receptor. These receptors are only present in human cells, that is why humans are the only reservoir of this virus (Mehar *et al.*, 2012; John *et al.*, 2013).

German physician Jakob Heine recognized poliomyelitis as a distinct condition for the first time in 1840. It is a disease that does not respect national borders or continental boundaries, and in that sense, it is truly a problem of global concern requiring large-scale international cooperation. From ancient times into the late 1800s, polioviruses were widely distributed in most of the world's populations, surviving in an endemic fashion by continuously infecting susceptible infants newly born into the community (Lester *et al.*, 2000; Shuguang *et al.*, 2013). The work of physicians Jakob Heine in 1840 and Karl Oskar Medin in 1890 led to it being known as Heine-Medin disease (Pearce, 2005). The disease was later called infantile paralysis, based on its propensity to affect children.

As was the case with smallpox virus, poliovirus causes acute non- persistent infections, virus survival in the environment is limited, and immunization with vaccine interrupts the transmission of virus. Collectively, these factors make poliovirus a candidate for eradication (Cochi, 2005). Poliomyelitis is a highly infectious disease caused by a virus that mainly affects children under five years of age (Shibuya and Murray, 2004; Qadar, 2014). It is one of the most thoroughly studied diseases. There is no cure for poliomyelitis, it can only be prevented (Wood and Thorley, 2003).

Even though paralytic poliomyelitis its chances increases with age, children are believed to play a dominant role in the transmission of polioviruses within populations (Fine and Ritchie, 2006), although the effect of age alone on the prevalence and severity of poliomyelitis is difficult to specify with confidence. Poor sanitation and hygiene are thought to contribute to the age profile of cases in developing countries (CDC, 2006a). In developed countries, population immunity, including historical deficiencies in vaccination coverage, is likely to be the most important determinant of the age distribution of poliomyelitis cases. Infection is spread most commonly by the faecal-oral route, particularly in the presence of poor hygiene and sanitation. Oral-oral and respiratory transmissions are thought to be more common in industrialized countries (Shibuya and Murray, 2004).

There are three clinical types of paralytic polio. But spinal polio is the most common and account for 79% of paralytic cases characterized by asymmetric paralysis that, most often involves the legs. While bulbar polio account for 2% of cases and leads weakness of muscles innervated by cranial nerves. And lastly bulbospinal polio account for 19% of cases and is a combination of bulbar and spinal paralysis (Atkinson *et al.*, 2007).

Cumulatively the number of poliomyelitis patients world-wide had reached an estimated 10 million cases by the beginning of this century (Baicus, 2012; Jacob and Vipin, 2013; Ousmane *et al.*, 2015). No effective vaccine for this disease existed before the 1950s and ignorance about the route of transmission often hampered attempts to control its spread. Following the successful eradication of small pox, World Health Assembly (WHA)

decided to target poliovirus eradication (Okonko *et al.,* 2009; Gregory and Kaufmann, 2010; Heymann, and Ahmed, 2014). By the efforts of the World Health Organization (WHO), paralytic poliomyelitis has been eradicated from America, Western Europe, and many other regions of the world (Masaud *et al.,* 2011). In its history of more than two decades, the Global Polio Eradication Initiative (GPEI), led by national governments and the World Health Organization, has made remarkable headway in its effort to eradicate the disease, punctuated by a few challenges particularly in the developing world.

The efforts of polio endemic countries were encouraged and supported by an effective coalition of partners including WHO, Rotary International, United Nation Children Fund (UNICEF) and the governments of Australia, Canada, Denmark, Finland, Germany, Japan, Norway, Sweden, the United Kingdom, the United States. The participation of Rotary International had been described as the largest contribution ever by a private sector organization to a public health initiative (Cochi, 2005).

The Global Polio Eradication Initiative was launched in 1988 when the estimated annual incidence of poliomyelitis was 350, 000 cases. The goal of global eradication of poliomyelitis by the year 2000 was then, approved by the World Health Assembly in 1988 and was adopted by the World Health Organization. The program had the clear objective of having no more cases of poliomyelitis caused by wild polioviruses or circulating vaccine derived poliovirus (Jagadish *et al.,* 2003; Okonko *et al.,* 2008; Hovi *et al.,* 2010). The global eradication program has emphasized the use of oral poliovirus vaccine (OPV), considering its numerous advantages, compared to the inactivated polio

vaccine (IPV), i.e. its low cost, the logistic ease of its administration, and its ability to induce local gut immunity.

The goal of global eradication of wild poliovirus was defined as: no cases of clinical poliomyelitis associated with wild poliovirus. The primary strategies for achieving this goal are two folds. One is the administration of polio vaccine in the manner most effective to the interrupt transmission of wild poliovirus. This includes attaining high routine immunization coverage with at least three doses of oral polio vaccine, conducting national immunization days (NIDs) and "mopping-up" immunization when polio is reduced to focal transmission. The other strategy is the implementation of action oriented surveillance for all possible cases of poliomyelitis. This includes case investigation and isolation of virus from stool specimens (Bassey *et al.,* 2011; Saraswathy Subramaniam *et al.,* 2014).

At the time the goal to global eradication of poliomyelitis was established in 1998, wild poliovirus (WPV) was endemic in more than 125 countries in five continents and paralyzing more than 350,000 children per annum. Today, only 3 countries in the world remain endemic and these include Nigeria, Pakistan and Afghanistan (WHO, 2010a; CDC, 2011a). Experience in the Americas, where polio has been eliminated since August 1991, demonstrates that the recommended strategies are effective and the global eradication of polio is feasible (Cochi, 2005). Cases of poliomyelitis had continued to drop from an estimated 350, 000 in 1988 approximately 416 in 2013 (GPEI, 2014; WHO, 2015).

The reduction in cases was due to extensive use of the two available vaccines, the live attenuated oral poliovirus vaccine (OPV) or the Sabin vaccine (Friedrich, 2000), and the inactivated poliovirus vaccine (IPV), or the Salk vaccine, (Combiescu *et al.*, 2007). Seroconversion to these vaccines has been found to be poor in most African countries (Aminu, 2000; Okonko *et al.*, 2008; Nicholas, 2013; Robert and Robin, 2013). Monovalent vaccines have been documented as being more effective than trivalent oral polio vaccine (tOPV) in providing protection against the corresponding WPV serotype. Additionally, bivalent oral polio vaccine (bOPV) has been found to be almost equivalent to monovalent oral polio vaccine (mOPV) and superior to tOPV in providing protection against wild polio virus type1 (WPV1) and wild polio virus type3 (WPV3) (WHO, 2009).

Some States in Northern Nigeria had remained a major reservoir for wild poliovirus (Jenkins *et al.,* 2008 and CDC, 2010a). Low trivalent OPV (tOPV) coverage in the RI program, suspension of SIAs in some states in 2003–2004 in the North West, and low coverage in SIAs have contributed to making them sanctuaries for WPV transmission in the region (CDC, 2006b).

Immuno-surveillance is not required by the World Health Organization as part of the strategies of polio eradication, but it will provide an insight into the protective immunity of targeted populations. It will be of added value in eradicating polio, particularly in countries with pockets of unvaccinated persons (Kimberly *et al.,* 2012; Saint-Victor and Saad, 2013). Immunization coverage across most States in the North is not satisfactory,

as such high (Oderinde *et al.,* 2012) and low (Gnanashanmugam *et al.,* 2011; Marta *et al.*, 2013) serum antibody prevalence levels against polioviruses had been reported.

Different techniques for measuring or detecting antibodies have been described by different workers (Thorpe, and Swanson, 2005; Ivanov and Dragunsky, 2005; Žigon *et al.,* 2011). Amongst which is Immunofluorescence, the technique utilizes fluorescent-labeled antibodies to detect specific target antigens. It is used widely in both scientific research and clinical laboratories. Its limitation include inability to detect substances of low quality and concentration (Ghosh and Sharma, 2000; Odell and Deborah, 2013). ELISA, Radio Immunosorbent Assay and Western Blot are other procedures that have been used commonly for the screening and detection of antibodies (Thorpe and Swanson, 2005). From the array of these methodologies ELISA was adopted because of its simplicity, rapidity and quick reproducibility (Odell and Deborah, 2013).

Very few seroprevalence studies have been conducted in what are now the persistent poliovirus reservoir States of North-Western Nigeria. This study was therefore carried out to determine the prevalence level of poliovirus antibodies amongst children aged 0-59 months old in Kano, Kebbi and Zamfara States. This is with a view to make available relevant data to authorities saddled with the responsibility of vaccinating children.

Many scholars had studied the association of some factors in respect of antibody prevalence in children. While some factors had been found to have statistically significant effect on antibody prevalence, others did not (Dashe *et al.,* 2010). Factors

such as gender, water source, nature of place of residence, in most researches, had no significant relationship, except in few instances like what (Dungumaro, 2009; Witsø *et al.,* 2010) reported. In the same manner, prevalence of polio-like paralysis had been found to be significantly higher in the urban population than in rural (Dalhatu *et al.*, 2015). Likewise immunity level had been found to be correlated with the number of vaccine doses taken (Allen *et al.,* 2010).

Poliomyelitis virus remains a major problem in the developing world. In non vaccinated populations, epidemics of poliomyelitis are mainly caused by poliovirus Type 1. The cases that do occur are caused mainly by wild type or vaccine-associated poliovirus, within groups that refuse vaccination (Sutter *et al.,* 2008). The cornerstone of the Global Polio Eradication Initiative is immunization of children with multiple doses of oral poliovirus vaccine, via both Routine Immunization and Supplementary Immunization Activities (CDC, 2010a). The key advantages of OPV are: ease of administration and efficient induction of mucosal immunity, thereby limiting poliovirus shedding and person-to-person transmission (Sutter *et al.,* 2008).

It is customary in any country where a comprehensive program to vaccinate children against polioviruses was undertaken, to carry out laboratory studies aimed at investigating the immune status of the eligible children or even the entire population from time to time (Gangolli *et al.,* 2005).

Precise methods of strain characterization of polioviruses are considered essential for understanding the epidemiology and for studies of vaccine efficacy. Different methods are used for biochemical analysis of the viral genomes, which is mainly restricted to specialized laboratories (Romanenkova *et al.,* 2006), the most widely used method for this purpose is intratypic differentiation (Georgopoulou *et al.,* 2000). Intratypic differentiation techniques have an important role in the epidemiological surveillance of poliomyelitis and in studies of the safety and efficacy of poliovirus vaccines. The value of strain-specific (absorbed) antisera for antigenic characterization of strains has been clearly established for the identification of both vaccine-like viruses and different epidemic wild strains (Yang *et al.,* 2003; Yakovenko *et al.,* 2006).

1.2 Statement of the Research Problem

Poliomyelitis is endemic in Northern Nigeria where there is continuous transmission of wild poliovirus 1 and 3 (WPV1 and 3) and circulating vaccine derived poliovirus 2 (cVDPV2) resulting in a high number of cases of children with acute flaccid paralysis. The seroprevalence of antibodies to polio serotypes which can be used to assess the immune status of children and the effectiveness of the vaccine against poliomyelitis is comprehensively unknown (Aminu, 2000; Giwa *et al.,* 2012).

Serologic studies on polio antibodies and virus characterization are undertaken to determine prevalence and virus circulation in different countries (Affanni *et al.,* 2005). Such studies have been carried out and documented in other geopolitical zones in much greater detail than North West (Dashe *et al.,* 2010; Baba *et al.,* 2012; Oladejo *et al.,*

2013). However, small scale studies covering some local government areas within a State have been done (Aminu, 2000; Giwa *et al.,* 2012).

As a result an approximate proportion of children covering two or more States in one study with antibodies to polio virus was not known, as such studies of this nature are needed to fill in the gap. Especially when such studies will try to linked some risk factors to poliovirus antibody prevalence in the area.

1.3 Justification

Rapid investigation of suspected poliomyelitis cases is critical to identifying possible wild poliovirus transmission and its route. Timely detection of cases permits the timely implementation of controls to limit the spread of imported wild poliovirus or circulating vaccine derive poliovirus (cVDPVs) and maintain the eradication of wild poliovirus. Moreover, rapid investigation of suspected cases will allow collection of specimens for poliovirus isolation, which is critical in some cases for confirming whether a case of paralytic poliomyelitis was due to poliovirus or any other cause (CDC, 2015).

Infections are not readily detected, because vaccine infected persons rarely develop clinical disease. For this reason, screening for cases of acute flaccid paralysis in a vaccinated population cannot be used as the sole method to determine the absence of poliovirus circulation (Herremans *et al.*,2002).

In the areas where this work was carried out, polio vaccines are being administered to children during routine immunization and campaign sessions but their effect with respect to antibody inducement is not periodically monitored to ascertain whether the vaccines are eliciting any antibody response or not. For effective monitoring, serosurvey has been recommended to be carried out at intervals so as to ascertain immunity level of those at risk (Abdel-Moety, 2008). This approach have been taken by various workers (Carlos *et al.,* 2002; Sabine *et al.,* 2002**;** Affanni *et al.,* 2005; Enza *et al.,* 2006).

The area where this work was conducted had remained a major reservoir for wild poliovirus for a long time (CDC, 2010a), and a leading source of extensive international spread in the years 2003–2006 (CDC, 2006a), 2008–2009 (CDC, 2010b) and limited WPV3 international spread in 2008–2009 (CDC, 2009).

The data that would be generated from the study could help the relevant authorities like NPHCDA, WHO in replanning, implementation and evaluation of what has been done so far.

1.4 Aim

The aim of the study was to carry out a poliovirus antibody prevalence survey amongst children in Kano, Kebbi and Zamfara States in North-Western part of Nigeria and to detect any poliovirus in the stool of the children.

1.5 Objectives

The main objectives of this study are to:

i) Determine antibodies to poliovirus serotypes 1, 2 and 3 in the three study areas (Kano, Zamfara and Kebbi States).

ii) Establish a base-line for poliovirus antibody prevalence in Kebbi and Zamfara States.

iii) Assess performance of the immunization programme in the three States.

iv) Determine the level of association between the risk factors and prevalence of poliovirus antibodies amongst the children.

v) Detect polioviruses in stool sample of children.

1.6 Research questions

1. What proportions of children in the study areas in the North Western Nigeria have Poliovirus antibodies?
2. To which serotype(s) do children have antibodies in the study areas?
3. Do the risk factors affect poliovirus antibody prevalence in the study area?
4. What are the circulating poliovirus serotypes in the study areas?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Introduction

Poliomyelitis was first recognized as a distinct condition by Jakob Heine in 1840 and its causative agent, poliovirus, was identified in 1909 by Karl Landsteiner (Trevelyan *et al.*, 2005). The disease is of major public health importance (Dutta, 2008). The term was derived from Greek: poliós (πολιός), meaning "grey", myelós (μυελός), referring to the "spinal cord", and the suffix -itis, which denotes inflammation (CDC, 2005a; Chamberlin and Narins, 2005). It is an acute viral infectious disease which is spread from person to person, primarily via the faecal-oral route (Longo *et al.*, 2004). Polio epidemics have crippled thousands of people, mostly young children in different countries (Trevelyan *et al.*, 2005).

Polioviruses are members of the *Picornaviridae*, a large family of small RNA viruses, consisting of nine genera: *Enterovirus*, *Rhinovirus*, *Cardiovirus*, *Aphthovirus*, *Hepatovirus*, *Parechovirus*, *Erbovirus*, *Kobuvirus*, and *Teschovirus*. The *Enterovirus* genus, to which the polioviruses belong, can be further subdivided into eight clusters: *Poliovirus*, *Human enterovirus A*, *Human enterovirus B*, *Human enterovirus C*, *Human enterovirus D*, *Simian enterovirus A*, *Bovine enterovirus* and *Porcine enterovirus B*.

Around 90% of poliovirus infections cause no symptoms at all (Robin *et al.*, 2013). However, infected individuals can exhibit a range of symptoms if the virus enters the

blood stream (Ryan and Ray, 2004). Different types of paralysis may occur, depending on the nerves involved. Spinal polio is the most common form, characterized by asymmetric paralysis that most often involves the legs. Bulbar polio leads to weakness of muscles innervated by cranial nerves. Bulbospinal polio is a combination of bulbar and spinal paralysis (Atkinson *et al.,* 2007).

2.2 The Poliovirus

It is a sub-microscopic intracellular, obligate, non-enveloped and icosahedral- shaped virus. It is composed of a single (+) sense ribonucleic acid (RNA) genome enclosed in a protein shell called a capsid (Ryan and Ray, 2004). In addition to protecting the virus's genetic material, the capsid proteins enable it to infect certain types of cells. Its diameter ranges between 27- 30nm and is made up of 60 copies of four proteins (VP1 to VP4). The 5′ and 3′ noncoding (5′NC and 3′NC) regions are involved in viral replication and translation (Belsham and Sonemberg, 2000). This demonstrates the complexity of their structure.

Polioviruses (PVs) are classified into three distinct serotypes Type 1, Type 2, and Type 3 (Katz *et al.,* 2004). All the three are virulent and produce almost the same disease symptoms (Ryan and Ray, 2004). Poliovirus Type 1 is the most commonly encountered form, and the one most closely associated with paralysis (Anthony, 2013). These viruses are found in the gastrointestinal tract (Longo *et al.,* 2004). They infect and cause disease in humans alone. Several sites involved in virus neutralization have been identified on the surface of the poliovirus (Brian, 2001; Klasse, 2014). The virus does not survive long in

the environment outside the human body and there is no long-term carrier state (Fatiregun, 2005).

Polioviruses are stable at acid pH and can survive for weeks at room temperature and for many months at 0°C to 8°C. As with other enteroviruses, polioviruses are resistant to ether, 70% alcohol and other laboratory disinfectants. Treatment with 0.3% formaldehyde, 0.1 N HCl, or free residual chlorine at a level of 0.3 to 0.45 parts per million rapidly inactivates the virus, as does exposure to a temperature of 50°C or higher or to ultraviolet light (Adley, 2006).

Isolation of wild polio virus from the stool samples of patients with acute flaccid paralysis (AFP) and healthy children does not always yield positive results (Kittigul *et al.*, 2000; WHO, 2013a). This is because shedding of the virus does not continue to occur for a long time (Pedro *et al.,* 2003; Laassri *et al.,* 2005). Amongst the poliovirus serotypes, Type 2 was the easiest to be eradicate followed by poliovirus Type 3 (Okonko *et al.,* 2009; Anda, 2012; Estı´variz *et al.,* 2013).

2.3 Transmission

Humans are the sole reservoir for poliovirus. It is spread directly or indirectly from person to person and is mostly shed by infected persons in their stools (Racaniello, 2006). The virus may occasionally be transmitted via the oral-oral route (Simon *et al.,* 2011; Vakili *et al.,* 2015). Human-to-human contact has also been established in areas with

poor sanitation and hygiene (Kew *et al.*, 2005). Direct neural spread may also occur in certain situations.

By the time poliomyelitis is recognized in any member of a family, almost all susceptible household contacts have already been infected (Vakili *et al.,* 2015). The virus can spread at any time of the year, but some results indicate that its circulation in temperate countries may be seasonal, and can peaks in winter (Abdulraheem and Saka, 2004; Kew, 2005). However, these seasonal differences which are apparent in temperate climates are far less pronounced in tropical areas (Miller *et al.,* 2006). Two peaks of transmission, Feb to May (low transmission period) and August to November (high transmission period) have been recognized in Africa.

Polio is most infectious between 7–10 days before and 7–10 days after the appearance of symptoms (Park, 2000; Racaniello, 2006). The incubation period is commonly 6 to 20 days with a range from 3 to 35 days (Fisher, 2014). Factors that increase the risk of polio infection or affect the severity of the disease include immune deficiency, malnutrition, tonsillectomy, physical activity immediately following the onset of paralysis, skeletal muscle injury due to injection of vaccines or therapeutic agents and pregnancy (Michael *et al.*, 2014). Although the virus can cross the placenta during pregnancy, the fetus does not appear to be affected by either maternal infection or polio vaccination (Salisbury *et al.,* 2006). But trans-placental transfer of the resulting antibodies to poliovirus infection had been document (Yorty and Robert, 2003; Palmeira *et al.,* 2012).

Infected asymptomatic persons shed virus in their stool, and are able to transmit it to others. No correlation has been established between the level of immunoglobulins and duration of virus shedding (Galal *et al.,* 2012). Transmission pathways can be inferred from the pattern of nucleotide variation among strains, and outbreaks can be traced using genomic analysis. The portal of entry is the mouth. Primary replication occurs in the pharynx and gastrointestinal tract (GIT). The virus invades local lymphoid tissue, enters the blood stream and may infect cells of the central nervous system (CNS). Replication in motor neurons of the anterior horn cells and brain results in cell destruction and causes the typical manifestation of poliomyelitis. The virus is usually present in the throat and in the stools before the onset of illness. One week after onset, there is little virus in the throat, it continues to be excreted in the stool for several weeks (CDC, 2005b). A gram of stool can contain several million virus particles (Dowdle *et al.*, 2002).

In settings with faecal contamination of the environment and water supplies the estimated basic reproduction number is very high approximating 10–15 (Nicholas, 2013). In 1988 there was endemicity in 125 countries and it has been estimated by WHO that more than 350, 000 children were paralyzed each year (Sutter *et al.*, 2008). At a certain time, in developing countries, 4 of every 1,000 children born annually had been found to be paralyzed as a result of polioviruses infection (Jacob and Vipin, 2013).

2.4 Pathogenicity

The disease is characterized by acute flaccid paralysis of any or in certain cases both of the limbs (Gideon and Stephen, 2015). There are two basic patterns of polio infection: a minor illness which does not involve the central nervous system, sometimes called abortive poliomyelitis, and a major illness involving the CNS, which may be paralytic or non-paralytic (Falconer and Bollenbach, 2000). In most people with a normal immune system, the viral infection is asymptomatic. Rarely the infection produces minor symptoms; these may include upper respiratory tract infection (sore throat and fever), gastrointestinal disturbances (nausea, vomiting, abdominal pain, constipation or, rarely, diarrhea), and influenza-like illnesses (Atkinson *et al.,* 2007).

Only about 1% of poliovirus infections result in a paralytic illness (Evans, 2013). The virus enters the body through the mouth, infecting the first cells it comes in contact with—the pharynx (throat) and intestinal mucosa. It gains entry by binding to an immunoglobulin-like receptor (He, *et al.,* 2003). It can survive and multiply within the blood and lymphatics (Todar, 2006; CDC, 2011b; Cann, 2012). In a small percentage of cases, it can spread and replicate in other sites. In most cases this causes a self-limiting inflammation of the meninges (Chamberlin and Narins, 2005). Penetration of the CNS provides no known benefit to the virus and the mechanisms by which, the spreads to the CNS are poorly understood (Mueller *et al.*, 2005).

2.5 Classification

Depending on the site of paralysis, paralytic poliomyelitis is classified as spinal, bulbar, or bulbospinal. Different types of paralysis may occur, depending on the nerves involved. Spinal polio is the most common form, characterized by asymmetric paralysis that most often involves the legs (Falconer and Bollenbach, 2000). Bulbar polio leads to weakness of muscles innervated by cranial nerves. Bulbospinal polio is a combination of bulbar and spinal paralysis

Outcome **Percentage of cases**

Asymptomatic……..……..90 – 95%

Minor illness…….…….....4 – 8%

Non paralytic aseptic meningitis…….…….**.....**1 – 2%

Paralytic poliomyelitis……0.1 – 0.5%

- Spinal polio…….……..79% of paralytic cases
- Bulbo – spinal polio..…….……19% of paralytic cases
- Bulbo polio…….2% of paralytic

Source: Atkinson *et al.,* 2007

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In most people with a normal immune system, a poliovirus infection is asymptomatic. The spectrum of disease ranges from benign febrile illness to meningitis, myocarditis, and neonatal sepsis. The virus enters the central nervous system in about 3% of infections. Most patients with CNS involvement develop non-paralytic aseptic meningitis, with symptoms of headache, neck, back, abdominal and extremity pain, fever,

vomiting, lethargy and irritability (Chamberlin and Narins, 2005). Approximately 1 in 200 to 1 in 1000 cases progress to paralytic disease, in which the muscles become weak, floppy and poorly controlled, and finally completely paralyzed; this condition is known as AFP (Cann, 2012). Encephalitis, an infection of the brain tissue itself, can occur in rare cases and is usually restricted to infants. It is characterized by confusion, changes in mental status, headaches, fever, and less commonly seizures and spastic paralysis (Wood *et al.*, 2005).

The paralysis is asymmetrical with no sensory losses or changes in cognition. Many affected persons recover completely and in most cases, muscle function returns to some degree. Patients with weakness or paralysis 12 months after onset will usually be left with permanent residuals (CDC, 2005a). The differential diagnosis of acute flaccid paralysis includes paralytic poliomyelitis, Guillain-Barre syndrome and transverse myelitis; less common aetiologies are injection neuritis, encephalitis, meningitis and tumors (Leis and Dobrivoje, 2012).

When a person susceptible to infection is exposed to poliovirus, one of the following responses may occur:

(i) Inapparent infection without symptoms,

(ii) Mild (minor) illness (abortive poliomyelitis),

(iii) Aseptic meningitis (nonparalytic poliomyelitis)

(iv) Paralytic poliomyelitis

(Leis and Dobrivoje, 2012).

2.5.1 Paralytic Polio

In around 1% of infections paralytic poliomyelitis occurs (Mueller *et al.,* 2005). Inflammation alters the color and appearance of the gray matter in the spinal column (Chamberlin and Narins, 2005) and other destructive changes (Mueller *et al.,* 2005). Paralysis generally develops one to ten days after early symptoms begin (Silverstein *et al.,* 2001).

The likelihood of developing paralytic polio increases with age (Nielsen *et al.,* 2002). In children under five years of age, paralysis of one leg is most common; in adults, extensive paralysis of the chest and abdomen also affecting all four limbs—quadriplegia—is more likely. Paralysis rates also vary depending on the serotype of the infecting poliovirus (Carp, 2015).

2.5.2 Spinal Polio

Spinal polio is the most common form of paralytic poliomyelitis (Cono and Alexander, 2002). The extent of spinal paralysis depends on the region of the cord affected, which may be cervical, thoracic, or lumbar (Hagerstown, 2005). Paralysis is often more severe proximally (where the limb joins the body) than distally (the fingertips and toes).

2.5.3 Bulbo Polio

This makes up about 2% of cases of paralytic polio (Atkinson *et al.,* 2007). Pulmonary edema and shock are also possible, and may be fatal (Hagerstown, 2005).

2.5.4 Bulbospinal Polio

Approximately 19% of all paralytic polio cases have both bulbar and spinal symptoms; this subtype is called respiratory polio or bulbospinal polio (Atkinson *et al.,* 2007). It affects breathing, making it difficult or impossible for the patient to breathe without the support of a ventilator. It can lead to paralysis of the arms and legs and may also affect swallowing and heart functions (Hoyt *et al.,* 2005).

2.6 Diagnosis

Definitive diagnosis of poliomyelitis is by viral isolation. Poliovirus may be recovered from stool or pharynx. Isolation of virus from the cerebrospinal fluid is diagnostic but rarely accomplished. Oligonucleotide mapping or genomic sequencing is required to differentiate wild like or vaccine-like virus (Fatiregun, 2005). Neutralizing antibodies appear early in the serum and may be at high levels. Paralytic poliomyelitis may be clinically suspected in individuals experiencing acute onset of flaccid paralysis in one or more limbs with decreased or absent tendon reflexes in the affected limbs, which cannot be attributed to another apparent cause, and without sensory or cognitive loss (CDC, 2015).

A laboratory diagnosis is usually made based on recovery of poliovirus from a stool sample or a swab of the pharynx. Antibodies to poliovirus can be diagnostic, and are generally detected in the blood of infected patients early in the course of infection (Atkinson *et al.,* 2007). Analysis of the patient's cerebrospinal fluid (CSF), which is

collected by a lumbar puncture ("spinal tap"), reveals an increased number of white blood cells (primarily lymphocytes) and a mildly elevated protein level. Detection of virus in the CSF is diagnostic of paralytic polio, but rarely occurs (Atkinson *et al.*, 2007).

If poliovirus is isolated from a patient experiencing acute flaccid paralysis, it is further tested through oligonucleotide mapping (genetic fingerprinting), or more recently by PCR amplification, to determine whether it is "wild type" (that is, the virus encountered in nature) or "vaccine type" (derived from a strain of poliovirus used to produce polio vaccine) (Millard, 2010). It is important to determine the source of the virus because for each reported case of paralytic polio caused by wild poliovirus, it is estimated that another 200 to 3,000 contagious asymptomatic carriers exist (Gawande, 2004).

2.7 Management

There is no cure for polio. The focus of modern management of the disease has been on providing relief of symptoms, speeding recovery and preventing complications. Supportive measures include antibiotics to prevent secondary bacterial infections in weakened muscles, analgesics for pain, moderate exercise and a nutritious diet (Wilburta, 2013). Management of polio often requires long-term rehabilitation, including physical therapy, braces, corrective shoes and, in some cases, orthopedic surgery (Hagerstown, 2005).

Today many polio survivors with permanent respiratory paralysis use modern jacket-type negative-pressure ventilators that are worn over the chest and abdomen (Goldberg, 2002).

Devices such as rigid braces and body casts, which tended to cause muscle atrophy due to the limited movement of the user were also touted as effective treatments.

2.8 Prevention

2.8.1 Passive Immunization

In 1950, William Hammon at the University of Pittsburgh purified the gamma globulin component of the blood plasma of polio survivors. He proposed that the gamma globulin, which contained antibodies to the virus, could be used to halt its infection, prevent disease, and reduce the severity of disease in other patients who had contracted polio. The results of a large clinical trial were promising; the globulin was shown to be about 80% effective in preventing the development of paralytic poliomyelitis (Amanna and Mark, 2009; David *et al.*, 2013). It was also shown to reduce the severity of the disease in patients that developed polio. The globulin approach was later deemed impractical for widespread use, due to the limited supply of blood plasma, and the medical community turned its focus to the development of a polio vaccine (Rinaldo, 2005).

2.8.2 Personal and Environmental Hygiene

High standards of personal and environmental hygiene especially sanitary disposal of sewage and provision of adequate and safe water supply are other proven primary preventive measures. These, in combination with community health education constitute the primary preventive package for control of poliomyelitis (Schlipköter and Flahault, 2010). Good nursing care, from the beginning of illness can minimize or prevent crippling. Physiotherapy initiated in the affected limb on time is of vital importance. It

helps the weakened muscles to regain strength, as is likely the child may have to be put on metal calipers.

2.8.3 Vaccines

The foundation for the development of poliovirus vaccines was laid in 1949. The two polio vaccines developed by Jonas Salk in 1952, inactivated polio vaccine (IPV) and Albert Sabin in 1962, oral polio vaccine (OPV), were credited with reducing the global number of polio cases per year from many hundreds of thousands to around a thousand in 2006 (Miller, 2004; Aylward, 2006). Vaccination with these two vaccines could result in global eradication of the disease (Heymann, 2006). Immunization with live-attenuated vaccine mimics natural infection and results in the induction of a local secretory antibody (IgA) response that is associated with a reduction in shedding of poliovirus from the intestine.

The two types of polio vaccines available differ with regards to administration and immunologic mechanism: injectable IPV and orally administered OPV. The two vaccines are used throughout the world to combat polio. Both vaccines induce immunity to polio, efficiently blocking person-to-person transmission of wild poliovirus, thereby protecting both individual vaccine recipients and the wider community (herd immunity) (Jacob, 2003).

In Nigeria, OPV was the sole vaccine that was used for the purpose of preventing poliomyelitis amongst the population at risk. But attempts have started to introduce the

inactivated polio vaccine into the routine immunization schedule. The attenuated poliovirus in the Sabin vaccine replicates very efficiently in the gut, the primary site of wild poliovirus infection and replication, but the vaccine strain is unable to replicate efficiently within nervous system tissue (Rhoades *et al.*, 2011). A single dose of oral polio vaccine produces partial immunity to all three poliovirus serotypes in approximately 50% of recipients. Three doses of live-attenuated OPV have been found to produce protective antibody to all three poliovirus types in more than 95% of recipients (Atkinson *et al.*, 2011).

In addition to the immunity produced, the vaccine virus competes with the wild polio virus to occupy the coupling sites in the intestinal lumen, and is therefore very efficient in blocking outbreaks. Vaccine viruses that colonize the intestines are excreted in large quantities in the faeces and may secondarily infect the vaccinated individual's susceptible contacts, producing immunity in them as well (Carvalho and Lily, 2006). The capacity of attenuated strains to spread contributes to a higher rate of immunization than that provided by vaccination coverage. This knowledge provides the basis for mass vaccination campaigns, successfully implemented in various regions of the world (Coates *et al.*, 2013).

As is the case with other live virus vaccines, OPV is thermo-unstable (Mrudula and Kamlesh, 2003), and great loss of potency occurs through heat. Of the three strains, poliovirus type 3 is the most thermo-unstable, losing potency some hours afterwards at temperatures above 10 ºC. Therefore, it is fundamental to maintain an adequate cold

chain to guarantee the vaccine's full immunogenicity. Plotkin and Vidor (2008) and Ozan *et al.,* (2014) have described the properties of oral polio and inactivated polio vaccines.

One of the problems noted with the use of the vaccine is the low rate of seroconversion obtained with oral vaccine administration in Tropical countries. Whereas, in temperate climate countries over 95% seroconversion is obtained, in Tropical climate countries various studies have shown seroconversion rates as low as 50% and additional doses are necessary for seroconversion. A review of oral vaccine immunogenicity studies in developing countries showed that on an average, after three doses of OPV, only 73%, 90% and 70% are protected against the polioviruses Type 1, Type 2 and Type 3 respectively (Hambleton, and Gershon, 2005; Flipse and Jolanda, 2015). Probably, oral polio vaccine thermo-instability and mainly, the interference of other enteroviruses contribute to this. Intestinal immunity that occurs after OPV uptake is serotype-specific, and is more immunogenic for serotype 2 (Samoilovich *et al.,* 2003) than for serotypes 1 and 3.

Oral polio vaccine is usually formulated as a trivalent product, but monovalent OPV (mOPV) formulation for each serotype (mOPV1, mOPV2, and mOPV3) was used in the United States from 1961 to 1964, and both monovalent (primarily mOPV1) and bivalent formulations have been used in other countries since the 1960s (Flipse and Jolanda, 2015). Trivalent OPV is used in many countries for routine immunization and supplemental immunization activities (mass campaigns); mOPV1 and mOPV3 were licensed in 2005 and bivalent OPV (Types 1 and 3) was licensed in 2009 for use in supplemental immunization activities in polio-endemic Countries (WHO, 2010b). WHO

advocated OPV exclusively for developing Countries both in the Expanded Programme on Immunization (EPI, established in 1974) and for polio eradication (from 1988) (Duintjer Tebbens *et al.*, 2013). The control of polio in endemic regions is heavily dependent on the use of the attenuated oral poliovirus vaccine as monovalent, bivalent or trivalent formulations (Aylward and Maher 2006).

Some of the advantages of OPV include its low cost, ease of administration, high vaccine efficacy for low number of doses in some cases, mucosal immunity to stop virus transmission and vaccine-related virus spread contributing to "contact immunization" (Aylward *et al.*, 2000; Hull, 2001). In the majority of susceptible children who receive oral vaccine, the virus persists in the oropharynx for 1 to 2 weeks and is excreted in the faeces for a period of up to 2 months, the excretion peak being in the first week after administration (Pickering, 2003).

Vaccines may be responded to poorly because of the following reasons (Michael, and Mark, 2012):-

i. Use of vaccines of low potency. This can be due to poor quality vaccine or unsatisfactory storage during distribution.
ii. Interfering activity of other enteroviruses and bacteria in the gut.
iii. Inadequate immune response due to malnutrition.
iv. Intestinal resistance produced by prior exposures to polio virus or related virus that were insufficient to elicit serum antibody
v. Presence of an inhibitor substance in saliva or the alimentary canal.

In 1988, the World Health Assembly resolved to eradicate poliomyelitis worldwide (CDC, 2011a). One of the main tools used in polio eradication efforts has been the live, attenuated oral poliovirus vaccine. This inexpensive vaccine is administered easily by mouth, makes recent recipients resistant to infection by wild polioviruses, and provides long-term protection against paralytic disease through durable humoral immunity (CDC, 2012).

The major disadvantage of OPV is the slight risk of genetic drift to a vaccine derived polio virus (VDPV), which can cause local outbreaks (CDC, 2012; Oliver and Olaf, 2013; GPEI, 2013). At least one case occurs per 750 000 doses of trivalent OPV for the first dose given (Mohammed *et al.*, 2010), compared with one case of poliomyelitis per 100 to 1000 infections with wild-type poliovirus, depending on the serotype (Ehrenfeld *et al.*, 2008; Oliver, 2009).

2.9 Eradication

Since the launch of the Global Poliomyelitis Eradication Initiative in 1988, considerable progress has been achieved globally. At present, the causative agent for the disease remains endemic in only three countries (Afghanistan, Nigeria and Pakistan). The poliovirus eradication plan, as outlined in the WHO strategic plan for 2004--2008, incorporates priority activities for each phase of the plan:

(i) Polio eradication certification for regions,

(ii) Oral poliovirus vaccine cessation phase,

(iii) Post-OPV phase (Dutta, 2008).

Following the widespread use of poliovirus vaccine in the mid-1950s, the incidence of poliomyelitis declined dramatically in many industrialized countries (Plotkin *et al,* 2012). In 2000 polio was officially eradicated in 36 Western Pacific Countries, including China and Australia (D'Souza *et al.,* 2002). In the European region, the last case of poliomyelitis caused by indigenous transmission of wild poliovirus was observed in Turkey (Vancelik *et al.,* 2008).

The World Health Organization has set different targets for the global eradication of wild polioviruses (Khuri-Bulos, 2004; WHO, 2013b). Good progress has been achieved toward meeting this goal. As polio eradication is approached, increased emphasis has been placed upon strengthening the field and virologic components of wild poliovirus surveillance, prompting the development of more specific and sensitive methods for the detection and identification of the virus in clinical specimens or environmental samples (GPEI, 2010; CDC, 2011b).

Although cases of polio have decreased by 99% worldwide since 1988, geopolitical conflicts have exacerbated its spread—Syria, Ethiopia, and Kenya have reported polio infections (Mohammadi, 2013; WHO, 2013c).

2.10 Antibodies

Antibodies are found in every human being. These molecules are essential to life, and play a very important role in the immune system. Each person has approximately one to

two billion different antibodies continuously flowing throughout the bloodstream, patrolling day and night to fight infections and diseases in their human host (NIH, 2007; Harris and Gause, 2011).

Individuals who are exposed to the virus, either through infection or by immunization with polio vaccine, develop immunity. In immune individuals, IgA antibodies against poliovirus are present in the tonsils and gastrointestinal tract and are able to block virus replication. IgG and IgM antibodies against the virus can prevent its spread to motor neurons of the central nervous system (Kew *et al.,* 2005). The antibody molecule itself has two separable functions. First, antibodies have the unique ability to recognize and attach themselves to the virus that causes disease. Second, in recognizing and attaching themselves to these pathogenic molecules, they act as markers, sending signals to other parts of the immune system to attack and eliminate the disease-associated viruses.

There are five different classes of immunoglobulins (IgG, IgM, IgA, IgD, and IgE) based on structural differences in the composition of their heavy chains. Some of the immunoglobulin classes contain subclasses. For example, IgG has four subclasses: IgG1, IgG2, IgG3, and IgG4 that display differences in their heavy chains. The antibody responses to most protein antigens are found primarily in the IgG1 subclass, although significant amounts of antiviral antibodies occur in IgG3 as well. Small amounts of anti-protein antibodies also occur in IgG4.

In contrast to nonspecific defense mechanisms, specific immune defense systems are not effective fully at birth and require time to develop after exposure to the infecting agent or its antigens. Specific immunity may be acquired naturally by infection or artificially by immunization. Following natural exposure, IgM and IgG appear in the serum about 7 to 10 days after infection. Sufficiently high levels can block Poliovirus entry into the central nervous system. Initially, the IgM response is 2 to 8 fold greater than the IgG response. IgM levels peak at about 2 weeks after exposure and disappear from the serum within about 60 days. IgG levels increase steadily and persisting serum antibody belongs to this class. IgA antibody appears in the serum 2 to 6 weeks after exposure and remains at low levels; in some individuals there is no rise in serum IgA. Serum antibodies are type specific.

There may be a low degree of heterotypic antibody induced by infection, especially with Type 1 and 2 polioviruses. It is believed that serum neutralizing antibodies (primarily IgG) persist for life. A survey carried out in an isolated Eskimo village showed that IgG antibodies produced from subclinical infection with wild virus persisted for a long time without subsequent exposure.

The persistence of secretory IgA antibody may be related to the virulence of the infecting virus and to the number of virus particles presented to the intestinal and nasal mucosa. Because it is rather easy to estimate immunity by measuring circulating antibody, there is a tendency to identify antibody with immunity. However, the antibody level does not reflect the total immunity of the body (Wilson-Welder *et al.*, 2009). The presence of

serum antibody does not always mean that immunity exists, but it does indicate that the individual has had a previous encounter with the microorganism.

Poliovirus infection also induces development of secretory IgA antibody. Secretory antibody is produced by plasma cells originating in gut-associated lymphoid tissues, mainly Peyer's patches. These cells localize in mucosal sites, including the intestine, the pharynx, and the mammary glands (Ogra *et al.*, 2001; Brandtzaeg, 2013).

Protective immunity against poliovirus infection develops following immunization or natural infection. Immunity following natural infection or administration of live oral polio vaccine is believed to be life-long. However, immunity to one poliovirus type does not protect against infection with the other serotypes. Therefore full immunity requires exposure to each serotype (Kew *et al.*, 2005). Infants born to mothers with high antibody levels against this virus are protected for the first several weeks of life.

2.11 Serology

Antiviral antibodies can be measured by neutralization, hemagglutination inhibition (HI) test, and enzyme-linked immunosorbent assay (ELISA). Even though several methods for viral antibody testing are available, the two standard methods for typing poliovirus isolates are neutralization and enzyme-linked immunosorbent assays (Bawage *et al.*, 2013; Yang *et al.*, 2013).

Detection of the neutralizing antiviral antibodies forms the basis for evaluation of protection. Neutralization of the poliovirus by the antiviral antibodies is believed to involve different mechanisms which block one or more of the viral functions of attachment to cells, penetration, and uncoating. In vitro virus neutralization is the main standard assay for the detection and measurement of the neutralizing poliovirus antibodies. Although the polioviruses induce clear visible cytopathic effects (CPE) in susceptible cell cultures, the interpretation of neutralizing titers is influenced by the subjective visual scoring of CPE and the long time required for low virus titers to develop CPE. For these reasons, a number of assays have been described and evaluated as alternatives to this standard assay (Wahby, 2000).

Comparison of results from ELISA and the neutralization test suggest that ELISA variants, based on the principle of blocking or binding inhibition that emulate the neutralization test, might offer an alternative to the neutralization test (Ivanov AP, Dragunsky, 2005). The main problem with cell culture is the long period (up to 4 weeks) required for a result to be available. Also, the sensitivity is often poor and depends on many factors, such as the condition of the specimen, and the condition of the cell sheet. Cell cultures are also very susceptible to bacterial contamination and toxic substances in the specimen. Lastly, many viruses will not grow in cell culture at all e.g. Hepatitis B and C, Diarrhoeal viruses and parvovirus (Mao *et al.*, 2013).

2.11.1 Overview of ELISA (Enzyme – linked immunosorbent assay)

ELISA is very similar to radioimmunoassay (RIA) but it has the advantage of safety and at the same time sharing the specificity, sensitivity and rapidity of RIA (Yeo and Brian, 2002; Žigon *et al.,* 2011; Reverté *et al.,* 2014). In an ELISA test the most commonly used enzymes are horseradish peroxidase (HRP) and alkaline phosphatase (AP). Any other enzyme may be used if it fulfills the following criteria: high stability and extreme specificity.

ELISAs are typically performed in 96-well polystyrene plates, which passively bind antibodies and proteins. It is this binding and immobilization of reagents that make ELISAs so easy to design and perform. Having the reactants immobilized to the microplate surface makes it easy to separate bound from unbound material during the assay. This ability to wash away nonspecifically bound materials makes the test a powerful tool for measuring specific analytes within a crude preparation. ELISA is thus a rapid and simple method that may be useful for mapping seroepidemiology of poliomyelitis and as a tool for effective surveillance of the disease by offering rapid diagnosis in acute cases (Harrington *et al.,* 2002; Tirimacco and Tideman, 2004; Hashimoto *et al.,* 2007).

A detection enzyme or other tag can be linked directly to the primary antibody or introduced through a secondary antibody that recognizes the primary antibody. It also can be linked to a protein such as streptavidin if the primary antibody is biotin labeled. A large selection of substrates is available for performing the ELISA with HRP or AP

conjugates. The choice of substrate depends upon the required assay sensitivity and the instrumentation available for signal detection.

ELISAs can be performed with a number of modifications to the basic procedure. The key step, immobilization of the antigen or antibody of interest, can be accomplished by direct adsorption to the assay plate or indirectly via a capture antibody that has been attached to the plate. The antigen is then detected either directly (labeled primary antibody) or indirectly (labeled secondary antibody). Another variant is the sandwich assay. This type of capture assay is called a "sandwich" assay because the analyte to be measured is bound between two primary antibodies – the capture antibody and the detection antibody. ELISA can also be performed as a competitive assay. This is common when the antigen is small and has only one epitope, or antibody binding site (Janeway *et al.,* 2001).

2.11.2 Direct ELISA

It is considered to be the simplest type of ELISA the antigen is adsorbed to a plastic plate, then an excess of another protein (normally bovine serum albumin) is added to block all the other binding sites. While an enzyme is linked to an antibody in a separate reaction, the enzyme-antibody complex is applied to adsorb to the antigen. After excess enzyme-antibody complex is washed off, enzyme-antibody bound to antigen is left. By adding in the enzyme's substrate, the enzyme is detected illustrating the signal of the antigen. It has high specificity and sensitivity (Noedl *et al.,* 2006).

2.11.2 Indirect ELISA

The indirect ELISA is used primarily to determine the strength and/or amount of antibody response in a sample. It is one of the most powerful qualitative and quantitative tools for antibody detection (Samuel *et al.*, 2000; Wielkopolska *et al.*, 2002; Ivanov *et al.*, 2005). Its discriminatory power lies in the fact that it is based on the extremely specific antibody-antigen complexation. ELISA is rapid and simple. And may be useful for sero-epidemiology mapping.

2.11.3 Sandwich ELISA

The sandwich technique is used to identify a specific sample antigen. The well surface is prepared with a known quantity of bound antibody to capture the desired antigen. After nonspecific binding sites are blocked using bovine serum albumin, the antigen-containing sample is applied to the plate. A specific primary antibody is then added that "sandwiches" the antigen. Enzyme-linked secondary antibodies are applied that bind to the primary antibody. Unbound antibody–enzyme conjugates are washed off. Substrate is added and is enzymatically converted to a color that can be later quantified. The Sandwich ELISA measures the amount of antigen between two layers of antibodies (Stephanie, and Kruti, 2013). The antigen to be measured must contain at least two antigenic sites capable of binding to antibody, since at least two antibodies act in the sandwich. Either monoclonal or polyclonal antibodies can be used as the capture and detection antibodies in Sandwich ELISA systems. The advantage of Sandwich ELISA is that the sample does not have to be purified before analysis, and the assay can be very sensitive (Canady *et al.*, 2013).

2.11.4. Competative ELISA

The key event of competitive ELISA is the process of competitive reaction between the sample antigen and antigen bound to the wells of a microtiter plate with the primary antibody. First, the primary antibody is incubated with the sample antigen and the resulting antibody–antigen complexes are added to wells that have been coated with the same antigen. After an incubation period, any unbound antibody is washed off. The more antigen in the sample, the more primary antibody will be bound to the sample antigen. Therefore, there will be a smaller amount of primary antibody available to bind to the antigen coated on the well. Secondary antibody conjugated to an enzyme is added, followed by a substrate to elicit a chromogenic or fluorescent signal. Absence of color indicates the presence of antigen in the sample (Stephanie, and Kruti, 2013). The main advantage of competition ELISA is its high sensitivity to compositional differences in complex antigen mixtures, even when the specific detecting antibody is present in relatively small amounts (Dobrovolskaia *et al.*, 2006).

2.12 Molecular detection

In recent years, serologic approaches have been supplemented or replaced with a variety of powerful molecular methods to detect and type polioviruses (Tansuphasiri *et al.*, 2000; Hamza *et al.*, 2011), Sabin related isolates or wild poliovirus. The molecular methods generally have the combined advantages of high specificities, good selectivities, rapid performance, and ease of use (Brown *et al.*, 2003). When molecular reagents are available, identification of poliovirus isolates is rapid and straightforward. The difficulty being experienced in molecular detection of wild polioviruses is that specific probes and

primer sets are not yet available for all contemporary wild poliovirus genotypes (Espy *et al.,* 2006; Shulman *et al.,* 2014).

Reliable laboratory techniques for the intratypic characterization of poliovirus Types 1, 2, and 3 isolates have an important role in the epidemiological surveillance of poliomyelitis and in studies of the safety and efficacy of poliovirus vaccines. The polymerase chain reaction (PCR) is the most sensitive of the existing rapid methods to detect microbial pathogens in clinical specimens. In particular, when specific pathogens that are difficult to culture in vitro or require a long cultivation period are expected to be present in specimens, the diagnostic value of PCR is known to be significant.

However, the application of this procedure to clinical specimens has many potential pitfalls due to its susceptibility to inhibitors, contamination and experimental conditions (Roux, 2009). In most cases, the effect of such products can be overcome by making a 1:20. It has also been known that the sensitivity and specificity of a PCR assay is dependent on target genes, primer sequences, techniques, DNA extraction procedures, and product detection methods (Yoshimasa, 2002).

Polioviruses can be distinguished from non polio enteroviruses (NPEVs) in PCR assays with poliovirus-specific primers (WHO, 2004). Specific RNA probes and PCR primers recognizing Sabin vaccine-related isolates or different wild poliovirus genotypes have been developed for routine diagnostic use (WHO, 2004; Bahri *et al.,* 2005). These tests hold great promise and are being introduced for use in routine diagnostic laboratories.

Poliovirus being an RNA virus, reverse transcription-polymerase chain reaction (RT-PCR) is being used to detect it (Casas *et al.,* 2001). Instead of testing a sample for a serotype per test round, a multiplex RT-PCR method that allows the simultaneous identification of serotypes 1, 2 and 3 in a single reaction is being used (Huseyin *et al.*, 2013).

2.13 Previous surveys for neutralizing antibodies

Surveys are done to assess the level of neutralizing antibody to the three poliovirus serotypes amongst children at risk and occasionally looking at different risk factors such as age, gender and location. Surveys show and exhibit different outcomes as operating conditions are not same (WHO, 2014). For instance, in one study where the immunity against poliomyelitis was evaluated, antibodies against poliovirus Type 1, 2 and 3 were detected as 97.6%, 95.8% and 70% respectively, in that study about 0.3% of the subjects were seronegative to the three serotypes. Whereas in another study conducted, antibodies detected to the three poliovirus types were 65.5%, 71.4% and 66.7% respectively to 1, 2 and 3 serotypes, and 19.5% were completely seronegative. This goes to show that surveys could have varying outcomes.

Maintaining polio-free status requires laboratory monitoring of the antibody level and nature of circulating poliovirus strains (Pacsa *et al.,* 1994; Jacob and Vipin, 2013). The demonstration of low antibody prevalence in an area could lead to the decision to administer additional boosters to sustain antibody protective level (Atkinson *et al.,* 2007; Andrew *et al.,* 2009). Some countries following an epidemic of paralytic poliomyelitis

and or on the basis of the results of some serological investigations had caused some immunization schedule to be altered or a new one designed (Mensi, and Pregliasco, 1998).

The seropositive rate required for attaining population immunity to polio has not been universally determined, by world health organization. However, had advanced vaccination coverage necessary to break transmission at around 80 – 85% (Tesovic, 2012). Routine immunization and national and sub-national campaigns were the means, through which National authorities are using to administer the polio vaccines to all eligible children. Re-introduction of poliovirus to areas which were previously poliovirus free is possible especially where immunity level is low (WHO, 2000; Carlos *et al.*, 2002; CDC, 2006b; CDC, 2009). So it is highly recommended that immunity level should be checked periodically, especially in high risk States which are very close to eradication, so that high quality immunization activities can be put in place if so desired (André, 2001; CDC, 2004a). Coordination, poor monitoring and supervision and limited use of local data for appropriate interventions are some of the issues hampering effective delivery of services (Muhammad *et al.*, 2010).

Seroprevalence studies have been performed in different countries, especially in countries that were nearing eradication. These studies have proven their importance as a tool in monitoring and evaluating the successes achieved or otherwise in the process of polio eradication (Diedrich *et al.*, 2000; Conyn-van Spaendonck *et al.*, 2001). Estimation of poliomyelitis immunity prevalence is mainly used as an auxiliary tool with the hope of finding areas in need of special focus or intervention in polio eradication programs.

Despite the important achievements worldwide in the polio eradication initiative, particularly the eradication of wild poliovirus in the Western Pacific region and global interruption of wild poliovirus Type 2 transmission (CDC, 2000; CDC, 2001). In 2014, just three countries still have endemic polio infection: Afghanistan, Nigeria, and Pakistan (IMB, 2014). Until they are rid of poliovirus, no other country is wholly safe from polio and the goal of global eradication will remain elusive. Maintenance of high levels of immunity to poliomyelitis is priority in India as transmission of wild polioviruses in its two states has never been interrupted. Determination of serological profile of vaccines in disease endemic as well as disease free areas is important for evaluation of efficacy to the extended polio vaccination program in the country (Moghe *et al.,* 2010).

2.14 Risk factors predisposing to polio

Several factors have been seen to affect poliovirus antibody prevalence in different ways. For example increasing the amount of vaccine virus in each dose does not translate to increasing antibody levels in children, but breast feeding and presence of maternal antibodies have been seen to be responsible for poor seroconversion (Linhares and Bresee, 2000; William *et al.,* 2002). Contaminated water had been linked with various illnesses, amongst which is diarrhea. Diarrhea had been seen to adversely affect seroconversionion rates of poliovirus serotypes 2 and 3 amongst infants in Bangladesh receiving the first dose of trivalent OPV. The same effect was observed in Brazil on children (Prevots *et al.*, 2000; Levine, 2010).

The low eficiency of trivalent oral polio vaccine in inducing protective antibody titres to poliovirus serotype 3 is a problem of great importance in many regions of the world, particularly in third world countries. Very high immunity level (>96%) is required to interrupt poliovirus transmission particularly in the last remaining reservoirs. Substantial regional differences in OPV immunogenicity in rural and urban centres have been observed (Nasr *et al.,* 2007).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study areas

The States from which children were randomly and stratifically enrolled are shown below in Figure 3.1 All the states: Kano, Kebbi and Zamfara were drawn from the North West geopolitical zone of the country. The states fall within the zone the National Primary Health Care Development Agency classified as high risk states in terms of poliovirus load and transmission (NPHCDA, 2012).

3.1.1 Kano State

Kano State with its capital in Kano is located in North-Western Nigeria. It borders Katsina State to the North-West, Jigawa State to the North-East, and Bauchi and Kaduna States to the South. The capital of Kano State is Kano. It originally included Jigawa State which was made a separate State in 1991. Historically, the State has been a commercial and agricultural State, known for the production of groundnuts as well as for its solid mineral deposits. The State has more than 18,684 square kilometers of cultivable land and is the most extensively irrigated State in the country. It lies approximately between latitudes 100 33'N and 120 23'N and longitudes 70 45'E and 90 29'E. It has an estimated land size of 21,276.872 km2 with 1,754,200 hectares agricultural and 75,000 hectares forest vegetation and grazing land (AIAE, 2007; Abaje, 2014). The mean annual temperature is about 26°C (Falola, 2002; Olofin, 2008).

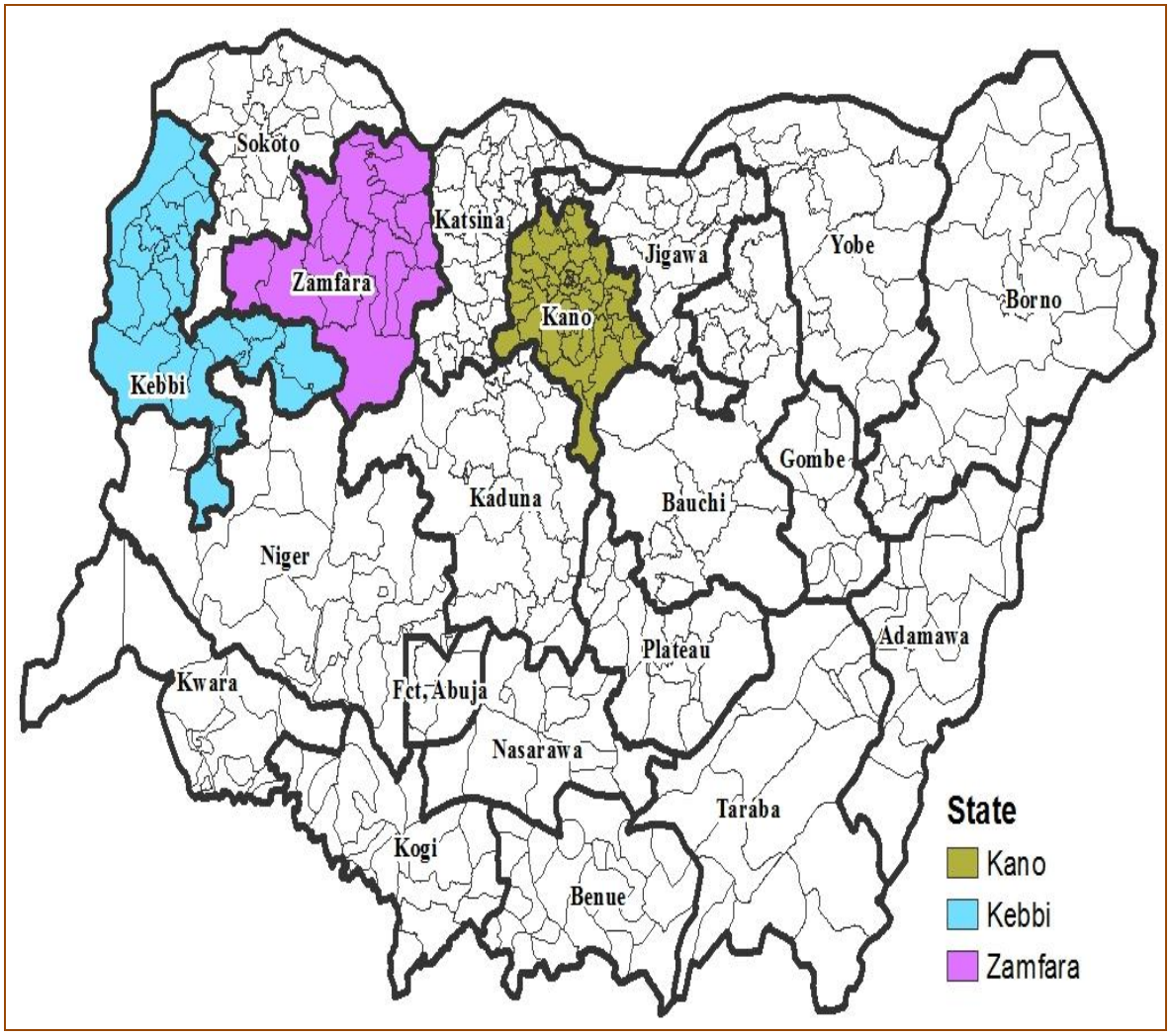

Figure 3.1 Map showing the study areas.

Foreign investments and investors can be seen all over the city. It is arguably within the first five States in terms of commercial activity in Nigeria. The official language of Kano State is English but the Hausa language is commonly spoken. The State is mostly populated by Hausa people.

The State has three senatorial districts made up of the following LGAs:-

- Kano South made up of Bebeji, Bichi, Karaye, Kibiya, Bunkure, Rano, Garko, Garun Mallam, Rogo, Kunchi, Tsanyawa and Tudun Wada.
- Kano North comprising of Kura, Madobi, Makoda, Minjibir, Gaya, Sumaila, Rimin Gado, Shanono, Warawa. Takai, Kiru, Kumbotso, Tofa and Gezawa and lastly
- Kano Central with Gwale, Gwarzo, Tarauni, Gabasawa, Nasarawa, Fagge, Kabo, Kano Municipal, Ungogo, Ajingi, Albasu, Bagwai, Dala, Dambatta, Dawakin Kudu, Dawakin Tofa and Doguwa.

3.1.2 Kebbi State

Kebbi State is situated in North-Western Nigeria with its capital at Birnin Kebbi. It is bordered to the East by Sokoto State, to South by Niger and Zamfara States, and to the west by Benin and Niger republics. It has a total area of 36,800 km². The State has Sudan and Sahel-savannahs. The southern part is generally rocky with River Niger traversing the State from Benin republic up to Ngaski LGA. The Northern part of the State is sandy with river Rima passing through Argungu to Bagudo LGA where it empties into river Niger. Agriculture is the main occupation of the people especially in rural areas. Crops

produced are mainly grains, animal rearing and fishing are also common. The State has four major tribes, which include: Hausa, Fulani, Dakarkari and Gungawa. Kebbi State is mainly populated by Hausa people, with some members of Bussawa, Dukawa, Kambari and Kamuku ethnic communities. The State enjoys tropical continental type of climate, which is largely controlled by two air masses namely; tropical maritime and tropical continental blowing from Atlantic and Sahara desert respectively. The air masses determined the two dominant seasons, wet and dry. Humidity is 27% while wind blow at 11Km/h in ESE direction. The State receive a mean annual rainfall of 800mm between May to September with a peak period in August, the remaining period of the year is dry. The average temperature is 26°C and can rise up to 40°C in the peak of hot season (March-July). However, during harmattan, (December – February) temperature can falls to 21°C. (Usman, 2013).

The State has three senatorial districts made up of the following LGAs:-

- Kebbi North comprising of Argungu, Arewa, Augie, Dandi and Bagudo.
- Kebbi Central with Birnin-Kebbi, Gwandu, Aleiro, Jega, Kalgo, Suru, Bunza, Koko-Besse and Maiyama.
- Kebbi South made up of Zuru, Ngaski, Danko-wasagu, Fakai, Sakaba, Shanga and Yauri.

3.1.3 Zamfara State

The area called Zamfara State today was one of the old Hausa city-States like Kano, Katsina, Gobir, Kabi and Zazzau. It extends up to the bend of River Rima to the North West and River Ka in the South West. Zamfara Kingdom was established in the 11th century and flourished up to 16th century as a city-State.

This State is mainly populated by Hausa and Fulani people, with some members of Gwari, Kamuku, Kambari, Dukawa, Bussawa and Zabarma ethnic communities, others living there include the Igbo, Yoruba, Kanuri, Nupe and Tiv. The State capital is an important commercial centre with a heterogeneous population of people from all over Nigeria. As in all major towns in Nigeria, all the major towns in Zamfara have a large population of other people from different parts of Nigeria.

Rainy season starts in late May to September while the cold season known as Harmattan lasts from December to February. The State has similar climatic condition with Sokoto State, which is dry Sahel, surrounded by sandy Savannah and isolated hills. With an annual average temperature of 28.3 degrees Celsius (Okunola and Ikuomola, 2010).

The State has three senatorial districts made up of the following LGAs:

- Zamfara West comprising of:- Maradun, Anka, Talata Mafara, Bakura, Gummi, Bukkuyum,.
- Zamfara Central with Bungudu, Gusau, Maru, Chafe, LGAs and
- Zamfara North having Birnin Magaji, Kaura Namoda, Zurmi and Shinkafi LGAs.

3.2 Study population

The study population consisted of children aged 0-59 months old residing in the states, whose mother has given consent for the collection of blood and stool samples. The population sample was drawn from children with their mothers or caregivers who went to one of the designated health facilities for any service. One of the health facilities was located in the rural area, while the other was located in the urban area. Enrolling children brought to the designated health facility throughout the three States offered a safer and more feasible method of enrolling children in the study. The parents or guardians were informed that participating children would be tested for protection from poliovirus infection. Any child whose illness contraindicates blood collection procedure (professional judgement of the health worker) was not sampled.

3.3 Ethical approval

Ethical approval from all the States (Ethical Research Review Committee) and consent from parents or guardians were sought before blood and stool samples were taken (Appendices 1, 2, 3 and 4).

3.4 Study design

A health facility based convenience sampling method was used. The method was designed in such a way to be as cross-sectional as possible, involving both quantitative and qualitative approaches. Three health facilities in each of the three senatorial districts

of each state were selected for enrolling children. The study was carried out between September 2010 to January 2014.

3.5 Inclusion and exclusion criteria

Children aged between 0 – 59 months were selected in the three states. Children were excluded in the study if: they were outside this age range, not resident in the state, mothers or caregivers did not give consent for their enrollment in the study and any child whose illness contraindicates blood collection procedure (based professional judgement of the health worker) was not sampled.

3.6 Sampling techniques

Mothers with eligible child/children attending one of the three clinics for services were contacted for possible participation. Enrolment was stratified by place of residence, age, gender, major source of household's drinking water, father's education, father's occupation, urban/rural, father's tribe and the number of OPV doses a child received.

3.7 Administration of structured questionnaires

The demographic data and other relevant information of each participant were obtained using a structured questionnaire (Appendix 5). All questionnaires were allocated a code that also identified the respective blood and stool samples, and the information obtained was confidentially kept. Those who were administered the questionnaire were either the parents or guardians of the children under study.

3.8 Sample size

The sample size was calculated based on polio antibody prevalence survey conducted in Kano State (NPHCDA, 2007). In the study 82% of the children sampled were found to have antibodies to poliovirus. Convenience sampling method was used to cross-sectionally sample children in the three States.

The sample size was calculated using the formular below

$N = Z^2pq/L^2$

(Ngowi *et al., 2007*)

Where

N is the sample size

Z is the standard normal distribution at 95% = 1.96

p is the probability (0.82)

q is 1-p which is 0.18

L^2 is the allowable error (5%) = 0.05

Therefore $(1.96)2 \times 0.82 \times 0.18 / (0.05)2$

$= 226.8$ samples

The sample size of 240 was used for this study, which enabled 80 children to be enrolled from each state.

3.9 Sample collection

Two types of samples, blood for investigating the presence of antibodies and stool for the detection of polioviruses were collected.

3.9.1 Blood sample collection

About 2ml of blood was collected by a Health Worker after swabbing the area of interest with 70% alcohol by venepuncture using sterile disposable 5ml syringe fixed with a 21 gauge (0.81mm) needle. Blood samples were collected into sterile collected into sterile blood collection tubes, allowed into a labeled specimen bottle and transported to the laboratory. Each sample was centrifuged at 1500rpm for 5 minutes and the serum was collected into a clean and dry labeled bottle using a clean and dry Pasteur pipette. The serum was either processed immediately or stored at -20°C until ready for use (Chessbrough, 2000). All blood samples were placed in sterile labelled containers free of anticoagulants or preservatives.

3.9.2 Stool sample collection

Stool sample was collected from the same child at the time of blood collection or later. The intervals between two specimen collections ranged from 1 day to 2 days. Stool specimens that were not immediately processed, were stored at -20°C.

3.10 Laboratory examination

All the samples collected were either immediately processed or stored at -20°C in deep freezers.

3.10.1 Blood examination

ELISA test was conducted to screen for poliovirus antibodies in children. The poliovirus IgG antibody monoclonal ELISA test Kit manufactured by Life Technologies (India) Pvt. was used for the detection of antibodies against poliovirus serotypes in the serum of the children according to the manufacturer's instructions. Three microplate types were used each coated with one of the three polio serotypes (Type 1, 2 and 3) to differentiation. Monoclonal secondary antibodies were used for the detection of the different serotypes. Briefly the procedure was as follows:

Approximately 100µl each of the diluted 1:100 (1ml of the test serum into 99ml of distill water) samples and the ready-to-use calibrators and controls were pipetted into the wells of a 96 coated microplate, one was left empty for the substrate blank. The plate was covered and incubated at room temperature (at the time of laboratory processing room fluctuated approximately between 23.8°C – 24.2°C) for 60 minutes. The wells of the plate were emptied and 300µl of diluted washing solution was added. This procedure was repeated three times. Washing buffer remnants were removed by gentle tapping of the microtiter plate on a tissue cloth. About 100µl of ready-to-use conjugate was pipeted into each well. The plate was covered with foil and incubated at room temperature for 30 minutes. The wells of the plate were emptied and 300µl of diluted washing solution was added. This washing procedure was repeated three times. About 100µl each of the ready-

to-use substrate was pipeted into each test wells. The substrate was also pipetted in to the blank well. The plate was covered with foil and incubated at room temperature for 20 minutes in the dark. After the incubation period about 100µl of the ready-to-use stop solution was pipetted into the test wells and that of the substrate blank. After thorough mixing by shaking gently, the absorbance was taken at 450 nm.

3.10.2 RNA extraction from stool

Only children, whose blood sample tested positive to all the poliovirus serotypes, were selected for RT-PCR. The stool samples of these children were subjected to a pre RNA extraction treatment as described by (Ahmed *et al.,* 2004; Petrich *et al.,* 2006; Shulman *et al.,* 2015). About 2g of each stool sample was vortexed for 15 seconds in 2ml stool suspension buffer (0.9% saline) with glass beads, and clarified by centrifugation at 2,500×g for 10min. The supernatant was kept for RNA extraction, which was performed with RNA extraction kit by Zymo Research, (Zymo Research Corporation 17062 Murphy Ave. Irvine, CA 92614, U.S.A.) and was used according to the manufacturer's instructions.

Approximately 200µl of Viral RNA buffer was added to 400µl of each supernatant sample. The mixture was transferred to a Zymo-Spin column in a collection tube and centrifuged at 12,000 × *g* for 2 minutes. The flow-through from the collection tube was discarded. Approximately 300µl of RNA wash buffer was added to the column. It was centrifuged at 12,000 × *g* for 30 seconds. The flow-through was discarded and the Zymo-Spi n column placed back into the collection tube. The washing was repeated. Zymo-Spin column was centrifuged at 12,000 × *g* for 2 minutes in an empty collection

tube to ensure complete removal of the wash buffer. The Zymo-Spin column was placed into RNase-free tube. Approximately 10µl of RNase-free water to the column and was left to stand at room temperature for 1 minute. Zymo-Spin column was centrifuged at 12,000 × *g* for 1 minute to elute RNA. Viral RNA extracted was used immediately.

3.10.3 Reverse Transcription (RT) and Multiplex Polymerase Chain Reaction (PCR)

Amplification reactions were carried out in 50µl reaction mixture containing 1.5mM $MgCl_2$, 50mM KCl_2, 20pmol of each primer set (Appendix 6), 200µM of each of the dNTPs (dATP, dCTP, dGTP, and dTTP), 1.5U of avian myeloblastosis virus reverse transcriptase, and 2.5 U of *Taq* DNA polymerase as described by (Limbach *et al.*, 1999; Egger *et al.*, 1995; Harrie, *et al.*, 1995).

RT-PCR was performed in a single step with a buffer compatible with both enzymatic reactions. RT-PCR amplification was performed by using the following program on a Molecular Diagnostic Laboratory thermal cycler. Reverse transcription cycle was carried out at 42°C for 45min, while PCR procedure was done as follows: first cycle of denaturation at 95°C for 3min, subsequent cycles were conducted at 94°C for 45s. Annealing was done at 50°C for 45s, and extension at 72°C for 1min followed by final cycle of elongation at 72°C for 7min. The procedure was completed after 30 cycles. A 96-well thermal cycler (Applied biosystems, Foster city, CA) was used for the amplification. A 100bp molecular was used to determine the size of the amplified products.

Ten microliters of amplified PCR products were loaded into wells of 2.0% agarose gel. The 2.0% agarose gel was prepared in this way: About 4.0 g of agarose (electrophoresis grade) was added to 200 ml 1X TAE electrophoresis buffer in a 600 ml beaker. The mixture was stired to suspend the agarose. The beaker was covered with aluminium foil, and was heated in boiling-water bath until all the agarose has dissolved. It was held in a hot-water bath (at about 60°C) until ready for use. Electrophoresis was carried in a medium containing 0.4mg/ethidium bromide in 0.53 Tris-borate-EDTA buffer. Finally, the products were visualized under UV light (Egger *et al.*, 1995).

3.11 Statistical analysis

The estimated prevalence was defined as the number of individuals having poliovirus antibodies to all the poliovirus serotypes (presence of anti-polio neutralizing antibodies to serotypes 1, 2 or 3) divided by the total sample size. The data was analyzed using Epi-Info version 5.3.4 computer software program. Tests for level of significance and association were done. Statistical significance was accepted at $P<0.05$ (95% confidence level).

CHAPTER FOUR

4.0 RESULTS

4.1 Detection of antibodies to poliovirus

Out of 240 blood samples collected from children and tested for the presence of poliovirus antibodies, 78.8% (189) were positive for all the three types of poliovirus serotypes. Antibody positive rates for Types I, 2 and 3 were 92.9%, 85.4% and 92.1% respectively, indicating that seropositive rates for Types I and 3 were considerably higher than for Type 2. About 21.2% of the children had no detectable antibody to any poliovirus serotype (Table 4.1).

4.2 Distribution of Poliovirus antibodies in children by State of residence

Approximately 56.3% of the children sampled in Kano State had antibodies to all the poliovirus serotypes (Table 4.2). The percentages of children who had antibodies to poliovirus types 1, 2 and 3 in the state were 65.2%, 86.5% and 68.2% respectively (Appendix, 7). Seropositivity prevalence to all the polioviruses in Kebbi State was 83.3% (Table 4.2). Children who had antibodies to poliovirus serotypes Types 1, 2 and 3 were about 89.3%, 89.3% and 88.2% respectively (Appendix, 7). Zamfara State children sampled had 96.3% antibody prevalence to all the three poliovirus serotypes. Overall, the children in the state had antibody prevalence to poliovirus serotypes 1, 2 and 3 as 97.5%, 98.7% and 97.5% respectively (Appendix, 7). Zamfara State children sampled, had the highest prevalence of poliovirus antibodies (96.3%), while Kano State children had the lowest (56.3%). The difference in the prevalence of antibodies between the States was statistically significant ($p < 0.05$).

Table 4.1: Percentage distribution of children with/without poliovirus antibodies in the study area.

Serotypes	**No. Tested**	**No. Positive**	**% Positive**	**No. Negative**	**% Negative**
Type 1 only	240	223	92.9	17	7.1
Type 2 only	240	205	85.4	35	14.6
Type 3 only	240	221	92.1	19	7.9
Types 1,2 & 3	240	189	78.8	51	21.2
Types 1 & 2	240	196	81.7	44	18.3
Types 1 & 3	240	214	89.2	26	10.8
Types 2 & 3	240	196	81.7	44	18.3

Table 4. 2: Distribution of Poliovirus antibodies in the children by State.

State	No. Tested	No. Positive	% Positive
Kano	80	45	56.3
Kebbi	80	67	83.8
Zamfara	80	77	96.3
Total	**240**	**189**	**78.8**

P = 0.0000, r = 0.16

The regression analysis revealed some level of association between prevalence of antibodies and the state where the children resides (r = 0.16). Children from Zamfara State had the highest poliovirus antibody prevalence to all the poliovirus serotypes, while those from Kano State recorded the lowest levels to all the serotypes (Table 4.2 and Appendix, 7 respectively).

4.3 Distribution of Poliovirus antibodies in children by age

Children in the age group 48 - 59 months had the highest poliovirus antibody prevalence (93.5%), while those in the 0 - 11 months age group had the lowest (66.0%). Generally, prevalence was seen to increase with age. Antibody difference between the age groups was found to be statistically significant ($p < 0.05$). However, regression analysis revealed that the degree of association between age and prevalence was not very strong (r = 0.04) as shown in Table 4.3

Children in the 36 – 47 and 48 – 59 months age brackets had the highest prevalence of poliovirus antibody (95.7%) to poliovirus Type 1. Likewise children in the 48-59 months age group had the highest antibody prevalence to Type 2 (97.8%) as well as to Type 3 (97.8%) as shown in Appendix, 8

In Kano State (Table 4.4), children in the 48 – 59 months age group had the highest antibody prevalence, the lowest occurred amongst 0 – 11 months age group. There was a slight difference in Kebbi State (Table 4.4), while children in the 48 – 59 months age group had the highest antibody prevalence, the lowest occurred amongst 12 – 2 3 months

Table 4.3: Percentage of children with Poliovirus antibodies by age groups.

Age (Months)	*No. Tested*	*No. Positive*	*(%)*
0 – 11	50	33	66.0
12 – 23	49	38	76.6
24 – 35	48	37	77.1
36 – 47	47	38	80.9
48 – 59	46	43	93.5
Total	**240**	**189**	**78..8**

P =0.0018, r = 0.04

Table 4.4: Percentage distribution of Poliovirus antibodies by age group in the States.

Age (Months)	**Kano (%)**	**Kebbi (%)**	**Zamfara (%)**
0 – 11	25.0	76.5	94.1
12 – 23	70.6	62.5	100.0
24 – 35	56.3	87.5	87.5
36 – 47	46.7	93.8	100.0
48 – 59	81.3	100.0	100.0
Total	**56.3**	**83.8**	**96.3**

age group. In Zamfara State (Table 4.4), children in three age groups 12 – 23, 36 – 47 and 48 – 59 months age groups had the highest antibody prevalence, the lowest occurred amongst 0 – 11 months age group.

Children in the age groups 48 – 59 months in Kebbi and Zamfara and 12 – 23 and 36 - 47 months in Zamfara States had the highest prevalence of poliovirus antibodies (100% in all) Table 4.4. Zamfara State had the highest prevalence of poliovirus antibody (94.1%) amongst the youngest age groups (0 – 11 months) when compared with other States. The poliovirus antibody prevalence between the age groups in all the States was statistically significant ($p < 0.05$).

4.4 Poliovirus antibodies distribution in children by gender

Female children sampled in the study area had higher polio antibody prevalence (86.0%) than their male counterparts (72.2%). The poliovirus antibody prevalence difference between the sexes was statistically significant ($p < 0.05$). However, regression to show association between antibody prevalence and the sexes was very weak ($r = 0.03$). The Female children also, had higher antibody prevalence to all the poliovirus serotypes in the study, this is shown in Table 4.5 while Appendix 9 shows prevalence to individual serotypes.

The Male and Female children in Kano State had the lowest poliovirus antibody prevalence amongst the children sampled in the three States (Table 4.6). Male and Female children in Zamfara State had the highest poliovirus antibody prevalence with

Table 4.5: Percentage of children with Poliovirus antibodies by gender in the study area.

Gender	***No. Tested***	***No. positive***	***Percentage***
Male	126	91	72.2
Female	114	98	86.0
Total	**240**	**189**	**78.8**

P = 0.0095, r = 0.023

Table 4.6: Distribution of Poliovirus antibodies among the sexes in the States.

Gender	***Kano (%)***	***Kebbi (%)***	***Zamfara (%)***
Male	50.0	79.5	95.1
Female	63.2	83.3	97.4
Total	**56.3**	**83.8**	**96.3**

95.1% and 97.4% respectively. The poliovirus antibody prevalence between the sexes was not statistically significant in all the States ($p \geq 0.05$) except in Kebbi State ($p < 0.05$). Females in all the three States had higher poliovirus antibodies prevalence than Males. The poliovirus antibody prevalence gap between the sexes was widest in Kano State and narrowest in Zamfara State

4.5 Urban and rural distribution of Poliovirus antibodies in children.

Children sampled in the urban areas had higher poliovirus antibody prevalence (75.2%) than their rural counterparts (48.7%). Although the difference was not statistically significant ($p \geq 0.05$) (Table 4.7). Similarly regression analysis revealed very weak association with location in the study area ($r = 0.01$). Children in the rural areas had higher poliovirus antibody prevalence to all the serotypes, this is shown in Table 4.7 for cumulative antibodies to all the serotypes and Appendix 10 for individual serotypes.

Poliovirus antibody prevalence was lowest in the two locations in Kano State which is reflected in (Table 4.8). Antibody prevalence was moderate amongst the children in Kebbi State. Poliovirus antibody prevalence was highest amongst the children in Zamfara State in both urban and rural areas. The poliovirus antibody prevalence was not statistically significant in the two areas in all the States ($p \geq 0.05$).

4.6 Distribution of Poliovirus antibodies by the number of OPV doses received

Children who were reported as not having received OPV vaccine had the lowest poliovirus antibody prevalence amongst the children sampled in the study areas (66.7%).

Table 4.7: Urban and rural distribution of Poliovirus antibodies in children.

Location	**No. Tested**	**No. Positive**	**% Positive**
Urban	129	97	75.2
Rural	111	92	48.7
Total	**240**	**189**	**78.7**

p = 0.1474, r = 0.01

Table 4.8: Distribution of Poliovirus antibodies in children in urban and rural areas in the States

Location	**Kano (%)**	**Kebbi (%)**	**Zamfara (%)**
Urban	53.5	76.7	95.3
Rural	59.5	91.9	
Total	**56.3**	**83.8**	**96.3**

Those reported to have received four OPV doses had the highest viral antibody prevalence (88.9%). The poliovirus antibody prevalence difference between children that have received different number of doses was statistically significant ($p < 0.05$), though regression analysis revealed very weak ($r = 0.02$) between recipients and prevalence.

Children who were reported to have received three, four and five OPV doses had the highest antibody prevalence to poliovirus serotypes 1, 2 and 3 respectively. Cumulative antibodies to all the serotypes is shown in Table 4.9, while specific antibody prevalence to individual serotypes is shown in Appendix 11.

The prevalence of poliovirus antibody as per the number of oral polio vaccine (OPV) administered to the children was statistically significant ($p < 0.05$) in Kano State only (Table, 4.10). The poliovirus antibody prevalence distribution in Kebbi State was fairly even. The highest poliovirus antibody prevalence (100%) was observed amongst the children who were reported to have received one, three and four doses of OPV all in Zamfara State (Table, 4.10). The lowest poliovirus antibody prevalence (21.4%) was observed amongst children who were reported as not having received any dose of OPV in Kano State (Table, 4.10).

.

Table 4.9: Distribution of Poliovirus antibodies in children as per OPV doses received

Doses received	**No. Tested**	**No. Positive**	**% Positive**
0	42	28	66.7
1	39	31	79.5
2	42	33	78.6
3	39	29	74.4
4	36	32	88.9
>4	42	36	85.7
Total	**240**	**189**	**78.8**

P = 0.0274, r = 0.02

Table 4.10: Distribution of Poliovirus antibodies in children in the States by doses of OPV received.

OPV doses received	**Kano (%)**	**Kebbi (%)**	**Zamfara (%)**
0	21..4	85.7	92.9
1	69.2	69.2	100.0
2	71.4	71.4	92.9
3	30.8	92.3	100.0
4	75.0	91.7	100.0
>4	71.4	92.9	92.9
Total	**56.3**	**83.8**	**96.3**

4.7 Distribution of Poliovirus antibodies according to Father's educational level

Children whose fathers' educational level was up to tertiary level had the highest antibody prevalence (100%) when compared with other educational level groups. The lowest antibody prevalence (56.0%) was observed amongst those whose fathers' educational level stopped at primary school level (Table 11). This difference was statistically significant ($p < 0.05$). The association between poliovirus antibody prevalence and education was low ($r = 0.19$).

The highest antibody prevalence (100%) to poliovirus serotypes 1, 2 and 3 was observed amongst children, whose father's had education up to tertiary level. While the lowest was found amongst the children whose father's education stopped at primary school level (Appendix 12).

The lowest prevalence of poliovirus antibody amongst children whose father's education were at primary school and secondary school levels was observed in Kano State when compared with Kebbi and Zamfara States, while the highest prevalence in both levels occurred in Zamfara State (Table, 4.12). All the states recorded 100% prevalence to poliovirus antibody prevalence amongst children whose father's education reached up to tertiary level. Poliovirus antibody prevalence was statistically significant ($p < 0.05$) in all the states as per father's educational level.

Table 4.11: Distribution of Poliovirus antibodies in children according to father's educational level

Father's educational level	**No. tested**	**No. Negative**	**% Positive**
Primary level	91	51	56.0
Secondary level	90	79	87.8
Tertiary level	59	59	100.0
Total	**240**	**189**	**78.8**

$p = 0.000$, $r = 0.19$

Table 4.12: Distribution of Poliovirus antibodies in children according to father's educational level in the States.

Father's educational level	**Kano (%)**	**Kebbi (%)**	**Zamfara (%)**
Primary	34.1	66.7	90.0
Secondary	78.6	88.9	96.2
Tertiary	100.0	100.0	100.0
Total	**56.3**	**83.8**	**96.3**

4.8 Distribution of Poliovirus antibodies by father's occupation

Children whose father's were civil servants had the highest antibody prevalence (80.2%) when compared with other groups. Children whose father's were traders had the lowest prevalence (76.2%) Table, 4.13. However, the poliovirus antibody prevalence difference between the groups was not statistically significant (p 0.05). There was no association between poliovirus antibody prevalence and the occupational groups (r = 0.02).

Children of the civil servants had higher antibody prevalence to all the poliovirus serotypes. The children of traders had the lowest viral antibody prevalence for serotypes 1 and 2, while the children of farmers had the lowest antibody prevalence to viral type 3 (Appendix 13).

Kano State children had the lowest prevalence of poliovirus antibodies amongst children of the different occupational cadres in the three states (Table 4.14). The highest poliovirus antibody prevalence was observed in Zamfara State (Table 4.14). Children of the famers, traders and civil servants all had lowest poliovirus antibody prevalence in Kano State. Prevalence of polio antibodies amongst children in the three states in respect of their Father's occupation was not statistically significant (p 0.05) in allthe states.

In Kano State, traders' children had the highest poliovirus antibody prevalence, children of the farmers had the lowest poliovirus antibody prevalence, while it was the reverse in Kebbi and Zamfara States.

Table 4.13: Distribution of Poliovirus antibodies in children in the study area by father's occupation

Father's occupation	**No. Tested**	**No. Positive**	**% positive**
Civil Servant	131	105	80.2
Farmer	67	52	77.6
Trader	42	32	76.2
Total	**240**	**189**	**78.8**

$p = 0.5437$, $r = 0.00$

Table 4.14: Distribution of Poliovirus antibodies in children according to father's occupation in the State.

Father's occupation	**Kano (%)**	**Kebbi (%)**	**Zamfara (%)**
Civil servant	55.3	85.1	95.7
Farmer	47.8	87.0	100.0
Trader	68.8	70.0	92.3
Total	**56.3**	**83.8**	**96.3**

4.9 Prevalence of Poliovirus antibody by Father's tribe

The highest poliovirus antibody prevalence was observed amongst the children of other tribes (83.1%), while the lowest prevalence was observed amongst the children of the Hausas (72.8%) Table 4.15. The poliovirus antibody prevalence difference amongst the three groups was not statistically significant (p 0.05). No association between poliovirus antibody prevalence and the groups was observed (r = 0.00).

The children of the Fulanis had higher poliovirus antibodies to poliovirus serotypes 1 and 2 with 94.4% and 90.1% respectively. Children of other tribes had the highest antibody to poliovirus Type 3 (96.1%), Appendix 14.

The children of the three groups in Kano State had the lowest poliovirus antibody prevalence when compared with other states (Table 4.16), while the highest poliovirus antibodies prevalence was observed in Zamfara State amongst the children of the three tribal groupings. Kebbi State children's prevalence were moderate. Prevalence of poliovirus antibodies was not statistically different between the groups in all the States (p 0.05). Children of other tribes in Kano and Kebbi States had the highest prevalence of poliovirus antibodies, while the Fulani children in Zamfara State had the highest poliovirus antibody prevalence (Table 4.16).

Table 4.15 Distribution of Poliovirus antibodies in children by father's tribe

Father's tribe	**No. Tested**	**No. Positive**	**% Positive**
Fulani	71	58	81.7
Hausa	92	67	72.8
Others	77	64	83.1
Total	**240**	**189**	**78.8**

p = 0.7830, r = 0.00

Table 4.16: Distribution of Poliovirus antibodies in children in the States according to father's tribe.

Father's tribe	**Kano (%)**	**Kebbi (%)**	**Zamfara (%)**
Fulani	55.6	83.3	96.6
Hausa	48.6	80.6	96.2
Others	66.7	88.0	96.0
Total	**56.3**	**83.8**	**96.0**

4.10 Prevalence of Poliovirus antibodies by household's major water source

Children whose household's water source was pipe borne water had the highest poliovirus antibody prevalence (83.6%), while children whose source was stream had the lowest prevalence level (75.0%) Table 4.17. The difference in prevalence was not statistically significant (p □ 0.05). The association between antibody prevalence and water source was very weak (r = 0.01).

Children whose source of water was pipe borne water had the highest prevalence to poliovirus serotype 1. Children whose water source was steam water had the highest poliovirus antibody prevalence to poliovirus serotypes 2 and 3 (Appendix 15).

Children in Kano State recorded the lowest prevalence of poliovirus antibodies, across the entire household's major water sources, (Table 4.18). Zamfara State children had the highest poliovirus antibody prevalence, across the entire household's water sources, with 96.3%, 93.5% and 100% against Well, Stream and Pipe borne water respectively. The difference in poliovirus antibody prevalence was not statistically significant (p □ 0.05) in all the States.

Table 4.17 Distribution of Poliovirus antibodies in children according to major source of household's water source.

Household's major water source	**No. Tested**	**No. Positive**	**% Positive**
Well	96	72	75.0
Stream	83	66	79.5
Pipe borne water	61	51	83.6
Total	**240**	**189**	**78.8**

p = 0.1940, r = 0.01

Table 4.18: Distribution of Poliovirus antibodies in children based on household's major water source in the States.

Household's major water source	**Kano (%)**	**Kebbi (%)**	**Zamfara (%)**
Well	50.0	81.4	96.3
Stream	61.5	80.8	93.5
Pipe borne	59.1	94.1	100.0
Total	**56.1**	**83.8**	**96.3**

4.11 Detection of polioviruses by PCR

The multiplex PCR used for the detection of polioviruses revealed that of the 189 stool samples screened from 240 samples, only 5(2.6%) yielded positive result (Figure 4.1) based on the predicted chain length of the amplification product, which was 70bp. No prominent band corresponding to 79bp or 240bp was observed during sample processing, indicating that poliovirus Types 2 and 3 were not detected.

The profiling of the detected viruses is shown in (Table 4.19). All the five polioviruses identified were detected in Kano State. Three were detected from male stools while two were from female stools. Three of the viruses were from urban children's stool while rural children's stool had the remaining. Two polioviruses each were detected from children in age groups 36 – 47 months and 48 – 59 months, while one was detected from a child of 24 – 35 month age bracket.

Table 4.19: Distribution of the detected Poliovirus type 1 in the study area.

States	**No. of poliovirus type 1**	**Male**	**Female**	**Urban**	**Rural**	**24-35 month**	**36-47 month**	**48-59 month**
Kano	5	3	2	3	2	1	2	2
Kebbi	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Zamfara	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

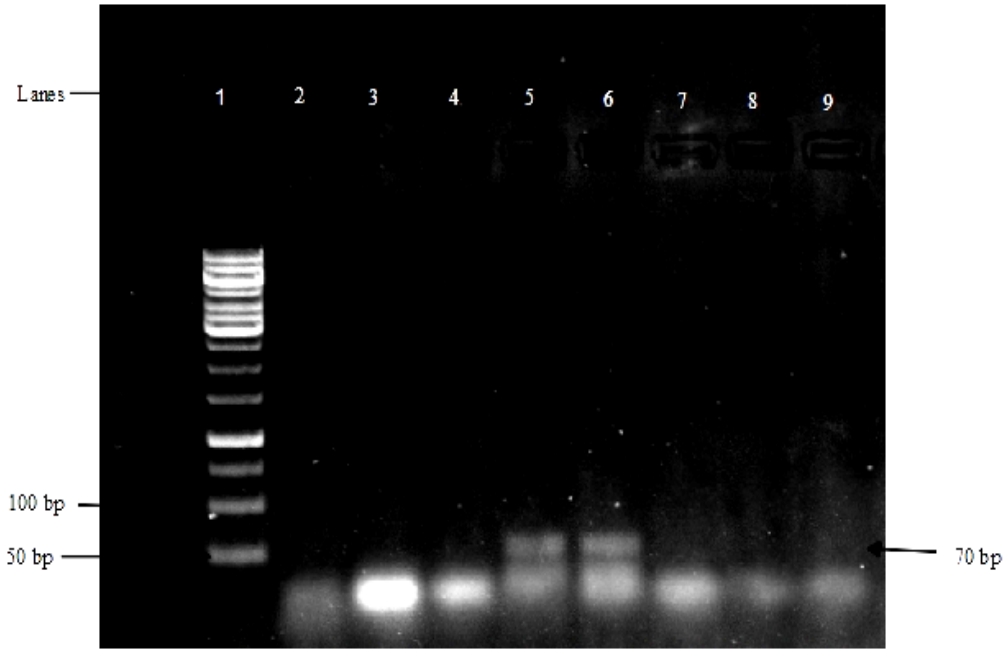

Figure 4.1: Agarose gel electrophoresis result for pcr after amplification of polio virus VP1 gene
Lane 1 50 bp molecular weight marker, lane 2 negative control and lanes 5 and 6 PCR amplicons

CHAPTER FIVE

5.0 DISCUSSION

Antibody prevalence in the study area against all poliovirus serotypes was high (78.8%), compared with previous works done within the country. Aminu (2000) obtained 42.2%, Adewumi *et al.,* (2006) obtained 53.8%, Giwa *et al.,* (2012) had 55.0% and Oladejo *et al.,* (2013) got 70%. Previous studies in the country have reported lower prevalence of 42.2% in Kaduna State (Aminu, 2000), 53.8% in Oyo, Ogun and Osun States (Adewumi *et al.,* 2006), 55.0% in Kaduna State (Giwa *et al.,* 2012) and 70.0% in Bida, North Central Nigeria (Oladejo *et al.,* 2013). The increase observed in this study of poliovirus antibody prevalence and sequential increase observed from the studies above, could possibly reflect steady increase in the immunization performance over the years. However, a prevalence of 97.8% which is higher than the one obtained in this study has been reported in Jos, Nigeria (Dashe *et al.,* 2010). Similarly other studies outside the country have reported much higher than that obtained in this study. For instance Orenstein *et al.,* (1988), Kelley *et al.,* (1991) and Jee *et al.,* (2004) obtained 85.0%, 85.1%, 82.2% respectively.

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The children sampled in the present study had 92.9%, 85.4% and 92.1% antibody prevalence against poliovirus serotypes 1, 2 and 3 respectively. Aminu in (2000) recorded 65.5%, 71.4% and 66.7%, Giwa *et al.,* (2012) observed 86.4%, 76.1% and 77.3%, while Oladejo *et al.* (2013) had 68.5%, 66.5% and 66% for poliovirus serotypes 1, 2 and 3 respectively. The percentage prevalence observed in the present study is higher when compared with the result obtained from the previous studies in the country. In studies

where such significant increases were observed, there was the notion that secondary spread of the virus could have contributed immensely (Wood and Thorley, 2003). Another factor that could have contributed was the mass vaccination campaigns, being launched periodically by the National Primary Health Care Development Agency as suggested by Aminu (2000).

About 81.7%, 89.2% and 81.7% had antibodies to combinations of serotypes 1 and 2, 1 and 3 and 2 and 3 respectively. Out of the children sampled in this study, 7.1%, 14.6% and 7.9% had no detectable antibodies to serotypes 1, 2 and 3 respectively. A decrease in the percentage of susceptible children is observed in the present when compared with the works of Aminu, (2000), Giwa *et al.,* (2012) and Oladejo *et al.,* (2013). However, studies from developed countries have had higher prevalence than what was obtained in this study (Jee *et al.,* 2004). Similarly in one study conducted in Spain, the findings indicated that prevalence to all the serotypes exceeded 94% (Pachón *et al.,* 2002). The reasons advanced to explain such a higher level, are; functionality of the health system, level of literacy, nutritional state of the children and environmental sanitation level.

High prevalence of poliovirus antibodies to serotypes 1 and 3 observed in this study (92.9% and 92.1%), was close to what Donbraye *et al.,* (2011) (89% and 90.5%) had in their studies conducted in the riverine areas of Delta State, Nigeria. Outside the country it was comparable to what Veiga Kiffer *et al.,* (2002) in São Paulo, Brazil got (94.6% and 91.9%) respectively. In South Africa and Italy, however lower values than what was found in this study have been reported (Signorini *et al.,* 2004; Waggie *et al.,* 2012). On

the other hand, higher levels than what was reported here were obtained from studies done in Israel, Netherlands and Germany which were close to 100% (Conyn-Van Spaendonck *et al.,* 2001; Carlos *et al.,* 2002; Affanni *et al.,* 2005). Vaccine specificity could have influenced the type of antibody developed by the recipients as a result of targeted campaigns in the area (Samoilovich *et al.,* 2003; Grassly *et al.,* 2006; Roberts, 2007; Grassly *et al.,* 2007; Jenkins *et al.,* 2008; GPEI, 2010).

Seronegativity (21.3%) in this study was higher than what Donbraye *et al.,* (2011) reported in South-South, Nigeria (4%) and Aminu *et al.,* (2003) (19.5%) obtained in Kaduna State. Therefore, comparing seronegativity from this study and that of the South-South showed the existence of a huge immunity gap between Northern and Southern part of the country. This could have provided the reason for absence of poliomyelitis cases from that region for a very long time. The seronegativity observed here, was comparable with what was obtained in other countries, which ranged from 26.5% to 50% (Serhat *et al.,* 2007; Vancelik *et al.,* 2008). In places where immunization services are working well, seronegativity can be zero or close to it (Pachón *et al.,* 2002; Affanni *et al.,* 2005).

Low poliovirus antibody prevalence to serotype 2 observed in this study, was not in agreement with what was seen in a survey conducted in India, where poliovirus serotype 2 had the highest prevalence (Azra *et al.,* 2004). The low poliovirus antibody prevalence observed here, could be as a result of reduced exposure to wild poliovirus type 2 which had been successfully eradicated since 1999 (CDC, 2001; Samoilovich *et al.,* 2003).

Overall, Zamfara State children had the highest poliovirus antibody prevalence (96.3%) amongst the children in the three States studied, while the lowest poliovirus antibody prevalence was observed in Kano State (56.3%). The poliovirus antibody prevalence difference observed between the States was statistically significant, even though the major determinant of antibody production which is the OPV vaccine, is given to States proportional to the number of eligible children to be vaccinated in each State (NPHCDA, 2012).

Therefore, other factors apart from vaccine availability could have aided in the difference. This finding was therefore indicative of the fact that some States might not be vaccinating the children as they are supposed to do, or it could be due to some technical issue associated with the vaccine such as potency. It is therefore, suggested that this aspect should be further exploited so as to find probable solution(s). Similar to what was observed in this study, the survey done by National Population Commission, on vaccination coverage in the country. The result showed a remarkable difference in terms of vaccination coverage in the three States as well (NDHS, 2013).

Refusal levels amongst the three States might have played a significant role as was the case in Pakistan between regions (Mukarami *et al.,* 2014). Aminu, (2000) similarly observed the same condition between local government areas in Kaduna state and attributed the outcome to socio cultural and religious beliefs. Another important factor which could have been responsible for this differential coverage, was commitment of the leadership in achieving results as also observed by Lashkevich (2013).

The three States covered by the study fall within a geographical area classified as high risk for poliovirus transmission. Therefore it was not surprising to observe higher antibody prevalence to Types 1 and 3 because of the frequent use of bOPV containing poliovirus serotypes 1 and 3 during campaigns. There was not much difference observed with regards to the prevalence of all the three serotypes, except in Kano State which had low prevalence to serotype 2. Low coverage with tOPV which contain Type 2 antigen might possibly account for that observation. Possibly, household visits during vaccination campaigns had significantly helped in boosting prevalence amongst the children, by eliminating the cost of taking these children to immunization centres as Masaharu *et al.*, (2007) equally observed. There was some level of association between antibody prevalence and State of residence of the children in the study area ($r = 0.16$). This goes to show that some States were doing better than others in terms of reaching all eligible children with OPV vaccines.

There appeared to be a gradual increase in poliovirus antibody prevalence, across the age groups of the children sampled in this study, as similarly observed by Aminu (2000) in a study conducted amongst school children in the Northern part of the country. The gradual increase might be as result of improved immunization services or natural/secondary spread of the virus could have played a significant role. Recently in a survey conducted by National Statistics Bureau to assess the percentage of children who had taken a particular number of OPV doses, it was reported that about 61.1% of the children

sampled had received 4 doses (NDHS, 2013). This coupled with mass campaigns could have aided the increased poliovirus antibody prevalence observed.

The relationship between age and poliovirus antibody prevalence had for long been found to exist (Roberto *et al.,* 2002; Simonetta *et al.*, 2004; Adewumi *et al.,* 2006). However, Donbraye *et al.,* (2011) did not observe such a correlation between age and antibody prevalence. He *et al.,* (2010) are of the view that even though prevalence increases with age, it cannot continue to do so indefinitely. In another scenario, Vincenzo *et al.,* (2012) and Simonetta *et al.* (2004) in two separate studies observed a decrease in antibody prevalence as age progressed. The difference in poliovirus antibody prevalence between the age groups in each State studied was statistically significant. Perhaps, increase in the number of Health Facilities, availability of Health Workers and vaccines could have been the major reason behind that fact (Vancelik *et al.,* 2008; NPHCDA, 2014). However a weak association ($r = 0.04$) was observed between age and antibody prevalence.

This study purposely placed emphasis on children below 5 years of age because of what had been reported by several researchers that, they are the ones mostly affected by poliomyelitis. As such immunity level checks targeting children under five years of age have been advised at different times (Sabine *et al.,* 2002). Based on this call, serologic surveys had been carried out to assess impact of immunization services in different parts of the world (Prevots *et al.,* 2004; Adewumi *et al.,* 2006; Donbraye *et al.,* 2011; Veronesi *et al.,* 2013).

Females in this study had higher prevalence of poliovirus antibody when compared with males. This finding was in agreement with what Donbraye *et al.,* (2011) similarly observed. The difference between male and female in poliovirus antibody prevalence was statistically significant. This was what Roberto *et al.,* (2002) reported in Brazil. However, other studies disagree with this findings (Williams and David-West, 2006; Adewumi *et al.,* 2006; Oladejo *et al.,* 2013). In reality, both sexes had equal chance of exposure to either natural infection or the vaccine either through routine immunization or campaigns. As such any difference between the two was not expected. Furthermore, vaccination in the country is not gender biased. The females were similarly found to have higher prevalence to all the serotypes. Perhaps certain cultural or religious peculiarities might possibly have an effect on the observed outcome as Aminu (2000) speculated. This could be a temporary issue which will possibly be resolved if the attention of concerned authorities are drawn to that fact. Further investigation is suggested to find out the real cause of the disparity between them.

The difference in poliovirus antibody prevalence between the sexes in Kano and Zamfara States did not exhibit any statistically significant difference. However in Kebbi State poliovirus antibody prevalence between the sexes displayed a significant statistical difference between the two. The regression analysis showing association between gender and poliovirus antibody prevalence ($r = 0.02$) was very weak. This could be because no special preference was given to either of the sexes, neither does the strong reason exist for the sexes to respond differently under the same conditions.

Children whose parents or guardians claimed that, they had received four OPV doses had the highest poliovirus antibody prevalence, while those who were reported as not having received any dose at all, had the lowest prevalence. Similar experience was reported in Kaduna state (Aminu, 2000). The difference in prevalence between the various OPV doses recipient groups was statistically significant. What was seen here should be taken with reservation, because it had been observed in India that taking additional oral polio vaccine did not significantly commensurate with increase in antibody level.

One of the major problems that workers had associated oral polio vaccine with, was that of low immunogenicity particularly in developing countries (Aminu, 2000; Diedrich *et al.,* 2000; Sutter *et al.,* 2000; Conyn-Van Spaendonck, *et al.,* 2001). This might explain the reason behind the weak association (0.02) observed in this study between OPV doses and poliovirus antibody prevalence. Presence of antibodies in children, who had not received a single dose of OPV, could be because of passive transfer of antibodies from mothers or because of exposure to the virus in nature, or the excreted vaccine virus (John, 2001; CDC, 2005; Bonnet *et al.,* 2008). Parents or guardians always had difficulty in recalling the actual number of OPV doses given to their children or wards. Not only in this study was this problem encountered, but was equally faced by Vancelik *et al.*, (2008). And registers at Health Facilities could not completely be relied upon to solve the problem as Stokley *et al.*, (2001) pointed out.

Children whose major household's water source was pipe borne water had higher (83.6%) poliovirus antibody prevalence than children of the other groups, while children

whose major water source was stream had the lowest prevalence (75%) This could be indirectly related to socioeconomic status of the parents. This goes to show that level of sanitation had improved significantly even in the area where this study was carried out. Therefore water is gradually losing some of its usefulness as transport medium for poliovirus transmission (Zgodzinski, 2011). Poor sanitation in the past and the effect of tropical climate have helped in the continued transmission of poliovirus some decades ago (Richard, *et al.*,2014). This goes to show that sewage, which in most cases had contaminated open surface water sources, is being gradually contained (Jagadish *et al.*, 2003). This study looked at the effect water source could have had because enteroviruses have been recovered from different water sources (Walter and Maureen, 1997).

Children in the urban areas had higher poliovirus antibody prevalence (75.2%) than their rural counterpart (48.7%). There was no statistically significant difference observed in poliovirus antibody prevalence between the children in urban areas and those in rural areas in this study ($p > 0.05$). Not only in this study was this phenomenon observed, but in other studies done in Asia (Khurana *et al.*, 2006), and Africa (Perzanowski *et al.*, 2002). Government policies and socio-economic factors have been observed to substantially aid variation in prevalence between urban and rural areas (Sabine *et al.*, 2002). Children in the rural areas were far behind in terms of prevalence when the cumulative data is carefully analyzed. This could be because most of the Health Facilities were located in the urban areas (NPHCDA, 2014). And the children's parents or guardians having been more educated or sensitized in the urban areas, sees the need to take their children for immunization services at the designated points.

Worthy of mention is the fact that outreach services which were aimed at bridging the gap between urban and rural communities were very effective in the rural areas to have achieved that modest level. In each of the three States the viral antibody prevalence was not statistically significantly different between the children sampled in the rural areas and those in the urban areas. The association between living in urban or rural area and having antibodies to poliovirus was very weak (r = 0.01). This could be because from the result of the study living in either place does not confer any advantage as regards to having poliovirus antibodies.

Children of other tribes residing in the study area had the highest antibodies to poliovirus when compared with the two major indigenous tribal groups. From many studies done on vaccine acceptance in the study area (NPI, 2006), some level of resistance amongst the Hausas and Fulanis were noticed. A lot of reasons have been advanced for the continued rejection of OPV vaccine not only in Nigeria but in different countries as well (Kishore *et al.,* 2003; Thacker and Shendurnikar, 2004; Mittal and Mathew, 2007; Dasgupta *et al.*, 2008; Chaturvedi *et al.,* 2009). The reasons which were maily advanced borders on safety, presence of infertility agents and religious believes.

Oral polio vaccine refusal rates in most cases had been found to be consistently lower for RI than for SIAs (Mangal *et al.,* 2014). The difference in poliovirus antibody prevalence between the children of the three tribal groups was not statistically significant. This might be due to close cultural linkage between the tribes in the study area. Likewise, in all the States prevalence difference was not statistically different between the tribes, and tribal

background did not exhibit any association with poliovirus antibody prevalence (r = 0.00). This could be because vaccination had no tribal links in the area studied.

This study had reaffirmed what was already known (Habib *et al.,* 2013), that father's educational level positively affects child's antibody prevalence. Illiteracy of fathers had been observed to be an important risk factor for polio eradication activities in the study area. This study, as one of its priorities wanted to find out to what extend does father's educational level affects child antibody's prevalence. The emphasis on father's education as against that of mother's educational level was because in the area, where this study was carried out, husbands had an upper hand in the affairs of the family.

Prevalence of poliovirus antibody was highly significant between the three groups. Educational and cultural differences in any community had been found to play a significant role in transforming attitudes and behaviours towards vaccination (Vincelik *et al.,* 2007). For example noncompliant heads of households compared to compliant heads of households in Sokoto State, Nigeria had low level of education (Mohammed *et al.*, 2014).

In a study conducted in Zaria, the level of education was found to be important in accepting OPV during mass campaigns (Obadare, 2005). The opposite had been observed in developed countries, where advanced educational status had been a major factor in vaccine refusals (Omer *et al.,* 2009). It had been observed that antibody prevalence in rural areas even amongst the children of un-educated parents can be as high as in children

of the educated parents in urban areas, this might be because of mass mobilization (Aboubakary *et al.,* 2009; Mitchell *et al.,* 2009). Children of the fathers who had education up to tertiary level had the highest antibody prevalence to all the serotypes, followed by the children of those who had education up to secondary school level. The association between poliovirus antibody prevalence and educational level was low (r = 0.19). This could be as a result of the fewer number of educated fathers in the study sample, as such their impact could not be well felt.

Children of the civil servants had higher prevalence than the children of parents in other occupational categories. This could be as a result of their background especially with parents that had been sensitized on the need to avail their children for immunization activities (Anjum *et al.,* 2004). Socio-economic factors could have aided the variation observed (Shafiqullah *et al.,* 2009). However, no significant poliovirus antibody prevalence difference was observed between the children in the three groups. There was no association observed between poliovirus antibody prevalence and occupation of the fathers. Also the effects of nutrition on children and their ability to produce poliovirus antibodies which can be crudely linked to the parent's occupation have been found to play a significant role on antibody prevalence (Nasr *et al.,* 2007; Estívariz *et al.,* 2012). What might have played a significant role could be household visits during vaccination campaigns by the teams, as it will eliminate the cost of taking these children to immunization centres (Masaharu *et al.,* 2007).

The use of polymerase chain reaction has made it possible to identify polioviruses rapidly and with a high degree of specificity and sensitivity in samples (Chowdhary *et al.,* 2005; Costa *et al.,* 2012). This study detected five polioviruses from stool samples of children, similarly other workers had similar experience of detecting very few polioviruses from the samples they worked with (Pedro *et al.,* 2001). This goes to show that detecting poliovirus in samples doesn't always yield positive result (Attoh *et al.,* 2014). That could be linked to the fact that as a country is approaching interruption and eradication getting a virus from the populace was not easy (Kofi *et al.,* 2014; Philip *et al.,* 2014; WPS, 2014). The detection of poliovirus in this study was an indication of continued viral circulation in the study area. Therefore susceptible children in the affected area could be at risk of coming down with the disease.

One of the limitations of this study is that it was Hospital/Health clinic based therefore children in this study may not be representative of all the children of the same age in the area. Children receiving care at clinics may have higher exposure to vaccination activities than children not being taken for medical care at all. Above all, this study was not population-based and thus it may be difficult to generalize these findings, unless with caution. Another limitation was data collection, because the study had to rely on parental recall for a number of variables, such as vaccination histories and age of a child.

CHAPTER SIX

6.0 CONCLUSION

6.1 Summary

This research was undertaken to determine the poliovirus antibody prevalence to serotypes 1, 2 and 3 in the three study areas of Kano, Zamfara and Kebbi States, to determine the effect of some probable risk factors of poliovirus antibody amongst the children and also to detect if present, the poliovirus in the stool sample of children positive to all the three serotypes.

A cross-sectional survey was designed to enroll children whose mothers had access to Health Facilities across the study area. Two hundred and fourty blood and stool samples each were collected and tested for the presence of poliovirus antibodies and poliovirus particles respectively. Indirect ELISA was used to detect the presence of poliovirus antibodies, while Reverse Transcription Polymerase Chain Reaction (RT-PCR) was used for poliovirus detection. Nine variables were evaluated for their role/contribution or otherwise to overall poliovirus antibody in children in the selected areas in the States.

The result showed that 189 children (78.8%) had antibodies to all the three poliovirus serotypes, while 223 children (92.9%), 205 children (85.4%) and 221 children (92.1%) had antibodies to poliovirus serotypes 1, 2 and 3 respectively. From the result obtained, about 21.2% of the children sampled had no detectable antibody to any of the poliovirus

serotypes. Children numbering 196 (81.7%), 214 (89.2%) and 196 (81.7%) had poliovirus antibodies to poliovirus serotypes 1 and 2, 1 and 3 and 2 and 3 respectively.

The study found out that about 56.3% of the children sampled in Kano State had antibodies to all the poliovirus serotypes. The percentages of children who had antibodies to poliovirus serotypes 1, 2 and 3 were 65.2%, 86.5% and 68.2% respectively. In Kebbi State, seropositivity prevalence to all the polioviruses was 83.3%. Children who had antibodies to poliovirus serotypes 1, 2 and 3 were about 89.3%, 89.3% and 88.2% of the entire children sampled respectively. Accordingly, Zamfara State children sampled had 96.3% antibody prevalence to all the three poliovirus serotypes. Overall, the children had antibody prevalence to poliovirus serotypes 1, 2 and 3 as 97.5%, 98.7% and 97.5% respectively.

The study revealed that older children 48 – 59 months had higher poliovirus antibody prevalence (93.5%) than the 0 – 11 months old (66.0%). Female children had higher poliovirus antibody prevalence (86.0%) than Male children (72.2%). Poliovirus antibody prevalence showed increase with the doses of oral polio vaccine taken by the children sampled in the study area. Children who were not administered any oral polio vaccine dose had the lowest poliovirus antibody prevalence (66.7%), those that received more than four doses of the vaccine had the highest poliovirus antibody prevalence with (85.7%).

Urban children had higher poliovirus antibody prevalence (75.2%) than their rural counterpart (48.7%). Children whose father's educational level was up to tertiary level had highest poliovirus antibody prevalence (100%) than those with either primary (56.0%) or secondary school (87.8%) educational levels. Children of the civil servants had the highest poliovirus antibody prevalence (80.2%) while children whose fathers were traders had the lowest prevalence (76.2%). Children of other tribes resident in the study areas had higher poliovirus antibody prevalence (83.1%) than the indigenous tribes (Hausa 72.8% and Fulani 81.7%).

Children whose household's major water source was pipe borne water had the highest poliovirus antibody prevalence (83.6%) while those with well water as their major water source had the lowest antibody prevalence (75.0%). Conclusively, this study found out that age of the children, sex, number of vaccine doses taken, state of residence and educational level of the fathers had a significant effect on the prevalence of poliovirus antibodies ($p < 0.05$). However, place of residence (urban/rural), father's occupation, father's tribe and household's water source had no statistically significant effect on poliovirus antibody prevalence ($p \square 0.05$). Detecting poliovirus in one of the study areas was an indication that the virus was still circulating in the area.

The high seroprevalence observed in this study showed the great and dramatic improvement that the immunization system had under gone. Identification of some children without detectable antibodies to one or more poliovirus serotypes, in their sera, showed that pockets of susceptible individuals to poliovirus are still present within the

study area. Virus circulation was evidenced through the detection of the virus in one of the three States. Thus, this finding had demonstrated a useful role, which laboratory surveillance can render to polio eradication program in its entirety.

The detection of this serotype agrees with the recent statistics released by NPHCDA and Partners which showed the continued circulation of poliovirus serotype 1 in Kano State. The result presented here could be used to guide outbreak investigations and enable the establishment of linkages with previous isolates so as to come up with the possible source(s) to the index case. Even though Global Polio Eradication Initiative has not made laboratory based surveillance for polio viruses as a requirement for eradication, its implementation could go a long way in speeding up eradication processes in endemic countries, particularly Nigeria. This finding had reinforced the fact that the high prevalence of poliovirus antibody found, amongst the children sampled could not be an indication that so many viruses would be detected.

6.2 Conclusion

The result obtained showed that antibody prevalence had improved compared to previous findings. This study found out that age of the children, sex, number of vaccine doses taken, state of residence and educational level of the fathers had a significant effect on the prevalence of poliovirus antibodies ($p < 0.05$). However, place of residence (urban/rural), father's occupation, father's tribe and household's water source had no statistically significant effect on poliovirus antibody prevalence (p □ 0.05). Detecting poliovirus in one of the study areas was an indication that the virus was still circulating in the area.

6.3 Recommendations

1. States should maintain vigilance, identify and actively vaccinate unreached/underserved populations, and develop the mechanisms for rapid detection and appropriate response to any area having appreciable number of susceptibles children.
2. To interrupt the transmission of wild polioviruses efforts should be made towards achieving and sustaining high levels of poliovirus vaccine coverage.
3. Delivery of poliovirus vaccine to hard-to-reach populations should be given top priority as the final stage of poliomyelitis eradication is nearing.
4. Re-evaluation of the vaccination programme and implementation should be under taken as to improve effectiveness and eventual eradication of polio from the country.
5. There should be a continuous monitoring of polio antibody to assess the level of immunity to Poliovirus serotypes among children at risk of the virus infection.
6. Inter State patrol units should be set up to check immunization status as earlier workers also suggested.
7. The seemingly low level of antibodies against some serotypes might necessitate the need for continued vaccination campaigns and intensification of routine immunization activities, targeting those observed gaps.

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APPENDICES

Appendix 1

ZAMFARA STATE HOSPITAL SERVICES MANAGEMENT BOARD, GUSAU.

OFFICE:
Gada Biyu
Along Gusau-Sokoto Road
Gusau, Zamfara State
☎: 063-202410

Your Ref No.:
Our Ref No.: **HSMB/SUB/135/VOL. 1**

Date: **25/10/2010**

ZAMFARA STATE
ZAMFARA STATE OF NIGERIA

The Head of Department,
Microbiology Department,
Ahmadu Bello University,
Zaria.

RE: ETHICAL CLEARANCE FOR KABIR YUSUF MAWASHI PHD/SCHEW/03639/08-09

I am directed to refer to the letter No. **PHD/SCENN/ 2008-2009** dated 17th Sept., 2010 to inform you that after going through the research proposal document, the Hospital Service Management Board Gusau has no objection to the conduct of the research in the state.

The candidate is therefore cleared to conduct his research within the limit of the proposal. It will be appreciated if the result of the survey is shared with Hospital Service Management Board as we believe it will help us in planning future programme.

HASSAN KABIRU TSAFE
Deputy Director Admin
For: Executive Chairman.

Appendix 2

MINISTRY OF HEALTH
(HEADQUARTERS)
GWADANGAJI SECRETARIAT COMPLEX, BIRNIN KEBBI, KEBBI STATE

P.M.B 1040

Tel: 068-320069
068-320074
063-201733

Ref:_____

Date:_____

30 March 2010

The Head of Department,

Microbiology Department,

Ahmadu Bello University Zaria.

RE: ETHICAL CLEARENCE FOR KABIR YUSUF MAWASHI PHD/SCIEN/03639/08-09

I am directed to refer to your letter no. PHD/SCIEN/03639/08-09 dated 26th March 2010 and inform you that after going through the research proposal document, the Ministry has no objection to the conduct of the research in the state.

The candidate is therefore cleared to conduct his research within the limit of the proposal. It will be appreciated if the result of the survey is shared with the MOH as we believe it will help us in planning future programmes.

Thank you

Sani Y Argungu

Director PHC

For: Hon. Commissioner

Appendix 3

KANO STATE
HOSPITALS MANAGEMENT BOARD
BOARD HEADQUARTERS
P.M.B 3540, Post Office Road, Kano

HMB/GEN/184/I/T

13/07/2010(1/8/1431AH)

Kabiru Yusuf Mawashi,
Dept. of Microbiology,
Ahmadu Bello University,
Zaria.

PROVISIONAL ETHICAL CLEARANCE

Sequel to your application to conduct research titled "***POLIO ANTIBODY LEVELS IN CHILDREN 0-59 MONTHS IN NORTH WESTERN PART OF NIGERIA***" dated 26/03/2010 and subsequent to your appearance before the Hospitals Management Board's ethical committee, I am under instruction to inform you that provisional approval has been granted to conduct your study on the following conditions:-

1. Your informed consent form should include that " I understand that the participation of my child in this research is entirely voluntarily and withdrawal from the study could be done without any negative response towards myself or my child
2. That consent of all participants must be obtained by filling and signing informed consent form.
3. That the blood sample must to be collected by the qualified lab scientist.
4. That a copy of the finding should be submitted to Ethical committee for final approval.

Best wishes.

ABDULLAHI TUKUR
Prin. Assistant Secretary
FOR: DIRECTOR GENERAL

Appendix 4

CONSENT FORM

Code…….Alh/Malam/Mr/Mrs……residing at….……..……..in…….……Ward……..Local Government of……State has agreed to let the researcher collect blood and stool from my child/ward for the purpose of conducting research on Poliovirus.

…… …….

Signature Thumb print

Appendix 5

QUESTIONNARE

CODE ……

SEX……..……

AGE……..…

DATE…….....

SENETORIAL DISTRICT……

L.G.A/WARD……..

RESIDENTIAL ADDRESS……..

FATHER'S OCCUPATION…….

FATHER'S EDUCATIONAL LEVEL……..…….

FATHER'S TRIBE……..…….

NUMBER OF DOSES OF OPV RECEIVED……..……

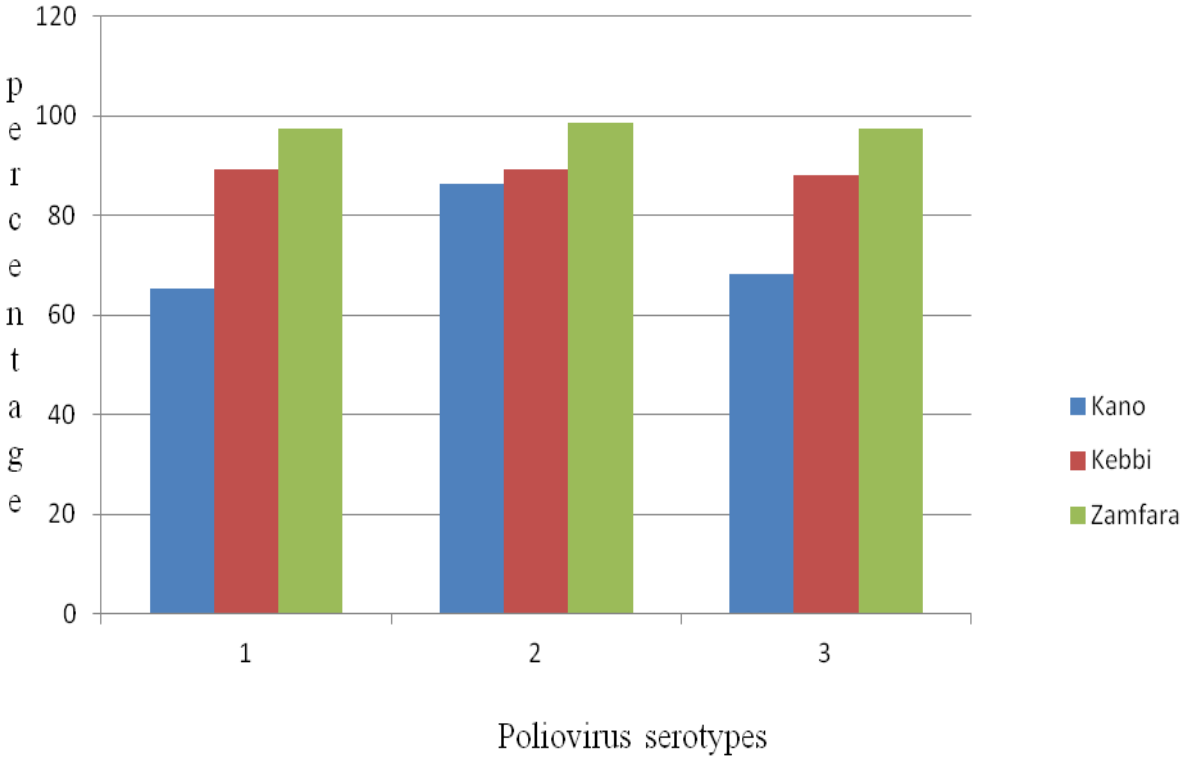
MAJOR SOURCE OF DRINKING WATER……...……

Appendix 6

PRIMERS USED

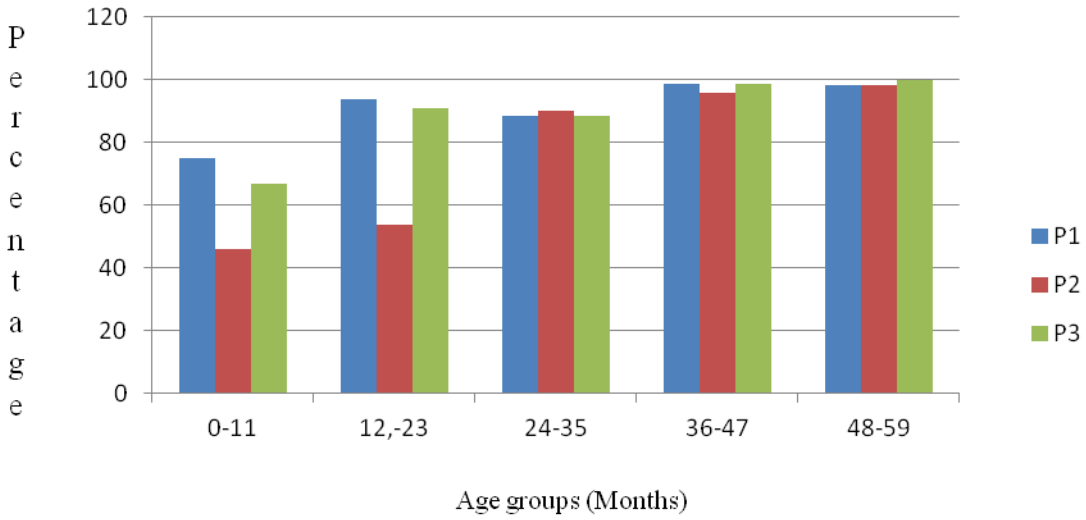
PRIMER	EXPECTED AMPLICON SIZE	REFERENCE
PV1,S 5′- ATCATIYTPTCIARPATYTG -3′ PV1A 3′- TGCGIGAYACIACICAYAT-5′	70 bp	WHO, 2004
VP2S 5′- AYICCYTCIACIRCICCYTC -3′ VP2A, 3′- TGCGIGAYACIACICAYAT-5′	79 bp	WHO, 2004
PV3S, 5′- CCCCIAIPTGRTCRTTIKPRTC -3′ PV3A, 3′- AAYCCITCIRTITTYTAYAC-5′	140 bp	WHO, 2004

Appendix 7



Percenage of children in the with Poliovirus antibodies to individual virus serotypes

Appendix 8



Percentage distribution of Poliovirus antibodies in the children in respect of the serotypes.

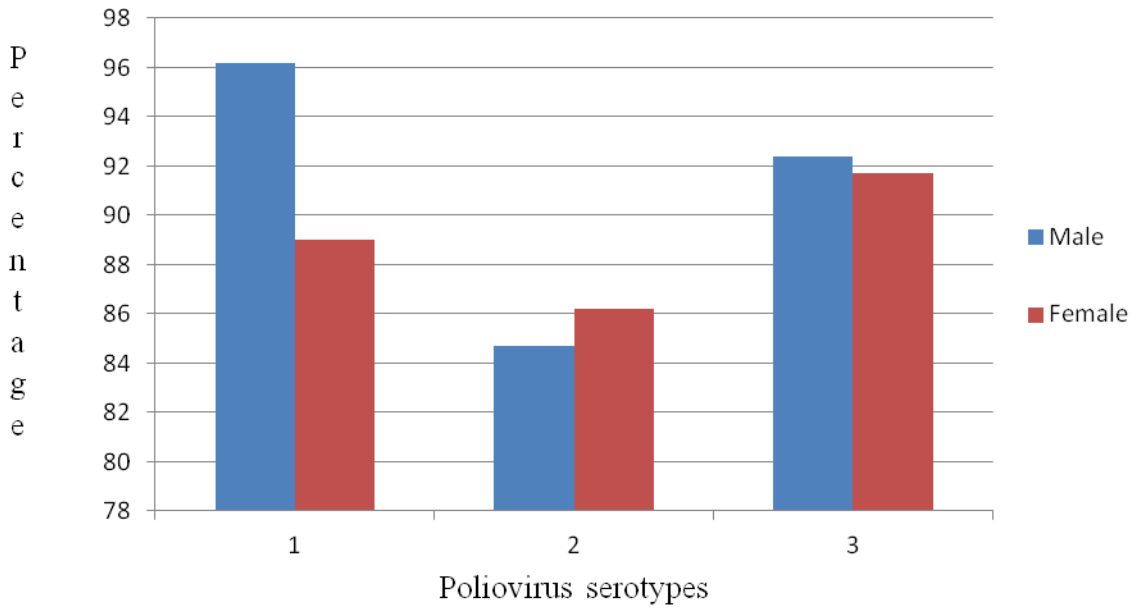
Key

P1= type 1

P2= type 2

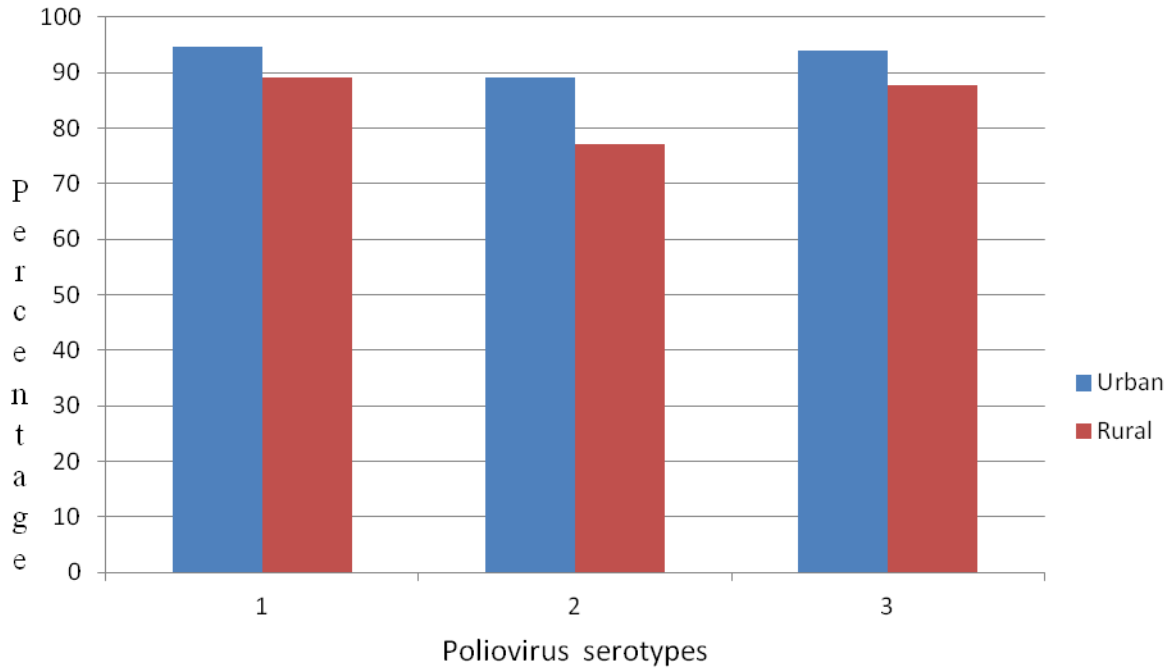
P3 = type 3

Appendix 9



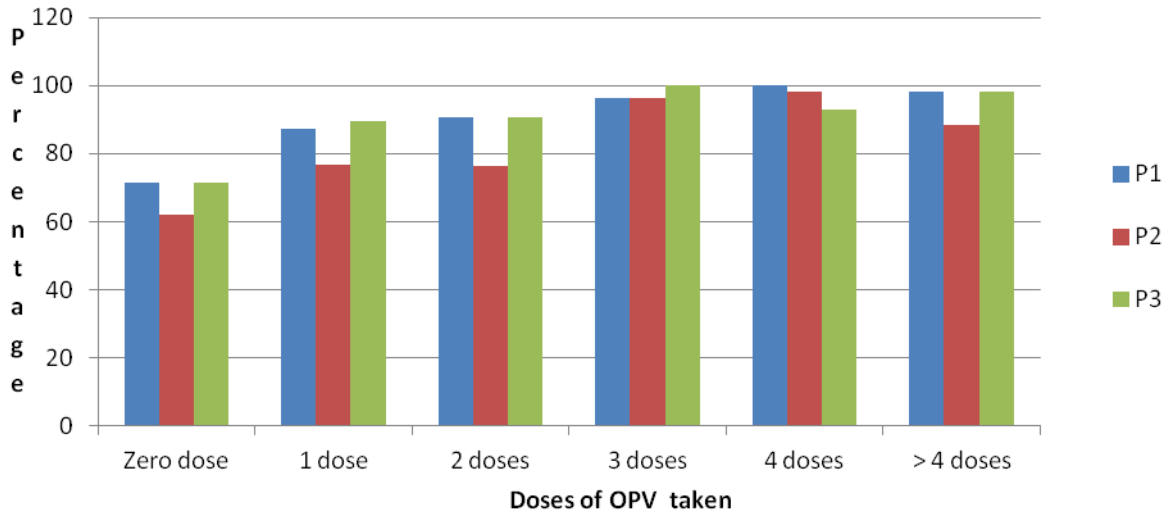
Distribution of poliovirus antibodies to individual serotypes by gender

Appendix 10



Urban /rural distribution of Poliovirus antibodies to individual serotype in children

Appendix 11



Distribution of Poliovirus antibodies to individual viral serotype in chlidren as per the number of doses taken

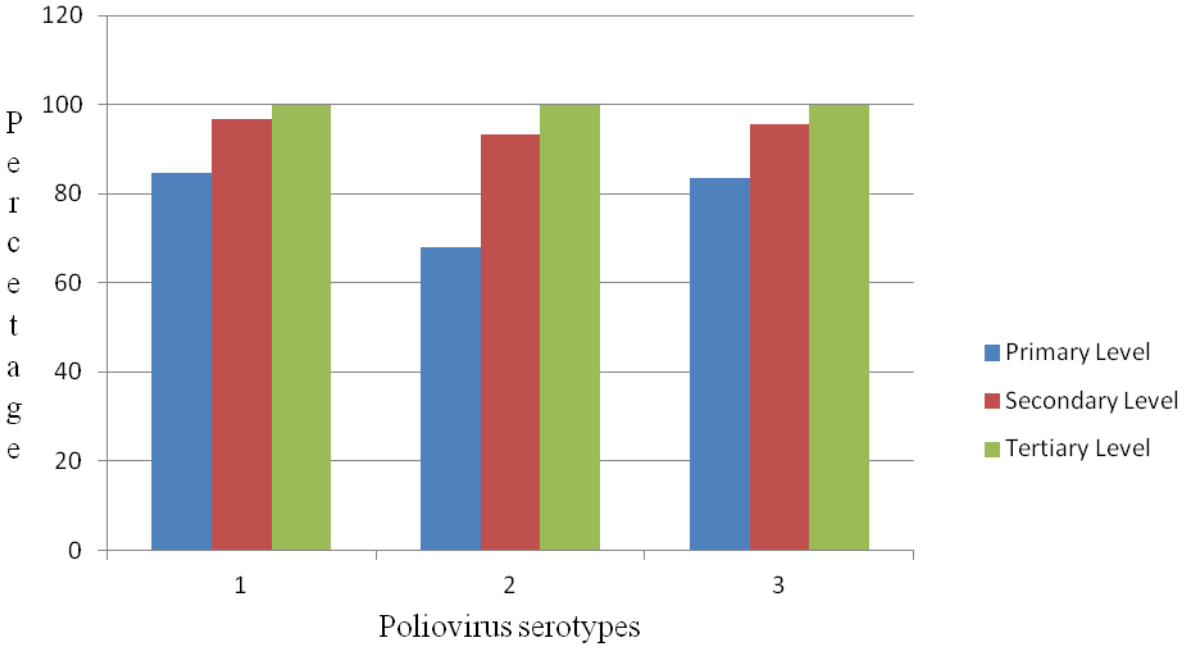
Key

P1 = serotype 1

P2 = serotype 2

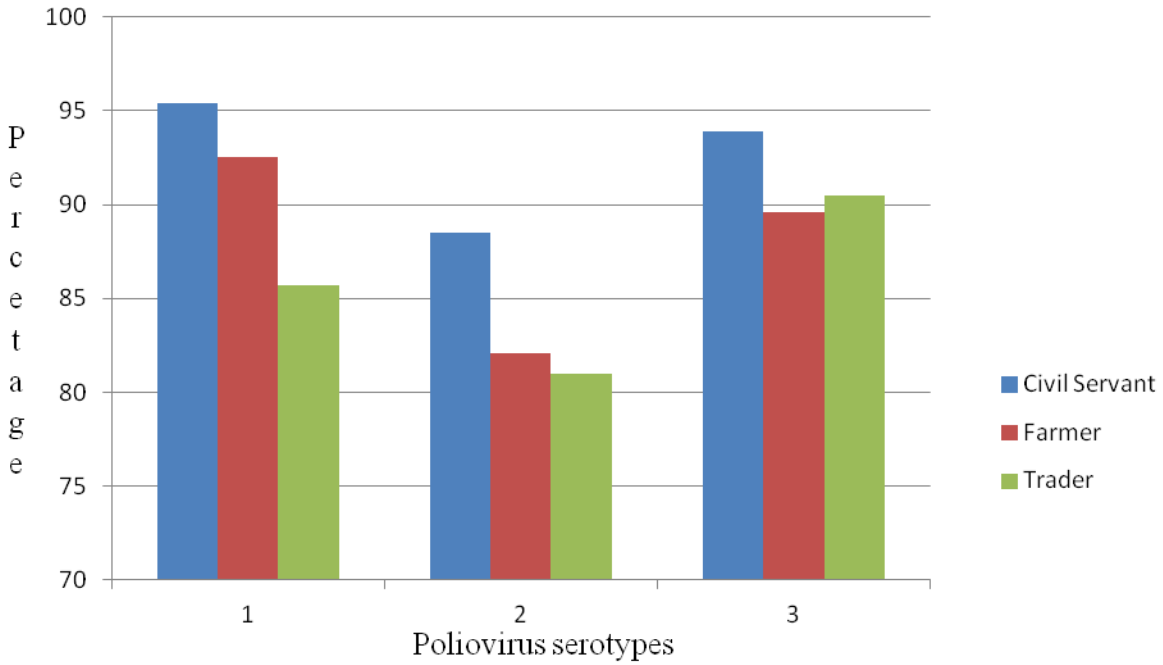
P3 = serotype 3

Appendix 12



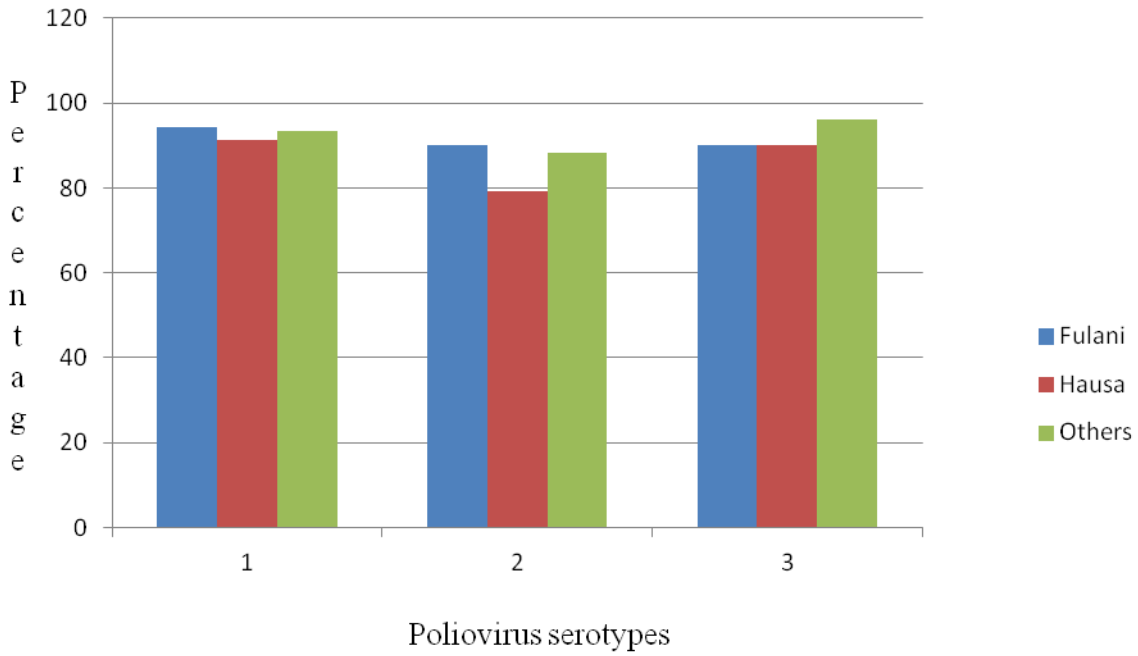
Distribution of Poliovirus serotype specific antibodies in children as per father's educational level

Appendix 13



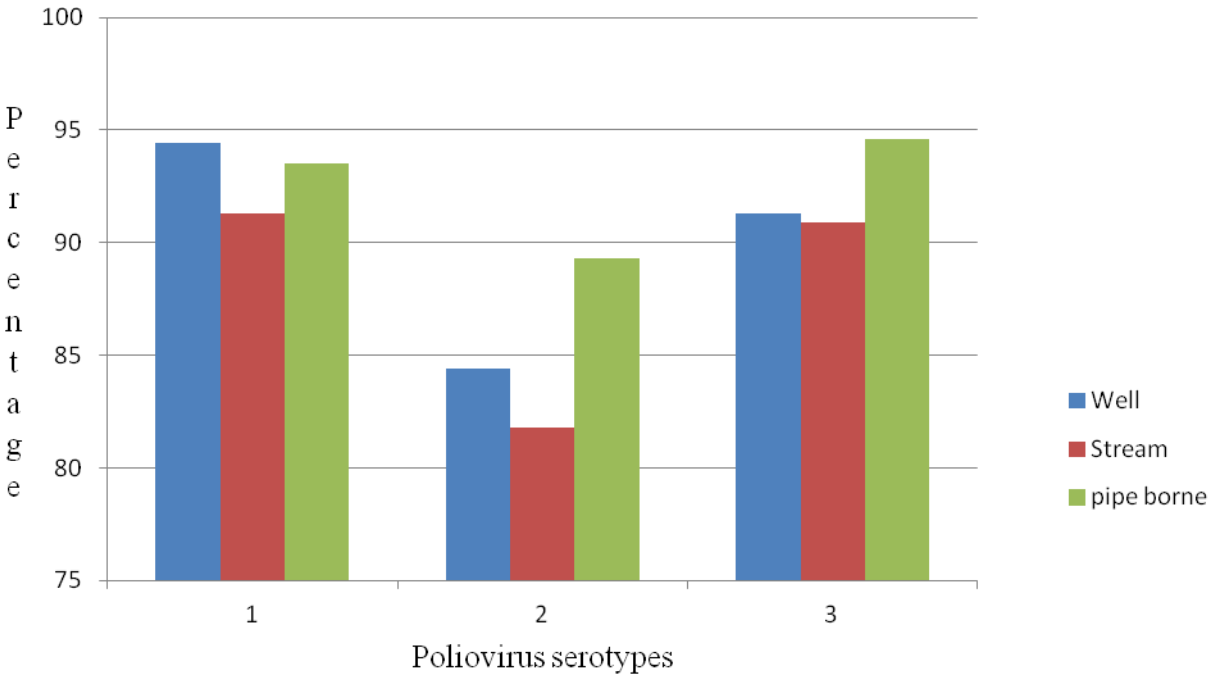
Distribution of Poliovirus antibodies in the children according to father's occupation

Appendix 14



Distribution of Poliovirus antibodies in children by serotypes according to father's tribe

Appendix 15



Distribution of Polivirus antibodies in children by serotypes as per major source of drinking