EPIDEMIOLOGICAL SURVEY OF THE LEVEL AND SIGNIFICANCE OF ANTIBODIES TO POLIOMYELITIS VIRUS IN KADUNA STATE, NIGERIA

BY

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A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, AHMADU BELLO UNIVERSITY, ZARIA, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE MICROBIOLOGY

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OCTOBER, 2000

DECLARATION

I declare that the work reported in this thesis was carried out solely by me in the laboratory of the Department of Microbiology, Ahmadu Bello University, Zaria, under the supervision of Professor A.A. Ahmad of the Department of Microbiology, and Dr. G.O. Ogunrinde of the Department of Paediatrics, Ahmadu Bello University Teaching Hospital, Zaria.

This thesis is an original work carried out by myself and no part of it has been presented elsewhere for the award of a degree or a diploma. The work of other researchers are duly acknowledged and referred to appropriately.



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CERTIFICATION

This thesis, entitled `Epidemiological Survey of the level and significance of antibodies to poliomyelitis virus in Kaduna State, Nigeria" by MARYAM AMINU, meets the regulations governing the award of the degree of Masters of Science (Microbiology) of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This thesis is dedicated to the memory of my late mother, Hauwa'u Aminu, who, by the Grace of Almighty God gave me the needed support and encouragement during my primary education, and stood by me thereafter until her departure to eternal rest. *May her gentle soul rest in perfect peace. Amin.*

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ABSTRACT

While data is available on the incidence of poliomyelitis and the vaccination coverage against the disease in Nigeria, there is a dearth of information on the herd immunity and the immune status of vaccinees. This study was therefore, undertaken in 1999/2000 to determine the immune status to poliomyelitis in primary school children aged 1-11 years in Kaduna State, Nigeria, an area where high vaccination coverage has been reported.

Two schools each were selected in eleven randomly selected local government areas (LGAs) of the state. Questionnaires were sent through the teachers to the parents of randomly selected children from these schools in order to obtain information concerning these children's vaccination status to poliomyelitis.

Blood samples were collected from 1200 respondents, but a total of 1087 sera from which clear and adequate quantity of sera could be obtained were analyzed according to LGA, age, sex and vaccination status. Serum levels of poliovirus neutralizing antibodies were investigated in the sera using poliovirus types 1, 2 and 3 in HEp-2 cells.

The results of the analysis of questionnaires indicated that, of the 161 (14.8%) children with no history of vaccination, 77 (47.8%) had demonstrable antibodies to poliovirus. And of the 926 (85.2%) children with a history of vaccination, 128 (13.8%) had no demonstrable antibodies to poliovirus. These results show that there was poor correlation between the history of vaccination and the presence and absence of antibody in the children, implying that vaccination history might not be an accurate index of immunity.

Children with antibody titre <1:10 were considered seronegative (susceptible). Antibody titration results indicated that 42.2% of the children had

antibodies to the three serotypes of poliovirus, 19.5% had no anti-poliovirus antibodies at all, and 65.5, 71.4 and 66.7% had antibodies to poliovirus types 1, 2 and 3 respectively. A total of 80.5% of the children had demonstrable antibodies to one or more serotypes of poliovirus. The proportion of potentially susceptible children to one or two poliovirus serotypes was 57.8%, while 34.5, 28.6 and 33.3% of the children were potentially susceptible to poliovirus types 1, 2 and 3 respectively. Statistical analysis of the results by Chi-square tests at $P<0.05$ showed that the prevalence varied significantly in the LGAs, with the highest in Kagarko LGA and the lowest in Zaria LGA.

Antibody prevalence was higher in the older age group (7-11 years) with lower geometric mean titre (GMT) values than the younger age group (1-6 years) that had higher GMT values. No significant difference was observed in the antibody distribution by sex. Seropositive rates were higher in children who received five doses of oral polio vaccine (OPV) and the observed difference in the prevalence rates by number of vaccine doses received was highly significant. The results of this study also confirmed the known higher immunogenicity of poliovirus type 2 with respect to the other two types.

The level of protection against the three poliovirus serotypes in Kaduna State is unacceptably low (42.2%), indicating low herd immunity to poliomyelitis. This finding is difficult to explain in view of the high (140%) level of vaccination coverage reported by Kaduna State Ministry of Health. It is concluded that the potential risk of an outbreak of poliomyelitis in the susceptible population in Kaduna State is real. This necessitates the need for improved vaccination efforts and the monitoring of vaccination efficacy by serological survey of the vaccinated population.

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CHAPTER ONE

1.0 INTRODUCTION

Poliomyelitis is on the verge of being eradicated from the face of the earth, but there are still endemic and "high risk" countries where the virus is being transmitted.

The term "poliomyelitis" is derived from the Greek words *polios*: grey, and *muelos*: marrow. The shortened form "polio" is often used to indicate the paralytic form of the disease (Collier and Oxford, 1998). Poliomyelitis is an infectious viral disease, which affects the central nervous system (CNS) and infrequently results in paralysis. The disease can occur at any age but mainly affects preschool children under three years of age worldwide (Hajar *et al.*, 1983; WHO, 1999). The virus destroys the motor neurons in the spinal cord following invasion of the gastrointestinal tract, resulting in acute flaccid paralysis (AFP). In some severe cases polio paralysis can lead to death by asphyxiation (Hewer, 1981; WHO, 2000a). The disease is caused by the three related serotypes (types 1,2 and 3) of poliovirus, a member of the genus *Enterovirus* in the family *Piconaviridae* (Bodian, 1959).

In industrialized countries during the 1940s and 1950s, large polio epidemics caused panic every summer and crippled thousands of children every year until the epidemic was brought under control in the late 1950s and early 1960s (WHO, 2000a). During this period over 76,000 cases of paralytic polio were reported yearly with between 10,000 and 21,000 of such cases coming from the United States of America (Cockburn and Drozdov, 1970). It took a

somewhat longer time for polio to be recognized as a major health problem in developing countries. The "lameness survey" during the 1970s and 1980s revealed that the disease was also a frequent public health problem in developing countries, crippling thousands of children every year (Ofosu-Amaah *et al.*, 1977; Kok *et al.*, 1992).

In Nigeria, it was estimated that between 200,000 and 300,000 children suffered poliovirus infection in the early pre-independence period, with about 100,000 cripples of all ages from poliomyelitis (Collis *et al.*, 1961). Also in 1968, 151 cases of poliomyelitis were reported in Nigeria (Cockburn and Drozdov, 1970).

The increase in the global cases of polio led to the development of inactivated poliovaccine by Salk in 1953 and subsequently the attenuated live polio vaccine by Sabin in 1955. As a result, during the 1970s routine immunization with oral poliovirus vaccine (OPV) as part of National Immunization Programme was introduced worldwide. This helped in controlling the disease and saved thousands of children from being crippled worldwide.

In May 1988, the 41st World Health Assembly (WHA) committed the member states of the World Health Organization (WHO) to the global eradication of polio by the year 2000 (Crayford and Hobbs, 1992; Fox, 1998) in ways which strengthen national immunization programmes and health infrastructure (Goodman *et al.*, 2000j). This initiative was reinforced by the World Health Summit for children in New York in 1990 and the Organization for African Unity

(OAU) summit of Heads of State in Yaounde, Cameroun in 1996 (National Programme on Immunization (NPI), 2000).

The global initiative to eradicate polio is the largest international disease control ever targeted at completely eliminating an infectious disease from the face of the earth, and it involves both halting the incidence of the disease and the worldwide eradication of the virus that causes it (WHO, 2000*i*). This is possible because polio is one of only a limited number of diseases that can be eradicated based on the premise that the virus will disappear if it is deprived of its human host through immunization, a strategy similar to that used for small pox eradication in 1977 (WHO, 2000c). Eradication of polio is at its final phase (Hovi and Stenvik, 2000) and there are now only 30 countries (mainly in Sub-Saharan Africa and South Asia) where the disease is still endemic (Fox, 1998; WHO, 1999).

Since the launching of polio eradication initiative in 1988, there has been remarkable progress. Polio has been eradicated from the Americas, Continental Europe, the Western Pacific and much of the Middle East. The disease has virtually disappeared from most of Northern and Eastern Africa (Polio News, 2000; WHO, 2000i).

While 30 countries worldwide are still considered polio endemic, intense activities are currently targeted towards interrupting virus transmission in the ten remaining "high risk" or priority countries facing particular challenges. These countries include;

1. Countries affected by conflict where eradication activities started relatively late: Afghanistan, the Democratic Republic of Congo (DR Congo), Somalia, Sudan and Angola;

2. Countries with large dense population and intense virus transmission accounting for more than 70% of the world's cases (Aylward *et al.*, 2000) - the "major global polio virus reservoirs": India, Bangladesh, Pakistan, Ethiopia and Nigeria (WHO, 2000). India consistently accounts for over 50% of the reported cases of poliomyelitis globally (Banerjee *et al.*, 2000).

In order to reach the global target of polio eradication in all the ten endemic global priority countries, major collaborative efforts are being undertaken between international organizations and these countries to stop the transmission of the virus by using house-to-house immunization. This will rapidly build up adequate levels of protection with additional intensification of surveillance for AFP and wild poliovirus (WHO, 2000i).

Nigeria is one of the remaining global priority countries and remains one of the largest reservoirs of poliovirus (Polio News, 1999). The Nigerian government is committed to the global goal of polio eradication and began a national effort known as the National Immunization Days (NIDs) in order to eradicate polio in 1996, with a coverage of 47% (1st round) and 75% (2nd round) of the target population of under fives. There were improvements in the coverage in 1997 (74%, 1st round; 95%, 2nd round) and in 1998 (100% 1st round, 108%, 2nd round) (NPI, 2000). In 1999, the government pledged the sum of 15 million US dollars to support five rounds of NIDs in the year 2000 (Polio News, 1999),

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which have since commenced with the implementation of more than three rounds. Only recently, the Guardian Newspaper of October 12, 2000 reported that the Nigerian government has voted N27.8 million to eradicate polio in the country.

In 1999, two rounds of Sub-National Immunization days (SNIDs) (house-to-house strategy) were conducted in fifteen endemic states, which included Kaduna State. The experience of the SNIDs showed that the house-to-house strategy detected and immunized 40% more children than the projected population and detected at least 5% of the children who had never received any dose of OPV before (NPI, 1999a).

In 1999 the Kaduna State Primary Health Development, Ministry of Health, reported the coverage level to be 140% in 1998, which also showed a 40% increase compared with the estimated target population. So far, statistical data available in Nigeria shows a high level of vaccination coverage of the target population of under fives.

It appears that in spite of increased levels of coverage, immunization campaign, and intensification of immunization in all parts of the country including Kaduna State, wild poliovirus still circulates in Nigeria (NPI, 1999a). Cases of AFP are also on the increase in many parts of the country every year amongst primary school children (NPI, Nigeria, 1999b). For instance, information received from the National Polio Laboratory in Ibadan, Nigeria, indicates that the number of AFP cases reported in 1996, 1997, 1998 and 1999 were 12, 24, 996 and 2,466 with 2, 2, 79 with 136 of the cases being confirmed as caused by the

wild poliovirus. However, increase in reported cases may be due to increase in awareness and surveillance of the disease.

Nonetheless, the increase in frequency of AFP cases reported raises the question of whether the acceleration of eradication activities really has any impact. Apart from the data available on immunization coverage and the disease incidence, there is a dearth of information on the degree or level of protection acquired by the general population (herd immunity) and the immune status of vaccinees, which is usually and better evaluated serologically. Serological surveillance of an immunisable disease usually presents a better position of the state of the disease in a population and can be used to evaluate the effectiveness of a vaccine (John, 1976; Orenstein *et al.,*1985; Deivanayagam *et al.,* 1993). It is an essential monitor of susceptibility to disease (Reid *et al.,* 1973) it constitutes a vital part of disease control (Evans, 1980; Harrison *et al.,* 1998) and can therefore be used with information available on immunization coverage to effectively monitor the success of control programmes (Adeiga *et al.,* 1994; Koza, 1996; Grasso *et al.,* 1999).

Poliomyelitis is a disease that spreads rapidly infecting hundreds of people, depending on the level of sanitation, before the first case of polio paralysis emerges. Hence, WHO considers a single confirmed case of polio paralysis to be evidence of an epidemic, particularly in countries where very few cases occur. The virus can also be re-imported into a polio free country with high levels of immunization coverage and spread rapidly leading to an epidemic. Therefore, so long as a single child remains infected with poliovirus, children

worldwide are at risk. So no country can be said to be polio free until all countries of the world are certified free of the disease.

This work was therefore designed with the following aims and objectives:

(1) To evaluate the immune status of children in Kaduna State of Nigeria in respect to poliovirus. This would be a measure of the efficacy of OPV vaccine being used for the polio eradication programme in Nigeria.

(2) To assess the levels of protection attained in the target population.

(3) To describe the demographic characteristics of children in Kaduna State with regards to polio vaccination.

(4) To validate record of immunization and the disease, by correlation of serological status and immunization history.

The data so obtained will provide an insight into the success or otherwise of the current accelerated efforts at eradicating polio in Kaduna State. The study will also define further immunization needs by identifying the age group at the highest risk in Kaduna State of Nigeria. Based on the results of this study, recommendations will be made that will help in promoting the current programmes aimed at eradicating polio in Kaduna State and Nigeria at large.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Picornaviridae

Picornaviruses are small icosahedral viruses, which include human and animal pathogens. They are associated with more than 20 clinically recognised syndromes. The name picornavirus (pico=small; rna=ribonucleic acid genome) was introduced in 1963 as a larger grouping to which the enteroviruses and rhinoviruses belong because of the fundamental similarities in many of their properties (Horstmann, 1965; Melnick, 1963). With the advancement of virus classification on the basis of further knowledge of biophysical and biochemical characteristics, the International Committee on Nomenclature of Viruses assigned into this larger taxon the family status *Picornaviridae* (Bodian and Horstmann, 1965; Cooper *et al.,* 1978).

The family *Picornaviridae* comprises one of the largest and important families of viruses (White and Fenner, 1994). The family is currently divided into six genera, viz (a) enteroviruses, of which poliovirus is the most extensively studied member, (b) rhinoviruses, the single most important etiological agent of the common cold, (c) hepatoviruses, including human hepatitis A virus (HAV), (d) the aphtho viruses [e.g., foot-and-mouth disease virus (FMDV)], (e) cardioviruses [e.g., encephalomyocarditis virus (EMCV)], and (f) the parechovirues [e.g. echovirus 22] (Rueckert *et al.,* 1996; Andino *et al.,* 1999).

Poliovirus was the first of the genus and indeed of the whole family to be isolated by Enders, Weller and Robins in 1948 (Collier and Oxford, 1998).

The picornavirus virion is a small symmetrical icosahedron which is only 20-30nm in diameter. They are non-enveloped and ether resistant viruses, appearing smooth and round in outline by electron microscopy. The capsid is constructed from 60 protein sub-units (protomers) each comprising a single molecule of four polypetides, VP1 (1D), VP2 (1B), VP3 (1C) and VP4 (1A), derived by cleavage of a single poly protein (Rueckert et al., 1996). The picornavirus genome is a single stranded ribonucleic acid of positive polarity which has a molecular weight of between 2.5 x 10^6 and 3.0 x 10^6 Daltons (Agol 1980). The single stranded RNA (ss-RNA) genome is made up of three regions: a 650-1300 nucleotide (nt) 5′ untranslated region (5′ UTR); a single long open reading frame (ORF) encoding the viral polyprotein; and a relatively short (~100nt) 3′ untranslated region (3′ UTR) followed by a poly adenylate (poly (A)) tract of variable length (Andino *et al.*, 1999). A small protein of 22 amino acids, Vpg (3B), is attached to the 5′ end of the RNA.

Members of the family *picornaviridae* cannot be distinguished on the basis of their morphology, even with an electron microscope. However, each of the genera has serotypes which may be distinguished based on the study of some physicochemical properties like buoyant density in caesium chloride, base composition of the genome and relative stability at different pH values.

Members of the genus *Enterovirus* include polioviruses, coxsackie viruses, echoviruses and the numbered enteroviruses, which are commonly found in the

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alimentary canal of humans (Horstmann, 1965; Melnick, 1989b; Brooks *et al.*, 1991). About 70 members are known to be human pathogens (Melnick, 1978) and they grow readily in stationary culture at 36°C-37°C. They are acid resistant which enables them to survive passage through the stomach.

Rhinovirus includes more than 100 antigenic types (Brooks *et al.*, 1991) and is classified into a major group and a minor group based on their specificities for cell receptors (Hewat *et al.*, 1998). The major group includes two types, H and M recognized on the basis of their optimal replication in human and monkey tissue culture respectively (Horstmann, 1965).

Most *Rhinovirus* strains can be recovered only in cells of human origin and are primarily isolated in human fetal kidney cultures. Cytopathic changes are favoured by media with low bicarbonate content (0.03%) and incubation of cultures at 33°C (Horstmann, 1965; Brooks *et al.*, 1991) Rhinoviruses are acid labile and can be stabilized by magnesium chloride against thermal inactivation. They are the major causes of coryza (common cold) in humans especially among adults (Horstmann, 1965; Smith *et al.*, 1998; Sanders *et al.*, 1998). In children, they cause upper and occasionally lower respiratory tract illnesses. Recent evidence also implicates rhinovirus infection as an important precipitating factor for the exacerbation of asthma (Nicholas *et al.*, 1993), chronic bronchitis, sinusitis, and otitis media (Sanders *et al.*, 1998). They are isolated chiefly from the nose and throat (Smith *et al.*, 1998) and they differ from enteroviruses by having a buoyant density in caesium chloride of about 1.4g/ml (Melnick, 1989b).

Cardioviruses are similar to the enteroviruses but are not stable at pH values below 4 and above 6. Some members like the encephalomyocarditis virus of rodent possess hemagglutinins and are capable of agglutinating human erythrocytes (Andrewes *et al.*, 1978).

Aphthovirus is a genus that has only one member virus, the foot and mouth disease virus (FMDV). Over 65 strains of FMDV have been isolated and grouped into seven serotypes: A, O, C, Asía 1, SAT1, SAT2 and SAT3 (Mateu *et al.*, 1988). Foot and mouth disease virus causes the most economically important viral disease of cattle (Brown *et al.*, 1996) and other cloven-hooved animals, and it is cytolytic in cell culture. Occasionally, however, FMDV can establish persistent infections in ruminants and cell culture (Sevilla and Domingo, 1996).

Picornaviruses causes disease in humans ranging from poliomyelitis and aseptic meningitis to the common cold (Brooks *et al.*, 1991) and their replication takes place in the host cytoplasm independent of the host genome.

2.2 Enteroviruses

Enteroviruses of human origin include the following:

1. Polioviruses: types 1-3;
2. Coxsackie viruses of group A: types 1-23;
3. Coxsackie viruses of group B: types 1-6;
4. Entero viruses: types 68-71 and
5. Enterovirus 72.

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The enteroviruses share many clinical and epidemiological features as well as physical and biochemical properties of the picornaviruses (Horstmann, 1965). The virion of the enteroviruses consists of a capsid shell of 60 subunits, with of four proteins (VP1-VP4) arranged in icosahedral symmetry. They are stable at acid pH (3.0-5.0) for 1-3 hours at freezing temperatures for many years, remain viable for weeks at 4°C and for days at room temperature. Enteroviruses are amongst the most stable of the human pathogenic viruses outside the animal body and have a buoyant density in caesium chloride of about 1.34g/ml (Melnick, 1989b; Brooks *et al.*, 1991). They are resistant to most laboratory disinfectants and lipid solvents. Treatment with 0.3 % formaldehyde, 0.1 N Hcl, or free residual chlorine at a level of 0.3-0.5ppm causes rapid inactivation, but the presence of extraneous organic matter protects the viruses from inactivation. They are stabilized by magnesium chloride against thermal inactivation (Melnick, 1989b; Wu *et al.*, 1995).

Most enteroviruses are inhibited from growth in cell culture by 2-(α-hydroxybenzyl) – benzimidazole (HBB), (Tamm and Eggers, 1962), hydantoin (Shimuzu *et al.*, 2000), and guanidine (Melnick, 1989b; WHO, 1997).

Exposure of these viruses to a temperature of 50°C destroys them rapidly and they are inactivated by ultra-violet light and by drying (Melnick, 1989b). Vital dyes when incorporated into the structure of these viruses render them rapidly susceptible to visible light (Wallis and Melnick, 1965).

Except for the coxsackie A group, enteroviruses grow rapidly in human and monkey tissue cultures (Stewart and Beswick, 1977) and about one third of

them, notably, coxsackie viruses and echo viruses have the ability to agglutinate human type O erythrocytes (Melnick, 1989b).

Humans are the only known reservoir for members of the human enteroviruses (VanLoon, 2000) and they are found in all parts of the world. The host range varies greatly from one type to the next and even among strains of the same type (Melnick, 1989b; Brooks *et al.*, 1991).

Enteroviruses are present in urban sewage and this may serve as a source of contamination of flies and water used for drinking and bathing. They have also been found in aerosols produced by sprinkler irrigation using recycled wastewater (Melnick, 1989b). The presence of human enteric viruses in water has been recognized for several decades but the full health significance of this has yet to be determined.

Most enterovirus infections are subclinical, particularly in young children. Nevertheless, they can cause a wide range of clinical syndromes involving many of the body systems. Rashes, upper respiratory tract (URT) infections and "undifferentiated summer febrile illnesses" are common. Neurologic diseases such as paralysis, meningitis, chronic meningoencephalitis and cardiac diseases including myocarditis and pleurodynia occur. Others include foot and mouth disease, herpangina, neonatal diseases, etc. (Collier and Oxford 1998).

2.3 The poliovirus

2.3.1 Structure

The poliovirus virion is approximately 27nm in diameter and consists of 60 copies of capsid protein VP1 and VP3, and an average of 58 to 59 copies of capsid protein VP2 and VP4, plus 1 and 2 copies of their capsid precursor protein Vpo (Hindiyeh *et al.*, 1999). These four capsid proteins are arranged in *icosahedral symmetry around the single strand –positive-sense* RNA *genome* (Brock and Madigan, 1988; Brooks *et al.*, 1991; Melnick, 1990; Robertson, 1993; WHO, 1997).

The RNA is a linear molecule of about 7500 nucleotide bases (Giachetti *et al.*, 1992; Xiang *et al.*, 1998) and has a molecular weight of 2.6x 10^6 daltons. At the 5´ terminals of the viral RNA is a protein (vpg protein) (Agol, 1980; Percy *et al.*, 1992) which attaches covalently to the RNA and is essential for the initiation of RNA synthesis (Xlang *et al.*, 1998). At the 3´ terminal of the RNA is a polyadenylic acid (Poly (A)) tail (Brock and Madigan, 1988). Investigations have revealed that RNA molecules which lack the Poly (A) tail are not essential for infectivity (Brock and Madigan, 1988).

The linear single stranded RNA genome is characterized by a long (742-nucleotide) 5´ non-translated region (Zhao *et al.*, 1999; Chapman *et al.*, 2000). This region contains two important cis-acting elements, the 5´ clover leaf-like structure and the internal ribosomal entry site element (Zhao *et al.*, 2000) that allows the translation of viral genomic mRNA in a 5´ and cap-independent manner. The four-capsid and non-capsid proteins are formed from the cleavage

of long 247-K Da polyproteins (Xiang *et al.*, 1998; Andino *et al., 1999*). The three largest proteins (VP1-VP3) are similar in core structure and contain a series of loops in their amino acid chain, which include the chief antigenic sites involved in neutralization of virus infections (WHO, 1997). The smallest (internal) protein, VP4 is functional in the encapsidation of the viral RNA in the mature virus (Melnick, 1989b; Brooks *et al.*, 1991; WHO, 1997).

2.3.2 Serotypes

Polioviruses are classified into three antigenically distinct serotypes originally called Brunhilde, Lansing and Leon (Collier and Oxford, 1998) but now known as poliovirus 1 (PV1), poliovirus 2(PV2) and poliovirus 3(PV3) based on their reactions with reference panels of neutralizing antisera (Bodian and Horstmann, 1965; Stewart and Beswick, 1977; Robertson, 1993). Although immunologically distinct, the three types appear to show some minor antigenic overlap, which is most readily demonstrable by complement fixation (CF) tests with virus which has been inactivated by heat or ultra-violet irradiation. Minor *et al.* (1982) have been able to demonstrate cross-reactivity among the serotypes by immune precipitation and immunologic studies of purified viral protein.

In more recent times, monoclonal antibodies and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) has been used for sero differentiation of poliovirus (Kew *et al.*, 1981).

2.3.3 Antigenic characteristics

Two type-specific antigens, the D and C antigens are present in the poliovirus preparation and can be detected by Enzyme Linked Immunosorbent Assay (ELISA) and CF tests (Stewart and Beswick, 1977; Robertson, 1993). The D form represents full particle containing RNA and can be converted to the C forms are (empty particles) by heating. The C antigens of different viral types are cross-reactive, but D antigens are not (Brooks *et al.*, 1991).

Clinical features presented by infections with the different serotypes may be similar but manifestation of infections with the same type varies widely. Wild polioviruses vary in their neurovirulence and it has been demonstrated by Nathanson and Martins (1979) that their ability to produce paralysis in monkeys differs markedly.

2.3.4 Physicochemical Properties

In addition to other shared properties of the enteroviruses, polioviruses can survive for weeks at room temperature, and for many months at 0°C to 8°C (Robertson, 1993). They have been shown to be stable at a temperature of -20°C and can last for about eight years at -70°C but cannot be preserved by lyophylization except when done in a mixture of 5% glucose and 5% dextran (Tyrell and Ridgewell, 1965; Duguid *et al.*, 1978).

Poliovirus exhibits similar resistance to lipid solvents such as ether, chloroform or sodium deoxycholate and bile salts like other picornaviruses (WHO, 1997). Poliovirus is sensitive to low dose of U.V irradiation without loss

of antigenicity but loss of infectivity occurs within two minutes of extensive irradiation due to virion disruption (Katagiri *et al.*, 1967).

Poliovirus like other enteroviruses is amongst the most stable of the human pathogenic viruses outside the animal body. Heat inactivation of the virus occurs at 50-60°C when exposed for 30 minutes, except if otherwise stablized with magnesium chloride (Melnick, 1989b; WHO, 1997).

2.3.5 ***Virus-cell Interaction***

Like other viruses, poliovirus initiates infection by binding to specific receptor molecules on the surface of susceptible cells (Karnauchow *et al.*, 1996) and it is delivered into the cytoplasm as viral RNA freed from its protein shell.

Dramatic morphological and biochemical alterations in the host cell accompany virus infection (Schlegel *et al.*, 1996). These alterations include enhanced membrane permeability (Carrasco, 1978), inhibition of DNA, RNA, and protein synthesis, blockade of membrane traffic (Cuconati *et al.*, 1998), increased intracellular calcium levels (Schlegel *et al.*, 1996) and modifications in phospholipase activity and lipid turnover (Barco and Carrasco, 1998). There are also dramatic morphological alterations in the chromatin structure as well as cytoskeleton (Lenk and Penmann, 1979).

Moreover, the Golgi apparatus is not recognizable as such in poliovirus-infected cell and numerous membranous vesicles (50 to 400nm) fill most of the cytoplasm later in infection (Dales *et al.*, 1965; Schlegel *et al.*, 1996). All of these changes are referred to as cytopathic effect (Caliguiri and Tamm, 1969;

Barco and Carrasco, 1998). The host cell materials are mobilized for the synthesis of viral proteins and nucleic acids, which takes place 2.5-5 hours after infection (Darnell *et al.*, 1961; Schlegel *et al.*, 1996).

In the replication of polioviruses, all steps are independent of the host DNA and occur in the cytoplasm of infected cell (Melnick, 1989b; Schlegel *et al.*, 1996). The steps include, adsorption, penetration, uncoating, synthesis of viral RNA and proteins, assembly and maturation and ultimately the release of infective virions. During the process of virus multiplication, the single stranded (SS) genomic RNA serves as its own mRNA for successful expression and duplication of genetic information (Melnick, 1989b). The production of single stranded RNA occurs within virus-induced lipid – containing cytoplasmic vesicles known as replicative complexes (Caliguiri and Tamm, 1970; Heinz and Vance, 1996, Towner *et al.*, 1998; Shimuzu *et al.*, 2000). Highly specific interactions between cellular and viral proteins associated with the virion RNA and cellular membranes result in the formation of these replicative complexes. These interactions involve tight membrane protein association by poliovirus non-structural proteins 3AB and 2C combined with specific RNA-protein interactions between the poliovirus 5′ non-coding region and the viral polypeptides (Tershak, 1984; Towner *et al.*, 1998, Xiang *et al.*, 1998). Other non-structural proteins, 2B and 3D, and their precursors have also been found to be involved in these interactions (Shimuzu *et al.*, 2000).

The VPg protein also known as 3B protein serves as a primer for the synthesis of positive-strand RNA (Xiang *et al*, 1998). It requires a hydrophobic

carrier to facilitate its attachment to membranes (Heinz and Vance, 1996). It is translated from a single initiation point to form a single large polypeptide that is subsequently cleaved in a stepwise fashion to produce the various capsid polypeptides (Agol, 1980). The genome is eventually cleaved to four structural and seven non-structural proteins (Shimuzu *et al.*, 2000).

The structural capsid proteins are located at the amino-terminal P_1 region of the polyprotein. The remainder contains viral non-structural proteins including two proteases ($2A^{pro}$ and $3C^{pro}$), and RNA dependant RNA polymerase ($3D^{pol}$) and several other proteins essential for viral RNA synthesis (Shimuzu *et al.*, 2000). The 2C-coding region is likely to be responsible for viral encapsidation (Shimuzu *et al.*, 2000).

Completion of encapsidation produces mature viral particles that are then released by exocytosis when the cell undergoes lysis (Cuconati *et al.*, 1998). The time required from initiation of infection to completion of viral assembly ranges from five to ten hours (WHO, 1997) depending on pH, temperature, host cell, and number of infective particles. Yields may be up to 100,000 particles per cell but only one in 10,000 particles may be infectious.

2.4 Route of transmission and mode of spread

Human beings are the sole reservoirs of polioviruses (Melnick, 1989b; VanLoon, 2000) and close contact appears to be the primary avenue of spread. Wild poliovirus is spread directly or indirectly from person to person (Robertson, 1993; WHO, 1997). Fecal-oral contamination (fingers, table, utensils, foodstuffs,

milk) is the usual source of infection, however, droplets or aerosol from coughing and sneezing can also be a source of direct or indirect contamination (Melnick, 1989b).

Warm weather favours the spread of the virus by increasing human contacts, susceptibility of the host, and the dissemination of the virus by extra human source (Melnick, 1989b). The viruses are readily spread within the family by young children (WHO, 1997).

Virus dissemination is also facilitated by poor hygiene and sanitation (Robertson, 1993; WHO, 1997). Houseflies act as mechanical carrier of poliovirus from faeces to food (Robertson, 1993; WHO, 2000b) during epidemic periods. Polioviruses have been shown to persist in flies for weeks but do not multiply (Melnick, 1989b).

2.5 Pathogenesis

Wild poliovirus enters through the mouth, attaches to receptors in the lymphoid tissue of the pharynx and gut, including Payer's patches, and replicates inside these cells. Incubation period is usually between seven and fourteen days but may range from two to thirty-five days (WHO, 1997; Collier and Oxford, 1998; WHO, 2000a).

After an initial and continuous viral multiplication in these cells, a viraemic phase is followed by extension to the CNS through viral spread along axons. Lytic infection of neurons with secondary degeneration of axons is the primary cause of paralysis (Collier and Oxford, 1998).

After initial infection with poliovirus, the virus is demonstrable in nasopharyngeal secretions thirty-six hours later and in the stool after seventy-two hours (Imperato, 1974). The virus persists in the throat for one week and occasionally for up to two weeks (Melnick, 1989b) and is shed intermittently in faeces for several weeks. During this time, polio can spread rapidly through the community (WHO 2000) and the greatest period of infectivity is a week before and a week after the onset of symptoms (Imperato, 1974).

Once established in the intestine, the virus can enter the blood stream via the lymphatics (Brooks *et al.*, 1991; Melnick, 1989b) and invade the central nervous system (CNS) unless sufficiently high levels of neutralizing antibodies are present to block it (Robertson, 1993; WHO, 1997).

Within the CNS, the virus spreads along nerve fibres and invades certain types of nerve cells (Melnick, 1989b). In the process of its intracellular multiplication, the virus may damage or completely destroy these cells, thus causing oedema and muscle paralysis (Cheesbrough, 1984). Since these nerve cells cannot be regenerated, the affected muscles no longer function (WHO, 1997; WHO, 2000a). The muscles of the legs are affected more often than the arm muscles (WHO, 2000a). The anterior horn cells of the spinal cord are most prominently involved, but in severe cases the intermediate grey ganglia and even the posterior horn and dorsal root ganglia are often affected resulting in flaccid paralysis (Melnick, 1989b) where the limbs become floppy and lifeless (WHO, 2000a).

More extensive paralysis involving the trunk and muscles of the thorax and abdomen can result in quadriplegia (WHO, 2000a). In most severe cases, (bulbar poliomyelitis) poliovirus attacks the motor neurons of the brain stem(bulbar poliomyelitis). This reduces breathing capacity and causes difficulty in swallowing and speaking, which can lead to death by asphyxiation (WHO, 2000a). Sensory neurons are not however, affected and poliovirus does not multiply in the muscles in vivo (WHO, 1997).

2.5.1 Pathology

As a result of the neuronal damage due to direct viral invasion, there is a lytic destruction of the neurons, inflammatory reaction and an initial polymorph response (Isma'il and Lal, 1993). Subseqently, there is perivascular infiltration (cuffing) characteristic of inflammatory reaction in the central nervous system, microglial proliferation, and oedema. Inflammatory foci in lymphoid tissue are also present (Collier and Oxford, 1998).

In poliomyelitis, the cerebrospinal fluid (CSF) shows an increase in protein and leucocyte count with a normal sugar level. Also the polymorphonuclear cell count is high in the pre-paralytic phase, averaging 185 cells per microlitre (μl), dropping to 50 per μl a week after onset of paralysis (Ismail and Lal, 1993).

2.6 Clinical presentations of poliovirus infection

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When an individual susceptible to polio infection is exposed to the virus, the response is variable. The incubation period is usually between 7-14 days with extremes of 2-35 days. There may be an inapparent infection, a minor illness with malaise fever and sore throat, or a major illness heralded by a meningitis phase. Each of these, except the major illness may resolve without squealae (Collier and Oxford, 1998).

It has been estimated that for every 100 persons infected by poliovirus, an average 90-95 percent do not develop clinical symptoms (Melnick, 1989b; Robertson, 1993), 4-8 percent suffer a minor illness, 1-2 percent suffer meningitis and 0.1-1 percent suffer major paralytic diseases (Cheesbrough, 1984). These figures are however, dependent on the virulence of the virus and the age of the person affected. Moreover, only about one percent of infections are recognized clinically (Brooks *et al.*, 1991).

The inapparent infection (without symptoms) is an acute form of poliovirus infection that presents with no visible clinical sign while the mild illness is a symptomatic acute phase that is the most common form of the disease. Patients here have only the minor illness characterized by fever, malaise, drowsiness, headache, nausea, vomiting, constipation or sore throat in various combinations. Recovery is within a few days and the nervous system may be involved here but with no apparent paralysis.

In addition to the aforementioned symptoms and signs, the patient with the non-paralytic form, otherwise known as aseptic meningitis, (because of the involvement of the meninges) presents stiffness and pain in the back and neck.

Meningeal irritation also accompanies this form. This disease lasts 2-10 days and recovery is rapid and complete. In a small percentage of cases, the disease advances to the paralytic form (Brooks *et al.,* 1991; Melnick, 1989b; WHO, 1997). It is important to emphasize that poliovirus is only one of many enteroviruses and there are other viruses that produce aseptic meningitis (WHO, 1997).

In patients with paralytic poliomyelitis, the onset of the major illness usually follows the minor illness described above, but it may occur without the antecedent first phase (Melnick, 1989b). Paralytic poliomyelitis is marked with pain and tenderness of muscles and muscle spasms epitomised by flaccid paralysis (Oyemade, 1981; Ismail and Lal, 1993) due to damage to lower motor neurons (Minor *et al.,* 1990). Paralysis starts on the 1st and 2nd day of major illness with pain in the lower back, often in association with drowsiness and irritability. Painful spasms of non-paralysed muscles, the loss of deep tendon reflexes and non - coordination also occurs (Ismail and Lal, 1993).

Fever often accompanies the classically asymmetrical paralysis that may reach its maximum within a few hours or advance for 4-5 days. In addition to the symptoms mentioned above, delirium, convulsion, hyperaesthesia and gastrointestinal disorders like diarrhoea, vomiting and abdominal pains are also seen in paralytic poliomyelitis (Volovskaya, 1990). Although recovery may occur up to 6 months after onset of illness, the paralysis is usually permanent (WHO, 1997) and deaths from paralytic poliomyelitis are between 2-10% of affected

individuals and are usually due to respiratory failure caused by paralysis of respiratory muscles (Cheesbrough, 1984).

Dulbecco and Ginsgery (1988) and Volovskaya (1990) recognized four different types of paralytic poliomyelitis based on the anatomical site of nervous disorder. They are: spinal, bulbar, encephalitic and pontine poliomyelitis. Spinal poliomyelitis often involves the anterior horn cells of the spinal cord, with lesser involvement of the posterior and intermediate horns. The cervical and the lumbar enlargement of the cord appear most susceptible (Ismail and Lal, 1993). Paralysis occurs in the muscles of the extremities, neck, diaphragm and trunk with a mortality rate of 1%. Bulbar poliomyelitis which is a very common in Africa (Collis *et al.,* 1961) involves the organs of respiration, speech and swallowing. It appears to be the most dangerous form and can lead to death by asphyxiation (WHO, 2000). The mortality rates of bulbar polio is 10% and up to one-third of cases show evidence of encephalitis, with altered consciousness and irritability or irrationality (Ismail and Lal, 1993). Pontine polio involves paralysis of cranial nerves (Volovskaya, 1990), while symptoms of encephalitic polio occur in the brain.

Apart from the three serotypes of poliovirus, other enteroviruses like echo viruses, coxsackie viruses and the numbered enteroviruses are known to cause paralysis (Khuri-Bulos *et al.,* 1984) but in low frequencies (Grist and Bell, 1984; Melnick, 1989b).

2.7 Progressive Post Poliomyelitis Muscle Atrophy

Since the nineteenth century, medical literatures have described the appearances of new weaknesses and muscle wasting in subjects many years after recovery from paralytic poliomyelitis (Campbell *et al.*, 1969; Jamison *et al.*, 1991; Melnick, 1989b). It is currently estimated that about 300,000 persons with the history of poliomyelitis who survived the last major polio epidemic of the 1950s are at risk of developing the progressive post polio muscle atrophy (PPMA) (Munsat, 1991). This usually begins 25-35 years after recovery from natural attack of paralytic poliomyelitis. PPMA is rare and causes of neurological deterioration in these patients frequently arise from the use of crutches or wheelchairs or from spinal deformity (Melnick, 1989b).

Pathologically PPMA is a disorder of the motor units in the nervous system, probably due to instability of previously damaged motor units during poliovirus infections. Melnick (1989) reasoned that the disorder could be as a result of the aging process, since it has been established that substantial loss of motor neurons and muscle fibres is common after the age of 55, even in people who have not suffered poliovirus infections.

2.8 Laboratory Diagnosis

Detailed description of principles and procedure for diagnosis of poliovirus infection have been described and published by Melnick (1985). The diagnosis rests on virus isolation, identification and the detection of type specific antibody responses (Melnick, 1989b).

2.8.1 Virus Isolation and Identification

The usual specimens are stool, rectal swabs and throat swabs. In severe cases, cerebrospinal fluid is used. Two of such specimens are collected on successive days (24-48 hours apart) as early as possible in the course of the disease to increase isolation rate, since virus shedding may be intermittent. Most specimens require pre-treatment before inoculation to remove gross debris and bacteria/fungi, which might interfere with virus isolation attempts. Faecal samples are treated with chloroform to which enteroviruses are resistant to remove potentially toxic lipids and help dissociate virus aggregates in addition to removing bacteria and fungi (WHO, 1997).

A 10% faecal suspension is made in a balanced salt solution (PBS) containing 100 units of penicillin with 10g of streptomycin/ml which is then centrifuged to remove coarse particles. The specimen extract is then used to inoculate the appropriate cell culture which is then and incubated at 36°C. Cell lines used for poliovirus isolation include RD cells derived from a human rhabdomyosarcoma and HE_P-2 (Cincinnati) cells derived from human epidermoid carcinoma (WHO, 1997).

If the virus is replicating, cytopathic effects are usually seen in the cells within 48 hours (Duguid *et al.*, 1978; WHO, 1997). Identification of poliovirus type is carried out by a neutralization test in which a measured dose of virus ($100TCID_{50}$) is exposed to the action of standard type-specific polyclonal antisera made in animals (Rabbits).

All types of infected materials are stored in the refrigerator at -20°C for further study and negative cultures are examined for a period of 14 days before being discarded. Any polio isolate may either be the wild type or the vaccine strain. They can be differentiated using, neutralization assays, polyclonal antibody test, monoclonal antibody panels, probe hybridisation, *in vitro* enzymatic gene amplification and Restricted Fragment Length Polymorphism (RFLP) (WHO, 1992).

2.8.2 Test for Antibodies

Paired serum samples are required. The first sample must be taken as early as possible in the course of the illness or infection and the second after an interval of 3 to 4 weeks (Dunguid *et al.*, 1978; Melnick, 1989b). The neutralization test is accurate and type specific and is at present the test commonly used for poliovirus antibody tests. Acute and convalescent sera are usually tested using various dilutions of serum against a constant amount of specific virus. Serum titres are calculated on the basis of the dilution of the serum that neutralizes a given amount of virus, and a four fold or greater rise in antibody titre is considered a significant indication of an infection during the period covered (Melnick, 1989b).

2.9 Epidemiology

Being enteric in their habitat and excreted for up to several weeks in faeces, polioviruses spread mainly via the faecal-oral route. Direct faecal contamination of hands, hence food or eating utensils, is probably responsible

for most case-to-case spread, especially under crowded conditions of poor hygiene and sanitation (White and Fenner, 1994). Uncommonly explosive epidemics have resulted from contamination of water supplies by sewage. Respiratory spread from the pharynx may also occur.

Children are generally more vulnerable to infection than adults; especially those between 5-7 years (Collis, 1961; Melnick, 1989b; WHO, 1997) and childhood paralytic poliomyelitis is twice as common in boys as in girls (Melnick, 1989b; Hajar *et al.*, 1983).

In spite of attention focused on the study of the epidemiology of the poliomyelitis, many of the salient epidemiological features of the infection have not been fully understood (Nathanson and Martins, 1979). However, one unique characteristic of poliovirus that has been uncovered over the years is its sporadic outbreak which results in an epidemic (Metselaar *et al.*, 1977).

Two major developments have greatly changed the epidemiology of poliomyelitis over the years. The first was the increase in the standard of hygiene and sanitation over the years and the second major influence has been the development and widespread use of a highly effective vaccines (White and Fenner, 1994).

It has been observed worldwide that improved hygiene and sanitation reduce the contact of children with wild poliovirus and subsequently reduce the spread of poliovirus by faecal-oral route. This limits the circulation of the virus and the incidence of infection in the community as a whole (Hajar *et al.*, 1983; White and Fenner, 1994). This has led to the increase in the average age of

primary infection and vulnerability (Stones, 1961; Nicholas *et al*, 1977) and has consequently shifted the age incidence of paralytic poliomyelitis to include young adults (Metselaar, 1976; Nicholas *et al.*, 1977; Nathasnson and Martins, 1979; Melnick, 1989b; White and Fenner, 1994; Collier and Oxford, 1998).

A frequently quoted statistic is that 1 in a 100 infections results in paralysis in older age groups, whereas only one in a 1000 occurs so in young children (Collier and Oxford, 1998) and it has been observed that the case fatality rate in older children may reach 5-10% (Brooks *et al.*, 1991)

However, by the age of two years, regardless of climate, geography or socio economic conditions, most children have already experienced several asymptomatic or mildly symptomatic polio infections (Melnick, 1989b) and by the age of 5 years over 50% have already been infected, while at age 25, practically all have experienced an infection (Cvjetanovic, 1982). The age distribution pattern has been consistent over the years and in tropical countries the age group most commonly affected still comprises those less than three years (Metselear, 1976; Guyer *et al.*, 1980). In Nigeria, Collis *et al.* (1961) found the maximum incidence of the disease in children between 1 and 2 years of age. Infants under one year of age are less susceptible than those in higher age group due to protection engendered by maternal antibodies (Melnick, 1989b; Robertson, 1993; WHO, 1997).

The development and widespread use of highly effective vaccines have reduced the incidence of paralytic poliomyelitis and the disease is no longer endemic in much of the world (Valente *et al*, 2000). It has been virtually

abolished in countries where a vigorous policy on immunization with OPV has been successfully pursued (White and Fenner, 1994).

Paralytic poliomyelitis still occurs in all countries of the world, but the annual incidence per million persons varies from a minute 0.026 in the USA to over 200 in certain African countries (Collier and Oxford, 1998).

Climate appears to be an important factor in the circulation and prevalence of poliomyelitis. In temperate climates infection with poliovirus occurs mainly during the summer and autumn, while in tropical climates virus circulation tends to occur all the year round with an increase during the rainy season (WHO, 1997). Seasonal rise in transmission has been observed by Yorke *et al.*, (1979) in their studies to coincide with the period of rise in temperature and humidity. Collis *et al.* (1961) have also observed a similar seasonal variation in transmission in the Southern part of Nigeria where they reported high incidence at the onset of rainfall between the months of February and April with the lowest incidence in September.

Throughout the world, there is a direct correlation between poor hygiene, poor sanitation, overcrowding and the acquisition of poliovirus infection and antibodies at an early age if not protected by immunization (WHO, 1997; Collis *et al.*, 1961). Therefore, socio-economic factors also play a significant role in determining the pattern of polio epidemics. It has been observed that children from families with poor socio-economic background are usually more susceptible at younger ages than other peers from economically buoyant homes.

It has been estimated that the number of officially reported poliomyelitis cases only represent 10% of the actual number of cases that are occurring (Nathanson and Martins, 1979; Collier and Oxford, 1998) and about 80% of poliomyelitis cases are caused by various genotypes within serotype 1 which is the most virulent and the most frequently isolated from patients with polio (Bell *et al.*, 1981; Cheesbrough, 1984). Type 2 is the least common and the least virulent (Imperato, 1974; White and Fenner, 1994).

2.10 Immunity

Immunity against poliomyelitis can be stimulated either through immunization with polio vaccines or by natural infection with poliovirus.

Upon infecting a susceptible host, a reasonable level of IgM and IgG neutralizing antibodies are often detectable 7-10 days after infection and sufficiently high level can block poliovirus entry into the CNS (Robertson, 1993). Thus, poliovirus infection provides lifelong immunity against the disease, but this protection is limited to the particular type of poliovirus (types 1, 2 or 3) involved (WHO, 2000c). Unfortunately, infection with one type does not protect an individual against infection with the other two types (WHO, 2000c).

Passive immunity is transferred from mother to foetus via the placenta and maternal antibodies protect infant for about 6 months (Imperato, 1974; Melnick, 1989b; Robertson, 1993).

The viral protein VP1 found in the capsid of the virus is the neutralizing epitope on the poliovirus (Hogle *et al.*, 1985) and it plays an important role in poliovirus antigenicity (Minor *et al.*, 1982; Robertson, 1993). VP2, and VP3 are also capable of inducing low levels of neutralizing antibodies while VP4 plays a vital role in enhancing virus attachment to receptor site on susceptible cells.

Antigenicity of poliovirus may be associated with the structural protein, because it has been observed that biochemical and immunological differences among the three serotypes occur more in the part of the genome encoding structural proteins (Romanova *et al.*, 1981).

Circulating serum antibody against poliovirus is not the only source of protection against the disease, secretory immunity provides protection against intestinal re-infection after recovery from a natural infection or after immunization with OPV (Ogra, 1984; Melnick, 1989b). The role of secretory antibody in the defence against infection by polioviruses is one of the reasons for the success of simultaneous mass immunization with OPV in interrupting the transmission of wild poliovirus (WHO, 1997).

Cell mediated immune response to poliovirus has also been reported to play a role in protection against intestinal re-infection (Leclere *et al.*, 1991). It is also known that the 3 serotypes are capable of inducing interferon production in infected tissues except the vaccine strain serotype 2 (Rytel, 1975; Andino *et al.*, 1999)).

2.10.1 ***Risk Factors***

It is not known why only a small percentage of infection lead to paralysis, but a number of factors may affect the potentials for infections with poliovirus or the severity of clinical poliomyelitis. These include immune deficiency, tonsillectomy, physical activity, age, injections, pregnancy and malnutrition. It has been observed that infection with poliovirus poses an increased risk for persons with primary B cell immune deficiency (Wyatt, 1973) and a decrease in resistance to poliovirus infection accompanies removal of tonsils and adenoids (WHO, 1997).

Excessive muscular fatigue during the prodromal period of infection may also predispose one to the paralytic form of the disease (Horstmann, 1950; Imperato, 1974). Intramuscluar inoculation has been observed to predispose young children to paralytic poliomyelitis (McCloskey, 1950; Collis *et al.*, 1961; Guyer *et al.*, 1980) with paralysis occurring mostly in the injected limbs and more frequently with caustic substances and multiple injections (Bradford-Hill and Knowelden, 1950; Bodian, 1954). Elevated vaccine-associated paralytic poliomyelitis risk is also caused by multiple intramuscular injections with antibiotics given within 30 days of onset of paralysis (Izurieta *et al.*, 1995).

Other host factors that influence the severity of poliovirus infection are age (particularly the very young), pregnancy and chronic malnutrition. Secretory IgA antibodies have been detected significantly less often at lower levels, and with delayed appearance in malnourished children (Chandra, 1981; Adeiga *et al.*, 1994).

2.11 Management Of PoliomyelitisTreatment:

There is no known specific treatment for poliomyelitis, but during the acute phase, management consists essentially of rest and sedation while refraining from active movement (Collis *et al.*, 1961). When paralysis occurs and fever has subsided, management involves the reduction of pain and muscle spasm by the use of moist heat, frequent changing of the position of the paralysed limb and physical therapy (Ajao and Oyemade, 1981; WHO, 2000a). Maintenance of hydration and respiration by using "iron-lungs" (respiratory tank) (Melnick, 1989b) or recently by positive pressure ventilator are employed in the event of respiratory insufficiency due to weakness or paralysis of chest muscles (WHO, 2000a). The limb must also be supported to avoid contracture (Jamison *et al.*, 1991).

Complete recovery can seldom be obtained and if permanent paralysis develops, surgery is the only alternative. However, residual lesions, like inequalities and/or lack of motion (ataxia) are not uncommon sequelae of surgery.

2.11.1 Management of Contacts

By the time the first case of poliomyelitis is clinically recognized, the virus is usually widespread among families and close contacts hence, their vaccination is of little value in terms of immediate control. The administration of human immunoglobulin (Ig) in a dose of 0.3ml/kg of body weight is of

considerable protective value if given within a few days after known exposure (Imperato, 1974).

2.12 Prevention and Control

2.12.1 Treatment of Waste and Sewage Water

Since the spread of the virus is from human to human via the faecal – oral – routes, maintenance of high sanitary condition in the environment to prevent food and water contamination is a necessary measure in poliomyelitis control. Effective water treatment especially where it is recycled is also important. Raw sewage should be treated effectively using bromine chloride and chlorine before discharging into receiving water (Keswick *et al.*, 1989). Investigations have revealed that chloramine-T can be viricidal against type 2 poliovirus under acidic conditions (Trieff *et al.*, 1981).

2.12.2 Use of Therapeutic Agents

Prior to and during the vaccine development period, a number of chemotherapeutic agents were investigated for their action on polioviruses. Tamm *et al.* (1952) reported that benzimidazole and its derivatives selectively inhibited poliovirus multiplication as reflected by its inability to produce cytopathic effect in susceptible tissue culture. Hydantoin has also been shown to inhibit polioviruses (Shimuzu *et al.*, 2000). The antiviral activity of guanidine on poliovirus has also been reported. Caliguiri and Tamm (1968) observed that guanidine inhibits RNA synthesis by specifically impairing the formation of SS

RNA and replicative intermediates during viral replication. Recent study by Shimuzu *et al.* (2000) has shown that guanidine hydrochloride specifically inhibits the function of the 2C protein and inhibits the growth of poliovirus at concentration of 0.1 to 2.0 mM and the target of this blockage is thought to be the initiation step of viral RNA synthesis.

2.12.3 Vaccination

Although improved sanitation and hygiene help to limit the spread of polioviruses, the only known specific means of preventing poliomyelitis is immunization with polio vaccines (WHO, 1997; WHO, 2000c). The development of effective vaccines to prevent paralytic poliomyelitis was one of the major medical break-throughs of the 20th century (WHO, 2000c) and vaccination has helped to reduce the incidence of infection (Wyatt, 1995) as well as increase the period between clinical cases of poliomyelitis (Eichner and Dietz, 1996).

Two different kinds of vaccines are available, an inactivated (killed) polio vaccine (IPV), developed in 1955 by Dr. Jonas Salk and a live attenuated (weakened) oral polio vaccine (OPV) developed by Dr. Albert Sabin in 1961. Both vaccines are highly effective against all three types of polioviruses. There are however, significant differences in the way each vaccine works (WHO, 2000c).

2.12.4 **Inactivated Polio Vaccine (IPV)**

This vaccine which was first licensed in the USA in 1955 and consists of viruses of all 3 antigenic types grown in monkey kidney tissue culture and inactivated by exposure to a low concentration of formalin (1/10,000 HCHO at 37°C for 10-12 days) (Stewart and Beswick, 1977). Inactivated polio vaccine needs to be administered parenterally and works by inducing protective circulating (humoral) antibodies in the blood thereby protecting the CNS against subsequent invasion by wild poliovirus (Melnick, 1989b).

However, IPV induces only very low levels of immunity to poliovirus in the intestinal tract and local secretory IgA antibodies and cell-mediated immunity is not induced. Hence, when a person immunized with IPV is infected with wild poliovirus, the wild virus can still multiply in the gut and shed in the stool, thus becoming a source of infection to others (Melnick, 1989b; WHO, 1997). Consequently, cases of polio outbreak among unvaccinated person have been reported in countries such as Netherlands, Sweden and Finland when such persons were exposed to the wild strain of the virus (Melnick, 1989b; Goldblum *et al.*, 1994).

Killed Salk vaccine contains no living virus, thus cannot mutate towards increasing virulence (Melnick, 1989b) and is known to trigger excellent response of the immune system in most IPV recipients (90%) (Banker, 1980; WHO, 2000). Antibody response to IPV is directly proportional to the dosage given (Salk, 1958) and therefore, repeated booster inoculations are required to maintain immunity with IPV (WHO, 1997).

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2.12.5 **Oral Polio Vaccine (OPV)**

Oral polio vaccine contains viruses grown in primary monkey cell culture or in human diploid cell culture and is stabilised by the addition of either magnesium chloride (*Wu et al., 1995*) or saccharose, with the former being used more often. Stabilized OPV can be stored at 0°C to 8°C for 6 to 12 months without significant loss of titre and up to two years at -20°C. OPV is cheap, administered orally and produces humoral antibodies in the blood to all 3 types of polioviruses thus protecting the CNS from subsequent invasion by wild poliovirus in the event of infection.

OPV infects, multiplies and immunizes in a manner that parallels natural infection (WHO, 1997) and in simulating the natural poliovirus infections, it confers long lasting (possibly life long) immunity in recipients (Mattion *et al.*, 1996; WHO, 2000c). It stimulates the production of IgG antibodies in the blood and as well as secretory IgA antibodies in the pharynx and mucus membrane of the intestine, the primary site for polio virus multiplication, which then becomes resistant to infection by wild virus (WHO, 1997).

Following administration of OPV, viral multiplication in the intestine takes place and excretion of vaccine virus in faeces is noticed after two weeks and subsequent serotype-specific neutralizing antibodies are detectable in the serum. This intestinal immune response is probably the main reason why mass immunization with OPV can rapidly stop person-to-person transmission of wild poliovirus.

The short-term shedding of vaccine virus in the stools of recently immunized children means that areas where hygiene and sanitation are poor and where the incidence of poliomyelitis is likely to be highest, immunization with OPV can result in the spread of OPV to unvaccinated persons within close contact (Kok *et al.*, 1992; WHO, 2000c; Fine *et al.*, 2000). Thus, OPV has been widely recommended and remains the vaccine of choice for the prevention of poliomyelitis in many countries (Kok *et al.*, 1992; Wood *et al.*, 2000).

The use of OPV through routine immunization or through mass immunization has resulted in a significant decrease in the number of poliomyelitis cases (Khuri-Bulos, 1984; Slonim *et al.*, 1995; Valente *et al.*, 2000). OPV is usually administered in three doses, OPV_1, OPV_2 and OPV_3 at the ages of 6, 10, and 14 weeks after birth respectively. A further dose given at birth otherwise known as OPV_{zero} (OPV_0) has also been introduced (Melnick, 1989a; WHO, 1997) to further enhance immune response in infants. Besides, when immunization against poliomyelitis is started at birth excellent seroconversion rates are obtained from the third dose onward (Weckx *et al.*, 1992).

Even though the use of OPV has been shown to be effective in the control of poliomyelitis in many countries and has helped significantly in reducing the incidence in tropical regions (Minor *et al.*, 1990), there have been reports of low rate of serological response (seroconversion) in children (Lee *et al.*, 1964; John and Jayabal, 1972; Domok *et al.*, 1974; Linkins *et al.*, 1995; WHO, 1996). Immunity induced by the conventional 3 doses of OPV has been incomplete, resulting in cases of paralytic poliomyelitis amongst recipients of 3 or more doses

of trivalent oral polio vaccine (TOPV) (Krishnan *et al.*, 1983; Deivanayagam, 1993). It is however, important to note that the degree of immunity obtained by an individual by vaccination varies greatly depending on the individual, and the quality and quantity of the vaccine (Brock and Madigan, 1988). The effectiveness of a vaccine however, depends on the vaccine being potent (Khrishnan *et al.*, 1983) and on its proper administration to individuals capable of responding (Orienstein *et al.*, 1985; Melnick, 1989a).

The poor seroconversion rates to TOPV in tropical countries have been associated with a break in cold chain, (Melnick, 1989a; Patriarca *et al.*, 1991; Wu *et al.*, 1995) resulting in loss of vaccine potency from exposure to heat (WHO, 1997), possible interference by other micro organisms (Cheesbrough, 1984), and enteroviruses in the intestinal tract of vaccinees (Sabin *et al.*, 1960; Ramos-Alvarez, 1967; Metselaar *et al.*, 1977; MasLago *et al.*, 1994; WHO, 1997) and other inhibitors present in the saliva and intestinal secretions (WHO, 1997).

However, studies by John and Jayabal (1972) could not detect any significant difference in seroconversion rates in children, whether or not found infected with viruses in their studies but they attributed these poor seroconversion rates to the perennial problem of malnutrition and under nourishment in the tropics. The high frequency of diarrhoea, bacterial, viral and parasitic infections of the gastro intestinal tract found in many children in the tropics could perhaps also lower their potential for intrinsic defence against pathogens and hence affect their seroconcersion rates (poliomyelitis

Commission of the Western Region, Ministry of Health, Nigeria, 1966; John and Jayabal, 1972).

Improper storage of vaccines, which could lead to loss in immunogenicity, coupled with the relatively hot climate and poor sanitary standards could also be responsible for poor seroconversion.

Recent studies have demonstrated that breast-feeding and high levels of maternal antibodies reduce the rate of seroconversion (Kok *et al.,* 1992; Osei-Kwasi *et al.,* 1995; WHO, 1996). Poor seroconversion rates are lowest for type 3 viruses, followed by type 1 in developing countries (Robertson, 1993). This may be due to type 2 vaccine virus and enteric pathogens interfering with the response to types 1 and 3 and may be also due to the time interval between doses. Continuing intestinal infections could also potentially interfere with the immune response to subsequent doses

The lower than expected rates of seroconversion to more than 3 doses of TOPV in the tropics resulting in incomplete immunity (Deivanayagam, 1993) has consequently led to unacceptable high incidence of paralytic poliomyelitis in vaccinated recipients (John, 1976; Bass *et al.,* 1978; Oduntan *et al.,* 1978; Krishnan *et al.,* 1983, WHO, 1997) and to outbreaks in highly vaccinated populations (John, 1976; Ramia and Arif, 1991; Sutter *et al.,* 1991). Countries that have been practically free of poliomyelitis for many years with high overall community vaccination level have also reported the occurrence of polio outbreaks (Subrahmanyan *et al.,* 1973; Kim-Farley *et al.,* 1984; Bóóthing *et al.,* 1990; Ismail and Lal, 1993). Thus, the absence of paralytic poliomyelitis in a

country does not imply eradication of wild paralytic poliovirus from circulation in the population of a country (Koza, 1996).

The main cause of an outbreak and the most important risk factor for paralytic poliomyelitis remains the failure to vaccinate rather than vaccine failure (Kim-Farley *et al.*, 1989; Robertson *et al.*, 1994; Deivanayagan *et al*, 1993). The recent massive outbreak of poliomyelitis in May 1999 caused by wild poliovirus type 3 in Angola reported by Valente *et al. (2000)* reiterates the above fact.

2.13 Vaccine Associated Paralytic Poliomyelitis (VAPP)

Although OPV is safe and effective, the live attenuated vaccine virus in OPV can cause paralysis (WHO, 2000c). One disadvantage associated with the sabin vaccine over the years is the tendency of the attenuated virus to regain neurovirulence in extremely rare cases, thus becoming pathogenic upon replication in humans and causing paralysis either in the vaccinated child or in close contacts (Sutter *et al.*, 1991, Kok *et al.*, 1992; Andrus *et al.*, 1995; WHO, 2000c). This has been attributed to mutations occurring in the attenuated virus as a result of serial passage through the human intestinal tract (Kok *et al.*, 1992) when transmitted from individuals to whom they are administered to others. It has also been observed that within a year, the genetic make-up of poliovirus can change by 2% (WHO, 2000h). Immune deficiency among recipients may also be among the causes (Brooks *et al.*, 1991; Andrus *et al.*, 1995). VAPP is more often associated with poliovirus type 3 than poliovirus types 1 and 2 (WHO,

1997; Robertson, 1993). Type 2 is associated with paralysis mostly among contacts of cases (Robertson, 1993).

Fortunately, the risks of Paralytic poliomyelitis associated with reversion of OPV are exceedingly rare. Based on several studies conducted, including a 10-year collaborative study conducted by WHO, it was estimated that one case of VAPP has occurred for every 2 to 4 million doses of TOPV distributed (Andrus *et al.*, 1995; WHO, 1997).

However, the relative frequency of paralysis associated with the first dose in the OPV series is one case per 700,000 doses compared with one case per 6.9 million subsequent doses (Robertson, 1993). This suggests that as OPV coverage level increases for infants who are likely to attain protection while they are still under the umbrella of maternal antibodies, the incidence of VAPP in recipients can be expected to decline.

12.14 ***Combined Immunization Schedule With OPV/IPV***

Even though Oduntan *et al.* (1978) and Khrishnan *et al.* (1983) reported high seroconversion rates following vaccination of infants in Nigeria and India respectively with IPV and thus recommended the use of the salk vaccine in tropical countries, it has been found that combined immunization schedule with OPV/IPV is highly effective and gives excellent seroconversion rates (WHO, 1996). Besides, socio-economic status or levels of maternal antibodies do not affect the response.

This combined immunization schedule with IPV/OPV has been found to be highly effective in high risk localities where re-importation of wild virus into such countries serves as a repeated challenge to the immunity of young children (Melnick, 1988; Rumke *et al.,* 1995). This schedule has helped to control poliomyelitis in Denmark, Gaza and West Bank areas of Israel (Goldblum *et al.*, 1994; WHO, 1996; Collier and Oxford, 1998). Therefore, combined OPV/IPV immunization schedule has been highly recommended by WHO and may offer countries that are well advanced in poliomyelitis eradication an opportunity to close the remaining gap in immunity, an important consideration when wild polio virus importation remains a threat until global eradication is achieved (WHO, 1996).

12.15 **Eradication**

Poliomyelitis can be eradicated because it only affects humans with no animal reservoir, and there is an inexpensive vaccine (OPV) that confers life long immunity to it (Aylward *et al., 2000*; WHO, 2000e). In addition to the above, the virus cannot survive for long outside the body and there is no long-term carrier for the virus.

The World Health Assembly adopted the goal of eradicating poliomyelitis by the end of the year 2000 in 1988. The strategy is based on the premise that poliovirus will cease to exist if it is deprived of its human host through immunization, a strategy that is similar to that used for small pox eradication in 1977 (WHO, 2000e). The first region to be certified polio-free in 1994 was that of the Americas, which provided the technical feasibility of polio eradication

(Macedo and Melgaard, 2000). The technical feasibility of polio eradication is well established and the operational feasibility of achieving it has been demonstrated under every circumstance imaginable (Aylward *et al.*, 2000).

Four principal strategies have been central to the success of poliomyelitis eradication initiative and they include high routine immunization programmes, supplementary immunization in the form of NIDs, effective surveillance of AFP and house-to-house "Mop-up" operations (Hull *et al.*, 1994; Aylward *et al.*, 2000; WHO, 2000d). These strategies have been introduced into every country of the world regardless of geography, climate, culture, civil strife or outright war.

The global initiative for polio eradication has been extraordinarily successful in interrupting the transmission of the virus (Tangermann *et al.*, 2000) and poliovirus has disappeared rapidly from large areas of the world (Alyward *et al.*, 2000). The number of poliomyelitis cases has fallen by over 95% around the world, from an estimated 350,000 in 1988 to an estimated maximum of 20,000 in 1999 with the elimination of wild polio virus from three of the five continents where it was endemic at the onset of the initiative (Alyward *et al.*, 2000). However, it is estimated that the true number of cases in some countries is five to ten times more than reported (Robertson *et al., 1994;* WHO, 1999).

The geographical extent of the remaining poliovirus circulation continues to shrink. At the end of 1999, wild poliovirus was circulating in a maximum of 30 countries primarily in Sub-Saharan Africa and South Asia (Fox, 1998; Alyward *et al.*, 2000; WHO, 2000a). Some technical factors that present obstacles to the eradication of poliomyelitis in Sub Saharan Africa and South East Asia include,

primitive transportation (Fox, 1998), financial and delivery system barriers (Melnick, 1989ba), information barrier (Atkinson and Cheyne, 1994; Mitchel and Franco, 1995), technical aspect of supply, vaccine efficacy and cold chain technology (Evans, 1980; Patriarca *et al.*, 1991).

The non-technical factors that pose challenges to the eradication of poliomyelitis and must be tackled in order to meet the goal of eradicating poliomyelitis include, the problem of poor seroconversion after vaccination with OPV in tropical regions, the circulation of the wild type strains in the tropics in spite of vaccination as observed by John and Jayabal (1972) and the current and recent armed conflicts in war torn countries which leaves limited space for health or humanitarian interventions (Bush, 2000). War is one of the greatest challenges to polio eradication (Tangermann *et al.*, 2000), and must be tackled in order to meet the goal of eradicating poliomyelitis from the face of the planet (Bush, 2000).

The ultimate success of eradication of poliomyelitis now hinges on 3 factors (Alyward *et al.*, 2000). First, the gains made to date must be rapidly consolidated through an acceleration of the programme, particularly in the ten global priority countries. In view of this the current level of supplementary immunization activities doubled in the five densely populated countries that are considered to be global reservoirs for poliomyelitis and account for more than 70% of the world's cases. The capacity to implement eradication strategy fully and consistently must be sustained in conflict affected areas (Tangermann *et al.*, 2000) In view of this the current level of supplementary immunization activities

doubled in the five densely populated countries that are considered to be global reservoirs for poliomyelitis and account for more than 70% of the world's cases.

Second, additional financial resources will be essential for implementing the acceleration called for by the WHA in May 1999. Finally, it will be essential to maintain and indeed enhance commitment from the highest possible level (polio news, 1999) among United Nations Agencies, poliomyelitis endemic countries and donor nations. The post eradication era presents new challenges such as a massive effort to ensure the safe containment of laboratory stocks of wild polioviruses, a final surveillance push prior to global certification and implementation of a globally coordinated strategy to stop OPV immunization once and for all (Alyward *et. al.*, 2000).

12.16 ***Introduction to Methodology***

There are numerous serological techniques used to diagnose viral infections. These techniques may be used to accurately identify an unknown virus isolate utilizing a known reference antibody, or may utilize a panel of known viral antigens in order to test for a viral antibody.

Serologic techniques presently in use include Complement Fixation, (CF), Neutralization (Neut), Passive Hemagglutination (PHA), Hemagglutination Inhibition (HI), Indirect Fluorescent Antibody (IFA), Immune Adherence Hemagglutination (IAHA), Immuno Electron Microscopy (IEM), Counter Immuno Electrophoresis (CIE), Radio Immuno Assay (RIA), Enzyme Linked Immuno

Sorbent Assay (ELISA) Flourescent Focus Inhibition (FFI), Anti Complement Immuno Fluorescence (ACIF) and S ngle Radial Haemolysis (SRH) (Drew *et al.*, 1990). Each of these tests can be adapted to demonstrate antibody response or to identify a particular agent. The applicability of each test depends on the nature and type of virus being diagnosed. The characteristics to be considered before selecting one of the above tests include sensitivity, specificity, simplicity, expense and time required (Burleson *et. al.*, 1992).

The complement fixation test is a highly satisfactory test used to demonstrate members of some virus groups that possess common antigens such as Adenoviruses, Influenza A viruses and Influenza B viruses (Drew, 1990).

The HI test can be performed with a variety of viruses that have the capacity to agglutinate selective red blood cells of various animal species. It has been used to detect certain serotypes of coxsackie and echoviruses in the enterovirus group but not useful in detecting polio antibodies because the virus has no hemagglutinating property.

Other serological tests such as agglutination, immune precipitation and ELISA can be used to detect polio virus antibodies but have not been found to be generally suitable for polioviruses as they measure both neutralizing and non-neutralizing antibodies. The value of the latter in protection against poliomyelitis is unknown, and may be irrelevant (Robertson, 1993; WHO, 1997).

The neutralization test can be performed with viruses which neither hemagglutinate nor react in CF tests. This test has been recommended for the sero surveillance of immune antibodies against polioviruses (WHO, 1997) and

the measurement of serum-neutralizing antibody was described by Halsey and Galazka (1985) to be one of several ways of assessing immunity against poliomyelitis. The serum-neutralizing test is considered the most specific method for detecting the protective antibody response to poliovirus infections (Robertson, 1993) and it is used to identify epidemiological important immunity gaps in the population as well as to measure vaccination response (WHO, 1997).

Neutralization assays are based on the principle that viral antibody will bind specifically to the virus with a resultant neutralization of viral infectivity in an indicator host system. Neutralization of viral infectivity therefore correlates with protection from disease (Duguid *et al.*, 1978; Burleson *et al.*, 1992). The viral neutralization assay is sensitive and specific, but also more complex, time consuming and expensive compared to the other assays (Robertson, 1993).

There are two methods of performing neutralization tests; the constant-serum-varying virus concentration which is also known as the alpha procedure, and the constant-virus-varying serum dilution or beta procedure (Rovozzo and Burke, 1973; Grist *et al.*, 1979).

The beta method is more generally used in tissue culture and in diagnostic virus laboratories because it is more economical in the use of sera and it gives a greater delicacy of test. It also measures a broader range of antibody titre (Rovozzo and Burke, 1973) and allows the antibody level to be expressed as a 'titre' (Grist *et al.*, 1979).

In the beta procedure, two fold serial dilutions of the inactivated serum are made in the wells of microtitre plates containing maintenance medium, which serve as diluents. A constant volume of virus suspension containing 100 tissue culture infective dose (100 $TCID_{50}$) is added to all wells on the plates except the serum control wells. The plates are incubated at 36°C for 3 hours. After the period of incubation, appropriate susceptible cell suspension containing about 1-2 x 10^4 cells per 0.1 ml of growth medium is added to all wells on the plates. This concentration should produce confluent monolayer in the microtitre plate wells within 24-48 hours (WHO, 1997).

The plates are wrapped in thin foil or cling film and incubated at 36°C for five days. The plates are observed daily beginning from the 3rd day. An inverted microscope is used to check for cytopathic changes in the wells that have no neutralizing antibodies. Controls are set up using reference sera.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Area of Study

This study was carried out using blood samples collected from randomly selected primary school children in Kaduna State of Nigeria. Kaduna State is one of the thirty-six (36) states of Nigeria and has an area of 46,053 square kilometres. It is situated between longitudes 06.00 to 09.00 east of the Greenwich Meridian and between latitude 09.00 to 11.00 north of the equator. The state shares borders with Sokoto, Niger, Katsina, Kano, Bauchi and Plateau States. To the southwest, the Federal Capital Territory, Abuja, borders the state. Kaduna state has a total number of 23 local government areas with Kaduna city (town) as the state capital (Ministry of Health and Social Development, Kaduna State, 1995). Kaduna State has a 1998 total projected population of 4, 872,185 with a population of 389,775 under 5 years old and 678,585 representing children 5-10 years old.

There are a total of 1,614 primary schools in the state with an enrolment of 693,655 comprising 413,092 males and 280,563 females (Primary Health Care Development, Ministry of Health, Kaduna State, 1999).

Eleven of the local government areas (LGAs) comprising of Chikun, Igabi, Jema'a, Kagarko, Kauru, Kubau, Kaduna North, Lere, Sabon gari, Zaria and Zangon Kataf in Kaduna State of Nigeria were randomly selected for the study.

In each of these LGAs, two schools each within and outside the local government headquarters were randomly selected.

Pupils in each primary school were selected at random across age, sex, and socio-economic status. Most of the children selected were in the age range of 1 to 10 years, with very few exceptions above the upper limit. Questionnaires were prepared to obtain information on the age, sex, vaccination history and socio-economic status of the selected subjects. About ninety one percent (91%) of the questionnaires issued out were returned. In addition, blood samples were collected from 1,200 respondents, but one thousand eighty seven (1087) samples from which clear sera could be obtained comprising Kubau (93), Chikum (119), Igabi (127), Jema'a (92), Kaduna North (139), Zaria (100), Zangon Kataf (120), Lere (117), Kagarko (86), Kauru (54) and Sabon gari (40) LGAs were analysed for the presence of neutralizing antibodies against poliovirus in the sera samples.

3.2 Collection of Blood Samples and Separation of Sera

Blood samples were collected from the study subjects from September to December 1999. Approximately five millilitres (≈5ml) of blood sample was obtained from each of the nursery and primary school pupils enrolled for the study by venepuncture using sterile disposable 5ml-capacity syringe fixed with a 21 gauge (0.81mm) needle. Blood samples were collected into sterile blood collection tubes or vacutainers, allowed to clot in a slanted position at room temperature and separated 6-10 hours after sampling in most cases. Where it was not possible, the blood was refrigerated at 4°C overnight and separated

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within the following 24 hours. The serum was separated from the clot after centrifugation at a low speed of 1000rpm for 10 minutes or at 500g for 10 minutes.

The serum was aseptically separated into a sterile Eppendoff tube by aspiration with a sterile Pasteur pipette and immediately stored at -20°C. The sera separation was handled in a safety cabinet.

3.3 Materials Used for the Study

3.3.1 Test Sera and Reference Sera

The sera were stored frozen at –20°C until they were analyzed at the National Polio Laboratory, Ibadan.

3.3.2 Poliovirus Challenge Sample

The poliovirus used for the neutralization test was obtained from the National Polio Laboratory, Ibadan. The isolates were adapted to HEp-2 (Cincinnati) cell line by three passages, but five passages were required to build up serotype 2 to the titre required for the assays.

3.3.3 HEp-2 (Cincinnati) Cell line

This cell line derived from human epidermoid carcinoma was used as the indicator system for the performance of the serum neutralization test and was provided by the National Polio Laboratory, Ibadan.

The susceptibility of HEp-2 and L20 cell lines were compared and found to be similar. However, the L20 cell lines were less stable than the HEp-2 cell lines. Though WHO (1997) recommends the use of only HEp-2 cell lines in the neutralization test for polio antibodies the relatively new L20 cell lines may be used it the HEp-2 cell lines are not available.

3.3.4 Media and Reagents

These include, phosphate buffered saline (PBS), Hanks basic salt solution (Hanks' BSS), sodium bicarbonate solution, Eagles growth medium and maintenance medium, (Sigma Chemical Company, U.S.A) penicillin and streptomycin antibiotics, trypsin and versene cell dispersing agents and crystal violet.

The stock solutions of the media and reagents were prepared according to standard specification. The composition of the media and reagents used in the analysis are presented in appendix 7.

3.4 Preparation of Hep-2 (Cincinnati) Cell Suspension

A healthy confluent monolayer of the cell line was propagated in a $25cm^2$ cell culture flask containing growth medium. The growth medium was decanted from the culture flask and the monolayer was gently washed twice with PBS (without magnesium and calcium ions) and pre-warmed to $36^{o}C$ to remove debris. The washed monolayer was evenly dispersed through the addition of 0.5ml of 0.25% working solution of trypsin-ersene in PBS, which was pre-

warmed to 37°C. The flask was then incubated for 2 minutes at 36°C until the cells were detached from the surface. The cells were checked for detachment using an inverted microscope. The flask was tapped a few times by the side to assist the dislodgement of cells from the flask surface. To the suspension, 0.4ml of growth medium was added to halt the action of trypsin. The suspension was then aspirated a few times through a fine Pasteur pipette to break up cell clumps after which the cells were re-suspended in growth medium.

The accurate number of cells in the suspension was calculated by carrying out a viable cell count using the dye exclusion test (improved Neubauer) in which 0.1ml of a 1:10 suspension of the cells in 0.9ml trypan blue was counted in a Neubauer hemocytometer. A cell suspension containing about 1-2 x 10^4 cells/0.1ml was prepared in growth medium and used in the tests.

3.5 Preparation of Poliovirus Stocks

The poliovirus used for the analysis was propagated by inoculating 1ml of the prototype poliovirus containing the three serotypes at $TCID_{50}$ into a bottle of cell culture containing a confluent healthy monolayer of HEp-2 cell lines. Prior to the inoculation, the culture bottle was carefully decanted to remove the growth medium and then rinsed in PBS (without calcium and magnesium ions). The virus was allowed to adsorb to the cell monolayer for 1 hour at 36°C.

Sufficient amount (about 15mls) of maintenance medium was then added to cover the cell layer and the bottle was incubated at 36°C and observed daily under an inverted microscope until almost complete cytopathic effect developed

on the cells. When cytopathic changes had occurred in about 75% of the cells, the flask was removed and stored at -70°C.

The virus was harvested by two cycles of freezing and thawing of the culture fluid (to release the virus in the suspension) and centrifuged at 2000rpm for 10 minutes or 500g for 20 minutes. The supernatant containing the stock poliovirus suspension was removed and 1-2ml each was distributed into sterile clearly labelled Eppendoff tubes or screw capped vials and stored at -70°C until required for titration. The same procedure was used for the preparation of stock virus suspension of each of the three serotypes of the poliovirus

3.6 Titration of Challenge Virus Stocks

The challenge virus stocks were titrated using the micro technique procedure described by WHO (1997). A serial ten-fold dilution of the virus stock was made from 10^{-1} to 10^{-8} in sterile Khan tubes using maintenance medium as diluent.

Appropriately labelled culture tubes containing previously prepared healthy monolayer cultures of HEp-2 cell lines were gently decanted to remove the growth medium. About 1ml of each of the viral dilutions was added to four culture tubes each (in order to expose the virus to the cell), followed by the addition of 0.1ml of maintenance medium to each of the culture tubes. On the other hand 0.2ml volume of maintenance medium was added to four tubes, which served as controls. All the tubes were covered, incubated at 36°C the

stationary slope position and observed daily using an inverted microscope until cytopathic changes fully developed in the incubated tubes.

The tubes of each virus dilution were scored on CPE (1+ to 4+)/no CPE (0) basis after the incubation period. The 50 percent tissue culture infective dose ($TCID_{50}$)(the highest dilution of the virus that produced CPE in 50% of the inoculated cell cultures), was calculated using the Kärber formula (Log $TCID_{50}$ = L-d(s—0.5) where L = Log of lowest dilution used in the test, d=difference between log dilution steps and s = sum of proportion of "positive" tests (i.e., cultures showing CPE). The results of the titration of the reference prototype poliovirus is presented in table 1.

Table A: **Result of virus titration in cell culture tubes**

Virus dilution	Proportion of cultures infected
10^{-1}	4/4=1
10^{-2}	4/4 = 1
10^{-3}	4/4 = 1
10^{-4}	4/4 = 1
10^{-5}	4/4 = 1
10^{-6}	2/4 = 0.5
10^{-7}	2/4 = 0.5
10^{-8}	0/4 = 0

Applying the formula:

Log $TCID_{50}$ = L – d (s – 0.5)

Log $TCID_{50}$ = -1 – 1 (6 – 0.5)

= -6.5

Log $TCID_{50}$ = $10^{-6.5}$ = Log $TCID_{50}$ (endpoint dilution). The $TCID_{50}$ titre is more correctly expressed as $10^{6.5}$ per inoculum's volume.The titre of the prototype or reference trivalent poliovirus used was thus $10^{6.5}$/0.1ml using the Kärber formula.

The above procedure was also used for the titration and preparation of 100$TCID_{50}$ virus stock of the three serotypes of the poliovirus used in the neutralization test. PV_1, PV_2 and PV_3 were used at $TCID_{50}$ titres of 10^{-4}, 10^{-2} and 10^{-3} respectively.

3.7 Serum Neutralization Test

The neutralization antibody titres against poliovirus types 1, 2 and 3 were measured by means of the modified micro neutralization assay described by Richardson *et al.* (1995) as recommended in the manual for the virological investigation of polio (WHO, 1997) with slight modifications described below.

3.7.1 Screening Test

A 1:10 dilution of each of the test serum was prepared in maintenance medium and inactivated at 56°C for 30 minutes. Growth medium on previously prepared monolayer HEp-2 cell lines containing 1-2 x 10^4 cells/0.1ml in flat bottom microtitre plates (Microtiter ® System, U.K) were removed using a

multichannel pipette fitted with disposable tips. About 0.05ml of each of the inactivated serum was transferred in to a pair of wells in the plate except the virus back titration well and serum control wells. In addition 0.05ml of a known reference serum-containing antibody to the three serotypes was added to the serum control wells.

About 0.05ml of the prototype reference virus suspension containing 100$TCID_{50}$ was added to all wells except the control and virus back titration wells. The plate was lightly tapped at the edge and incubated for 1 hour at 36°C,after which 0.1m of maintenance medium was added to all the wells.

The plate was sealed with a non-toxic plate sealer, incubated at 36°C for 5 days after which, the plate was observed for CPE. The plate was then stained with crystal violet and read visually. Sera in wells that did not show CPE after the period of incubation were considered as having demonstrable (neutralizing) levels of antibody to the poliovirus serotype. The presence of stained cells indicated the presence of antibody while the absence of such cells showed lack of antibody in the serum.

3.7.2 Serum Toxicity Test

This was set up along with the neutralization test to ensure that the test samples were not toxic to the cell lines used. The procedure described for the screening test was used except that poliovirus suspension was not added to any of the wells and the plates were incubated for seven days(instead of five) and observed daily using an inverted microscope.

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3.7.3 Titration of Antibody (Neutralization Test)

A serial two-fold dilution of each of the test serum was made in the wells from rows 1-10 of a microtitre plate with 0.025ml transfers in maintenance medium diluent. The transfer for each sample was made from row A to row H and each transfered until the serum dilution ranged from 1:10 to 1:640. A calculated amount of the challenge virus suspension was prepared at $100TCID_{50}$ in maintenance medium for the number of sera to be tested.

About 0.025ml of the suspension of PV_1 serotype at $100TCID_{50}$ was added to all wells except wells H_1-H_{12} that received 0.05ml of maintenance medium. To rows 11 and 12 serving as antiserum control wells, about 0.025ml of known antiserum dilution of PV_1 serotype antiserum were added.

A back titration of the suspension of the PV_1 serotype from $100TCID_{50}$ was made in wells H_1-H_7 to check the titre of the test virus. The plate was gently agitated to ensure complete mixing of serum-virus or virus-diluent mixtures, covered with perplex lid, and incubated at 36°C for 3 hours.

During this time a HEp-2 cell suspension was prepared in growth medium to contain approximately 1-2 x 10^4 cells/0.1ml and 0.1ml of the cell suspension was added to all the wells in the plate using a multichannel pipette fitted with sterile disposable tips.

The above procedure was repeated using PV_2 and PV_3 as challenge virus strains respectively. All tests were carried out in duplicates and appropriate controls were included in the test run as in above.

The plates were sealed with adhesive sealers, covered with lids, wrapped in cling film and then incubated at 36°C for five days. They were observed daily until CPE was noticed in the virus control wells. The presence or absence of CPE was then recorded. The level of serum neutralizing antibody present in each sample was expressed as the reciprocal of the lowest dilution of the serum at which antibody was detected (titre). For example, if antibodies are detected at a dilution of 1:10 then the titre is 10.

3.7.4 Statistical Analysis

The results of the neutralization tests on the blood samples from the primary school children in the various LGAs were analysed on the basis of age, sex and number of vaccine doses received by vaccinees.

Type specific geometric mean titres (GMTs) of the antibodies were calculated using the following relationship: GMT = antilog [(log X_1+log X_2+ log X_3 ...)/n] as stated by Harper, (1991). Chi-square tests were used to detect statistically significant differences in percentages and GMTs obtained for all the comparisons.

CHAPTER FOUR

4.0 RESULTS

4.1 Results of Analysis of Questionnaire

Results of the analysis of questionnaires based on the information provided by parents of the children on their history of vaccination showed that a total of 926 (85.2%) of the 1087 children enrolled in the study had a history of vaccination i.e., have received one or more doses of OPV while 161 were not vaccinated. Of the 161 with no history of vaccination, 77 (47.8%) had demonstrable antibodies to poliovirus. And of the 77 (47.8%) that had demonstrable antibodies to poliovirus, 56 (72.6%) had antibody to just one serotype, while 12 (15.6%) and 9 (11.7%) had antibodies to two serotypes and three serotypes respectively.

Amongst the 926 (85.2%) children with a history of vaccination, 128 (13.8%) had no demonstrable antibodies to the poliovirus while 798 (86.2%) had demonstrable antibodies to it.

4.2 Results of Neutralization Test

These serological results were supported by questionnaire information on the history of vaccination provided by parents of the children enrolled in the study. The neutralization results were examined and an index of seropositivity or prevalence to poliovirus antibody was taken as the number of children with demonstrable antibodies to at least one of the polio serotypes, while index of

susceptibility to polio was taken as lacking demonstrable antibody at the lowest dilution tested (1:10) with the results presented in percentages.

The results of the initial screening test carried out on the serum samples showed that 875 (80.5%) of the 1087 primary school children had demonstrable levels of antibodies to at least one of the polio virus serotype. The other 212 (19.5%) subjects had no demonstrable antibodies to any of the three poliovirus serotypes. The total prevalence rate was therefore 80.5% while the total susceptibility rate of the children to poliovirus infection in Kaduna State was 19.5% (Table 1). The seropositivity ranged from 74.0% in Zaria LGA to 87.2% in Kagarko. Zaria LGA had the highest susceptibility rate with the value (26.0%) twice that of Kagarko (12.8%) which had the lowest number of children susceptible to poliovirus (Figure 1).

Table 1: Number and percentages of children seropositive for one or more poliovirus types and their susceptibility rate in the various LGAs of Kaduna State, Nigeria (at a titre ≥ 10).

Subjects Primary School Children (LGA)	Number of sera samples tested	Number of seropositive (percentage)	Number Seronegative-susceptibility rate (percentage)
Kubau	93	70 (75.3)	23 (24.7)
Chikun	119	99 (83.2)	20 (16.8)
Igabi	127	96 (75.6)	31 (24.4)
Jema'a	92	75 (81.5)	17 (18.5)
Kaduna North	139	115 (82.7)	24 (17.3)
Zaria	100	74 (74.0)	26 (26.0)
Zangon Kataf	120	97 (80.8)	23 (19.2)
Lere	117	96 (82.1)	21 (17.9)
Kagarko	86	75 (87.2)	11 (12.8)
Kauru	54	47 (87.0)	7 (13.0)
Sabon Gari	40	31 (77.5)	9 (22.5)
Total	1087	875 (80.5)	212 (19.5)

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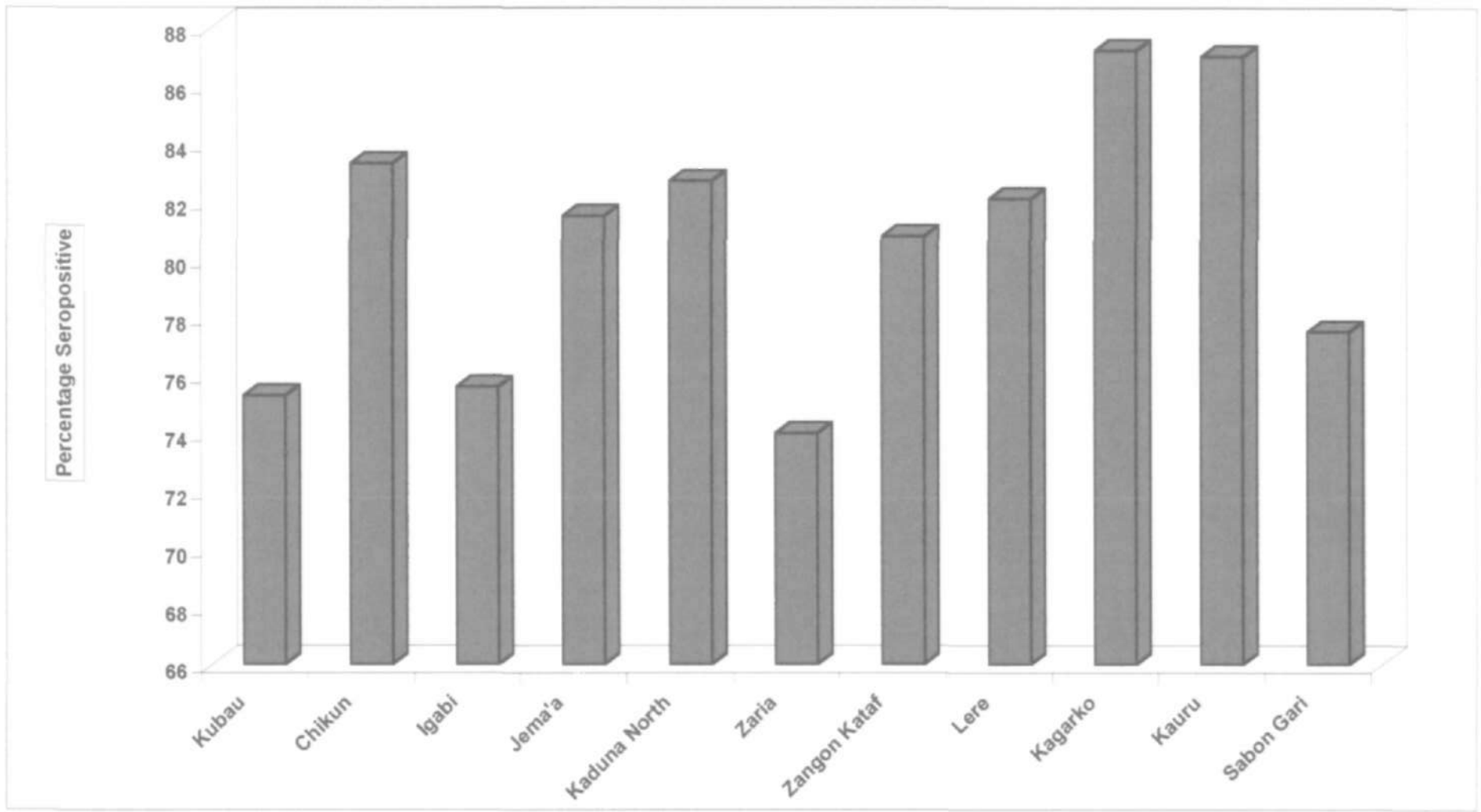


Fig. 1: Percentage distribution of poliovirus neutralizing antibodies (at Titre ≥ 10) among primary school children in Kaduna State,Nigeria.

The results of the antibody titration indicated that 712 (65.5%), 776 (71.4%) and 725 (66.7%) of the children had neutralization antibodies to poliovirus types 1, 2 and 3 respectively (Table 2). The titre ranged from 10-640 for all the 3 serotypes in children in Kaduna State.

The number of primary school children who were seronegetive to the three serotypes at the lowest dilution at which the sera were tested (1:10) were calculated and the susceptibility rate was found to be 34.5%, 28.6% and 33.3% for types 1, 2 and 3 respectively.

The cumulative GMT for each serotype in the children were calculated and found to be 37.6, 48.2 and 50.2 for serotype 1, 2 and 3 respectively. Serotype 1 had the lowest value while the other two had values very close to each other.

Table 2: Percentage distribution of antibodies to poliovirus by type (at a titre ≥ 10) and geometric mean titres among children in the various LGAs of Kaduna State, Nigeria.

Subjects / Primary school children (LGA)	Number of sera samples tested	Serotype 1		Serotype 2		Serotype 3	
		Percent positive	GMT	Percent positive	GMT	Percent positive	GMT
Kubau	93	63.4	45.5	66.7	49.5	63.4	48.8
Chikun	119	68.1	38.6	69.7	53.3	68.9	65.9
Igabi	127	61.4	28.5	63.8	39.7	58.3	37.8
Jema'a	92	69.6	48.6	72.8	49.2	70.7	60.0
Kaduna North	139	74.1	36.9	75.5	41.3	70.5	46.4
Zaria	100	58.0	38.1	66.0	49.9	69.0	73.1
Zangon Kataf	120	67.5	47.1	68.3	42.4	71.7	42.3
Lere	117	61.5	29.7	77.8	60.8	61.5	51.9
Kagarko	86	66.3	32.9	84.9	60.7	61.6	42.7
Kauru	54	64.8	26.4	70.4	38.6	74.1	38.0
Sabon Gari	40	60.0	59.9	70.0	46.4	67.5	55.8
Total	1087	65.5	37.6	71.4	48.2	66.7	50.2

The seropositivity ranged from 58.0% in Zaria LGA to 74.1% in Kaduna North LGA for serotype 1, 63.8% in Igabi LGA to 84.9% in Kagarko for serotype 2 and 58.3% in Igabi to 74.1% in Kauru LGA for serotype 3. For serotypes 2 and 3, Igabi LGA had the lowest prevalence rate hence, the highest number of children susceptible to types 2 and 3 poliovirus infection. Children in Zaria LGA were more susceptible to poliovirus type1 and children in Kaduna North LGA were least susceptible to this serotype. Children in Kauru and Kagarko LGAs were least susceptible to serotype 3 and 2 respectively (Figure 2).

The GMTs were calculated for the three serotypes in the LGAs and were found to be lowest for type1 in all the LGAs except Zangon Kataf and Sabon gari LGAs where the GMTs were higher. For serotype 2, Kubau, Igabi, Lere, Kagarko and Kauru LGAs had the highest GMTs while the remaining LGAs had the lowest values. Chikun, Jema'a, Kaduna North and Zaria LGAs showed highest GMTs for type 3 while lower values were recorded for the remaining LGAs.

The GMT ranges were between 26.4 in Kauru LGA to 59.9 in Sabon gari LGA for type 1, between 38.6 in Kauru LGA to 60.8 in Lere LGA for type 2 and between 37.8 in Igabi LGA to 73.1 in Zaria LGA for type 3. The highest GMT value (73.1) was recorded for Zaria LGA while Kauru LGA had the lowest value (26.4).

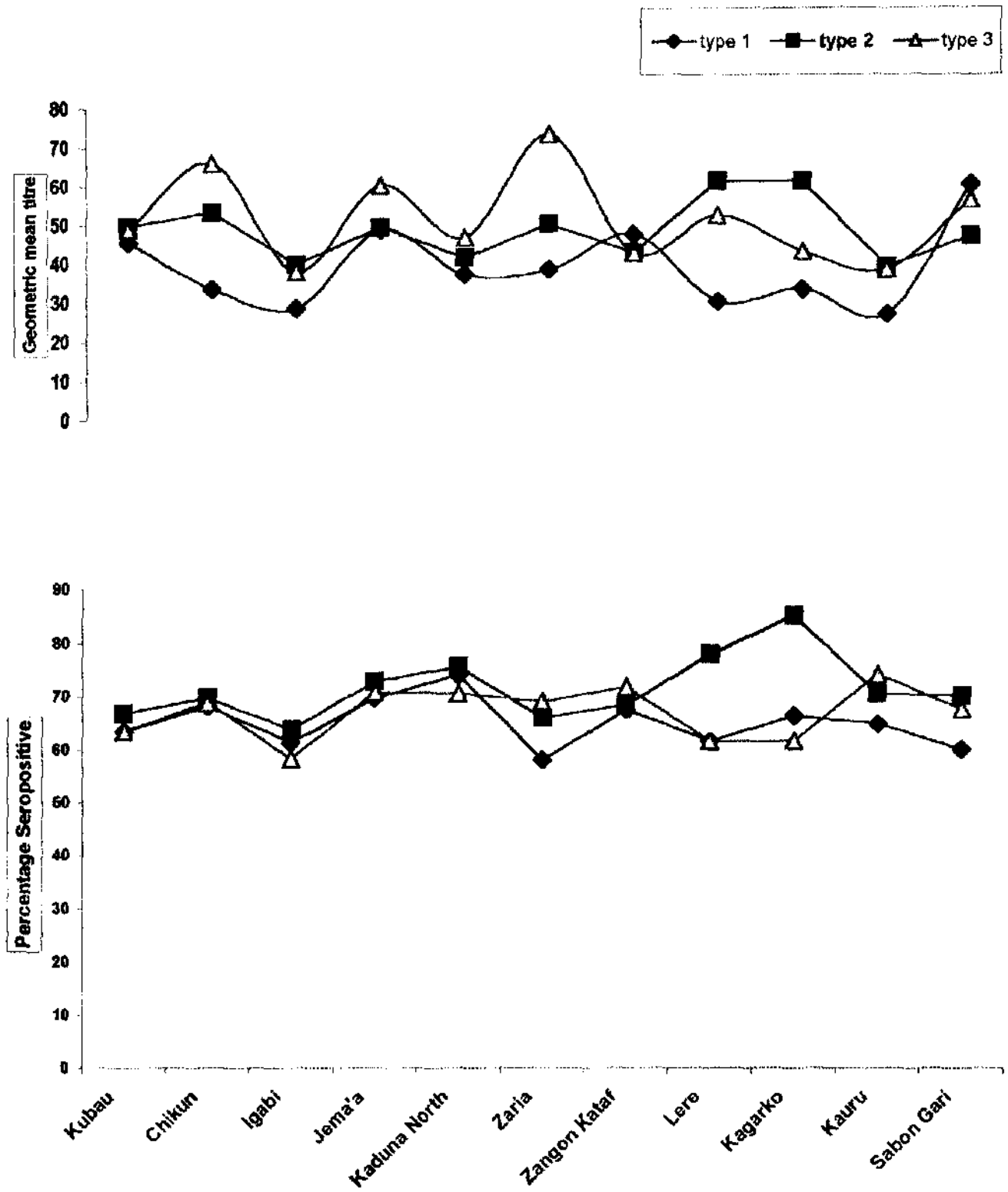


Fig. 2: Percentage distribution of antibodies to poliovirus by type (at a titre ≥ 10) and geometric mean titres among children in the various local government areas of Kaduna State,Nigeria.

The results were further analyzed on the basis of children who had antibodies to one, two or to three types of poliovirus. The results shows that 459 (42.2%) of the 1087 children had antibodies to the three serotypes, 243 (22.4%) had antibodies to two serotypes and 385 (35.4%) had antibodies to one serotype. The highest (48.9%) percentage of children with neutralizing antibodies to the three serotypes of poliovirus was recorded in Jema'a LGA while the lowest (33.1%) was in Igabi LGA (Table 3). Jema'a LGA therefore had the highest number of protected children while Igabi had the least number of protected children to poliovirus infections.

Figure 3 shows that Jema'a and Kagarko LGAs had more children susceptible to one serotype of poliovirus by having the lowest number of seropositive children to two types while Kauru LGA had the least by having the highest percentage of children seropositive to two serotypes. Kaduna North LGA had the least (28.1%) percentage seropositive to one serotype while Kagarko had the highest (43.0%). This shows that Kaduna North LGA had more children susceptible to two serotypes while Kagarko has the least.

Table 3: **Distribution of children seropositive to one, two or three serotypes of poliovirus neutralizing antibodies in the LGAs of Kadna State, Nigeria.**

Subjects	Number of sera samples tested	Number seropositive (percentage)		
Primary school children (LGA)		One serotype	Two serotypes	Three serotypes
Kubau	93	31 (33.3)	21 (22.6)	41(44.1)
Chikun	119	36 (30.3)	34 (28.6)	49 (41.2)
Igabi	127	54 (42.5)	31 (24.4)	42 (33.1)
Jema'a	92	32 (34.8)	15 (16.3)	45 (48.9)
Kaduna North	139	39 (28.1)	36 (25.9)	64 (46.0)
Zaria	100	36 (36.1)	21 (21.0)	43 (43.0)
Zangon Kataf	120	40 (33.3)	25 (20.8)	55(45.8)
Lere	117	47 (40.2)	22 (18.8)	48 (41.0)
Kagarko	86	37 (43.0)	14 (16.3)	35 (40.7)
Kauru	54	18 (33.3)	17(31.5)	19 (35.2)
Sabon Gari	40	15 (37.5)	7 (17.5)	18 (45.0)
Total	1087	385 (35.4)	243 (22.4)	459 (42.2)

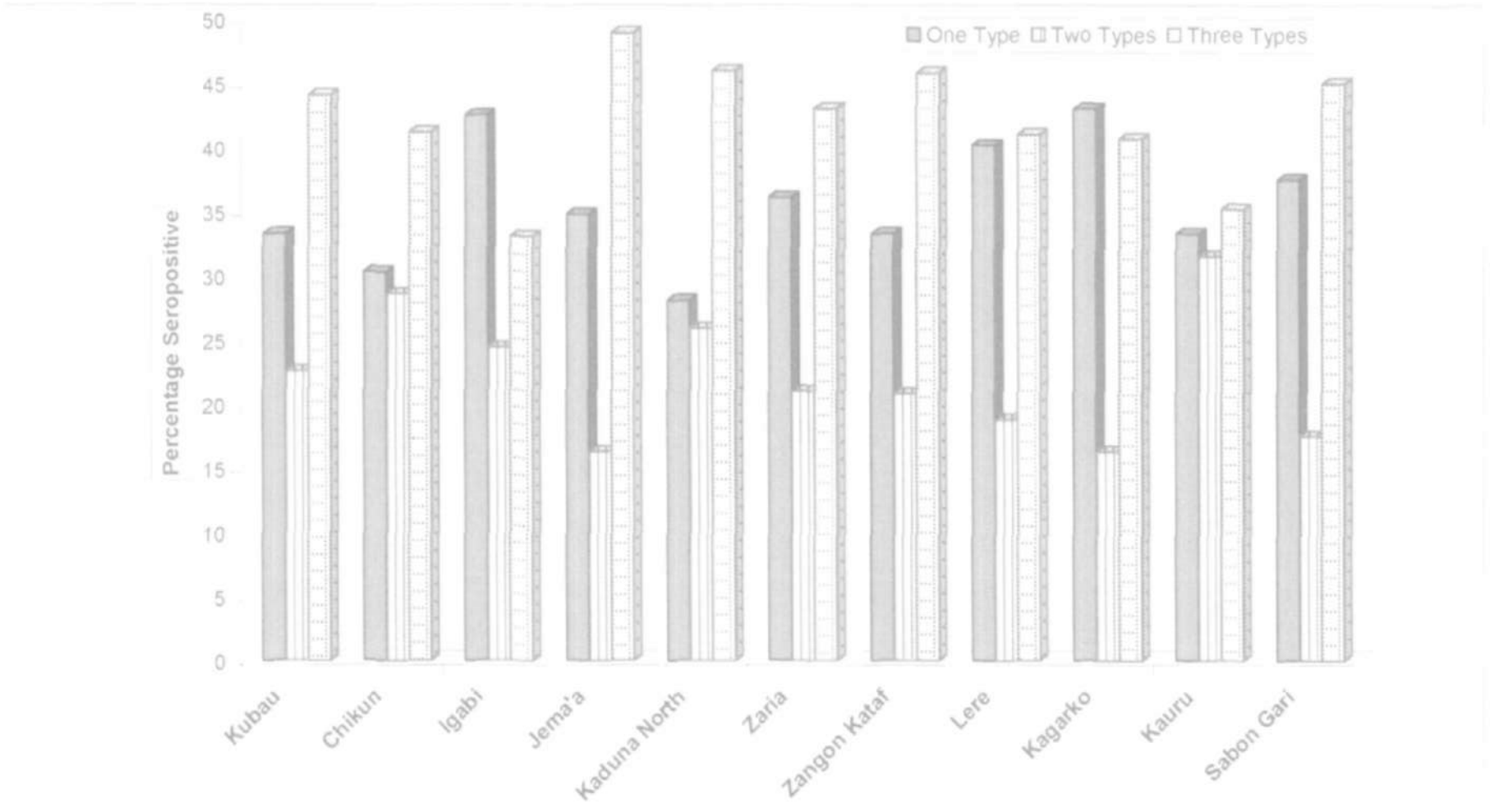


Fig. 3: Distribution of children with antibodies to one,two and three serotypes of poliovirus *in the local government areas of Kaduna State, Nigeria.*

Antibody titres ranged from 10-640 in Chikun, Jema'a, Kaduna North, Zaria, Zangon Kataf and Sabon gari LGAs, 10-320 in Kagarko, Kauru, Kubau and Igabi LGAs and 10-160 in Lere LGA for type1. For serotype 2, the antibody titre ranged from 10-640 for all the LGAs except Lere, Kauru and Sabon gari LGAs that had a range of 10-320. The antibody titre range for serotype 3 was between 10-640 for all the LGAs except Lere and Kauru LGA that had a range of 10-320 (Appendix 1). The lowest antibody titre range (10-160) was observed in Lere LGA while the highest was in Zaria LGA (10-640).

It is of interest to note that many of the children with demonstrable anitbodies and thus judged "not susceptible" had in fact low titres of 1:10 to 1:40 (Appendix 1). The result were thus further analysed and the percentage of children with antibody to poliovirus at titre ≤ 40 and > 40 were deduced. The results showed that 684 (62.9%), 604 (55.6%) and 618 (56.9%) of the 1087 children in Kaduna State had antibodies at titre ≤40 to serotype 1, 2 and 3 respectively while those with antibodies at titre above 40 were 403 (37.1%), 483 (44.4%) and 469 (43.1%) to serotype 1, 2 and 3 respectively. Table 4 shows the percentage of children with antibody to the three serotypes. at titre ≤ 40 and > 0.

The results generally showed that most of the children had antibody levels to poliovirus type 1 at a titre ≤ 40. Children in Kagarko LGA had very high percentage of antibody at titre > 40 for type 2 poliovirus, while all (100%) the children in Zangon Kataf LGA had antibodies to this serotype at titre > 40 (Figure 4).

Table 4: **Percentage distribution of poliovirus neutralizing antibodies at titres ≤ 40 and > 40 among children in the various LGAs of Kaduna State, Nigeria.**

Subjects / Primary school children (LGA)	Number of Sera samples tested	Percentage seropositive at titre ≤ 40			Percentage seropositive at titre > 40		
		Type 1	Type 2	Type3	Type 1	Type 2	Type 3
Kubau	93	57.0	59.1	60.2	43.0	40.9	39.8
Chikum	119	63.9	50.4	52.1	36.1	49.6	47.9
Igabi	127	70.1	66.9	66.9	29.9	33.1	33.1
Jema'a	92	53.3	53.3	51.1	46.7	46.7	48.9
Kaduna North	139	58.3	56.8	54.0	41.7	43.2	46.0
Zaria	100	67.0	60.0	51.0	33.0	40.0	49.0
Zangon Kataf	120	55.8	-	55.8	44.2	100.0	44.2
Lere	117	70.9	44.4	58.1	29.1	55.6	41.9
Kauru	54	75.9	57.4	59.3	24.1	42.6	40.7
Kagarko	86	60.5	37.2	62.8	39.5	62.8	37.2
Sabon Gari	40	65.0	62.5	52.5	35.0	37.5	47.5
Total	1087	62.9	55.6	56.9	37.1	44.4	43.1

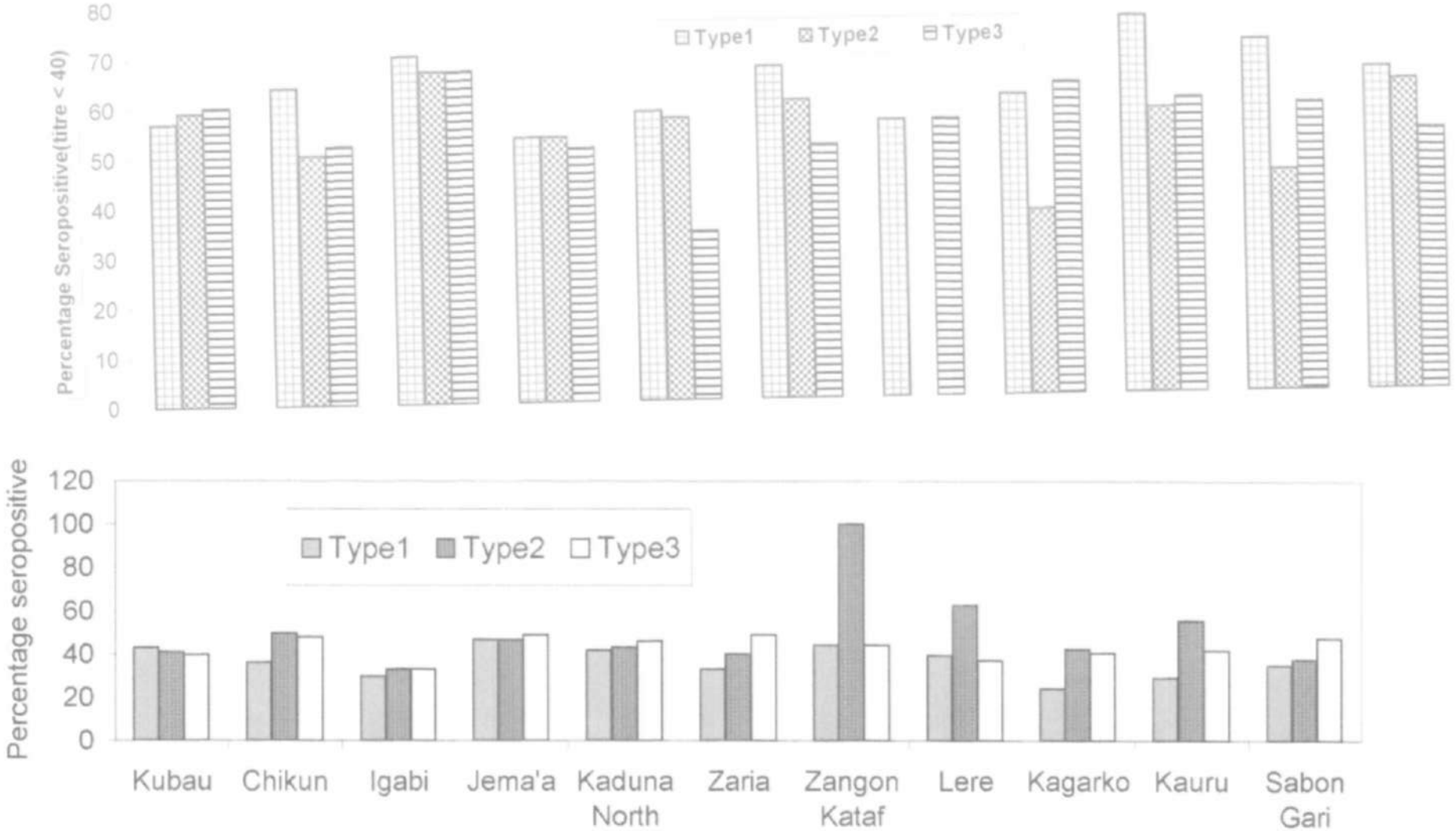


Fig.4:Percentage distribution of children with antibodies to poliovirus at titre <40 and >40 in the local government areas of Kaduna State, Nigeria.

Statistical analysis by Chi-square tests revealed that differences observed in the antibody prevalence and the GMT values amongst the school children in the LGAs were highly significant ($P < 0.05$) (Appendix 3).

The distribution of poliovirus neutralizing antibodies amongst the primary school children by age showed that the percentage of seropositive children ranged from 50% in the 1 year old to 87.4% in the 9 year old.

The number of sera tested by age together with the percentage of subjects seropositive to type 1, 2 and 3 poliovirus and their GMTs are presented in table 5. The antibody prevalence was highest in the 9 year old for the 3 serotypes. The lowest antibody prevalence rate was observed in the 2 years old for type 1 and in the one year old for types 2 and 3.

Though there were slight variations in the observed prevalence rate amongst the children according to their ages, statistical analysis using chi-square showed that the observed difference in the percentage of seropositive children to the three serotypes were not significant, but there was significant difference in the GMT values observed for the three serotypes at $P < 0.05$ (Appendix 4).

Table 5: **Distribution of children seropositive to types 1, 2, and 3 poliovirus by age and GMTs (at a titre ≥ 10) in Kaduna State, Nigeria**

Age (years)	No of sera tested	Percentage seropostive	Proportion seropositive to the three serotypes					
			Type 1		Type 2		Type 3	
			Percentage seropositive	GMT	Percentage seropositive	GMT	Percentage seropositive	GMT
1	2	50.0	50.0	79.8	50.0	160.0	50.0	160.8
2	13	69.2	30.8	160.0	53.8	72.4	53.8	32.8
3	68	83.8	67.6	40.0	72.1	39.4	66.2	43.2
4	147	80.5	64.4	34.9	70.5	52.1	67.1	50.3
5	177	74.6	65.0	38.2	66.7	57.4	67.2	46.3
6	202	79.2	67.3	21.9	69.3	49.1	65.8	51.9
7	188	83.0	67.6	39.1	73.9	43.3	71.3	54.8
8	112	80.4	59.8	35.7	73.2	43.9	60.7	61.4
9	103	87.4	72.8	34.2	78.6	47.1	69.9	45.8
10	58	84.5	60.3	34.1	75.9	40.9	65.5	42.2
11	12	75.0	66.7	47.6	66.7	80.0	58.3	72.4

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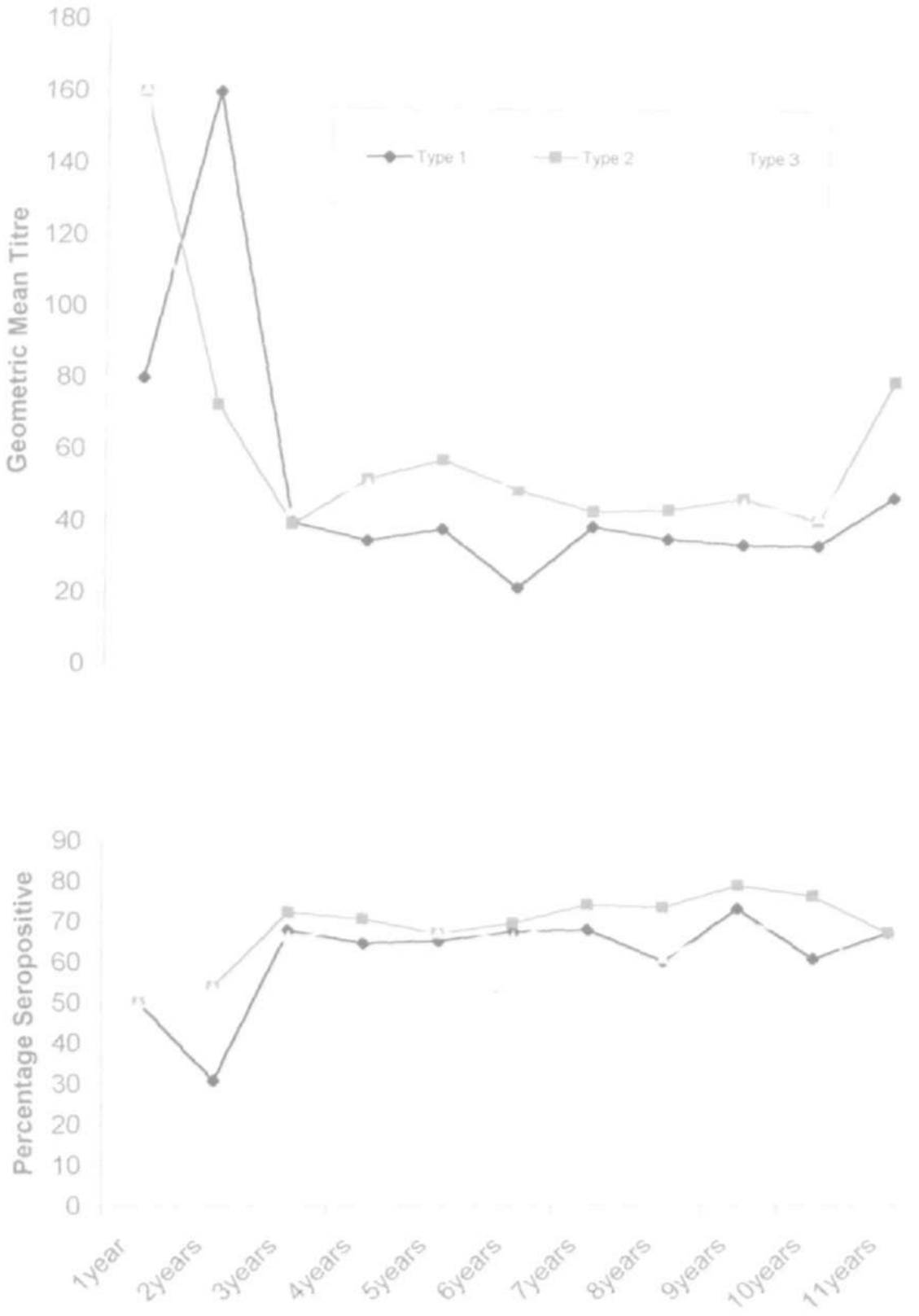


Fig.5: Distribution of children seropositive to poliovirus serotypes (at titre >10) by age and geometric mean titres in Kaduna State, Nigeria.

The GMT values ranged between 21.9 in the 6 year old to 160.0 in the 2 years for serotype 1, 39.4 in the 3 years old to 160.8 in the one year old for serotype 2 and between 32.8 in the 2 years old to 160.8 in the 1 year old for serotype 3.

Table 6 shows the number of sera tested by age together with the percentage of subjects seronagative and those seropositive to 1, 2 or 3 types of poliovirus. From the results obtained, the 7-year-old group had the highest percentage (49.5) of children having antibodies to three serotypes while the 2 year olds had the lowest percentage (23.1). The one year old were not considered because the number of sample tested from the group compared to the others was significantly small. Most of the children in the various age categories were susceptible to just one serotype of poliovirus (Figure 6) with a considerably high percentage in the 5 year olds because the age group had the lowest (16.4%) seropositive rate. Lower percentages of children were susceptible to two serotypes with the lowest rate in the 2 year olds that had a high prevalence rate of 53.8%. It is important to note that more than 50% of the children in all the age groups were found to be susceptible to one or two poliovirus serotypes

Table 6: **Percentage of children by age seronegative and those seropositive to 1, 2 and 3 types of poliovirus (at a titre ≥ 10) in Kaduna State, Nigeria.**

Age (years)	No. of sera tested	Susceptibilities rate (percentage seronegative)	Percentage seropositive		
			One type	Two types	Three types
1	2	50.0	50.0	-	50.0
2	13	30.8	53.8	23.1	23.1
3	68	16.2	36.8	17.6	45.6
4	147	19.5	34.9	22.1	43.0
5	177	25.4	41.2	16.4	42.4
6	202	20.8	35.6	21.8	42.6
7	188	17.0	30.3	20.2	49.5
8	112	19.6	37.5	25.9	36.6
9	103	12.6	28.2	33.9	38.8
10	58	15.5	37.9	29.3	32.8
11	12	25.5	33.3	25.0	41.7

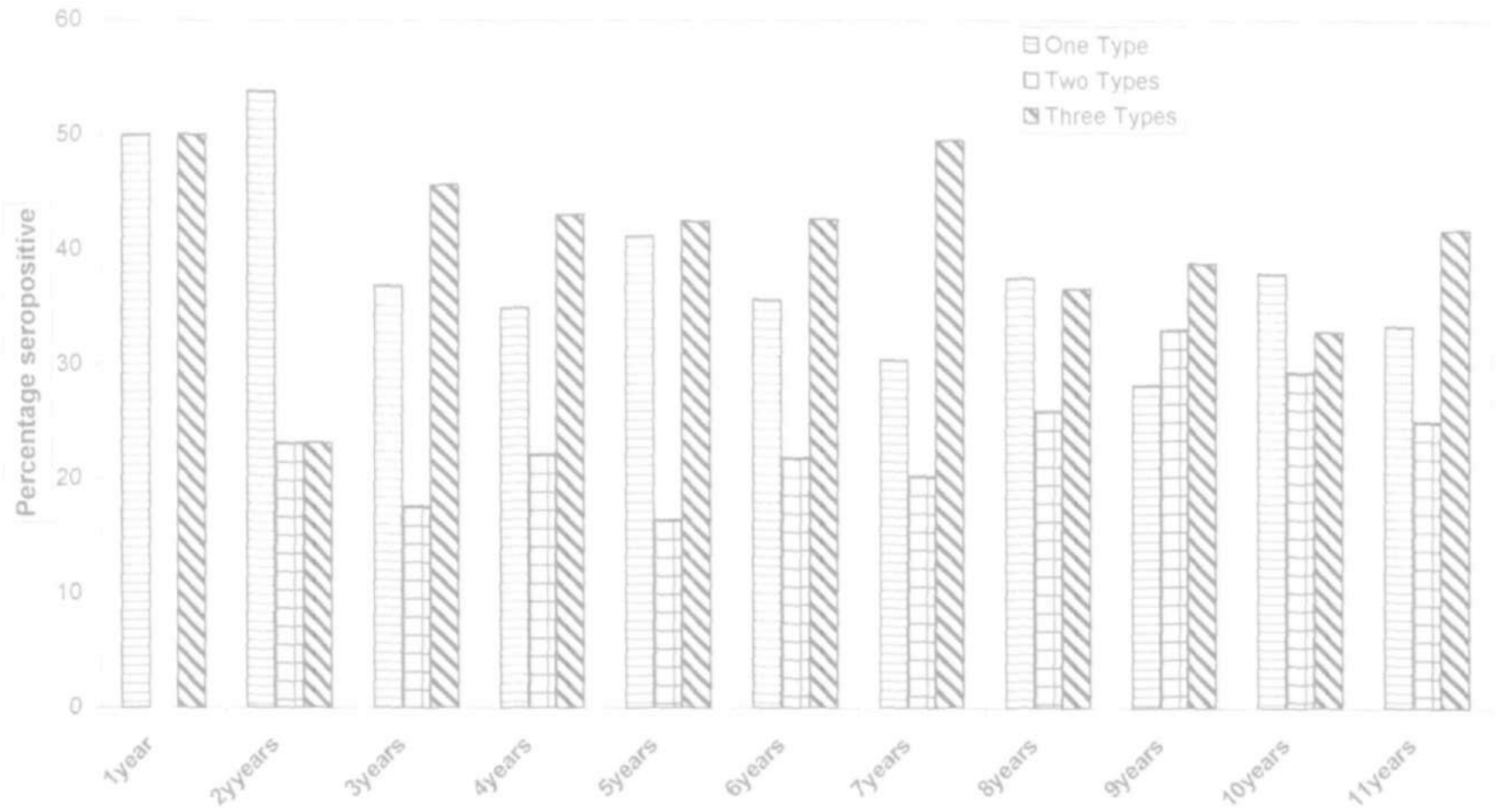


Fig. 6: Percentage distribution of children by age seropositive to one, two and three serotypes of poliovirus (at *titre* $\geq$ 10 in Kaduna State, Nigeria.

The younger children were more susceptible to poliovirus infection than the older children. This is so because the one and two year old had very high seronegative rates (50.0% and 30.8% respectively) while the 9 years old had very low seronegative rates (12.6%).

The percentage of children by age that had antibodies to poliovirus at titre ≤ 40 and at titre > 40 were also computed and the result showed that a greater proportion of all the age group had antibody at titre ≤ 40 than at titre > 40 (Table7). More children also had antibody to serotype 2 at titre > 40 than at titre ≤ 40, with all the five year old children having antibodies to this serotype at a titre > 40 (Figure 7).

Table 7: Percentage distribution by age of children with antibody at titres ≤ 40 and > 40 to poliovirus serotypes in Kaduna State, Nigeria.

Age (Years)	Percentage seropositive at titre ≤40			Percentage seropositive at titre > 40		
	Type 1	Type 2	Type3	Type 1	Type 2	Type 3
1	50.0	50.0	50.0	50.0	50.0	50.0
2	69.2	46.2	76.9	30.8	53.8	23.1
3	64.7	63.2	57.4	35.3	36.8	42.6
4	62.4	51.0	55.0	37.6	49.0	45.0
5	62.7	-	61.0	37.3	100	39.0
6	62.7	58.2	61.0	37.3	41.8	39.0
7	60.4	56.9	55.9	39.6	43.1	44.1
8	67.9	32.1	57.1	52.7	47.3	42.9
9	65.0	53.4	57.3	35.0	46.6	42.7
10	70.7	58.6	62.1	29.3	41.4	37.9
11	50.0	33.3	50.0	50.0	66.7	50.0

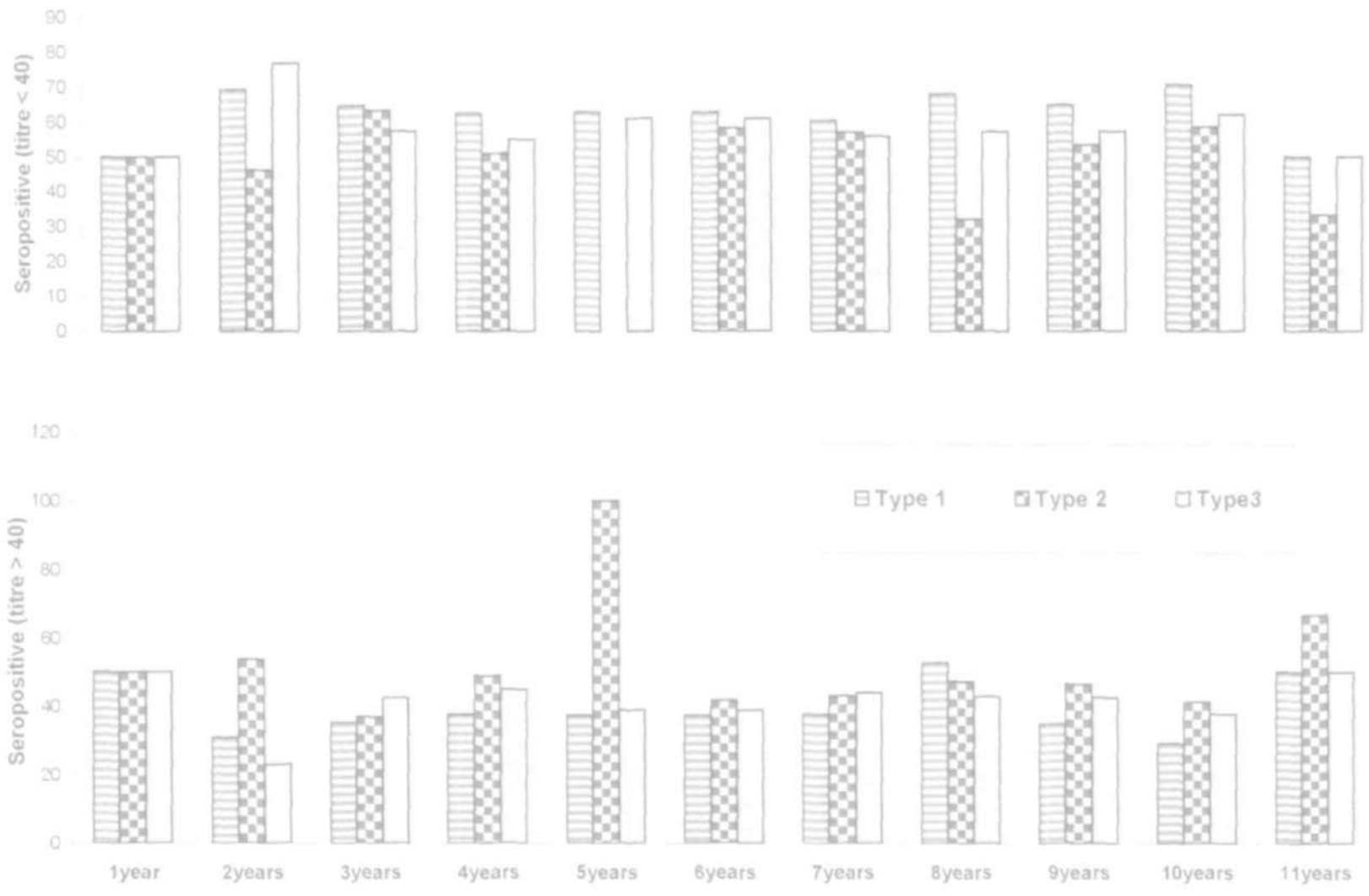


Fig. 7: Percentage distribution of children by age *with antibody at titre* < 40 and > 40 to poliovirus serotypes *in Kaduna State Nigeria*

The pattern of the sex distribution of polio antibodies among the children is presented in table 8. The table also shows the number of sera tested by sex together with the percentage of seropositive subjects to each of the three serotypes and the GMTs.

Of the 604 males included in the study, 477 (79.0%) had demonstrable antibodies to one or more polioviruses while 398 (82.4%) of the 483 females enrolled in the study had demonstrable antibodies to thevirus.

The percentage of children by sex that had antibody to the three serotypes of poliovirus at titres ≤ 40 and > 40 were also computed and found to be higher at titre ≤ 40 than at titre > 40 (Appendix 2).

The percentage of male children who were seropositive to one, two and three serotypes was 36.1, 22.2 and 41.7 percent respectively, while those of the female children were 34.6, 22.6 and 42.9 percent respectively. Although the female subjects had slightly higher antibody prevalence and mean titres, (Figure 8) the difference in the sex distribution was statistically insignificant.

Table 8: Sex distribution of children with antibodies to poliovirus by type and GMTs in Kaduna State, Nigeria.

Subjects	No of sera tested	Percentage seropositive	Percentage seropositive for each serotype					
			Type 1	GMT	Type 2	GMT	Type 3	GMT
Male	604	477 (79.0)	397 (65.7)	36.3	424 (70.2)	48.6	397 (65.7)	51.0
Female	483	398 (82.0)	315 (62.2)	39.3	352 (72.9)	47.6	328 (67.9)	493

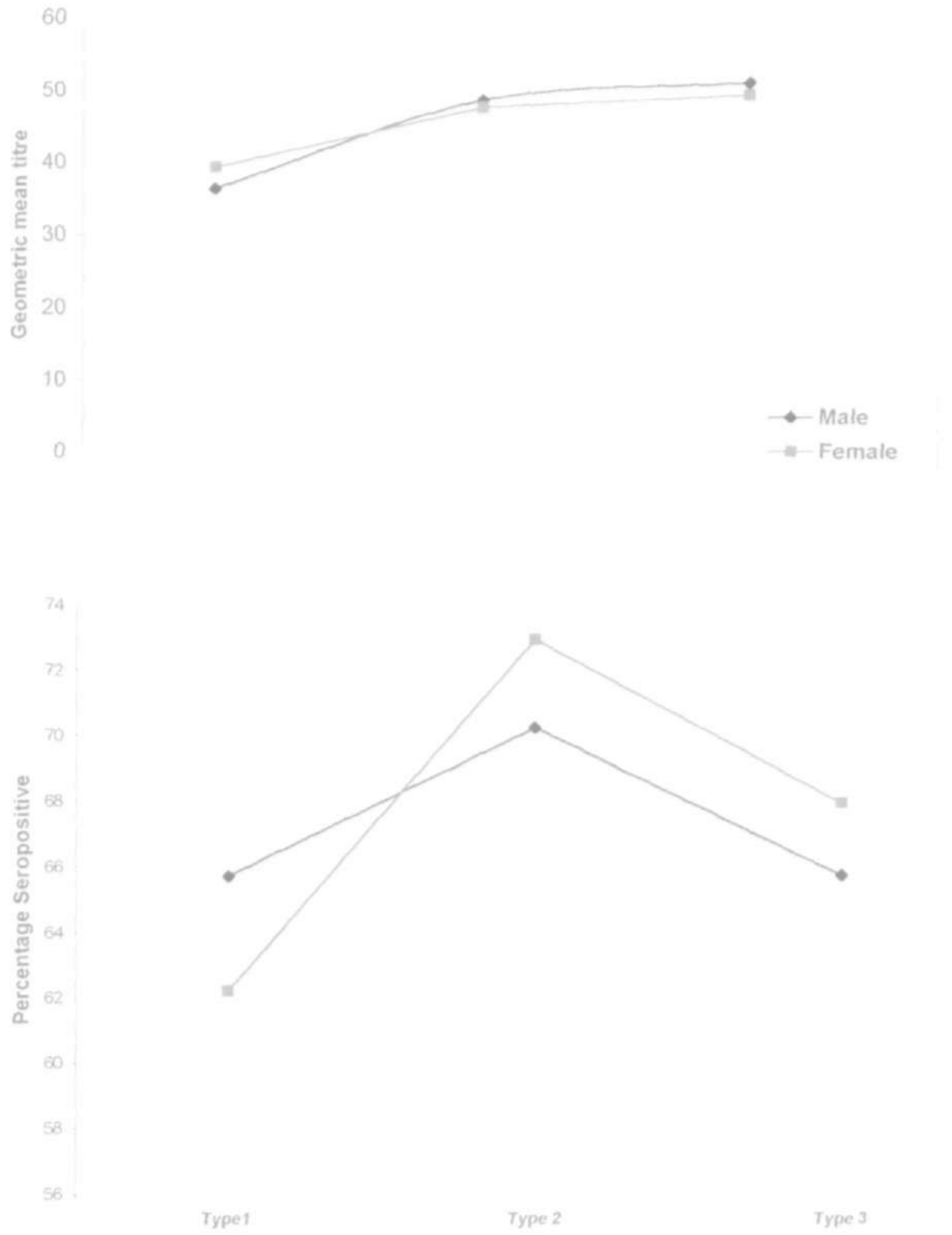


Fig.8: Sex distribution of children with antibodies to poliovirus by type and geometric mean titres in Kaduna State, Nigeria.

Further analysis of the immune status of the primary school children to estimate the effect of 1-6 doses of poliovirus vaccine received on seroprevalence rates as well as the seroprevalence rates associated with zero OPV doses was carried out. The results showed that children that received five doses of polio vaccine had the highest prevalence rates while the lowest was observed in those that received six doses.

The antibody prevalence was highest (93.9%) in children who received five doses of vaccine and was however lower (78.3%) in children who received six doses of vaccine. The result also shows the antibody prevalence to be highest in the children who received five doses for the three serotypes (81.6%, 89.8% and 91.8% for types 1, 2 and 3 respectively) and lowest in those who received 2 doses (63.4%, 67.5% and 60.2% for types 1, 2 and 3 respectively) (Table 9). Similar results were also obtained for the GMTs with the highest value in those children who received five doses and lowest value for those who received 2 doses.

Table 9: Pattern of antibody distribution among children that received 1 to 6 doses of oral polio vaccine and GMTs in Kaduna State, Nigeria.

Vaccine doses	Number tested	Number seropositive (%)	Number. seronegative (%)	Percentage seropositive					
				Type 1	GMT	Type 2	GMT	Type 3	GMT
1	67	55 (82.1)	12(17.9)	68.7	36.0	71.6	36.7	76.1	44.7
2	123	97 (78.9)	26(21.1)	63.4	25.9	67.5	29.4	60.2	32.4
3	263	241 (91.6)	22(8.4)	77.2	37.2	82.5	48.0	77.2	53.8
4	401	341 (85.0)	60(15.0)	72.8	38.1	77.8	55.8	72.1	55.7
5	49	46 (93.9)	3(6.1)	81.6	85.7	89.8	93.6	91.8	67.5
6	23	18 (78.3)	5(21.7)	65.2	42.4	73.9	53.8	65.2	40.0

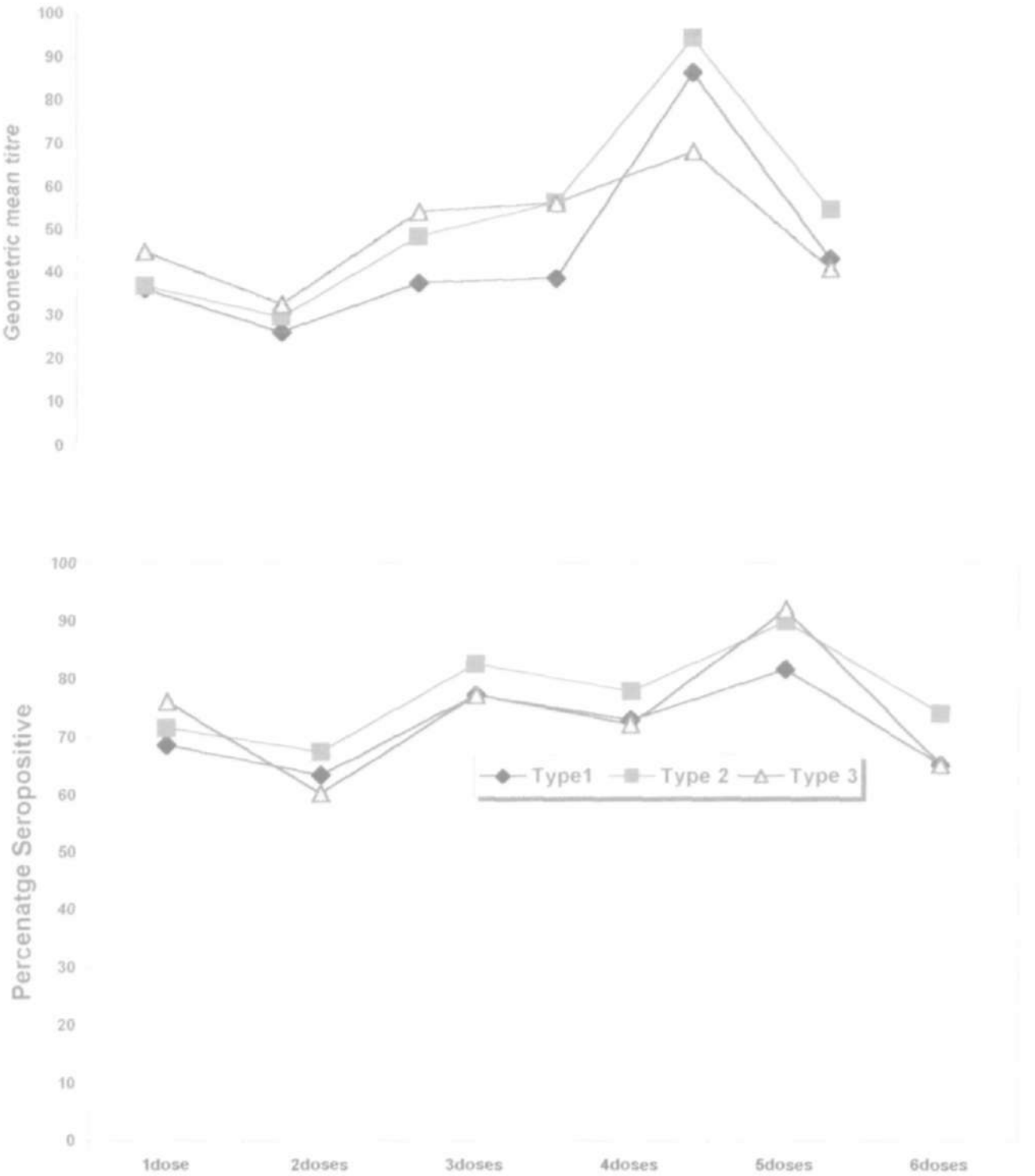


Fig. 9: Pattern of antibody distribution amongs children that received 1 to 6doses of oral polio vaccine and geometric mean titres in Kaduna state, Nigeria.

The seroprevalence rate associated with zero doses was also analyzed. The results showed that of the 161 children that were not vaccinated i.e., received no vaccine, 77 (47.8%) had demonstrable antibody to poliovirus, while of the 77 (47.8%) with antibodies to poliovirus, 38 (23.6%), 55 (34.2%) and 55 (34.2%) had antibodies to types 1, 2 and 3 respectively. Thus, the seroprevalence rate associated with zero doses was 47.8% in Kaduna State.

The proportion of children according to vaccine dose received and who had antibodies to one, two or three serotypes of poliovirus were analyzed and the result showed that most (71.4%) of the children who received five doses had antibodies to three serotypes while only, 28.5% of those who received two doses had antibodies to three serotypes (Figure 10). Only 18.4% of the children who received five doses had antibodies to two serotypes, while 27.6% of those who received two doses had antibodies to two serotypes. Also 10.2% of the children who received five doses had antibodies to one serotype while 43.9% of the children who received 2 doses had antibodies to one serotype (Table 10).

Table 10: **Percentage of children by number of vaccine dose received who had antibodies to one, two and three serotypes of poliovirus in Kaduna State, Nigeria.**

No of doses	No. tested	Proportion seropositive		
		One serotype	Two serotypes	Three serotypes
1	67	18 (26.9)	17 (25.4)	32 (47.8)
2	123	54 (43.9)	34 (27.6)	35 (28.5)
3	263	61 (23.2)	71 (27.0)	131 (49.8)
4	401	123 (30.7)	78 (19.5)	200 (49.9)
5	49	5 (10.2)	9 (18.4)	35 (71.4)
6	23	8 (40.0)	5 (25.0)	7 (35.0)

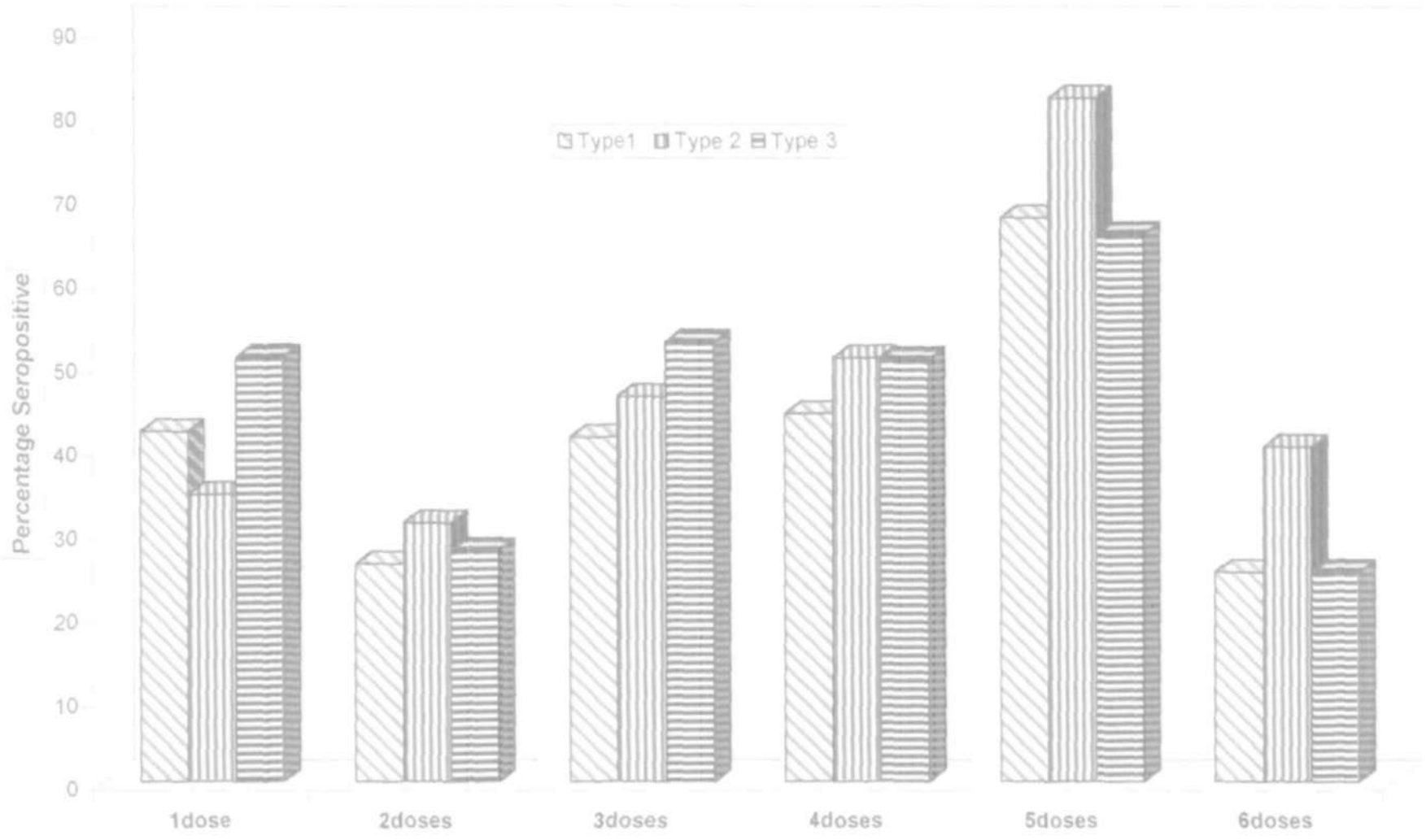


Fig. 10 : percentage distribution of children with antibodies to poliovirus serotypes by number of vaccine doses received in Kaduna State, Nigeria.

Further analysis of the immune status of the children by number of vaccine doses received on the basis of percentage with antibodies at titre ≤40 and at titre > 40 showed that those who received fives doses had the highest prevalence rate at titre > 40 while those who received 2 and 6 doses had the lowest prevalence rate at titre > 40 (Figure 11). Most of the children who received 2 and 6 doses had very high prevalences rate at titre ≤ 40 (Table 11).

Statistical analysis showed that the observed difference in the antibody distribution pattern according to the number of doses received were highly significant at $p < 0.05$ (Appendix 6).

Table 11: Percentage distribution of poliovirus antibodies at titres ≤ 40 and > 40 amongst primary school children by number of vaccine doses received in Kaduna State, Nigeria.

No of doses	Percentage seropositive at titre ≤ 40			Percentage seropositive at titre > 40		
	Type 1	Type 2	Type3	Type 1	Type 2	Type 3
1	58.2	65.7	49.3	41.8	34.3	50.7
2	74.0	69.1	72.4	26.0	30.9	27.6
3	58.9	46.0	47.5	41.1	46.0	52.5
4	56.1	49.4	49.5	43.9	50.6	50.4
5	32.7	18.4	34.7	67.3	81.6	65.3
6	75.0	60.0	75.0	25.0	40.0	25.0



Fig. 11: Percentage distribution of poliovirus antibodies at titres ≤ 40 and > 40 amongst children by number of vaccine doses received in Kaduna State, Nigeria.

CHAPTER FIVE

5.0 DISCUSSION

Antibody titration results indicated that 42.2% of the children had antibodies to the three serotypes of poliovirus, 19.5% had no antipoliovirus antibodies at all and 65.5, 71.4 and 66.7% had antibodies to poliovirus types 1, 2 and 3 respectively. A total of 80.5% of the children had demonstrable antibodies to one or more serotypes of poliovirus.

Children with an antibody titre less than 1:10 were considered unprotected and susceptible to the disease. The proportion of potentially susceptible children was 34.5%, 28.6% and 33.3% against poliovirus types 1, 2 and 3 respectively, while the proportion of potentially susceptible children to one or two poliovirus sertotypes was 57.8%.

The high prevalence of poliovirus antibodies among school children in Kaduna State could be attributed to the mass immunization being conducted by NPI, and to previous infections either with wild virus strains or with the vaccine strain (OPV) which is currently in use. The latter becomes more likely in view of the reported high rates of excretion of vaccine virus in stool following the use of OPV vaccine (Melnick, 1989b), which results in the contamination of food and water and subsequently the dissemination of the OPV virus in the community.

The seropositivity to one or more serotypes though high, is not as high as the rate reported in other countries. In a similar study in Saudi Arabia, Farag *et al.* (1995) observed an immunity level of 98.0%, 96.0% and 94.0% for serotype 1, serotype 2 and serotype 3 respectively in the studied population.

It is not yet possible to define the antibody titre necessary to give protection against the paralytic manifestation of poliomyelitis and indeed it might vary in different individuals (Böttiger *et al.*, 1972; Robertson, 1993). Persons are presumed to be protected against the disease caused by a particular type of poliovirus if they develop type specific serum neutralizing antibody. Since vaccine licensing requirement states that over 90% of susceptible persons should have attained titre 1:10 or higher following vaccination, a certain correlation between the level of antibody and immunity is implied. At this index of immunity, 34.5%, 28.6% and 33.3% of children in Kaduna State were found to be susceptible to poliovirus types 1, 2 and 3 respectively (Table 2).

If susceptibility to clinical disease is defined as having an antibody titre less than 1:10 to at least one of the 3 types the of polioviruses then a dangerously high percentage (57.8%) of the pupil tested in the present study were at potential risk. It is of interest to note that many of those with antibodies and thus judged "not susceptible" had in fact, low titres of 1:10 and 1:20. Further investigations of children with antibodies at titre ≤ 40 and > 40 showed that more or higher number of children had antibodies to poliovirus at titre ≤ 40 than at titre > 40 (Figures 4, 7 and 11). This suggests that these children are probably not at the risk of developing clinical poliomyelitis, which may lead to the paralytic form of the disease, but they may be re-infected with poliovirus and possibly provide a source of infection for others who are not immunized. Studies carried out by Gelfand *et al.* (1959) in the state of Louisiana (USA) have confirmed this possibility.

The majority of children in this study were susceptible to just one serotype of poliovirus, some to two serotypes, while 19.5% of them were found to be completely susceptible to the three serotypes. In view of the finding of low percentage (42.2%) of subjects with antibodies to the three serotypes and the percentage of children without antibodies especially to serotypes 1 and 3 (Tables 3, 6 and 10), it is possible that the disease could be readily introduced into such population in Kaduna State. Whether this finding represents incomplete vaccination coverage or defective vaccine procedure or simply lack of persistence of antibody after vaccination is not known. However, the fact remains that 19.5% of the children in the study area are without demonstrable antibody and a large proportion of the remainder have relatively low titres even against the ubiquitous type 2 virus.

Furthermore, the finding that not very high percentage (42.2%) of the subjects had antibodies to the three serotypes indicates that only 42.2% of the subjects were truly protected to poliovirus because infection with one type does not protect an individual against infection with the other two types since the antibodies do not cross protect.

These results support the findings of Oberhofer *et al.* (1975) in their study of an American community in which they suggested that if the trend of susceptibility becomes more widespread, the disease could be readily introduced into such population and lead to a serious condition.

A further analysis of the results of the current study showed that there was significant differences (Appendix 3) in antibody prevalence and GMTs

among the LGAs. Kagarko LGA had the highest percentage (87.2%) of seropositive primary school children while Zaria LGA had the lowest (74.0%) proportion of seropositive children. Zaria LGA therefore had the highest number of children susceptible to poliovirus infection while Kagarko LGA had the lowest number of susceptible children.

In a similar seroepidemiological study of poliomyelitis in Mexico, Ruiz-Gomez *et al.* (1992) reported a significant variation in the seroprevalence of poliovirus antibodies from one area to another which they attributed to differences in vaccination coverage. Meseda (1993) in his study of the significance of antibodies to polio in some parts of Kaduna State in Nigeria had also observed a similar variation in the prevalence rate in the area of study. He attributed the variations to possible differences in vaccination coverage and the presence of more health centres in the LGA with the highest sero-prevalence rate.

A comparatively high GMT value (73.1) was observed for poliovirus type 3 in Zaria LGA even though it had the lowest prevalence rate. This may be attributed to circulation of type 3 virus in the LGA, probably due to a previous outbreak caused by the serotype.

The sero-prevalence of antibodies to the three serotypes in the LGAs was fairly high with the lowest percentage observed for type 1 and the highest percentage for type 2 (Figure 2). The low seroprevalence observed in Zaria LGA could be attributed to socio cultural/religious beliefs. In certain countries parents out of ignorance do not allow their children to be vaccinated, neither do they

allow blood taken from them. The high seropositivity observed in Kagarko LGA could be due to a relatively low population, high level of vaccination coverage, and greater awareness of the immunization programme.

The variations observed in the GMT values amongst the children by age were statistically significant (Appendix 4). Generally, the prevalence rate was higher in the older children than in the younger children (Figure 6) with the 9 year old having the highest percentage (87.4%) while the 2 years old had the lowest percentage (69.2%). This implies that the 2-year-old children were more susceptible to poliovirus infection than the 9 year olds, but the antibody titres and GMT values were higher in the younger children suggesting higher immunity to poliovirus.

Most of the children in the various age groups were susceptible to just one serotype than 2 serotypes (Figure 6) but more of the older children had antibodies to the 3 serotypes than the younger children. These findings of children susceptibility to one type of poliovirus in all age groups seem to suggest a failure of the OPV vaccines being used in Kaduna State to induce complete immunity in the children. This finding has been widely reported in the tropics (Domok *et al.*, 1974; Khrisnan *et al.*, 1983; Deivanayagam, 1993; Linkins *et al.*, 1995). The OPV vaccine failure may be either primary i.e. lack of initial response to the vaccine virus or secondary, where the antibodies developed wane out resulting in low seroconversion rates. This low seroconversion in the younger children might have been caused by the perennial problem of malnutrition peculiar to this age group in the tropics, which could perhaps lower their intrinsic

potential for defence against pathogens. Adeiga *et al.* (1994) made this same observation their study of immune response in malnourished children in Nigeria. It is also most likely that diarrhoea in these children during immunization could be responsible for these low rates of seroconversion. This is because diarrhoea can lead to mal-absorption and erosion of secretory IgA from the intestinal tract and since secretory IgA is important in poliovirus immunization, its gross defects can affect immunity at the epithelial and secretory surfaces (Chandra, 1981; Adeiga *et al.,* 1994). This lower-than-expected serologic response to OPV may also be due to loss of vaccine potency when the live virus is inactivated due to exposure to heat.

The observed high seropositive rate in the older children may be attributed to antibody boosting due to passive immunization of these children as a result of the dissemination of OPV in the community. It is often thought that protection of older non-immunized children from contracting disease is largely dependent upon well-immunized younger ones who were at one time the principal reservoir of infection before vaccines were developed (Oberhofer *et al.,* 1975).

It is expected that children between the age of 1-5 years should have high percentage of seropositivity and high titre values owing to the ongoing house-to-house mass immunization being conducted but the result is contrary to this because this study showed that a greater proportion of the children in these age groups had antibody to poliovirus at a titre ≤ 40 than at titre > 40. All the 5-year-old children amazingly had antibody to serotype 2 at a titre > 40 (Table 7)

suggesting that higher immunity is conferred to type 2 in these age groups. The 11-year-old children were highly immunized to serotypes 2 and 3 while the 2 year olds were highly immunized to serotype 1 as observed in their GMT values. In view of the lower immunity in the younger children it can be inferred that these age groups are at risk of becoming reinfected and they may serve as a source of infection to the unvaccinated children in the population.

There was no significant difference in the sex distribution of polio antibodies amongst children in the study area (Appendix 5). Williams and David-West (1990) and Meseda (1993) observed a similar pattern in their studies of a population of vaccinated children in the Southern and Northern parts of Nigeria respectively. Thus, gender may not be important in determining immune response to poliovirus challenge.

Immunization with five doses of OPV as confirmed from vaccination history gave significantly high rates of sero-conversion and GMT values than immunization with 2 or 3 doses of OPV. This high prevalence (93.9%) of poliovirus antibodies in children who had received five doses could be out rightly attributed to immunological response to the vaccine virus challenge. Of the children who had received five doses 81.6%, 89.8% and 91.8% had antibodies to serotypes 1, 2 and 3 respectively (Figure 9). These findings are in agreement with previous reports by Osei-kwasi *et al.*, (1995) in Ghana and also more recently with results from Abidjan by Akuoa-Koffi *et al.* (1995), with almost similar seropositive rates.

In a parallel study of pre-school children in the United States, Chen *et al.* (1996) reported higher antibody prevalence with levels above 90% for all the three serotypes among children who had received 3 or more doses. This difference in percentage prevalence between this study and their study also shows that seroconversion is lower amongst Nigerian children. Seroprevalence levels after 1 or 2 doses of OPV were also much lower than those reported in developed countries (Chen *et al.*, 1996) but similar to those previously reported in developing countries (Akoua-Koffi *et al.*, 1995; Osei-kwasi *et al.*, 1995) and some subtropical countries (Hanna *et al.*, 1995).

In this study, subjects with no vaccination record as obtained from the questionnaires had demonstrable levels of antibody to poliovirus with seropositive rates of 28.6% to serotype 1, 34.2% to serotype 2 and 29.8% to serotype 3. Surprisingly, more children (47.8%) had antibodies following a history of inadequate than adequate immunization against one or more poliovirus serotypes. This finding agrees with earlier observations by Oberhofer *et al.* (1975). The study of Chen *et al.* (1996) also revealed that some children in their study confirmed not to have received polio vaccine included in their studies had demonstrable levels of polio antibodies.

In view of the finding of moderate prevalence and hence moderate susceptibility of subjects to all serotypes amongst children with no history of vaccination, it is conceivable that immunity in this group is related to subclinical infections with wild strains of poliovirus and might also be partly due to the spread of the vaccine virus (OPV) after administration beyond immunized

subjects. OPV has community effects and its multiplication in the intestine after infection leads to its dissemination beyond the individual being immunized in areas with poor hygiene and sanitation (Kok *et al.*, 1992; Fine, 2000).

The finding in this study therefore suggests that secondary spread of Sabin type virus currently plays a detectable but modest role in increasing seroprevalence levels in primary school children in Kaduna State. These findings are also consistent with those of earlier studies (Santoro *et al.*, 1984).

When the results of the GMTs of the seroanalysis of the vaccinated children were further analyzed, it was observed that the difference in the antibody distribution according to the number of doses received was highly significant at $P < 0.05$ (Appendix 6). This finding suggests that the most important predictor of seropositivity in this study was the number of OPV doses received.

From the analysis of questionnaires on vaccination history obtained from parents of subjects, it was confirmed that of the 1087 subjects enrolled in the study 161 (14.8%) had no history of immunization but 77 (47.8%) of these children had demonstrable antibodies to poliovirus. This suggests that percentage exposure following excretion of vaccine virus in stool or due to circulating wild virus is 47.8%. Interestingly enough, most (56 or 72.7%) of the children had antibody to one type of poliovirus, which possibly implies exposure to circulating wild virus due to subclinical infections, while 12 (15.6%) and 9 (11.7%) had antibodies to 2 serotypes and 3 serotypes respectively, implying

23.3% secondary spread due to exposure to vaccine virus circulating in the community.

However, 84 (52.2%) out of the 161 children with no history of immunization had no antibodies to poliovirus. This percentage constitutes the pocket of unimmunized children in the population. This 7.7% of the unprotected children that had no history of immunization suggests an over report of the immunization coverage levels in Kaduna State.

In areas where immunization coverage is >100%, the chance of wide spread dissemination of vaccine virus is higher than in areas where no marked coverage has been achieved, more so, the spread of vaccine virus is enhanced by crowding and poor hygiene. In Nigeria, the coverage level as reported by NPI (2000) stood at about 108% in 1999. The Ministry of Health, Kaduna State also reported a coverage level of 140%. It is expected therefore, that a higher parcentage of children should have had demonstrable antibodies to at least a serotyope of poliovirus because of the chance of widespread dissemination of the vaccine virus where high coverage has been achieved and the poor standard of hygiene and sanitation in this area of study. It can therefore be inferred from the results of this study that the coverage might not be as high as reported by the Ministry of Health in Kaduna State.

Of the 926 subjects confirmed by history of immunization to have been vaccinated, 128 (13.8%) had no demonstrable antibodies, suggesting immunization failure following vaccination. However, 798 (86.2%) seroconverted following vaccination, suggesting immunity following vaccination. Therefore,

13.8% of the children failed to sero-convert while 86.2% of the children seroconverted following vaccination.

A number of subjects who had a history of receiving 3 or more doses and presumed to be adequately immunized were found to be without antibodies. However, there was poor correlation between the history of immunization and the presence and absence of antibody in the subjects enrolled in this study. It can therefore be inferred that history of immunization obtained using questionnaires is obviously a relatively unreliable index of immunity. This is because parents tend to under report (Gold *et al.*, 1973) or over report the number of doses received by their children (Evans, 1980). This lack of agreement between serological results and historical data, therefore, may probably be in part responsible for the gap between coverage and immunity observed in this study.

One pattern that was observed in the results was the relatively higher prevalence of type 2 poliovirus antibody (Tables 2, 4,5,7, 8, 9 and 11) than types 1 and 3. This reflects the higher immunogenicity of this poliovirus type with respect to the other two that has been reported worldwide (Oduntan *et al.*, 1978; Meseda, 1993; Hanna *et al.*, 1995; Akuoa-Koffi *et al.*, 1995; Chen *et al.*, 1996).

This was further confirmed by the almost two fold higher GMT of antibodies to type 2 compared to the other types and the immunity to this virus type at titre > 40 for all the 5 year old children and all subjects in Zangon Kataf LGA.

The precise cause of lower sero-conversion rates to types 1 and 3 in some parts of the developing world is not clear, but available data suggest that type 2 vaccine virus and enteric pathogens often interfere with the response of types 1 and 3 with the type1 giving the lowest sero-conversion rates (Robertson, 1993).

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions and Recommendations

The present investigation has shown that the seropositive rate to the three poliovirus serotypes in primary school children in Kaduna State is unacceptably (42.2%). This finding will be dificult to explain in view of the high (140%) vaccination coverage with respect to poliomyelitis reported by the Ministry of Health, Kaduna State in 1999. The 19.5% completely susceptible children and the 84 (52.2%) of the 161 children with no history of vaccination that had no demonstrable antibody to any of the serotypes further confirm this. With the relatively high seropositivity to one or more serotypes of poliovirus observed in the children in Kaduna State, the acceleration of the mass immunization eradication activity in the state can be said to be an effective way of arresting poliomyelitis in the state and the country in general. Thus the acceleration of eradication activities should be pursued with vigour and the efforts of NIDs need to be further accelerated to close the gap in immunity in the state due to the remaining pockets of completely susceptible children.

In view of the differences observed in the seropositive rates to the three serotypes of poliovirus in the various LGAs, uniformly high immunization coverage should be maintained in these LGAs. If this is not done, pockets of non-immunized children will build up, thus favouring the spread of the disease which may lead to an outbreak.

Even though the seropositive rate to one or more serotypes of poliovirus is high, the immunity level is low and not as high as expected because only 42.2% of the children had antibodies to the 3 serotypes indicating low herd immunity to poliovirus in the state. These 42.2% are the truly protected children to poliomyelitis in Kaduna State. This low level of protection to poliovirus despite the high verified OPV coverage raises the question of whether this "gap" between coverage and immunity is caused by either failure of OPV, hence low seroconversion, or a greater difficulty in confirming actual OPV doses received by the children and the number of children immunized. This is because the secondary spread of vaccine in this study restricted the analysis to individuals more likely to have truly received no previous dose of OPV.

The overall results suggest that if the current trend of susceptibility to one or more serotype especially to types 1 and 3, and the relatively lower antibody titre even against the ubiquitous type 2 virus except in the 5 year old children and children in Zangon Kataf LGA are maintained, then more children may be at risk of becoming reinfected and serve as source of infection to the 19.5% completely susceptible children in Kaduna State. This may lead to an outbreak/epidemic just like the situation caused by wild poliovirus in Akwa Ibom State reported only recently in the Punch Newspaper of Saturday October 21, 2000.

Considering the number of AFP cases being reported in the National Polio Laboratory daily and those confirmed as caused by wild poliovirus, it must be borne in mind that wild polioviruses have not yet been eliminated and that an appropriate level of immunity should be maintained. The immune status of the

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population should be tested periodically to measure the induction of immune response in the vaccinees, which would also indicate persistence of antibody, and immunity in the absence of the disease. The presence of satisfactory levels of durable antibody in most immunized persons is a better indicator of the degree of protection in a community and this has also been used to determine the effectiveness of a vaccine, although the apparent absence of a common disease after the use of a vaccine is also a good indicator.

Seroconversion following vaccination was 86.2%, which was relatively high. However, younger children in this study were more susceptible to poliovirus than the older children while the older children had lower antibody titres (GMTs). In view of the problems of the relatively poor seroconversion often reported in the tropics amongst children given OPV and the significant difference in antibody levels among the children in this study according to OPV dose received, booster dose should be given to build up high immunity in these children, especially those below 5 years old. Moreso, the older children above 5 should also be given OPV booster dose, which may as well serve as a "starter dose" for the vaccinees that failed to respond immunologically to earlier challenge with OPV, or to those who were never immunized and were missed before the mass immunization started (to augment immunity to poliomyelitis). In addition more emphasis should be placed on the routine immunization programme than the mass immunization programme. This is because the routine immunization is likely to build up higher levels of immunity in children

since the latter programme tends to use vaccines, which may have lost their potency because of exposure to heat in the field.

Secondary spread of OPV virus was found to have played a modest role in increasing polio immunity in the primary school children in Kaduna State especially against types 1 and 3, while the most important predictor of immunity was the number of doses of OPV received irrespective of age. The findings of this study also suggests that the rate of seroconversion produced using the TOPV are still less than optimum in the tropics therefore, it is recommended that booster dose should be given to these children. The history of vaccination using questionnaires is an unreliable index of immunity, which does not give a true picture of the immune status of vaccinees, thus does not effectively help to monitor the success of control programmes. It is therefore recommended that vaccination cards certifying a child as having been vaccinated should be presented as a prerequisite for admission into nursery and primary schools. This will not only encourage mothers to immunize their children but also will be a measure to encourage proper record keeping by parents and it will really assist in obtaining adequate information concerning the history of immunization of vaccinees when required.

Finally with the implementation of the suggested measures and the tripling of the effort of the accelerated eradication activities, the goal of eradicating poliomyelitis can be reached in Nigeria, though not in the year 2000.

6.2 National Immunization Days (NIDs) (House-To-House) Observations

When the vaccinators involved in NIDs checked the vaccine vial monitor (VVM) upon reaching their offices, they discovered that some of the OPV vaccines used had lost their potency. Thus, a second dose was re-administered to the same children on the same day. Although the vaccines had not expired, they had lost their potency probably due to a break in the cold chain as indicated by the colour change on the vaccine vial monitor. This implies that in a situation where the vaccinator fails to check the VVM, the loss of potency may go unnoticed and the children may not be revaccinated, and thus may fail to seroconvert.

During the recent NIDs (October, 2000) it was also observed that the OPV vaccines being used were kept in food flasks with melted ice blocks wrapped in transparent nylon bags. This suggests a temperature of above 0°C which may rise further before the end of the day's immunization. This situation could lower the titre of the OPV vaccines below the recommended minimum concentration per dose leading to possible loss of potency of the vaccines, which in part could contribute to poor seroconversion rates. It is therefore suggested that adequate facilities, especially special vaccine carriers and ice packs for maintaining cold chain be provided for use during NIDs. In addition, the vaccinators and local guides involved in the NIDs should be well educated on the need to maintain cold chain.

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APPENDIX 1

Distribution of polio antibody titres by type among children in Kaduna State, Nigeria.

Subject primary school children	Total no. of samples tested	Antibody titre																							
		Type 1								Type 2								Type 3							
		0	10	20	40	60	160	320	640	0	10	20	40	80	160	320	640	0	10	20	40	80	160	320	640
Kut'au	93	34	9	10	15	17	5	-	3	31	6	18	13	12	5	5	3	34	6	16	12	12	9	1	3
Chikun	119	38	18	20	13	18	5	4	3	36	12	12	26	12	10	6	5	37	13	12	9	18	17	7	6
Igabi	127	49	22	18	22	9	6	1	-	46	16	23	13	14	7	6	2	53	15	17	21	8	7	5	1
Jema'a	92	28	15	6	12	14	1	2	2	25	10	14	16	11	6	9	1	27	9	11	13	13	8	6	5
Kaduna North	139	36	15	30	26	21	2	3	1	34	19	26	16	26	12	3	3	41	22	12	20	22	13	7	2
Zaria	100	42	10	15	16	10	2	3	2	34	11	15	11	12	6	10	1	31	10	10	14	10	5	10	10
Zangon Kataf	120	39	15	13	20	17	5	9	2	38	12	26	19	8	7	5	5	34	17	16	20	19	7	4	3
Lere	117	45	15	23	15	15	9	-	-	26	6	20	15	21	21	8	-	45	11	12	14	15	14	6	-
Kagarko	86	29	15	8	21	7	3	3	-	13	11	8	19	13	9	9	4	33	11	10	14	8	4	3	3
Kauru	54	19	12	10	6	3	2	2	-	16	4	11	13	4	5	1	-	14	8	10	9	6	4	3	-
Sabon Gari	40	16	4	6	1	4	5	2	2	12	3	10	4	3	5	3	-	13	4	4	8	4	1	5	1
Total	1087	341	141	149	167	135	527	29	15	311	110	183	165	136	93	65	23	362	126	130	154	135	85	57	34

APPENDIX 2

Percentage distribution of antibodies to poliovirus by sex at titre ≤ 40 and > 40 in children in Kaduna State, Nigeria.

Sex	Percentage seropositive at titre ≤ 40			Percentage seropositive at titre > 40		
	Type 1	Types 2	Type 3	Type 1	Types 2	Type 3
Male	63.4	56.3	57.3	36.6	43.7	42.7
Female	62.3	54.7	56.3	37.7	45.3	43.7

APPENDIX 3

STATISTICAL ANALYSIS OF GEOMETRIC MEAN TITRE (GMT) VALUES AMONGST PRIMARY SCHOOL CHILDREN IN THE VARIOUS LGA IN KADUNA STATE, NIGERIA.

LGA	Observed and expected GMT values			
	Type 1	Types 2	Types 3	Total
Kubau	46 41.0	50 50.6	49 53.5	145
Chikun	37 44.1	53 54.4	66 57.6	156
Igabi	28 29.9	40 37.0	38 39.1	106
Jema'a	49 44.6	49 55.1	60 58.3	158
Kaduna North	37 35.0	41 43.2	46 45.7	124
Zaria	38 45.5	50 56.1	73 59.4	161
Lere	30 40.4	61 49.9	52 52.8	143
Zangon Kataf	47 37.0	42 45.7	42 48.3	131
Kagarko	33 38.7	61 47.8	43 50.5	137
Kauru	26 29.1	39 35.9	38 38.0	103
Sabon gari	60 45.8	46 56.5	56 59.8	162
Total	431	532	563	1526

Chi-square =	0.62+	0.01+	0.38+
	1.13+	0.04+	1.24+
	0.13+	0.25+	0.03+
	0.43+	0.64+	0.05+
	0.11+	0.11+	0.00+
	1.23+	0.67+	3.11+
	2.67+	2.49+	0.01+
	2.70+	2.29+	2.83+
	0.84+	3.67+	1.13+
	0.33+	0.27+	0.00+
	4.43+	1.94+	0.24= 32.06

Expected GMT values are printed below the observed values

From appendix 3, degree of freedom (df) = 20

At degree of freedom (df) = 20, the observed value is 32.06 i.e., the Chi-square value, while the table value is 31.4, at $P < 0.05$. Since the Chi-square values (32.06) is greater than the table value (31.4) it means there is a significant difference in the GMT values observed in the LGAs.

APPENDIX 4

STATISTICAL ANALYSIS OF GEOMETRIC MEAN TITRE (GMT) VALUES BY AGE AMONGST PRIMARY SCHOOL CHILDREN IN KADUNA STATE, NIGERIA.

Age (years)	Observed and expected GMT values			
	Type 1	Types 2	Types 3	Total
1	76 117.0	161 143.1	161 137.9	398
2	160 77.9	72 95.3	33 91.8	265
3	40 35.9	39 43.9	43 42.3	122
4	35 40.3	52 49.3	50 47.5	137
5	38 41.5	57 50.7	46 48.8	141
6	23 36.8	50 44.9	52 43.3	125
7	37 39.7	43 48.5	55 46.8	135
8	36 21.5	44 50.7	61 48.8	141
9	34 37.3	47 45.7	46 44.0	127
10	34 34.4	41 42.1	42 40.5	117
11	48 58.8	80 71.9	72 69.3	200
Total	561	686	661	1908

Chi-Square = 14.35+ 2.24+ 3.88+
86.47+ 5.69+ 37.67+
0.48+ 0.54+ 0.01+
0.69+ 0.15+ 0.14+
0.29+ 0.78+ 0.16+
5.15+ 0.57+ 1.75+
0.18+ 0.63+ 1.45+
0.72+ 0.88+ 3.02+
0.30+ 0.04+ 0.09+
0.00+ 0.03+ 0.05+
1.99+ 0.91+ 0.11= 171.44

df = 20

Expected GMT values are printed below the observed values

At degree of freedom (df) = 20, table value is 31.4 which is lower than the Chi-square (observed) value (171.44).

Since the Chi-square value (171.44) is greater than the table value 31.4, there is a significant difference in the GMT values observed in the various age groups at $P < 0.05$

APPENDIX 5

STATISTICAL ANALYSIS OF GEOMETRIC MEAN TITRE (GMT) VALUES BY SEX AMONGST PRIMARY SCHOOL CHILDREN IN KADUNA STATE, NIGERIA.

	Observed and expected GMT values			
	Type 1	Types 2	Types 3	Total
Male	36 37.5	49 48.5	51 50.0	136
Female	39 37.5	48 48.5	49 50.0	136
Total	75	97	100	272

Chi-Square = 0.06+ 0.01+ 0.02+
0.06+ 0.01+ 0.02 = 0.17

df = 2

Expected GMT values are printed below the observed values

Observed value ≡ Chi-square value 0.17.

At df = 2, table value at $P < 0.05$ is 5.99. The table value (5.99) is greater than the Chi-square value (0.17). Therefore, there is no significant difference in the sex distribution of polio antibody among the primary school children.

APPENDIX 6

STATISTICAL ANALYSIS OF GEOMETRIC MEAN TITRES (GMTS) ACCORDING TO THE NUMBER OF OPV DOSE RECEIVED BY CHILDREN IN KADUNA STATE, NIGERIA.

No of doses	Observed and expected GMT values			
	Type 1	Types 2	Types 3	Total
1	36 37.5	37 42.4	45 38.0	118
2	26 27.7	29 31.3	32 28.1	87
3	37 44.2	48 50.0	54 44.8	139
4	38 41.3	36 46.7	56 41.9	130
5	86 78.9	94 89.2	68 80.0	248
6	42 43.3	54 48.9	40 43.9	136
7	166 153.6	161 173.6	161 155.7	483
8	80 79.5	113 89.9	57 80.6	250
Total	506	572	513	1591
Chi-Square	0.06+ 0.10+ 1.18+ 0.27+ 0.64+ 0.04+ 0.36+ 0.00+	0.69+ 0.17+ 0.08+ 2.47+ 0.26+ 0.53+ 0.92+ 5.95+	1.27+ 0.56+ 1.88+ 4.73+ 1.79+ 0.30+ 0.18+ 6.92= 31.37	

df = 14

Expected GMT values are printed below the observed values

At df = 14, and $P < 0.05$. Table value is 23.7

The Chi-square value (31.37) is greater than the table value (23.7) therefore; there is significant difference in terms of GMT according to the number of vaccine doses received.

APPENDIX 7

COMPOSITION OF MEDIA AND OTHER REAGENTS USED

1. **Hanks' basic salt solution (Hanks' BSS)**

It comprises of two solutions: Solution A and B and it was used as the basis of the growth media, but its main use is in the preparation of virus transport medium (VTM)

Solution A (Stock)

NaCl	-	40.00g
KCl	-	2.00g
$MgSo_4.7H_20$	-	0.50g
$MgCl_2.6H_20$	-	0.50g

These reagents were dissolved in 200ml-distilled water and 0.70g $CaCl_2$ dissolved separately in 30ml distilled water was added, mixed thoroughly and the volume made up to 250ml with distilled water.

Solution B (Stock) This contains

$Na_2HPO_4.12\ H_20$	-	0.76g
$K\ H_2PO_4$	-	0.30g
Dextrose	-	5.60g

These reagents were dissolved in 200ml-distilled water and the volume was made up to 250ml with distilled water.

The two solutions were prepared aseptically in sterile bottles, sterilized for 15 minutes at 10PSI. To each solution, 0.5ml chloroform was added and stored at 4°C.

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Working Solution

The working solution contains

Solution A	50ml
Solution B	50ml
Distilled H_20	870ml
0.4% Phenol red	2ml

The working solution was prepared by mixing solution A and B as indicated and adding 870ml distilled water and phenol red as pH indicator. The solution was distributed in bottles and autoclaved at 10PSI for 15 minutes. Before use, the pH was adjusted to 7.2 by the addition of 7.5% solution of sodium bicarbonate.

2. Sodium bicarbonate solution

Together with gaseous CO_2 this provided the buffering system for the cell culture media used. It is also an essential metabolite.

A 7.5% of $NaHCO_3$ was prepared and used for the adjustment of pH of media and reagents when necessary. It was prepared as follows:

In clean 200ml capacity bottle containing 50ml distilled water, 7.5g of $NaHCO_3$ was dissolved and 0.2ml of 0.4% phenol red was added and the volume was made up to 100ml with distilled water. The solution was then saturated with CO_2 until orange in colour. The bottle was autoclaved at 10PSI for 15 minutes and stored at 4°C.

3. **Phosphate buffered saline, pH 7.2 to 7.4 (PBS)**

This is the simplest of basic solutions and was used for cell dissaggregation and washing. An incomplete solution of PBS contains no calcium or magnesium ions. A complete solution of PBS is used mainly in the preparation of specimen extracts and as diluents for viruses. The presence of calcium or magnesium ions sterilizes viruses, particularly polioviruses.

The preparations of Solution A, B and C:

Solution A

NaCl	-	8.00g
KCl	-	0.20g
Na_2HPO_4	-	1.15g
KH_2PO_4	-	0.20g

The reagents were dissolved in distilled water, and 2ml of 0.4% phenol red was added as pH indicator. The solution was made up to 800ml, to give a working solution of incomplete PBS.

Solution B

$MgCl_2.6\ H_20$	-	0.10g
Distilled H_2O	-	100ml

Solution C

$CaCl_2$	-	0.10g
Distilled H_20	-	100ml

Solutions B and C were also prepared and all the solutions were autoclaved at 10PSI for 15 minutes.

Working Solution of Complete PBS

This was prepared to a pH of 7.2 by mixing solution A, B and C in the ratio of 8:1:1.

4. **Eagle's growth medium (GM) and Eagle's Maintenance Medium (MM) used for culturing Hep-2 (Cincinnati) cell lines.**

	GM	MM
Eagle's minimum essential medium (Earle's salts base)	89.0ml	91.5ml
L-glutamine 200mM	1.0ml	1.0ml
Fetal calf serum	5.0ml	2.0ml
$NaHCO_3$ solution 7.5%	1.5ml	2.5ml
HEPES 1M	2.0ml	2.0ml
Penicillin/streptomycin solution	1.0ml	1.0ml
0.4% phenol red	0.2ml	0.2ml

The constituents were dissolved in 500ml of distilled water and made up to 1 litre, sterilized, by membrane filtration through membrane filter of 0.22μm millipore size and stored at 4°C for each type of medium.

5. **Cell dispersing agents**

Trypsin and versene are commonly used either separately or in combination.

a. **Trypsin**: This proteolytic enzyme is particularly suitable for the digestion of cells from whole organ, but was used here to remove cells from glass or plastic flasks.

The solution was prepared by dissolving 1g of Difco 1:250 trypsin in 400µl PBS (without Ca, Mg). The solution was placed on a magnetic stirrer for 30 minutes at 36°C with constant stirring until all the trypsin dissolved. The volume was made up to 1000ml with distilled water and the pH adjusted to 7.3. The solution was sterilized by positive pressure filtration through membrane filter of pore size 0.22µm and stored at –20°C. Working solution (0.3%) of trypsin during trypsinization was obtained by mixing 2.5% solution above with PBS in a ratio of 1:9.

b. **Versene**: This chelating agent was also used to remove cells from glass and flasks. The solution was prepared by dissolving 0.1g of versene in 10ml-distilled water. The solution was distributed in 0.5ml volumes in sealed bottles, autoclaved at 10PSI for 15 minutes and stored at room temperature. For use, 0.4ml of this 1% solution was added to 20ml PBS deficient in calcium and magnesium ions (Solution A) to give a final concentration of 1/5000.

6 **Phenol Red Indicator**: Stock solution (0.4%) in distilled water was prepared and distributed into sealed bottles, autoclaved at 10PSI for 15 minutes and stored at room temperature. For media preparation, 1-2ml of this solution per litre was used.

7. **Penicillin/Streptomycin Solution**: This solution was prepared by dissolving 1 x 10^6 units crystalline penicillin G and 1g streptomycin sulphate in 100ml sterile PBS, the solution was distributed into 5ml volumes and stored at –20°C. For use, 1ml of this stock solution is added to 100ml medium to give a final concentration of 100-unit penicillin and 100μg streptomycin.

APPENDIX 8

CRYSTAL VIOLET STAINING PROCEDURE

The crystal violet solution used for staining the cells was prepared in advance by dissolving 2g of crystal violet powder in 3 litres of water. The solution was mixed thoroughly and 20ml of Tween 20 was added. To this solution, a litre of 95% ethanol was added and the solution was stored at room temperature until used. The staining procedures was carried out as follows:

The fluid from the wells of the plates were carefully aspirated completely using multichannel pipettes to which disposable sterile pipette tips are attached without movement that could disrupt the cell monolayer. Crystal violet solution was added to the wells using Pasteur pipettes leaving the wells approximately half full. The plates were incubated for 40 minutes at room temperature and washed three times with tap water using multichannel pipette. The plates were then air dried under a hood.

DEPARTMENT OF MICROBIOLOGY,

AHMADU BELLO UNIVERSITY, ZARIA,

QUESTIONNAIRE FOR SEROLOGICAL SURVEY OF POLIO ANTIBODIES IN KADUNA STATE.

Name: .....

Address: .....

.....

Date of Birth: ..... Sex .....

Tribe: .....

Religion: .....

Local Government Area: .....

Community (Urban/Rural) .....

Educational Status of Parents: .....

.....

Occupation: .....

Number of Sibling in Family: .....

(Dead or Alive)

Type of Water Supply: .....

Types of Sewage System: .....

Vaccination History: .....

BCG: .....

DPT: .....

Measles: .....

Others: .....

OPV_0 ..... Date .....

OPV_1 ..... Date .....

OPV_2 ..... Date .....

OPV_3 ..... Date .....

OPV_4 ..... Date .....

OPV_5 ..... Date .....

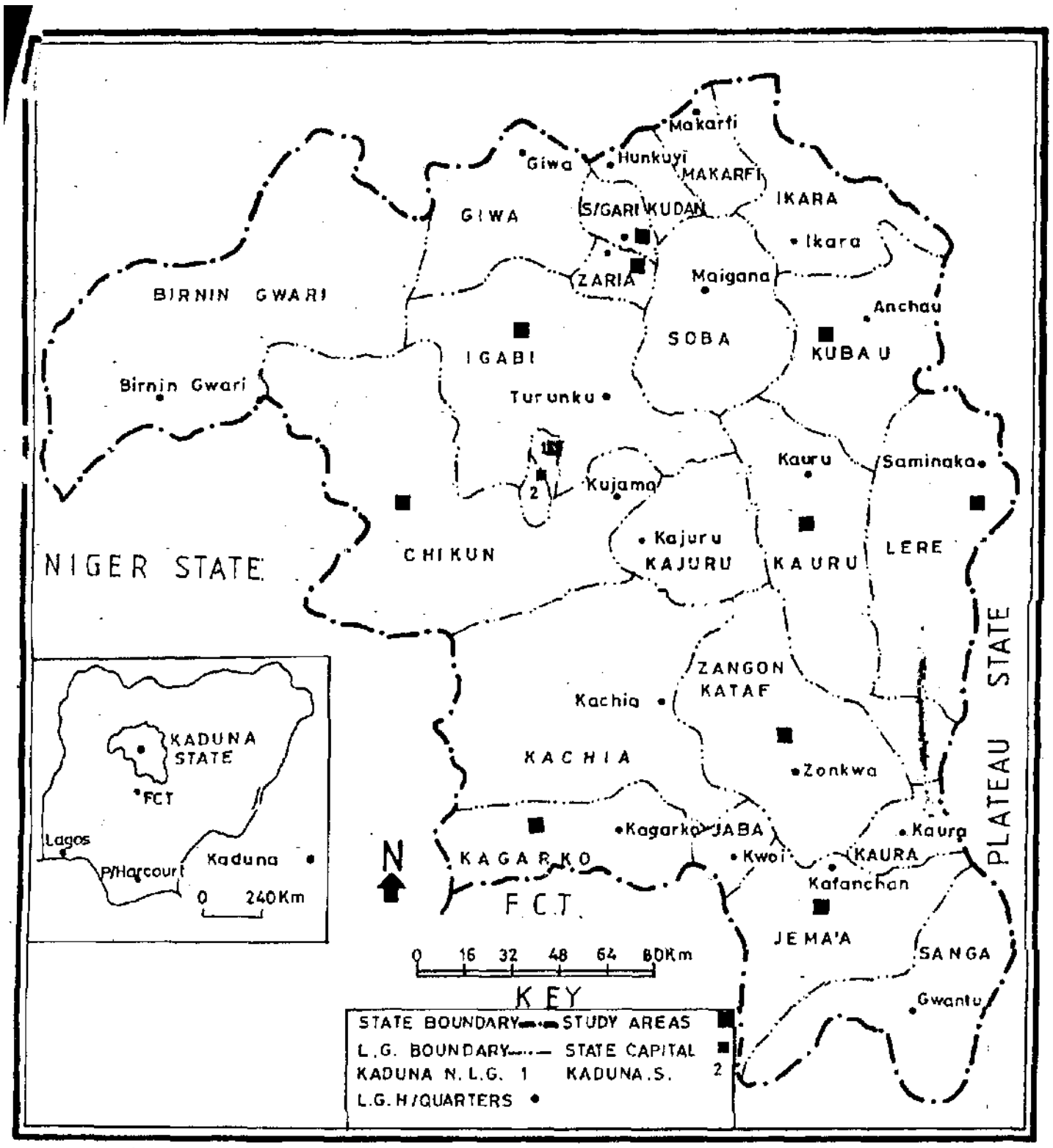


FIG.12 KADUNA STATE SHOWING STUDY AREAS