DETECTION OF IMMUNOLOGLUBULIN G TO POLIOVIRUS IN CHILDREN 5-10 YEARS OLD IN MINNA, NIGERIA

BY

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ABSTRACT

In the Minna, data on the seroprevalence rate of antibodies to poliovirus serotypes which can be used to determine children's immune status and the vaccine efficacy against poliomyelitis is sparse. This study was aimed at detecting immunoglobulin G to poliovirus in children aged 5-10 years old in Minna, Nigeria. About 2 mL of blood was collected by venepuncture from 91 children selected randomly from various health care facilities across Minna. Blood samples were centrifuged at 3000rpm for 5 minutes in order to separate the sera. The detection of poliovirus specific immunoglobulin G (IgG) antibodies from the processed blood samples was done using polyclonal enzyme linked immunosorbent assay (ELISA) detection test kits. In this study, all the children had detectable level of antibodies, 85 (93.4%) children consisting of 49 (53.8%) males and 36 (39.6%) females had protective level of antibodies (seropositive) with optical density of sera greater than standard cut-off and concentration of antibodies greater than 10U/mL. Seropositivity rate of 96.8% (30/31), 94.0% (31/33) and 88.9% (24/27) were recorded among children aged 9-10, 7-8 and 5-6 years old respectively. About 74.7% (68/91) of the participants were weak responder (concentration of antibodies <50 U/mL) to the vaccines received with low seroconversion rates while 6.6% (6/91) of the children had sub-protective level of antibodies (seronegative). Age, sex, parents' occupation, mothers' educational status and source of drinking water have no significant association (p>0.05) with seroprevalence rates while fathers' educational status showed significant statistical association with seroprevalence rate (p<0.05). High seropositivity and low seroconversion rate was recorded in this study, state-wide seroprevalence is recommended to comprehensively evaluate the progress made so far in sustaining polio-free status.

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CHAPTER ONE

1.0   **INTRODUCTION**

1.1 Background to the Study

Critical activities necessary to interrupt transmission and maintain a polio-free world becomes more crucial as the world draws closer than ever to eradicating the notorious wild poliovirus (WPV). Poliomyelitis (polio) caused by poliovirus is a disease that has caused permanent disability and even death to thousands of children, especially those between the age of 0-15 years (Bulama and Goodman-Brown, 2019). Polioviral infection results in clinical manifestations which ranges from in-apparent infection, nonspecific febrile illness, aseptic meningitis to paralysis and even death (John *et al*., 2017). Poliomyelitis is one of the most thoroughly studied diseases, though it has no cure but can be prevented through vaccination (Baicus, 2012).

There are three types of WPV (WPV1, WPV2, and WPV3); all of which can cause severe paralysis or death. The WPV2 and WPV3 have been certified as globally eradicated, while WPV1 remains endemic in Afghanistan and Pakistan. The live attenuated oral poliovirus vaccine (OPV) and the inactivated poliovirus vaccine (IPV) are the two main vaccines used to prevent poliomyelitis (Jehan *et al*., 2017). The polio vaccine, OPV is the most commonly used in the world. It contains live, weakened vaccine viruses that give a vaccinated individual immunity to wild polioviruses in their gut, preventing the viruses from entering the bloodstream and causing paralysis when it invades the nervous system (Centre for Disease Control and Prevention (CDC), 2020a).

Estimated 350,000 cases of polio recorded globally in 1988 reduced gradually to about 291 cases in 2012, representing a 99% reduction in the number of cases. In 2013 and 2014, there was an increase in number of cases in three countries; Nigeria, Afghanistan and Pakistan

(World Health Organisation (WHO), 2014). While in 2015, only two countries (Afghanistan and Pakistan) remains where the disease is endemic (WHO, 2015a).

The first case of poliomyelitis was identified in Nigeria in 1961, WPV1 was found to be the most common strain. In the first nine months of 1962 and April to October of 1963, WPV1 predominance was swapped for WPV2. The re-emergence of WPV2 resulted in a sharp increase in incidence rate of paralysis, prompting mass vaccination for children under the age of three in 1996 (Renne, 2012). The number of confirmed wild polioviral cases in Nigeria has decreased dramatically over the last eight years, from 122 cases in 2012 to 53 cases in 2013, down to 6 cases in 2014, zero cases in 2015, and six cases in 2016, although there has been no report of WPV for the last four years (National Primary Health Care Development Agency (NPHCDA), 2020). Nigeria has interrupted transmission of wild poliovirus, making African region polio-free and bringing the World closer than ever to being polio free (Scherbel-Ball, 2020; CDC, 2020a).

Poliomyelitis is on the verge of been eradicated globally, the cases that do occur are caused mainly by WPV1 or vaccine-derived poliovirus, within groups that are under-immunized (Mehndiratta *et al*., 2014). Globally, the reported cases of poliomyelitis caused by WPVs are on the rise and it is expected to rise further in the coming months. As of May 5, 2021, more than 200 cases of WPVs and about 900 cases of circulating vaccine-derived polioviruses (cVDPVs) have been reported globally (Global Polio Eradication Initiatives (GPEI), 2021). Circulating vaccine-derived polioviruses are caused by excreted vaccine viruses which circulate and can cause paralytic cases in populations with poor immunity (Ming *et al*., 2020).

Countries that have implemented a systematic program to vaccinate children against polioviruses are required to conduct serological studies on the immune status of the eligible

children, or even the whole population, on a regular basis (Wanjiku *et al.,* 2018). Seroprevalence studies may provide valuable information on the effectiveness of immunization programs, groups susceptible to polio infection and populations at risk of potential outbreaks (Opare *et al*., 2019). Low poliovirus antibody seroprevalence in a population can lead to a polio outbreak in a community. In the Democratic Republic of the Congo, a wild poliovirus type 1 outbreak occurred between 2010-2011 among persons aged more than 15 years old, the seroprevalence assessment indicated that antibodies against WPV1 and WPV3 were lower (<80%) in women aged 15-28 years old (Alleman *et al*., 2014; Voorman *et al*., 2017). There are several reports on the possibility of high seroprevalence reducing the chances of poliomyelitis outbreak. WPV1, WPV2, and WPV3 seroprevalence were found to be 91.0 percent, 94.2 percent, and 75.0 percent respectively in a sample of mainland Portuguese residents. In 2002, the Portuguese government declared the country polio-free (Opare *et al*., 2019). The seroprevalence of WPV1, WPV2, and WPV3 was recorded as 99.3%, 98.6% and 99.3% respectively, when determining the immunity status of migrant workers in Israel. This finding revealed that the workers have a high level of immunity, which explains why they have a low chance of contracting polio (Opare *et al*., 2019).

Nigeria continues to implement routine immunization activities to vaccinate against polio and other vaccine preventable diseases. In Nigeria, polio is prevented by giving four doses of the oral polio vaccine (mOPV) at 0, 6, 10 and 14 weeks after birth as well as two doses of IPV at 6 weeks and 14 weeks (NPHCDA, 2020).Nigeria was declared polio-free by the Regional Polio Certification Committee in 2020. In most countries, routine polio vaccine coverage has remained consistently above 90% (Pauly *et al*., 2018). Despite the issue of imminent global eradication, two countries in Asia still have inherent transmission (WHO, 2021). As a result, there is a risk of bringing wild poliovirus into countries that have previously been declared

polio-free. To achieve total eradication of polio globally, surveillance and immunization programs must be strengthened in all countries (GPEI, 2020). The vaccinations used to combat polio are safe and reliable, but 95 percent of the population must be vaccinated and immunized (Voorman and Lyons, 2016).

1.2 Statement of the Research Problem

Any country that has implemented a systematic program to vaccinate children against poliovirus, is required to conduct serological studies on the immune status of the eligible children, or even the whole population, on a regular basis (Wanjiku *et al*., 2018). Countries can risk losing their polio-free status if they are not careful, as was seen in the case of South Africa, Congo, China and Niger Republic (Alleman *et al*., 2014).

In the study area, data on the seroprevalence rate of antibodies to poliovirus serotypes, which can be used to determine children's immune status and the vaccine's efficacy against poliomyelitis, is sparse (Aminu, 2000; Giwa *et al.,* 2012).

Children above the age of 5 years are exempted from routine immunization and supplemental immunization activities and also, most of the studies conducted in the country placed emphasis on children below the age of five, even though children above the age of five years can be susceptible to poliomyelitis.

1.3 Justification for the Study

Nigeria has been certified as polio-free for the first time, preventing the introduction of wild polioviruses into the country and sustaining measures to curb the spread of circulating vaccine-derived polioviruses becomes paramount. In the study area, polio vaccines have been administered to children during routine immunization and campaign sessions but their effectiveness with respect to the children's immune status was not ascertained. For effective monitoring, seroprevalence survey has been recommended to be carried out at intervals so as to ascertain immunity level of those at risk (Razafindratsimandresy *et al*., 2018). Low

poliovirus antibody seroprevalence could result in polio outbreak. The data generated from this study may serve as an immunity benchmark for the study area against any polio infection to enable identification of the populations at risk of future polio outbreaks and also to determine the effectiveness of the vaccination and generate the prevalence data for monitoring, re-planning, implementation and evaluation to ensure total eradication with no possible recurrence.

1.4 Aim and Objectives of the Study

The aim of this study was to detect immunoglobulin G to poliovirus in children 5-10 years in Minna, Nigeria.

The objectives of this study were to:

i)   determine the seroprevalence of poliovirus immunoglobulin G in children aged 5-10 years old in Minna, Nigeria;

ii)   determine the concentration of poliovirus immunoglobulin G in the serum samples;

iii)   determine the socio-demographic factors associated with poliovirus immunoglobulin G seropositivity and seronegativity in children.

CHAPTER TWO

2.0   LITERATURE REVIEW

2.1 Classification of Polioviruses

Poliovirus is a virus that is sub-microscopic, obligate, non-enveloped, and icosahedral in form. It is made up of a single (+) sense ribonucleic acid (RNA) genome enclosed in a capsid, which is a protein shell (Romero and Modlin, 2015). The capsid proteins protect the virus's genetic material while also allowing it to infect specific cell types. Poliovirus has a diameter of 27- 30 nm and it is made up of60 copies of four proteins (VP1, VP2, VP3 and VP4) (Shaffer, 2019). Polioviruses are divided into three types: type 1, type 2, and type 3; they are extremely virulent and produce similar symptoms (Njile *et al*., 2019). The most common type is Type 1, which is also the one most closely linked to paralysis (Anthony, 2013). The gastrointestinal tract is home to these viruses, polioviruses are more infectious for the human gut than for the gut of lower primates (Romero and Modlin, 2015). Outside of the human body, the virus does not live long and there is no long-term carrier state (CDC, 2019a). Polioviruses can survive at both room temperature and temperatures ranging from 0 to 8 degrees Celsius. Polioviruses, like other enteroviruses, are not susceptible to commonly used disinfectants (ether and alcohol). Formaldehyde (0.3 percent), hydrochloric acid, free residual chlorine, exposure to high temperatures or ultraviolet light rapidly inactivates the virus (Chiossone *et al*., 2018).

2.2 Transmission of Poliovirus

Poliovirus has only one reservoir (humans), it is transmitted from person to person, either directly or indirectly, and is often shed in the stools of infected people. Oral-oral transmission of the virus is possible on rare occasions (Vakili *et al*., 2015). In areas with poor sanitation and hygiene, human-to-human contact has been reported (Kew *et al*., 2005; Kew and Pallansch, 2018) and under certain condition, direct neural spread is also possible.

By the time poliomyelitis is recognized in any member of a family, almost all susceptible household contacts have already been infected (Vakili *et al.,* 2015). The virus can spread at any time of the year, but some results indicate that its circulation in temperate countries may be seasonal, and can peak in winter (Estivariz *et al*., 2020). However, these seasonal differences which are apparent in temperate climates are much less pronounced in tropical climates. In Africa, two poliovirus transmission peaks have been identified: February to May (low transmission period) and August to November (high transmission period) (Mehndiratta *et al.,* 2014). Around 7–10 days before and after the onset of symptoms, polio is most infectious. The incubation period is usually between 3 and 35 days (Fisher, 2014). Factors that affect the severity of the disease includes; immune deficiency, pregnancy, muscle injury, tonsillectomy, malnutrition, and vigorous physical activity immediately after the onset of paralysis. While the virus can cross the placenta during pregnancy, neither maternal infection nor polio vaccination seems to affect the foetus. But trans-placental transfer of the resulting antibodies to poliovirus infection had been documented (Palmeira *et al.,* 2012). Asymptomatic people who are infected shed the virus in their stool and may transmit it to others. No association has been established between the level of immunoglobulin and duration of virus shedding (Galal *et al.,* 2012). Transmission pathways can be inferred from the pattern of nucleotide variation among strains, and outbreaks can be traced using genomic analysis. The mouth is the portal of entry, and then primary replication of the virus takes place in the pharynx and gastrointestinal tract. The virus infects the lymphoid tissue in the region, enters the bloodstream, and infects central nervous system (CNS) cells. Replication in motor neurons of the anterior horn cells and the brain causes cell death, resulting in the typical poliomyelitis symptoms. Before the onset of disease, the virus is normally found in the throat and stools. There is no virus in the throat one week after onset, and it is excreted in the stool for several weeks (CDC, 2015). Several million viral particles can be found in a

gram of stool (Mehndiratta *et al*., 2014). The estimated simple reproduction number in settings with faecal pollution of the environment and water sources is about 10–15. In 1988, endemicity of the virus was found in 125 countries, with the WHO estimating that more than 350, 000 children were paralyzed each year (WHO, 2019). At a particular period, in developing countries, 4 of every 1,000 children born annually was estimated to be paralyzed as a result of poliomyelitis (John and Vipin, 2013).

2.3 Pathogenicity of Poliovirus

Acute flaccid paralysis of one or both limbs is the major characteristics of poliomyelitis; a disease caused by poliovirus. Polio infection can manifest itself in two ways: a mild illness that has no effect on the central nervous system, known as abortive poliomyelitis, and a major illness that directly affects the central nervous system; it can be non-paralytic or paralytic case (Shaffer, 2019). The viral infection is asymptomatic in majority of individuals with a healthy immune system. Possible signs of polioviral infection include upper respiratory infections (such as sore throat and fever), stomach disturbances (diarrhoea, vomiting, nausea, constipation or abdominal pain) and influenza-like illnesses (WHO, 2019). Paralytic disease can result from about one percent of polio cases. The virus gains entrance into the human host via the mouth and infects the pharynx and intestinal mucosa, which are the first cells it comes into contact with. It enters the body by binding to a receptor similar to an immunoglobulin. It has the ability to thrive and replicate in the blood and lymphatics (Cann, 2012). It can spread and reproduce in other places in a limited number of cases. In most cases, this spread results in a self-limiting meningeal inflammation. The virus gains little advantage from CNS penetration, and the mechanisms through which the virus spreads to the central nervous system has not been well understood (Shaffer, 2019).

2.4 Diagnosis of Poliovirus

Virus isolation is used to make a definitive diagnosis of poliomyelitis. Poliovirus may be found in stool, the pharynx or cerebrospinal fluid (CSF). To distinguish between wild-type and vaccine-type viruses, oligonucleotide mapping or genomic sequencing is required (Sahoo *et al*., 2017). High concentration of neutralizing antibodies can be found in the serum at early stage. Individuals with acute onset of flaccid paralysis in one or both limbs having reduced or absent tendon reflexes in the affected limbs with no sensory or cognitive loss, may be clinically suspected of paralytic poliomyelitis (CDC, 2015). The presence of virus in the cerebrospinal fluid can also be used in the diagnosis of paralytic polio, although it appears infrequently. A lumbar puncture (spinal tap) is used to extract the patient's CSF, which shows an increased number of white blood cells (primarily lymphocytes) and slightly elevated protein content (CDC, 2019b). Polymerase chain reaction amplification or oligonucleotide mapping (genetic fingerprinting) is usually used to determine the type of virus isolated from AFP cases. It is crucial to verify the type of virus isolated, as wild poliovirus paralytic case has been associated with about 200 to 3,000 infectious asymptomatic carriers (CDC, 2015).

2.5 Management of Poliovirus

Polio has no known cure, the priority of current disease control has been on prevention, symptom relief, accelerating healing, and avoiding complications. Antibiotics are used to prevent secondary bacterial infection of affected muscles, analgesics for inflammation, regular exercise, and a healthy diet are among the supportive steps. Polio treatment also necessitates long-term recovery, which can require physical therapy, use of braces or corrective shoes, and in some cases, orthopaedic surgery (Kishner, 2019). Individual suffering from permanent respiratory paralysis use modern jacket-type negative-pressure ventilators (Chandrasekaran and Shaji, 2021).

2.6 Prevention of Poliovirus

2.6.1 Personal and environmental hygiene

Established primary prevention measures include adequate personal and environmental hygiene; sanitary sewage disposal and portable water supply, and community health education (Schlipköter and Flahault, 2010). At the onset of the disease, good nursing care and physiotherapy can minimize muscle damage or prevent paralysis.

2.6.2 Vaccines

Inactivated polio vaccine (IPV) and oral polio vaccine (OPV) developed by Jonas Salk in 1952 and Albert Sabin in 1962 respectively, were credited with reducing the global number of polio cases per year from many hundreds of thousands to around a thousand in 2006 (WHO, 2019). Immunization with IPV mimics normal infection and induces a local secretory antibody (IgA) reaction, which is linked to a decrease in poliovirus shedding from the intestine. Vaccines, when administered correctly, protect against polio and shield the patient for the rest of their lives. The World Health Organization recommends four doses of the oral polio vaccine for children at birth, 6, 10, and 14 weeks through the Expanded Program on Immunization (EPI). More than four doses of the OPV should be given in situations where the polio virus is more likely to spread due to hot weather or inadequate hygiene. Inactivated polio vaccine and the trivalent OPV protects against the three types of poliovirus whereas, the bivalent OPV protects against poliovirus types 1 and 3; while the monovalent oral polio vaccines 1 and 3 (mOPV1 and mOPV3) protect against poliovirus types 1 and 3 respectively (GPEI, 2018).

The two types of vaccines vary greatly in term of immunologic process and method of administration. Inactivated polio virus is usually injected while OPV is administered orally. Both vaccines provide adequate individual immunity and herd immunity by inducing immune

response against poliovirus (WHO, 2019). Inactivated polio vaccine was added to the regular immunization program in Nigeria in 2015. The vaccine virus replicates and reproduces in the intestine, but it is unable to reproduce effectively in nervous system tissue (Rhoades *et al.*, 2011). Majority of those who receive a dose of OPV usually develop partial immunity to all the three serotypes. Three doses of OPV have been reported to produce protective level of antibody against all the three poliovirus serotypes in more than 95% of the recipients (Atkinson *et al.,* 2011). In addition to providing immunity, the vaccine viruses compete with wild poliovirus for binding sites in the intestinal mucosa, making it very effective at preventing outbreaks. Vaccine viruses present in the intestines are usually excreted in vast amounts in the faeces which can infect the vaccinated individual's vulnerable contacts, giving them protection (Mehndiratta *et al.*, 2014). The ability of attenuated strains to propagate leads to a higher degree of immunization than vaccine coverage provides. This knowledge has been used to effectively introduce mass vaccine programs in different parts of the world (Coates *et al.,* 2013).

Oral polio vaccine, like other live virus vaccines, is thermo-unstable, meaning it loses a lot of its effectiveness when exposed to sunlight. Poliovirus type 3 is the most thermostable of the three strains, losing potency after a few hours at temperatures above 10°C. As a result, maintaining an effective cold chain is critical to ensuring the vaccine's maximum immunogenicity. The properties of oral polio and inactivated polio vaccines were identified by Ozan *et al.* (2014). Poor rate of seroconversion has been associated with oral poliovirus vaccine in tropical countries. About 95% seroconversion rate has been recorded in temperate zone countries, however, seroconversion rates as low as 50% have been recorded in different studies in tropical climate countries, thereby necessitating additional doses. According to a study of oral vaccine immunogenicity trials in developed nations, only 73%, 90% and 70% of people vaccinated with three doses of OPV are immunized against polioviruses Type 1, Type

2, and Type 3 respectively (Flipse and Jolanda, 2015). Thermo-instability of the oral polio vaccine, as well as interference from other enteroviruses, are likely factors. Serotype-specific intestinal immunity develops after OPV absorption, with serotype 2 being more immunogenic than serotypes 1 and 3 (Babji *et al*., 2020).

The use of attenuated oral poliovirus vaccines in monovalent, bivalent, or trivalent forms is critical for polio prevention in endemic areas (GPEI, 2016). Some of the advantages of OPV include its cost effectiveness, high vaccine efficacy, ease of administration and induction mucosal immunity which protects the vaccinated individual and susceptible contacts (GPEI, 2016). The virus remains in the oropharynx for 1 to 2 weeks in the majority of susceptible children that received oral vaccine and is excreted in the faeces for up to 2 months, with the peak excretion occurring in the first week following administration. Vaccines may be responded to poorly because of the following reasons (Michael and Mark, 2012);

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

The World Health Assembly declared poliomyelitis to be eradicated globally in 1988 (CDC, 2011). Oral poliovirus vaccine is one of the most important tool used in global polio eradication efforts. This low-cost vaccine is conveniently delivered by mouth, offers long-term protection against paralytic disease by durable humoral immunity, and renders recent recipients' immune to infection by wild polioviruses (CDC, 2012).

The main limitation of OPV is the possibility of genetic drift to a vaccine-derived poliovirus, which may result in local outbreaks (CDC, 2012; Oliver and Olaf, 2013). According to Mohammed *et al*. (2010), per 750 000 doses of first dosage of trivalent OPV administered, at least one case of poliomyelitis occurs. One case of poliomyelitis occurs per 100 to 1000 cases of wild-type poliovirus infection (Mach *et al*., 2017).

2.7 Polio Eradication

Globally, significant progress has been made since the start of the Global Poliomyelitis Eradication Initiative in 1988. At present, polio remains endemic in only two countries (Afghanistan and Pakistan). The GPEIpolio eradication strategy plan for 2022-2026underscores the urgency of getting eradication efforts back on track and offer a comprehensive set of actions that will position the GPEI to achieve a polio-free world (GPEI-WHO, 2021). These actions, many of which are underway in 2021, include:

(i) further integrating polio activities with essential health services including routine immunization,

(ii) applying a gender equality lens to the implementation of programme activities,

(iii) strengthening advocacy to urge greater accountability and ownership of the program at all levels,

(iv) implementing innovative new tools.

In many developing nations, the prevalence of poliomyelitis decreased significantly after the extensive use of poliovirus vaccine in the mid-1950s (Plotkin *et al*., 2012). Polio was declared eradicated in 36 Western Pacific countries, including China and Australia, in the year 2000 (Adams *et al*., 2014). The last case of poliomyelitis caused by indigenous transmission of wild poliovirus in Europe was discovered in Turkey (Nathanson and Kew, 2010). The World Health Organization has set various goals for eradicating wild polioviruses around the world (GPEI-WHO, 2021). Significant progress has been made, as the goal of

polio eradication approaches, more focus is being put on improving the field and virologic components of wild poliovirus surveillance, prompting the creation of more precise and effective methods for detecting and identifying the virus in clinical specimens or environmental samples (CDC, 2011).

2.8 Poliovirus Specific Antibodies

Antibodies can be present in any individual. These molecules are essential for survival and play a critical role in the immune system. Each individual has one to two billion antibodies that are constantly circulating through their bloodstream, patrolling day and night to combat pathogens and diseases in the humanbody (Harris and Gause, 2011). Individuals that are infected with the virus or that have been immunized with the polio shot build protection. Immunoglobulin A antibodies to poliovirus are found in the tonsils and gastrointestinal tract of immune people and can stop the virus from replicating (Weldon *et al*., 2016). The antibody molecule has two roles that can be separated. Antibodies have the unusual capacity to recognise and bind themselves to the disease-causing virus. Secondly, they serve as markers by identifying and binding themselves to these pathogenic molecules, signaling other members of the immune system to target and kill the disease-associated viruses.

Immunoglobulins are classified into five groups depending on the structural variations in their heavy chains (IgG, IgM, IgA, IgD, and IgE). Subclasses exist for some immunoglobulin classes. Immunoglobulin G has four sub-classes that vary in their heavy chains; IgG1, IgG2, IgG3, and IgG4. Antibodies to most protein antigens are predominantly present in the IgG1 subclass, though antiviral antibodies are also found in large quantities in IgG3. Anti-protein antibodies are also used in small concentrations in IgG4. Relevant immune response systems, in contrast to nonspecific defense mechanisms, are not completely functional at birth and take time to mature following exposure to the infecting agent or its antigens (Jia *et al*., 2020).

Specific immunity may be acquired naturally by infection or artificially by immunization. Immunoglobulin (IgM and IgG) present in the serum 7 to 10 days after infection after normal exposure. Poliovirus entry into the central nervous system can be blocked at sufficiently high levels. The IgM response is initially 2 to 8 times greater than the Immunoglobulin G response. Immunoglobulin M levels reach a plateau two weeks after exposure and then fade out in the serum after 60 days. Immunoglobulin G levels rise gradually, and persistent serum antibodies fall into this category. Immunoglobulin A antibody occurs in the serum 2–6 weeks after exposure and persists at low levels; in certain people, serum IgA does not increase at all. Antibodies in serum are class specific. Infection can induce a low level of heterotypic antibody, especially with Type 1 and 2 polioviruses. Serum neutralizing antibodies (primarily IgG) are thought to last a lifetime. Immunoglobulin G antibodies formed from sub-clinical infection with wild virus lasted for years without subsequent exposure, according to a survey conducted in an isolated Eskimo village (Mehndiratta et al., 2014; Lengkat et al., 2019).

The virulence of the infecting virus and the amount of virus particles delivered to the intestinal and nasal mucosa can influence the survival of secretory IgA antibody. Since measuring circulating antibody, makes estimating immunity very simple, there is a propensity to associate antibody with immunity. Antibody levels, on the other hand, do not represent the body's overall immunity (Kroger et al., 2015). While the presence of serum antibody does not necessarily imply immunity, it does suggest that the human has had prior contact with the microbe. Following immunization or natural infection, protective immunity to polioviral infection develops. Immunity is thought to last a lifetime after a normal infection or the delivery of a live oral polio vaccine. Immunity to one poliovirus serotype, however, does not protect against infection by other serotypes. As a result, complete immunity necessitates susceptibility to all serotypes (CDC, 2020b). For the first few weeks of life, infants born to mothers who have elevated antibody levels against this virus are safe.

CHAPTER THREE

3.0   MATERIALS AND METHODS

3.1 Study Area

The study was carried out in Minna, the capital of Niger State. It is located 9.62 latitude and 6.55 longitudes and it is situated at elevation 243 meters above sea level. Minna is the biggest city in Niger State, it has an estimated population of 462,743of which majority are in the age group 0-10 years old (World Population Review, 2021). The inhabitants of Minna are predominantly Nupe, Gbagyi, Hausa and Fulani with a mixture of other ethnic groups in Nigeria. Local indigenes are mainly farmers, traders, artisans and civil servants.

3.2 Study Design

This was a four months cross sectional descriptive and health facilities based study conducted from July – November 2020. Subjects were healthy children aged between 5-10 years.

3.3 Study Population

The study population consists of children aged 5-10 years old residing in Minna, whose parent/guardian consented to the collection of their blood sample. The population sample was drawn from children with their parents or caregivers who attended one of the designated health facilities for any service during the course of the study.

3.4 Sampling Techniques

Major health facilities in Minna Metropolis such as; General Hospital Minna, Ibrahim Babangida Specialist Hospital Minna, Bosso Primary Health centre, Asibitin Mata Town Primary Health centre, Primary Health care Tunga, Standard Hospital Minna and Old Airport Road Primary Health Centre were selected for the study. Children were selected randomly from each health facility until the number of study sample size was obtained. Parents/guardians with eligible child/children attending designated health facility for services

were contacted for possible participation. Informed consent forms were issued out to the parents/guardians. Social demographic data, vaccination history and other relevant information of each participant was obtained using a structured questionnaire. All questionnaires (appendix A) and informed consent forms (appendix B) were allocated a code that was also written on the respective blood samples containers.

3.5 Sample Size

Sample size was calculated based on polio antibody prevalence survey conducted by Aminu *et al*. (2017). In the study, 94.1% of the children sampled were found to have antibodies to poliovirus. The sample size was calculated using the formular (Ngowi *et al*., 2007) below:

$$n = \frac{Z^2 pq}{d^2}$$

Where
n = sample size

Z = confidence interval (1.96)

p = the probability (0.941)

q= 1-p which is 0.059

L= the allowable error (5%) = 0.05

$$n = \frac{(1.96)^2 \ X \ 0.941 \ X \ 0.059}{(0.05)^2}$$

= 85.3 samples

Therefore, sample size = 85.3

3.6 Sample Collection and Processing

About 2 mL of blood was collected by venepuncture from each subject, after swabbing the skin area of interest with 70% alcohol using sterile disposable 5ml syringe fixed with a 21 gauge (0.81mm) needle. Blood samples were collected into sterile plain blood collection tubes free of anticoagulants, labelled and kept in cooler. Blood samples were centrifuged at

3000rpm for 5 minutes in order to separate the sera. Serum of each sample was aspirated using a sterile Pasteur pipette and transferred aseptically into labelled sterile tubes (Appendix C) and stored at -20˚C until required for use.

3.7 Detection of Antibodies to Polio Virus

The detection of immunoglobulin G (IgG) specific for Poliovirus from the processed blood samples was carried out using polyclonal Enzyme linked immunosorbent assay (ELISA) detection test kits manufactured by Demeditec Diagnostics GmbH, Germany (Appendix D). It detects immunoglobulin G antibodies against the three types of poliovirus simultaneously. Sera and ready-to-use controls (negative control, standard cut off, weak positive, and positive control) were pipetted into the wells of the microtiter plate, leaving a well substrate-blank This caused the serum's unique IgG antibodies to react to the immobilized poliovirus antigen bound to the surface of the wells. The microtiter plate was sealed and incubated at room temperature for one hour, after which the wells were aspirated and diluted wash solution was added using automated microplate washer, in order to remove unbound material, this procedure was repeated thrice. The remaining washing buffer was then lightly tapped off the microtiter plate with a tissue rag. The plate was then coated with ready-to-use anti-human-IgG peroxidase conjugate in each well except the substrate-blank well and incubated for 30 minutes. The tetramethylbenzidine solution was then applied to all wells, including the substrate-blank well, after a third washing and blotting process. The plate was then covered and incubated in the dark for 20 minutes to induce the formation of a blue dye in the wells (Appendix E). By adding a stop solution to all wells after the incubation, the colour switches from blue to yellow (Appendix F). The absorption of the resulting dye were determined spectrophotometrically at the wavelength of 450 nm using a microtitre plate reader after the bottom of the microtitre plate was thoroughly mixed and cleaned with tissue cloth. For at

least 60 minutes, the colour remains unchanged. The optical density of the samples is directly proportional to the concentration of IgG antibodies (Demeditec Diagnostics, 2015).

3.7.1 Qualitative evaluation

The calculated absorption (OD value) for the children sera (Appendix G), as mentioned above, are compared with the value for the cut-off standards shown in Table 3.1. Positive result was recorded for the samples with OD value higher than the cut-off standard while for the values below the cut-off standard, negative result was recorded. A range of +/-0.2 around the value of the cut-off was taken as grey zone.

Table 3.1: Standard absorption values for result interpretation

Standards	OD values (n)	Corrected OD values (n-0.0551)
Substrate Blank	0.0551	
Negative Control	0.0879	0.0328
Cut-Off Standard	0.7204	0.6653
Weak Positive Control	1.607	1.5519
Positive Control	2.7076	2.6525

3.7.2 Quantitative evaluation

The Poliovirus IgG antibody kit's ready-to-use standards and controls are specified and expressed in arbitrary units (U/mL). As a consequence, a precise and repeatable quantitative evaluation was generated. On the quality control data sheet issued by the manufacturer, the values for controls and standards were written (Table 3.2). The absorption of the criteria and controls were graphically drawn point-to-point toward their concentrations using automated computer programs for a quantitative assessment. The concentration values for each patient population were then extracted in comparison to their absorption from the resultant reference curve using the equation of the graph (Appendix H).

Table 3.2: Standard concentration values for result interpretation

Standards	Concentration (U/mL)
Substrate Blank	0
Negative Control	1
Cut-Off Standard	10
Weak Positive Control	50
Positive Control	100

3.8 Ethical Consideration

The Ethical Committee of Hospital Management Board, General Hospital Minna, as well as the heads of each primary health care unit, gave their approval to conduct this study. The guardians or parents of participants in the study also signed a written informed consent form.

3.9 Data Analysis

Data generated from the study was analysed using SPSS version 25 Software. Results generated was presented as percentages, while chi-square and student T-test was used in determining any significant association with regards to socio-demographic and other associated risk factors among the studied children. A p-value of 0.05 or less was considered significant at 95% confidence interval.

4.0   **RESULTS AND DISCUSSION**

4.1 Results

4.1.1 Distribution of immunoglobulin G to poliovirus among children 5-10 years in relation to gender

A total of 91 serum samples obtained from children aged 5-10 years was analysed for the presence of polio specific immunoglobulin G using IgG ELISA test kit, 85 (93.4%) children consisting of 49 (96.1%) males and 36 (90.0%) females showed protective level (seropositive) of antibodies with absorbance greater than standard cut-off and concentration greater than 10U/mL . There was no significant association (χ^2 =1.345 df =1, p =0.399) between gender and seropositivity to the polio virus (Table 4.1).

Table 4.1: Distribution of immunoglobulin G to poliovirus among children 5-10 years in relation to gender

Immunoglobulin G	Frequency (%)			p-value
	Male	Female	Total	
*Seropositive	49 (96.1)	36 (90.0)	85 (93.4)	
**Seronegative	2 (3.9)	4 (10.0%)	6 (6.6%)	0.399
Total	51	40	91	

(χ^2 = 1.345 df =1)
*Seropositive implies protective level of poliovirus specific IgG in the serum
**Seronegative implies sub-protective level of poliovirus specific IgG in the serum

4.1.2 Proportion of children with poliovirus immunoglobulin G based on age group

Table 4.2 presents the proportion of children with poliovirus immunoglobulin G based on age group. The highest seropositive rate was recorded among age group 9-10 years (30/31, 96.8%) followed by 7-8 years (31/33, 94.0%) and the least was recorded in age group 5-6 (24/27, 88.9%).

Table 4.2: Proportion of children with poliovirus immunoglobulin G based on age group

Age group (Years)	Number examined	Number positive	Percentage positive (%)
5-6	27	24	88.9
7-8	33	31	94.0
9-10	31	30	96.8
Total	91	85	

(χ^2 = 1.450; df =2;p = 0.505)

4.1.3 Distribution of the concentration of poliovirus immunoglobulin G in relation to age group

According to the guideline of the ELISA kit manufacturer concentration of antibodies <10 U/mL is seronegative while those ≥10 U/mL is seropositive. The result for concentration <10 U/mL was 6 (6.6%) while 10-49 U/mL was 62 (68.1%), 50-89 U/mL was 17(18.7%) and concentration ≥90 U/mL was 6 (6.6%). There was no significant statistical association (χ^2 = 3.372; df =6;p = 0.789) between concentration of antibodies and age group of children. Table 4.3 showed that concentration of <10U/mL was recorded among 5-6 years (11.1%), 7-8 years (6.89%) and 9-10 years (3.2%). Concentration of 10-49 U/mL was recorded among 5-6 years (59.3%), 7-8 years (72.7%) and 9-10 years (71.0%). Most of the concentration recorded was within the range of 10-49 U/mL (62/91, 68.1%). Concentration of antibodies within the range of 50-89 U/mL was recorded among 18.5%, 18.2% and 19.4% of children within the age group of 5-6, 7-8 and 9-10 years respectively. Highest concentration of antibody recorded which is ≥90 U/mL was observed among 11.1%, 3.0% and 6.5% of children within age group 5-6 years, 7-8 years and 9-10 years respectively.

Table 4.3: Distribution of concentration of poliovirus immunoglobulin G in relation to age group

Concentration (U/mL)	Age groups (years)			
	5-6 Frequency (%)	7-8 Frequency (%)	9-10 Frequency (%)	Total Frequency (%)
<10	3 (11.1)	2 (6.1)	1 (3.2)	6 (6.6)
10-49	16 (59.3)	24 (72.7)	22 (71.0)	62 (68.1)
50-89	5 (18.5)	6 (18.2)	6 (19.4)	17 (18.7)
≥90	3 (11.1)	1 (3.0)	2 (6.5)	6 (6.6)
Total	27	33	31	91

$(\chi^2 = 3.372; df =6; p = 0.789)$

4.1.4 Distribution of concentration of poliovirus immunoglobulin G in relation to gender

There was no association ($\chi^2 = 5.42$; df =3; p = 0.163) between the distribution of concentration of antibodies and the gender of the children examined, highest concentration of antibodies (≥90 U/mL) was recorded among 7.8% (4/51) males and 5.0% (2/40) females. Concentration of antibodies within the range of 50-89 U/mL was recorded among 17.7% (9/51) males and 20.0% (8/40) females, concentration within range of 10-49 U/mL was recorded among 70.6% (36/51) males and 65.0% (26/40) females while the least concentration of <10 U/mL was recorded among 3.9% (2/51) males and 10.0% (4/40) females as presented in Table 4.4.

Table 4.4: Distribution of concentration of poliovirus immuboglobulin G in relation to gender

Concentration (U/mL)	Frequency (%)			p-value
	Male	Female	Total	
<10	2 (3.9)	4 (10.0)	6 (6.6)	
10-49	36 (70.6)	26 (65.0)	62 (68.1)	
50-89	9 (17.7)	8 (20.0)	17 (18.7)	0.163
≥90	4 (7.8)	2 (5.0)	6 (6.6)	
Total	51	40	91 (100)	

$\chi^2 = 5.42$; df =3;   <10 U/mL = seronegative, ≥ 10 U/mLl = seropositive

4.1.5 Distribution of poliovirus immunoglobulin G among children in relation to father's educational status

Table 4.5 presented the distribution of poliovirus immunoglobulin G among children in relation to father's educational status. Highest seropositive rate (97.5%) was recorded among children whose father had tertiary education while seropositive rate of 94.1%, 91.7% and 80.0% was recorded among children whose father had secondary level of education, primary level of education and no formal education respectively. There was a significant statistical association (χ^2 = 9.679; df =3; p=0.012) between the level of education of the subjects' father and seropositivity rate.

Table 4.5: Distribution of poliovirus immunoglobulin G among children in relation to father's educational status

Educational status	Number tested	Number positive (%)	p-value
None	24	22 (91.7)	
Primary	10	8 (80.0)	
Secondary	17	16 (94.1)	0.012
Tertiary	40	39 (97.5)	
Total	91	85	

(χ^2 = 9.679; df =3)

4.1.6 Distribution of poliovirus immunoglobulin G among children in relation to mother's educational status

High seropositivity rate of 100.0%, 96.2% and 93.3% was observed among children whose mothers had secondary, tertiary and primary level of education respectively while the least seropositivity rate (88.6%) was observed among children whose mothers had no formal education. There was no significant statistical relationship between seropositivity rate and the mothers' educational status (χ^2 = 2.101; df =3; p =0.608) as shown in Table 4.6.

Table 4.6: Distribution of poliovirus immunoglobulin G among children in relation to mother's educational status

Educational status	Number tested	Number positive (%)	p-value
None	35	31 (88.6)	
Primary	15	14 (93.3)	
Secondary	15	15 (100)	0.608
Tertiary	26	25 (96.2)	
Total	91	85	

$(\chi^2 = 2.101; df = 3)$

4.1.7 Distribution of poliovirus immunoglobulin G among children in relation to father's occupation

The distribution of poliovirus immunoglobulin G among children in relation to father's occupation is presented in Table 4.7. Children whose fathers were traders had the highest seropositivity rate (95.8%). Seropositivity rate of 94.7%, 94.1% and 85.7% was observed among children whose fathers were artisans, civil servants and farmers respectively, though there was no statistical relationship ($\chi^2 = 1.780$ df =3; p =0.709) between the fathers' occupation and seropositivity rate.

Table 4.7: Distribution of poliovirus immunoglobulin G among children in relation to father's occupation

Occupation	Number tested	Number positive (%)	p-value
Civil servant	34	32 (94.1)	
Artisan	19	18 (94.7)	
Trader	24	23 (95.8)	0.709
Farmer	14	12 (85.7)	
Total	91	85	

$(\chi^2 = 1.780; df = 3)$

4.1.8 Distribution of poliovirus immunoglobulin G among children in relation to mother's occupation

Seropositivity rate of 100.0%, 95.0%, 91.3% and 91.3% was observed among children whose mothers were traders, farmers, civil servants and housewives respectively, also there was no statistical relationship between the mothers' occupation and seropositivity rate (χ^2 = 2.673; df =3; p=0.525) as shown in Table 4.8.

Table 4.8: Distribution of poliovirus immunoglobulin G among children in relation to mother's occupation

Occupation	Number tested	Number positive (%)	p-value
Civil servant	23	21 (91.3)	
Trader	24	24 (100.0)	
Farmer	21	19 (95.0)	0.525
Housewife	23	21 (91.3)	
Total	91	85	

(χ^2 = 2.673; df =3)

4.1.9 Distribution of poliovirus immunoglobulin G among children in relation to source of drinking water

There was no significant statistical relationship between source of drinking water and seropositivity rate (χ^2 = 5.507 df =3; p=0.091). Highest seroprevalence rate of 100.0% was observed among children whose source of drinking water was bore hole while seropositivity rate of 94.3% and 88.0% was observed among children whose source of drinking water was tap and well respectively. The least seropositivity rate of 75.0% was observed among children whose source of drinking water was other source (apart from borehole, well and tap) as presented in Table 4.9.

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Table 4.9: Distribution of poliovirus immunoglobulin G among children in relation to source of drinking water

Source of drinking water	Number tested	Number positive (%)	p-value
Bore hole	27	27 (100.0)	
Tap	35	33 (94.3)	
Well	25	22 (88.0)	0.091
Others	4	3 (75.0)	
Total	91	85	

$\chi^2 = 5.507$; df =3

4.1.10 Distribution of poliovirus immunoglobulin G among children in relation to type of toilet facility

Seropositivity rate of 97.2%, 93.0% and 83.3% was observed among children who used water cistern, pit latrine and open dumping as their toilet facility. There was no significant association between seropostivity rate and type of toilet facility ($\chi^2 = 2.828$; df =2; p=0.181) as shown in Table 4.10.

Table 4.10: Distribution of poliovirus immunoglobulin G among children in relation to type of toilet facility

Type of toilet facility	Number tested	Number positive (%)	p-value
Water cistern	36	35 (97.2)	
Pit latrine	43	40 (93.0)	0.181
Open dumping	12	10 (83.3)	
Total	91	85	

$\chi^2 = 2.828$; df =2

4.2 Discussion

Sustainability of high population immunity to poliovirus is crucial for polio eradication, but poliovirus immunity remains poorly understood despite decades of research (Duintjer-Tebbens *et al*., 2013). In this study, 85 children (93.4%) were found to have immunoglobulin G against poliovirus. This high seropositivity indicated high efficacy of the polio vaccine administered in the study area. This finding is similar with the earlier finding in Kano by Aminu *et al*. (2017), who reported 94.1% seropositivity among children aged 1-10 years. However, the percentage is lower than the rate obtained in the findings of Dashe *et al*. (2011) and Aliya *et al*. (2015), who recorded seropositivity rate of 97.8% and 98.8% respectively. It is noteworthy to say that the seropositivity rate obtained in this study is higher than that of similar study (70.0%) conducted in Bida, Niger State (Oladejo *et al.,* 2013). The variation in this report and earlier studies may be due to differences in geographical location and the study population.

The result showed that 6.6% of the children in this study were seronegative in spite of history of complete routine immunization. This implied that these children have sub-protective level of poliovirus immunoglobulin G. This may be due to the facts that the children failed to seroconvert, received vaccines that may have lost its immunogenicity or they did not receive vaccination. Seronegativity rate recorded in this study agrees with the previous report of 5.9% and 7.9% by Aminu *et al*. (2017) and Adeniji *et al*. (2015) respectively.

Gender has no significance (P>0.05) on the seroprevalence of poliovirus antibodies, even though the percentage of seropositive male 96.1% (49/51) was higher than that of the female 90.0% (36/40). This could be as a result of both gender equal chance of vaccination and seroconversion. Oladejo *et al*. (2013) also recorded a higher prevalence of antibody in male while Mawashi *et al*. (2015) observed that females have higher seropositivity rate. The findings of this study also confirmed an earlier observation by Aminu *et al*. (2017) that

recorded no significant (p>0.05) association between gender and seropositivity rate. However, this was in contrast with other studies (Donbraye *et al*., 2011; Mawashi *et al*., 2015) who recorded significant (p <0.05) association between gender and seropositivity rate.

About 88.9% (24/27), 94.0% (31/33) and 96.8% (30/31) seropositive rate was recorded for the age group 5-6, 7-8 and 9-10 respectively. The seropositive rate seems to increase with age though there was no statistical association (P>0.05) between seropositivity rate and age group. This agrees with the findings of Adewumi *et al*. (2006) and Dashe *et al*. (2011) that stated seropositive prevalence rate increase with age in children. Contrary to this observation, Opare *et al*. (2019) reported a decline in seroprevalence with increasing age.

Majority of the participants (74.7%) in the study had low seroconversion rates (those having concentration of antibodies below 50 U/mL). Low seroconversion rate suggested the existence of children that are susceptible to poliovirus virus infection in the population and might be due to a number of reasons such as incomplete vaccination, simultaneous enteroviral infection, interloping between serotype of oral polio vaccines and unavailability of good water supply as well as inadequate sewage treatment (Tao *et al*., 2013; Alattas, 2017). Poor maintenance in cold chain and sub optimal processing of vaccines could affect the quality of the vaccine and ultimately result in low seropostivity (CDC, 2021). Previous studies by Magrath *et al*. (1981) and Nishio *et al*. (1984) have shown that children with low concentration of antibodies could be re-infected with wild or vaccine derived virus. Children in this category may not be in danger of developing poliomyelitis but may be re-infected with poliovirus and possibly provide a source of infection for other children who have not been vaccinated (Thompson *et al*., 2013; Adeniji *et al*., 2015).

Immunization coverage as well as the level of awareness on the need to get children immunized against the poliovirus has increased tremendously among the populace. This

study reiterates the facts that the more educated a parent is, the more the seropositive rate, though children whose parents were uneducated also had high seropositivity rate of antibodies to the poliovirus. There was significant association (P<0.05) between seropositivity rate and fathers' educational level.

Furthermore, poor sanitation has always been a major driver of poliovirus infection (Richard *et al.,* 2014). Unavailability of portable water and inadequate sewage treatment have also been attributed with lack of seropositivity or low seropositivity (Tao *et al*., 2013). However, toilet facilities and drinking water source were found not to influence the seropositivity of the participant in this study.

All the children had detectable antibodies to poliovirus even though some were not up to protective level. This might be explained by a number of reasons such as failure to receive vaccines, acquisition of antibodies due to natural infection or exposure to excreted vaccines (Mawashi *et al*., 2015). In Nigeria vaccine failure is likely, due to sub-optimal storage of vaccine, inadequate cold chain maintenance during transportation and storage or incorrect vaccination protocol. Host factors such as the failure of the subjects to develop an immune response to vaccination due to a number of conditions; immunosuppressive therapies and recognised immunodeficiency illness or blood transfusion may have impaired immune response.

Likewise, persistent of passively acquired maternal antibody may have attenuated immune response. Post vaccination immune response against poliovirus may wane over time, especially if boosting from exposure to natural infection does not occur, so the longer the duration since vaccination the more likely is secondary vaccine failure to occur.

CHAPTER FIVE

5.0   CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Sero-epidemiological surveys are useful tools for assessing population immunity and identifying areas with low immunity. The study recorded high seropositivity rate of poliovirus immunoglobulin G among vaccinated children aged 5-10 years residing in Minna, Niger State. Though, polio eradication programme in Minna achieved high serologic performance, immunity gaps in young children remains as majority of the population had low concentration of poliovirus immunoglobulin G. This immunity gaps might pose risk for polio re-infection and emergence of vaccine-derived polioviruses. Socio-demographic factors such as age, gender, mother's education, parents' occupation, source of drinking water and type of toilet facilities have no influence on the distribution of seropositivity and seronegativity rate in children.

5.2 Recommendations

It is critical to avoid the risk of reintroduction of wild poliovirus into the country. Improved interventions aimed at monitoring and enhancing coverage against poliomyelitis in children would help achieve this target. The chance of wild polio transmission would persist until wild poliovirus transmission has been halted globally.

Though, significant progress in global polio eradication has been recorded, the number of polio cases in the world is on the rise in comparison to previous years. Nigeria must preserve its polio-free status by maintaining sufficient public protection to polio.

Since low seroconversion rate has been recorded in children aged 5-10 years, it is advisable that Nigeria introduces booster dose of OPV for children above the age of five.

Further studies involving larger samples size in the study area is recommended, to comprehensively evaluate the progress made so far in sustaining polio-free status.

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APPENDIX A: SAMPLE OF INFORMED CONSENT FORM

CODE NO…………………

INTRODUCTION

This study is aim to detect immunoglobulin G poliovirus in children 5-10 years old in Minna, Nigeria. The data that would be generated from this study will help in the determination of the seroprevalence of polio specific IgG in children and the socio-demographic factors influencing seropositivity and seronegativity rate in children in the study area.

I agree to allow the participation of my child/ward in the above study. I understand that the blood sample of my child/ward will be collected for the study. I make this consent willingly without being subjected to any pressure.

Participant signature   **Researcher's signature**

CODE NO…………………

1. Bio Data

Age: …………………………………………….

Residential Area: ………………………………

Gender: …………………………………………

Tribe: ……………………………………………

2. Education status of parents:

Father   (a) Quranic (b) Primary (c) Secondary (d) Tertiary

Mother   (a) Quranic (b) Primary (c) Secondary (d) Tertiary

3. Occupation of Parent

Father   (a) Civil servant (C/S) (b) Farmer (c) Trader (d) Artisan

Mother   (a) Civil servant (C/S) (b) Farmer (c) Trader (d) Housewife

4. Type of water supply: (a) Tap (b) Well (c) Borehole (d) Stream (e) Others

5. Type of Toilet Facilities: (a) pit latrine (b) Open dumping (c) Water cistern (d) Others

6. Are you aware of vaccination: (a) Yes (b) No

7. History of vaccination:
Complete routine immunization schedule for children: (a) Yes (b) No
Recieved doses of OPV during supplementary immunization activities: (a) Yes (b) No
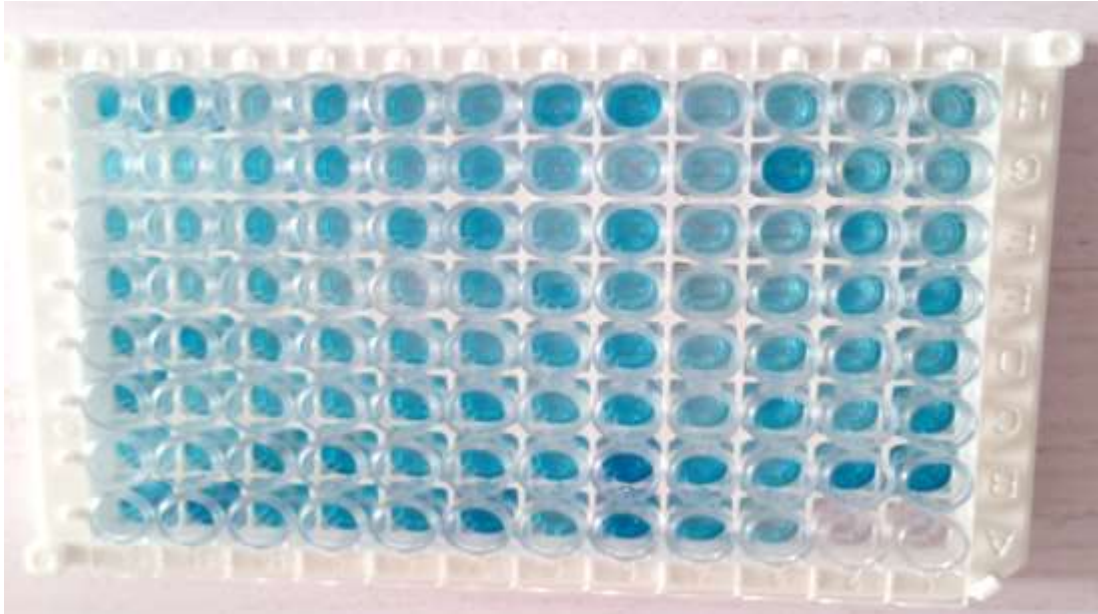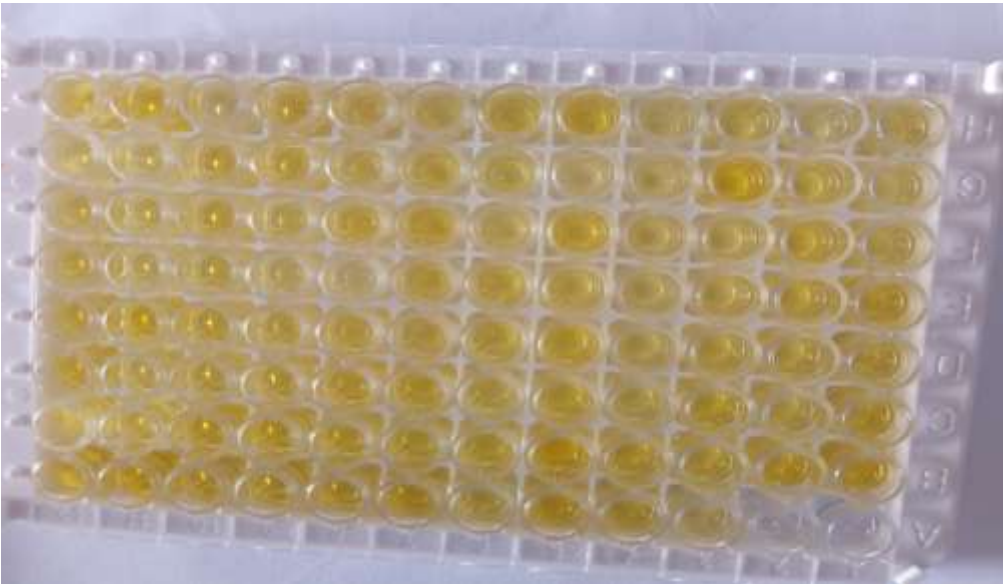
APPENDIX C: SERUM SAMPLES

APPENDIX D: POLYCLONAL POLIOVIRUS IMMUNOGLOBULIN G ELISA DETECTION TEST KIT:

APPENDIX E: FORMATION OF A BLUE DYE IN THE WELLS OF MICROTITRE PLATE

**APPENDIX F: COLOUR CHANGE FROM BLUE TO YELLOW ON ADDITION OF
A STOP SOLUTION TO ALL WELLS**

APPENDIX G: ABSORPTION (OD VALUE) OF THE CHILDREN SERA

Session:

Session:	Microtitre Plate Reader; Absorption values	
Plate format:	96 wells	
Area:	A1 - H12	
Measurement type:	0	Single
Measurement mode:	1	Precision
Wavelength (nm):	450	
Required temp (°C):	No	
Shaking:	1	Single
Duration (hh:mm:ss):	00:00:00	
Shaking speed:	1	Medium

Run:

|	
Instrument:	Multiskan GO 1.00.40
Serial number:	1510-01685C
Start time:	14.10.2020 06:06:38 PM
Stop time:	14.10.2020 06:07:04 PM
Start temperature:	33.7
Stop temperature:	33.7

Data: 450 nm

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.0551	0.0879	0.7204	1.6070	2.7076	1.1821	2.8314	1.8016	1.7936	1.7425	3.3174	2.4396
B	2.6687	2.2240	1.4058	1.6724	3.3332	1.6979	1.6200	1.5182	2.0183	1.9746	1.1144	1.3165
C	1.7405	1.0598	1.4888	0.8825	1.4629	1.0942	1.4814	1.4887	1.2013	1.1019	0.8590	1.9507
D	1.3619	1.2114	1.0927	0.8131	1.5155	1.1400	1.1119	1.1323	1.0366	1.2588	1.8451	1.4491
E	1.6304	1.2183	0.8922	0.7544	1.0914	1.2161	1.1193	0.5402	0.6864	0.9655	0.7059	1.3510
F	1.0964	1.4859	0.8209	0.7890	1.4697	0.7851	1.6043	1.2967	0.8814	1.2496	0.8164	1.0491
G	0.9179	1.1517	2.5035	0.7603	0.6495	0.9668	1.2801	0.8915	1.3928	1.2777	0.7612	0.9265
H	1.0044	0.6239	1.0453	0.7444	2.0320	1.6380	1.0530	1.2371	1.4972	0.6014	2.1897	1.6778

Checksum 47492

APPENDIX H: RESULTANT REFERENCE CURVE

Rational Function Equation: $y = (a + bx)/(1 + cx + dx^2)$

Coefficient Data:
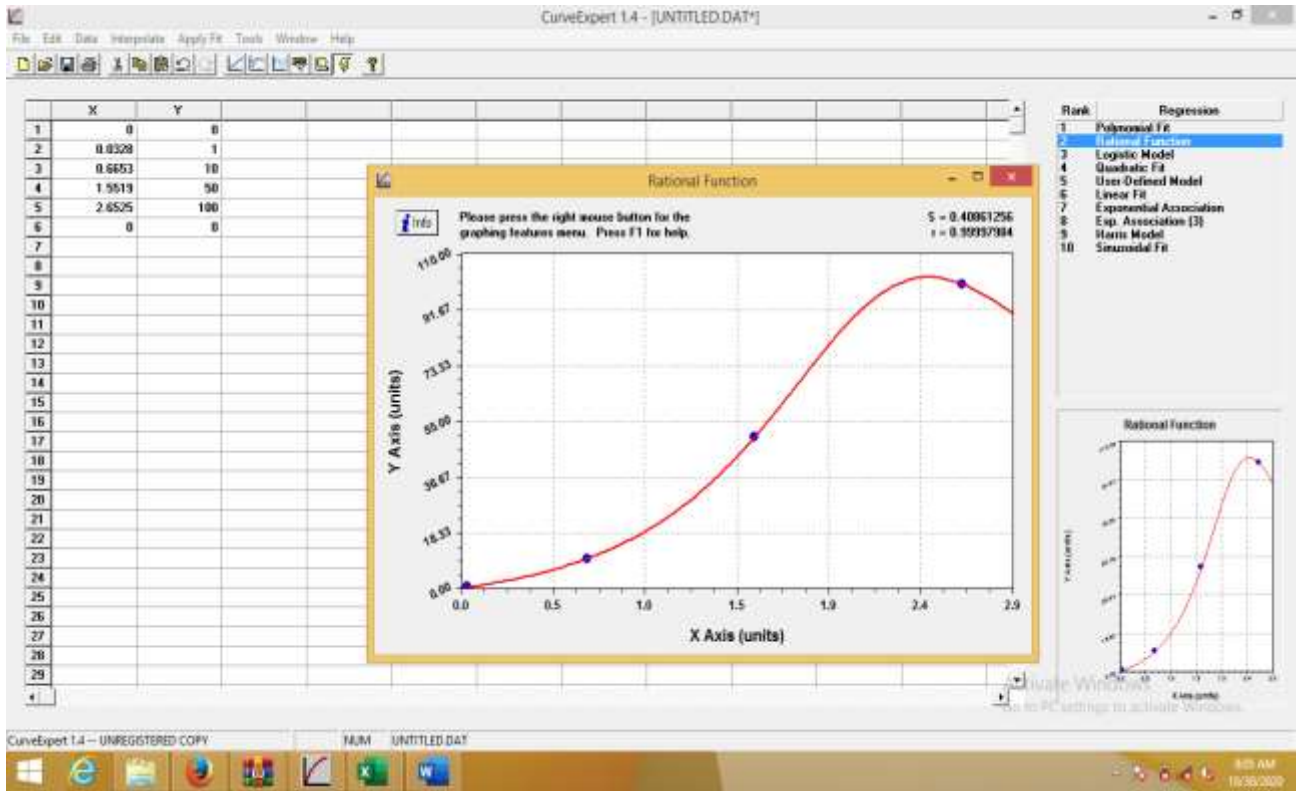
$$a = 2.30017040200E - 001$$
$$b = 8.55026593888E + 000$$
$$c = -7.23873402285E - 001$$
$$d = 1.63332811176E - 001$$

x	x^2	bx	cx	dx^2	$a + bx$	$1 + cx + dx^2$	y
1.127	1.270129	9.63615	-0.81581	0.207454	9.866167	0.391648	25.19139
2.7763	7.707842	23.7381	-2.00969	1.258944	23.96812	0.249254	96.15951
1.7465	3.050262	14.93304	-1.26424	0.498208	15.16306	0.233963	64.80964
1.7385	3.022382	14.86464	-1.25845	0.493654	15.09465	0.2352	64.17789
1.6874	2.847319	14.42772	-1.22146	0.465061	14.65774	0.243597	60.17216
3.2623	10.6426	27.89353	-2.36149	1.738286	28.12355	0.376794	74.63914
2.3845	5.68584	20.38811	-1.72608	0.928684	20.61813	0.202608	101.7636
2.6136	6.830905	22.34698	-1.89192	1.115711	22.57699	0.223795	100.8823
2.1689	4.704127	18.54467	-1.57001	0.768338	18.77469	0.198329	94.66424
1.3507	1.82439	11.54884	-0.97774	0.297983	11.77886	0.320247	36.78056
1.6173	2.615659	13.82835	-1.17072	0.427223	14.05836	0.256502	54.8079
3.2781	10.74594	28.02863	-2.37293	1.755165	28.25864	0.382235	73.92999
1.6428	2.698792	14.04638	-1.18918	0.440801	14.27639	0.251622	56.73745
1.5649	2.448912	13.38031	-1.13279	0.399988	13.61033	0.267198	50.9372
1.4631	2.140662	12.50989	-1.0591	0.34964	12.73991	0.290541	43.8489
1.9632	3.854154	16.78588	-1.42111	0.62951	17.0159	0.208402	81.64958

1.9695	3.87893	16.83975	-1.42567	0.633557	17.06977	0.207888	82.11044
1.0593	1.122116	9.057297	-0.7668	0.183278	9.287314	0.416479	22.29958
1.2614	1.59113	10.78531	-0.91309	0.259884	11.01532	0.34679	31.76368
1.6854	2.840573	14.41062	-1.22002	0.463959	14.64064	0.243943	60.01674
1.0047	1.009422	8.590452	-0.72728	0.164872	8.820469	0.437596	20.15664
1.4337	2.055496	12.25852	-1.03782	0.33573	12.48853	0.297913	41.92012
0.8274	0.684591	7.07449	-0.59893	0.111816	7.304507	0.512883	14.24204
1.4078	1.981901	12.03706	-1.01907	0.323709	12.26708	0.30464	40.2674
1.0391	1.079729	8.884581	-0.75218	0.176355	9.114598	0.424178	21.48766
1.4263	2.034332	12.19524	-1.03246	0.332273	12.42526	0.299813	41.44344
1.4336	2.055209	12.25766	-1.03774	0.335683	12.48768	0.297938	41.91366
1.1462	1.313774	9.800315	-0.8297	0.214582	10.03033	0.384879	26.06102
1.0468	1.09579	8.950418	-0.75775	0.178978	9.180435	0.421228	21.79447
0.8039	0.646255	6.873559	-0.58192	0.105555	7.103576	0.523633	13.56595
1.8956	3.593299	16.20788	-1.37217	0.586904	16.4379	0.214729	76.55177
1.3068	1.707726	11.17349	-0.94596	0.278928	11.4035	0.33297	34.24785
1.1563	1.33703	9.886673	-0.83701	0.218381	10.11669	0.381366	26.5275
1.0376	1.076614	8.871756	-0.75109	0.175846	9.101773	0.424755	21.42827
0.758	0.574564	6.481102	-0.5487	0.093845	6.711119	0.545149	12.31061
1.4604	2.132768	12.48681	-1.05714	0.348351	12.71683	0.291206	43.66948
1.0849	1.177008	9.276184	-0.78533	0.192244	9.506201	0.406914	23.36171
1.0568	1.116826	9.035921	-0.76499	0.182414	9.265938	0.417425	22.19786
1.0772	1.16036	9.210346	-0.77976	0.189525	9.440364	0.409768	23.03829
0.9815	0.963342	8.392086	-0.71048	0.157345	8.622103	0.446864	19.29471
1.2037	1.448894	10.29196	-0.87133	0.236652	10.52197	0.365326	28.80163
1.79	3.2041	15.30498	-1.29573	0.523335	15.53499	0.227601	68.2553
1.394	1.943236	11.91907	-1.00908	0.317394	12.14909	0.308315	39.40483
1.5753	2.48157	13.46923	-1.14032	0.405322	13.69925	0.265004	51.6945
1.1632	1.353034	9.945669	-0.84201	0.220995	10.17569	0.378985	26.84982
0.8371	0.700736	7.157428	-0.60595	0.114453	7.387445	0.508499	14.52795
0.6993	0.48902	5.979201	-0.5062	0.079873	6.209218	0.573668	10.82371
1.0363	1.073918	8.860641	-0.75015	0.175406	9.090658	0.425256	21.3769
1.161	1.347921	9.926859	-0.84042	0.22016	10.15688	0.379743	26.74673
1.0642	1.132522	9.099193	-0.77035	0.184978	9.32921	0.414632	22.49998
0.4851	0.235322	4.147734	-0.35115	0.038436	4.377751	0.687285	6.369632
0.6313	0.39854	5.397783	-0.45698	0.065095	5.6278	0.608113	9.254524
0.9104	0.828828	7.784162	-0.65901	0.135375	8.014179	0.47636	16.82377
0.6508	0.423541	5.564513	-0.4711	0.069178	5.79453	0.598081	9.688532
1.2959	1.679357	11.08029	-0.93807	0.274294	11.31031	0.336227	33.63894
1.0413	1.084306	8.903392	-0.75377	0.177103	9.133409	0.423333	21.57498
1.4308	2.047189	12.23372	-1.03572	0.334373	12.46374	0.298655	41.73288
0.7658	0.58645	6.547794	-0.55434	0.095787	6.777811	0.541444	12.51802
0.7339	0.538609	6.27504	-0.53125	0.087973	6.505057	0.556722	11.68457
1.4146	2.001093	12.09521	-1.02399	0.326844	12.32522	0.302853	40.69707

0.73	0.5329	6.241694	-0.52843	0.08704	6.471711	0.558612	11.58533
1.5492	2.400021	13.24607	-1.12142	0.392002	13.47609	0.270578	49.80491
1.2416	1.541571	10.61601	-0.89876	0.251789	10.84603	0.353028	30.72286
0.8263	0.682772	7.065085	-0.59814	0.111519	7.295102	0.513382	14.20988
1.1945	1.42683	10.21329	-0.86467	0.233048	10.44331	0.368381	28.34918
0.8063	0.65012	6.894079	-0.58366	0.106186	7.124096	0.522527	13.63393
0.994	0.988036	8.498964	-0.71953	0.161379	8.728981	0.441849	19.7556
0.8628	0.744424	7.377169	-0.62456	0.121589	7.607186	0.497031	15.30526
1.0966	1.202532	9.376222	-0.7938	0.196413	9.606239	0.402613	23.85971
2.4484	5.994663	20.93447	-1.77233	0.979125	21.16449	0.206794	102.346
0.7052	0.497307	6.029648	-0.51048	0.081227	6.259665	0.570751	10.96742
0.5944	0.353311	5.082278	-0.43027	0.057707	5.312295	0.627437	8.46666
0.9117	0.831197	7.795277	-0.65996	0.135762	8.025294	0.475806	16.86672
1.225	1.500625	10.47408	-0.88674	0.245101	10.70409	0.358356	29.86997
0.8364	0.699565	7.151442	-0.60545	0.114262	7.381459	0.508814	14.50718
1.3377	1.789441	11.43769	-0.96833	0.292274	11.66771	0.323949	36.01712
1.2226	1.494751	10.45356	-0.88501	0.244142	10.68357	0.359134	29.74813
0.7061	0.498577	6.037343	-0.51113	0.081434	6.26736	0.570307	10.98945
0.8714	0.759338	7.450702	-0.63078	0.124025	7.680719	0.493242	15.57192
0.9493	0.90117	8.116767	-0.68717	0.147191	8.346784	0.460018	18.14449
0.5688	0.323533	4.863391	-0.41174	0.052844	5.093408	0.641104	7.944741
0.9902	0.980496	8.466473	-0.71678	0.160147	8.69649	0.443368	19.61462
0.6893	0.475134	5.893698	-0.49897	0.077605	6.123715	0.578639	10.58296
1.9769	3.908134	16.90302	-1.43103	0.638327	17.13304	0.207301	82.64805
1.5829	2.505572	13.53422	-1.14582	0.409242	13.76423	0.263423	52.25147
0.9979	0.995804	8.53231	-0.72235	0.162647	8.762327	0.440294	19.90107
1.182	1.397124	10.10641	-0.85562	0.228196	10.33643	0.372578	27.74301
1.4421	2.079652	12.33034	-1.0439	0.339675	12.56036	0.295778	42.46554
0.5463	0.298444	4.67101	-0.39545	0.048746	4.901027	0.653294	7.502028
2.1346	4.556517	18.2514	-1.54518	0.744229	18.48141	0.199049	92.84877
1.6227	2.633155	13.87452	-1.17463	0.430081	14.10453	0.255451	55.21419

APPENDIX I: OPTICAL DENSITY AND CONCENTRATION OF THE

STANDARDS AND SERUM SAMPLES

Well Number	Sample type	OD value	corrected OD value	Outcome	Concentration
1A	Blank	0.0551	0	-	0
2A	Negative	0.0879	0.0328	NEG	1
3A	Cut-off	0.7204	0.6653	CUT-OFF	10
4A	Weak positive	1.607	1.5519	POS (w)	50
5A	Positive	2.7076	2.6525	POS	100
6A		1.1821	1.1270	POS (w)	25.19139
7A		2.8314	2.7763	POS	96.15951
8A		1.8016	1.7465	POS	64.80964
9A		1.7936	1.7385	POS	64.17789
10A		1.7425	1.6874	POS	60.17216
11A		3.3174	3.2623	POS	74.63914
12A		2.4396	2.3845	POS	101.7636
1B		2.6687	2.6136	POS	100.8823
2B		2.2240	2.1689	POS	94.66424
3B		1.4058	1.3507	POS (w)	36.78056
4B		1.6724	1.6173	POS	54.8079
5B		3.3332	3.2781	POS	73.92999
6B		1.6979	1.6428	POS	56.73745
7B		1.6200	1.5649	POS (w)	50.9372
8B		1.5182	1.4631	POS (w)	43.8489
9B		2.0183	1.9632	POS	81.64958
10B		1.9746	1.9695	POS	82.11044
11B		1.1144	1.0593	POS (w)	22.29958
12B		1.3165	1.2614	POS (w)	31.76368
1C		1.7405	1.6854	POS	60.01674
2C		1.0598	1.0047	POS (w)	20.15664
3C		1.4888	1.4337	POS (w)	41.92012
4C		0.8825	0.8274	POS (w)	14.24204
5C		1.4629	1.4078	POS (w)	40.2674
6C		1.0942	1.0391	POS (w)	21.48766
7C		1.4814	1.4263	POS (w)	41.44344
8C		1.4887	1.4336	POS (w)	41.91366
9C		1.2013	1.1462	POS (w)	26.06102
10C		1.1019	1.0468	POS (w)	21.79447
11C		0.8590	0.8039	POS (w)	13.56595

12C		1.9507	1.8956	POS	76.55177
1D		1.3619	1.3068	POS (w)	34.24785
2D		1.2114	1.1563	POS (w)	26.5275
3D		1.0927	1.0376	POS (w)	21.42827
4D		0.8131	0.7580	POS (w)	12.31061
5D		1.5155	1.4604	POS (w)	43.66948
6D		1.1400	1.0849	POS (w)	23.36171
7D		1.1119	1.0568	POS (w)	22.19786
8D		1.1323	1.0772	POS (w)	23.03829
9D		1.0366	0.9815	POS (w)	19.29471
10D		1.2588	1.2037	POS (w)	28.80163
11D		1.8451	1.7900	POS	68.2553
12D		1.4491	1.3940	POS (w)	39.40483
1E		1.6304	1.5753	POS (w)	51.6945
2E		1.2183	1.1632	POS (w)	26.84982
3E		0.8922	0.8371	POS (w)	14.52795
4E		0.7544	0.6993	POS (w)	10.82371
5E		1.0914	1.0363	POS (w)	21.3769
6E		1.2161	1.1610	POS (w)	26.74673
7E		1.1193	1.0642	POS (w)	22.49998
8E		0.5402	0.4851	NEG	6.369632
9E		0.6864	0.6313	NEG	9.254524
10E		0.9655	0.9104	POS (w)	16.82377
11E		0.7059	0.6508	NEG	9.688532
12E		1.351	1.2959	POS (w)	33.63894
1F		1.0964	1.0413	POS (w)	21.57498
2F		1.4859	1.4308	POS (w)	41.73288
3F		0.8209	0.7658	POS (w)	12.51802
4F		0.789	0.7339	POS (w)	11.68457
5F		1.4697	1.4146	POS (w)	40.69707
6F		0.7851	0.7300	POS (w)	11.58533
7F		1.6043	1.5492	POS	49.80491
8F		1.2967	1.2416	POS (w)	30.72286
9F		0.8814	0.8263	POS (w)	14.20988
10F		1.2496	1.1945	POS (w)	28.34918
11F		0.8164	0.8063	POS (w)	13.63393
12F		1.0491	0.9940	POS (w)	19.7556
1G		0.9179	0.8628	POS (w)	15.30526
2G		1.1517	1.0966	POS (w)	23.85971
3G		2.5035	2.4484	POS	102.346
4G		0.7603	0.7052	POS (w)	10.96742
5G		0.6495	0.5944	NEG	8.46666

6G		0.9668	0.9117	POS (w)	16.86672
7G		1.2801	1.2250	POS (w)	29.86997
8G		0.8915	0.8364	POS (w)	14.50718
9G		1.3928	1.3377	POS (w)	36.01712
10G		1.2777	1.2226	POS (w)	29.74813
11G		0.7612	0.7061	POS (w)	10.98945
12G		0.9265	0.8714	POS (w)	15.57192
1H		1.0044	0.9493	POS (w)	18.14449
2H		0.6239	0.5688	NEG	7.944741
3H		1.0453	0.9902	POS (w)	19.61462
4H		0.7444	0.6893	POS (w)	10.58296
5H		2.0320	1.9769	POS	82.64805
6H		1.6380	1.5829	POS	52.25147
7H		1.0530	0.9979	POS (w)	19.90107
8H		1.2371	1.1820	POS (w)	27.74301
9H		1.4972	1.4421	POS (w)	42.46554
10H		0.6014	0.5463	NEG	7.502028
11H		2.1897	2.1346	POS	92.84877
12H		1.6778	1.6227	POS	55.21419