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POLIOVIRUS ANTIBODY LEVELS AND LAMENESS AMONG INDIVIDUALS IN THREE REGIONS OF GHANA

BY

JOSEPH KWADWO LARBI OPARE

(10292370)

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DECLARATION

I, Joseph Kwadwo Larbi Opare, author of this thesis do hereby declare that except for references to other authors' work, which have been duly acknowledged, this work is the result of my own research work, carried out solely as partial fulfillment of the requirements for the award of Doctor of Philosophy in Public Health and has neither in part nor in whole been presented elsewhere for another degree.

…… **Date: ……**

Joseph Kwadwo Larbi Opare

(Candidate)

ACADEMIC SUPERVISORS

…… **Date: ……**

Dr. Patricia Akweongo

(Primary Supervisor)

…… **Date: ……**

Professor Col. Edwin Afari (Rtd)

(Secondary Supervisor)

…… **Date: ……**

Dr. John Kofi Odoom

(Supervisor)

DEDICATION

This study is dedicated to God, my able supervisors, my wife, children and family for the moral and emotional support given me during the conduct of this study.

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ABSTRACT

Introduction: In 2008, Ghana recorded the last case of poliomyelitis or polio and the causative agent was the wild polio virus. The country has been certified free of polio and it is at the verge of polio eradication. High levels of polio neutralizing antibodies (immunity) must be maintained to prevent the importation of wild poliovirus. We determined the seroprevalence of polio viruses (P1, P2, and P3) antibodies and the risk factors for seronegativity in Greater Accra, Ashanti and Northern regions of Ghana in order to identify any gaps for system improvement.

Methods: A cross-sectional, hospital-based seroprevalence study supplemented by a school lameness survey was undertaken in the three study regions in 2016. Individuals, who visited the three teaching hospitals of the regions and needed to give out blood samples for laboratory investigation, were invited to partake in the survey. Micro-neutralization test for poliovirus serotypes, 1, 2 and 3 antibodies was performed following WHO-standard procedures. Antibody titers of ≥ 1:8 were considered positive. Bivariate and multivariate analyses were conducted on subject characteristics to assess for potential factors for failure to sero-convert. Statistical significance was set at *P* -values <0.05

In the school lameness survey, clinical and epidemiological data were obtained from parents and their lamed children using a semi-structured questionnaire in the same three regions. School lameness data was descriptively analysed by person, place and time by employing frequency distributions, percentages, means, standard deviation and rates. Data was analysed using STATA version 13.

Results: Neutralizing-antibodies against poliovirus types 1, 2 and 3 were detected in 86.0% (264/307), 84% (258/307) and 75% (230/307) of samples respectively. Overall, 60.1%

(185/307) were seropositive and 2.9% (9/307) were seronegative for the three polio serotypes. Seroprevalence of polio-neutralizing antibodies among males (P1=51.9%, P2=51.6% and P3=52.6%) were higher than females. Seroprevalence rates of polio-neutralizing antibodies (P1, P2 and P3) were highest in the Northern Region (91.8%, 82.4%, and 77.4%). Polio neutralizing-antibodies (P1 and P2) decreased with age [p<0.001]. Low seroprevalence of polio-neutralizing antibodies was significantly associated with low school attendance of mothers [p=0.003 for PV1 and p<0.001 for PV2]. Prevalence of residual paralysis was 0.58/1,000 or 5.8/10,000 children aged 0-15years in schools of the study regions.

Conclusion: This study revealed a moderate level of seroprevalence of neutralizing antibodies to the three polio serotypes with some regional differences. Seropositivity was generally low with increasing age and the mother's education level was crucial to seronegativity. The drastic reduction of paralytic poliomyelitis may be attributed to the moderate level of polio-neutralizing antibodies.

To further strengthen the gains made in polio eradication, the Ghanaian government needs to increase and sustain budgetary allocation on polio eradication awareness and mass immunizations. The Expanded Programme on Immunization (EPI), Ghana, may consider young-adult booster-dose of polio vaccine, introduce inactivated polio vaccine (IPV) to supplement the oral polio vaccine and conduct a nationwide seroprevalence and community based lameness survey to gauge programme performance. Female child education and career counseling for Junior High School pupils and those older may be intensified by all District Assemblies and by churches.

Keywords: Polio, Immunization, Seroprevalence, Microneutralization, Lameness, Ghana

TABLE OF CONTENTS

CHAPTER THREE

LIST OF TABLES

LIST OF FIGURES

LIST OF ACRONYMS

AFP	Acute flaccid paralysis
ANOVA	Analysis of variants
CDC	Centres of Disease Control and Prevention
CPE	Cytopathic effect
EPI	Expanded Programme on Immunization
HEP2C	Cell line derived from human epidemoid carcinoma
GM	Growth medium
GMT	Geometric mean titres
MEM	Minimal essential medium
MM	Maintenance medium
NIDS	National immunization days
OPV	Oral poliovirus vaccine
PBS	Phosphate buffer saline
SIA	Supplementary immunization activity
WHA	World Health Assembly
WHO	World Health Organization
EDTA	Ethylene diamine tetra acetic acid
WPV	Wild Polio Virus
PV	Polio Virus

DEFINITION OF KEY TERMS

Seroprevalence

The number of persons in a population who test positive for a specific disease based on serology (blood serum) specimens; often presented as a percent of the total specimens tested or as a proportion per 100,000 persons tested.

Seropositivity

Respondents with detectable antibody levels at ≥ 1:8 dilution for that specific poliovirus serotype.

Acute flaccid paralysis

Acute: rapid progression of paralysis, (from onset to maximum paralysis)

Flaccid: loss of muscle tone, "floppy" (as opposed to spastic or rigid)

Paralysis: weakness, loss or diminution of motion.

Lameness

A physical handicap that prevents a person from walking normally.

CHAPTER ONE

1.0 INTRODUCTION

This chapter highlights background information on polio, the seroprevalence of polio virus antibodies, and polio eradication. It also provides the statement of the problem and justification, while further describing the conceptual framework, research questions, study objectives and hypothesis.

1.1 Background

This section describes the polio disease, eradication, seroprevalence and the progress towards polio eradication in Ghana.

1.1.1 The burden of poliomyelitis

Poliomyelitis, which is caused by the poliovirus serotypes 1, 2 and 3 is a highly infectious viral disease which can have crippling effects. It mainly affects children who are less than five years of age. According to Robert [Robert, 2010], in developing countries, in one out of 200 polio infections, paralytic polio is observed, while fatality is normally observed in 5-10% of paralytic polio cases. Since 1988, there has been a decrease of over 99% in polio cases from an estimated 350,000 cases spread across 125 countries. In 2016, only 37 cases of polio were recorded worldwide. The global effort to eradicate the disease has resulted in this reduction [WHO, 2016].

1.1.2 Global polio eradication

Global immunization campaigns against poliomyelitis promoted by the World Health Organization (WHO), with the aim of making the world “polio free” have resulted in the elimination of this disease from several regions [WHO, 2015]. In 1988, polio eradication

was a topical issue on the agenda of the World Health Assembly, and there was a decision to eradicate polio by 2000. Two decades have passed since this resolution and great strides have been made towards achieving this goal [WHO, 2015]. The Global Polio Eradication initiative has reduced by 99% the incidence of paralytic polio with the use of Oral Polio Vaccine and eradicated the polio virus serotype two (P2) [Kew et al., 2005]. Since the last reported case of polio virus serotype three (P3) in Nigeria in 2013, there has not been any case [WHO, 2015].

The first WHO region to be certified free from poliovirus circulation was the Americas in 1994 [CDC, 1999], followed by the Western Pacific in 2000, the European Region in 2002 and South East Asia in 2014 [WHO, 2015]. Nigeria, the only polio endemic country in Africa had the last WPV case on July 24, 2014 until the recent outbreak in August, 2016, in the northern Borno state [WHO, 2016].

Since the Global Polio Eradication Initiative was launched in 1988, it has succeeded in reducing the number of poliomyelitis cases significantly (WHO, 2003). The main challenges to the complete interruption of poliovirus circulation worldwide (CDC, 2000) and eradication of poliomyelitis in the remaining endemic countries include inadequate service delivery of oral poliovirus vaccine (OPV) and suboptimal OPV efficacy in the densely populated tropical reservoirs [Grassly, 2006; 2007].

The circulation of neurovirulent vaccine-derived polioviruses and the importation of wild polioviruses into polio-free countries are some of the key adverse factors complicating the eradication initiative. To block future introduction of wild polio viruses into any country, it is necessary to maintain a high level of population immunity by giving repeated doses of

oral polio vaccine. Sero-surveillance is therefore of value in countries where there are pockets of non-vaccinated individuals.

1.1.3 Seroprevalence for polio virus antibodies

Seroprevalence of antibodies to polio serotypes assesses the immune status of the individual and the effectiveness of the vaccine against poliomyelitis. For a specific disease in any given population, the number of persons testing positive based on serology is known as the seroprevalence. Seroprevalence provides important data on performance of immunization programmes, susceptible groups and populations at-risk of future outbreaks. To achieve polio eradication, high levels of polio antibodies are needed.

Immunity to the three different polio viruses, 1, 2 and 3 differ in different populations. To achieve eradication of polio in any population, it is essential to maintain high levels of immunity (high sero-positivity) to all the three serotypes of the poliovirus in the population.

1.1.4 Progress towards polio eradication in Ghana

The most common clinical manifestation of paralytic poliomyelitis is the syndrome Acute Flaccid Paralysis (AFP). In 1996, active surveillance for AFP cases was established with full laboratory support to help in detecting poliovirus in Ghana. Cases of wild poliovirus increased from two in 1996 to 23 in 1998 and reduced to one in 1999 when the last indigenous strain was seen. Since then the country has experienced two major wild poliovirus outbreaks, in 2003 and 2008. Eight wild poliovirus type-1 cases from acute flaccid paralysis (AFP) and 15 from apparently healthy children were isolated in 2003 after three years of the country being polio-free. Eight more wild type-1 polioviruses were detected in 2008 from AFP cases. Additional National Immunisation Days and mop-up activities followed and no wild poliovirus has been detected in Ghana since that date. To

prevent future importations, it is necessary to maintain a high level of population immunity (polio neutralizing antibodies) against the polio virus. Until polio is completely eradicated worldwide, it is necessary to carry out continuous surveillance of immunization levels in the population to help maintain a polio-free status. Surveillance of the circulation of enteric viruses is also important for confirming the absence of poliovirus. Ghana especially needs this level of surveillance since many immigrants who are from several countries having similarly low levels of sanitation have made the country their destination. In 2000, almost three-fifths (58.9%) of residents who were non-Ghanaian were Economic Community of West African States (ECOWAS) nationals, whiles almost a quarter (23%) of immigrants to Ghana were from non-ECOWAS African countries [GIS, 2008]. The presence of infected and receptive people could represent serious danger for the country.

1.2 Statement of the problem

Since the last case of wild polio virus in 2008, continuous Supplemental Immunization Activities (SIAs) coupled with quality acute flaccid paralysis (AFP) surveillance with the appropriate documentation to the Regional Certification Committee had declared Ghana a polio-free country in 2015 [GHS, 2015].

Despite the good of impending global eradication, there persist few areas of inherent transmission in Africa (Nigeria) and Asia. A danger of importing wild poliovirus to countries that are almost polio-free therefore still exists [CDC, 2001; 2006].

Ghana is at risk if protective immunity levels are not sufficiently high, given its closeness to Nigeria where the poliovirus is endemic. Ghana experienced wild poliovirus outbreaks in 2003 and 2008. Whereas the 2003 outbreak covered the entire country, the 2008

outbreak was confined more to the northern part of the country. Recently, D.R. Congo—which had been polio-free for several years—experienced wild polio outbreaks that resulted in several deaths. The majority (74%) of the deceased were adults who were expected to have some protection against the virus [Patel et al., 2012].

Weaknesses in health system infrastructure, inadequate service delivery of oral poliovirus vaccine (OPV), suboptimal OPV efficacy, social cultural beliefs and low seroprevalence to polio antibodies are some of the possible explanations for these observations. High levels of immunity in the form of neutralizing polio antibodies need to be maintained for the prevention of wild poliovirus importation into the country, which may lead to residual paralyses in the population. With persisting transmission of endemic wild poliovirus and outbreaks recurring in polio-free countries after the year 2000, which was the target date originally set for polio eradication [CDC, 2005; CDC, 2009; CDC, 2010], a declaration of the completion of the eradication of polio was made by the World Health Assembly in 2012 as a programmatic emergency [WHO, 2012].

The level of neutralizing polio antibodies is a surrogate marker of protective immune response to PV [Bahl, 2002; Savy, 2009; MMW, 2002] and neutralizing polio antibodies above 66-80% provides adequate protection against polio infection [Sutter et al, 2004]. There is inadequate information on the seroprevalence of neutralizing polio antibodies and risk factors of low seroprevalence in the Ghanaian population. Ghana is at the verge of polio eradication and the success gained through the routine and mass vaccination against polio should reflect in the population immunity. Valuable information can be gleaned from surveys on seroprevalence which have been conducted in other places.

This study determined the seroprevalence of neutralizing antibodies against poliovirus serotypes 1, 2 and 3 by evaluating the neutralizing antibodies in the Ghanaian population of three regions, and exploring the factors that predict low poliovirus antibody seropositivity.

1.3 Conceptual Framework

Figure 1.1 shows the conceptual framework for seroprevalence of polio antibodies and factors associated with it. The level of antibodies in the blood depends on the polio vaccination coverage, which is also dependent on vaccine efficacy, health system, communication, social cultural beliefs, malnutrition, gender and other factors. The threat of polio outbreak depends on the immunity provided by the level of antibodies against polio in the blood.

The major cause for vaccination failure globally is malnutrition, which on average causes one child death every 15 seconds, culminating in 2.3 million deaths per year among children. According to WHO, developing countries are home to the majority of children who are expected to be malnourished. Approximately a third (30%) of them are less than five years old while as much as half of them suffer from protein energy malnutrition [Jack, 2013].

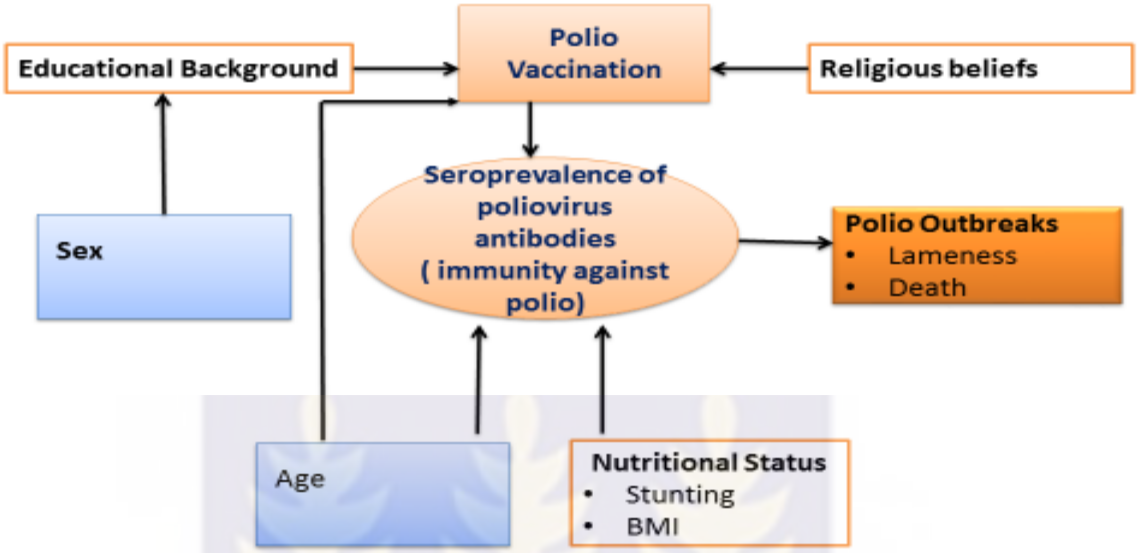

Figure1.1: Conceptual frame for understanding seroprevalence of antibodies to polio viruses

Education plays a crucial role in polio vaccination. It has been recently found out in a study in Pakistan among 768 people in Quetta and Peshawar Division that people with no formal education were less likely to have adequate knowledge on polio vaccination. This idea has been corroborated by many other studies [Jajoo et al., 1985; Mansuri & Baig, 2003; Fotso, 2006]. Maternal education in part determines the socioeconomic status and autonomy of the woman. High maternal education may imply an improved autonomy in decision-making power of the woman, which increases the possibilities of a woman being able to make confident decisions in sending her child for childhood immunization services.

Many vaccines provide protection through the stimulation of production of antibodies. The extent of the antibody production is highly dependent on many factors and the crucial ones are the age and sex of the vaccine recipient. Compared to the aged, antibody response in

general is higher in the younger age groups. The conceptual framework also explains that age of the child has an effect on their immune status. As the child is given more doses of oral polio vaccine through the years, these repeated doses boost the immune protection of the child.

The appropriate vaccine formulation, production and storage conditions make it efficacious. These conditions affect the level of antibodies in the blood which ensure the immune protection of the individual.

Although vaccination is a strategy for controlling and preventing some infectious diseases, there has been an increase in negative concerns among some religious groups about the use of vaccines worldwide [Mugerwa et al., 2002]. Many health workers face resistance to childhood vaccination, especially among some religious groups, and many parents find it difficult to ensure that their children are given the necessary vaccines [Omer et al., 2009; Streefland et al., 1999]. For example, in a protestant church in the Netherlands, 40% of the 250,000 church members had a religious objection to vaccination. There was then an outbreak of polio, measles and rubella among this group which spread to the relatives who lived in Canada [Oostvogel et al, 1994; Van Den Hof et al., 2002; Hahne et al, 2009]. Additionally, the boycott of polio vaccination in Nigeria was a great set back to polio immunization in the country, and cases of paralytic polio increased from 202 in 2002 to 1143 in 2006 [Ghinai et al., 2013].

The Expanded Programmes on Immunizations continue to prevent many vaccine preventable diseases. Improvement in the vaccination coverage among women and children has contributed to the reduction in morbidity and mortality from vaccine preventable diseases. The understanding of parents and caregivers is crucial in the delivery

of vaccination service. They should understand the need of the vaccines for their children and make the right informed choices on behalf of their children. This may dispel any false beliefs on vaccination and create a positive attitude toward immunization services. When the vaccines are given to the children at the appropriate times, the vaccines have an effect on the production of antibodies in the blood. This would ensure an adequate protection for the child against any poliomyelitis infection. When the serum level of polio virus antibodies is below a certain threshold the person could contract polio virus infection. There is less than one percent chance of developing paralytic polio. However, there is a probability of two and five per cent that people who developed paralytic polio may die and half of those who survive may have permanent paralysis.

1.4 Justification for the study

Seroprevalence studies provide important information on the performance of immunization programmes, vaccine efficacy and serve as an auxiliary tool for supporting poliomyelitis eradication [Bahl, 2002; Savy, 2009; CDC, 2002]. Despite the importance of seroprevalence findings, there has been insufficient current information on population immunity against polio, which provides an immunity benchmark to reflect program performance and to guide future program action to interrupt polio transmission in Ghana. Since the first lameness survey was conducted in Ghana, there had not been any known repeat survey [Ofosu-Amaah et al., 1977].

The data provided from this study may serve as an immunity benchmark for the three regions of Ghana against any polio infection to enable identification of the populations at-risk of future polio outbreaks. The risk factors affecting seroconversion of the oral polio

vaccine (OPV) need to be identified to help the programme on polio eradication to adopt other strategies to increase seroprevalence, to guide future programme action.

1.5 Research questions

1. What are the levels of neutralizing polio antibodies among Ghanaians against poliovirus types 1, 2, and 3 with specific micro neutralization assay in the Northern, Ashanti and Greater Accra regions of Ghana?
2. What is the distribution of neutralizing antibodies against the three polioviruses with relation to person and place?
3. Is there any association between age and neutralizing antibody mean titres among individuals in the three regions of Ghana?
4. What are the risk factors for low seroprevalence against polio virus antibodies among Ghanaians in the three regions?
5. What is the prevalence of lameness among school children in the three regions of Ghana?

1.6 Research hypothesis

1. The seroprevalence of the three serotypes of polio virus antibodies in three regions of Ghana is less than 90% (Zubairu , 2013)
2. There is no association between age and neutralizing antibody mean titres among Ghanaians in Northern, Ashanti and Greater Accra regions of Ghana

1.7 Study Objectives

1.7.1 General Objective

To assess the level of polio neutralizing antibodies against poliovirus serotypes 1, 2, and 3 in the Northern, Ashanti and Greater Accra regions of Ghana, and to identify factors predicting low poliovirus seroprevalence among individuals in the study areas.

1.7.2 Specific objectives

1. To determine the level of polio neutralizing antibodies against poliovirus serotypes 1, 2, and 3 with specific micro neutralization assay in Northern, Ashanti and Greater Accra regions of Ghana
2. To determine the distribution of antibodies that neutralize the three polioviruses with relation to person (sex, age) and place (region)
3. To determine the association between age and neutralizing antibody mean titres among individuals in the three regions
4. To determine the risk factors for low seroprevalence against polio virus antibodies in the three regions
5. To estimate the prevalence of poliomyelitis associated lameness among school children in the three regions of Ghana

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Introduction

This chapter covers a review of a broad scope of literature relating to the topic under consideration. It aims at uncovering critical facts and findings identified previously by various studies and other researchers.

Poliomyelitis, often called polio or infantile paralysis, is an acute, viral, infectious disease spread from person to person, primarily via the fecal-oral route [Cohen et al, 2004]. The term derives from the Ancient Greek, meaning "grey", *myelós* (marrow), referring to the grey matter of the spinal cord, and the suffix *-itis*, which denotes inflammation [Chamberlin SL and Narins B, 2005]. Thus poliomyelitis is the inflammation of the spinal cord's grey matter. Even though there are no symptoms presenting in approximately 90% of polio infections, individuals who are affected can show a range of symptoms once the virus gains entry into the blood stream [Ryan and Ray, 2004].

The virus preferentially infects and destroys motor neurons in about one percent of cases, when it enters the central nervous system. This leads to muscle weakness and acute flaccid paralysis and depending on the nerves that are involved, different types of paralysis may occur. The form that occurs most commonly is spinal polio, which is characterized by asymmetric paralysis involving the legs most often. Another occurring form is bulbar polio which leads to a weakness of the muscles innervated by cranial nerves. A combination of the bulbar and spinal forms of paralysis described is known as bulbospinal polio [Atkinson et al., 2009].

2.2 Epidemiology of polio

Polio remains endemic in places like Pakistan, Nigeria and Afghanistan though it is rare in the Western world; just as in many industrialized countries where the incidence of poliomyelitis has substantially reduced after the widespread use of poliovirus vaccine in the mid-1950s. The WHO, UNICEF and the Rotary Foundation led a global effort in 1988 to eradicate polio [Mastny and Lisa, 1999]. From an estimated 350,000 cases in that year, these efforts have led to a 99% reduction in the number of cases diagnosed annually. Thus in 2001, as low as 43 cases were recorded, with this much lower level of about 1,000 cases a year being sustained for a while (1,606 in 2009) [CDC, 2006; 2008]. In 2015, confirmed polio cases globally decreased to 74 [Figure 2.1]. As at September 20th, 2016, 26 wild polio cases had been reported globally; in Afghanistan (9), Pakistan (14) and Nigeria (3) [WHO, 2016].

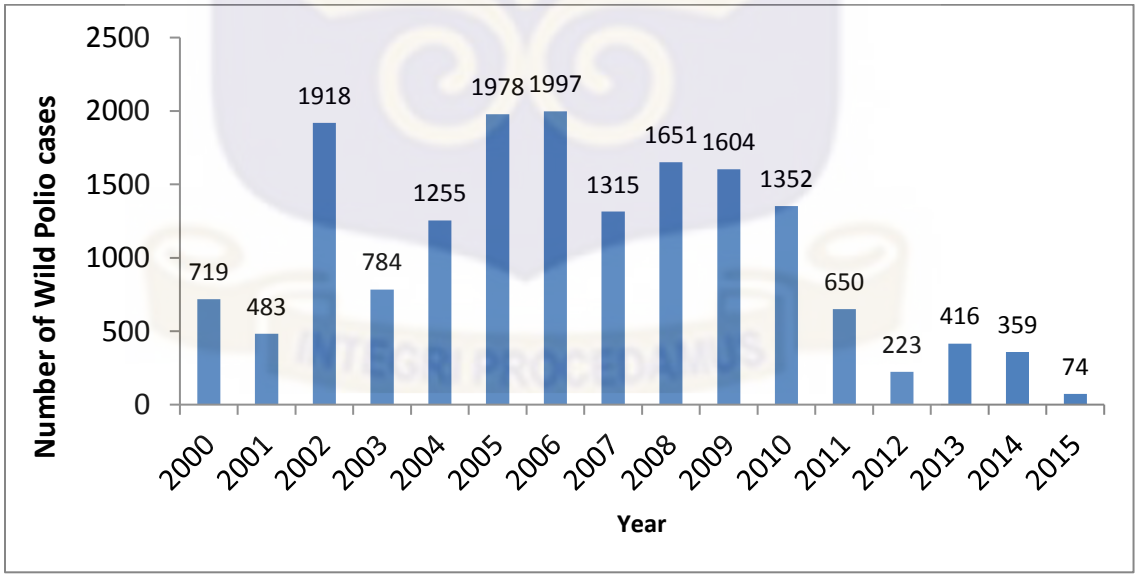

Figure 2.1: Global trend of wild polio cases, 2000-2015

2.3 History of polio

Karl Landsteiner in 1908 identified poliovirus as the causative agent of poliomyelitis even though earlier in 1840, poliomyelitis had been recognized as a distinct health condition [Paul, 1971]. Major epidemics beginning to occur in Europe and subsequently in the United States in the 1880s brought polio into the limelight. Until then, polio had quietly existed for millenniums as an endemic pathogen [Trevelyan, 2005]. Of the most feared childhood illnesses of the 20th century, polio was prominent. For much of human history, the disease polio has affected thousands of people including both the young and adults, causing paralysis and in some cases, death.

By 1910, many people in the world had been infected by polio, with polio outbreaks becoming a regular event in the cities during the hot weather. Epidemics that left thousands of young and old paralyzed became a catalyst for a "Great Race" to develop a vaccine. Polio vaccines were developed in the 1950s and these have reduced the number of polio cases recorded globally from hundreds of thousands to below a thousand. Health partners like Rotary International, the World Health Organization and UNICEF initiated enhanced vaccine efforts which led to eradication of the disease in some continents [Heymann, 2006].

2.4 Nature of polio

Poliomyelitis or polio has three serotypes and any of them can cause the polio disease. Polio has two basic clinical patterns. The first is abortive poliomyelitis which causes a minor illness not involving the central nervous system. The second causes major illness involving the central nervous system which may be paralytic or non-paralytic [Falconer and Bollenbach, 2000]. Poliovirus infection does not cause any symptoms in people who

have a normal immune system. In a few instances symptoms such as upper respiratory tract infection (sore throat and fever), gastrointestinal disturbances (nausea, vomiting, abdominal pain, constipation or, rarely, diarrhea), and influenza-like illness can occur. These are minor symptoms of polio [Atkinson, 2009]. In about 3% of cases, the virus gains access to the central nervous system. Most patients affected this way present with symptoms of headache, neck, back, abdominal and extremity pain, fever, vomiting, lethargy, and irritability. This is called non-paralytic aseptic meningitis [Chamberlin and Narins, 2005; Leboeuf, 1992]. Polio infection can progress to acute flaccid paralysis in about one to five out of a 1000 cases. People affected this way experience muscle weakness, the muscles become floppy and poorly controlled and finally complete paralysis may occur [Frauenthal, 1914]. With regards to the site of paralysis, paralytic poliomyelitis can fall into any of the following three clinical categories: spinal, bulbar or bulbospinal. In rare cases among infants, the brain tissue can also be infected. This is known as encephalitis and it portrays symptoms such as confusion, changes in mental status, headaches, fever, and less commonly, seizures and spastic paralysis [Wood et al., 2005].

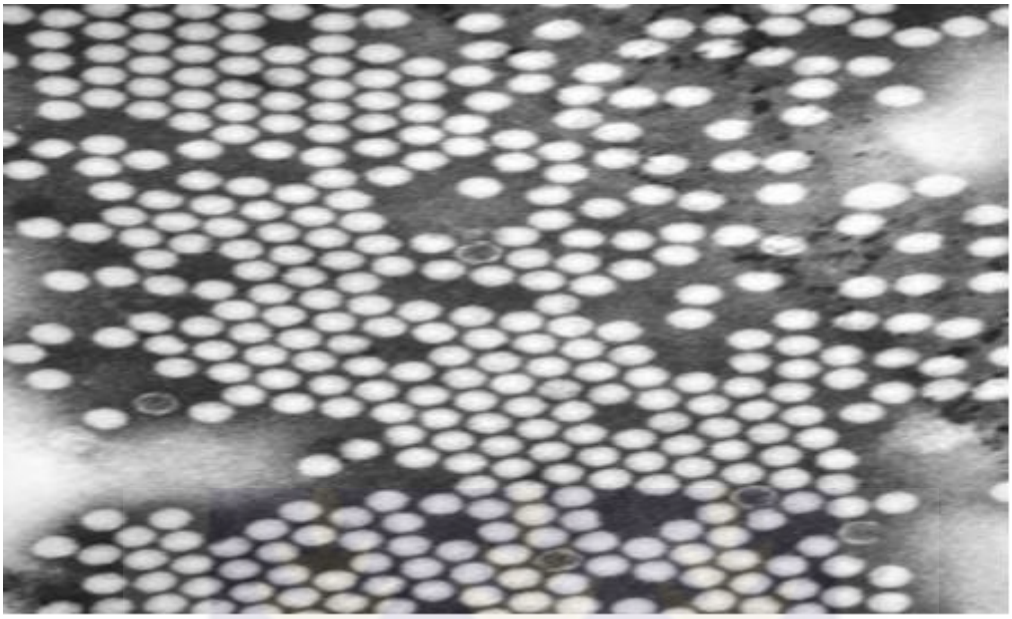

Figure 2.2: Transmission electronic microscopy micrograph of the polio virus

The poliovirus—which is a member of the genus *Enterovirus*—is the causative agent of poliomyelitis. These are RNA viruses and they live in the oropharynx and intestines of the gastrointestinal tract [Cohen, 2004]. Poliovirus infects only human beings. The disease does not affect animals and other living things [Ryan and Ray, 2004]. A single (+) sense RNA genome composes the simple structure of the poliovirus which is enclosed in a capsid (a protein shell) [Ryan and Ray, 2004] [Figure 2.2]. The capsid proteins enable the poliovirus to protect the genetic material of the virus as well as infect some specific types of cells. Poliovirus type 1 (PV1), type 2 (PV2), and type 3 (PV3) are the three identified serotypes of poliovirus. Each of them has a slightly different capsid protein [Katz, 2004]. All the three polio serotypes are exceptionally harmful and produce the same disease symptoms [Ryan and Ray, 2004]. PV1 occurs most frequently and it is the serotype which mostly causes weakness and paralysis of the extremities [Ohri et al., 1999].

Immunity to poliovirus serotypes is achieved by individuals who get exposure to the virus after immunization with polio vaccine or through polio infection. Those who are protected or immune to the poliovirus have IgA antibodies colonized in the tonsils and intestinal tract. These IgA antibodies are able to inhibit the replication of the virus. Similarly, IgM and IgG poliovirus antibodies also can inhibit transmission of the virus to motor neurons of the central nervous system [Kew et al., 2005]. Polio vaccination or infection with one serotype provides immunity for only that particular serotype but not the others. Thus for an individual to get full protection from all the serotypes, there must be an exposure to all the serotypes [Kew, 2005].

2.5 Transmission of polio

Poliomyelitis is highly contagious via the fecal-oral (intestinal source) and the oral-oral (oropharyngeal source) routes [Kew et al., 2005]. In endemic areas, wild polioviruses can infect virtually the entire human population [Parker, 1998]. It is seasonal in temperate climates, with peak transmission occurring in summer and autumn [Kew et al., 2005]. In many parts of Africa and South Eastern Asia, where the weather is generally warm, differences in seasonal transmission may not therefore be usually observed [Parker, 1998]. The incubation period, which is the time between first exposure to the virus and the onset of symptoms usually, ranges between six to 20 days. The maximum incubation period is three to 35 days [Racaniello, 2006]. Poliomyelitis may infect another person primarily through the fecal-oral route. This normally occurs when an individual consumes contaminated food or drinks. Occasionally, poliomyelitis is transmitted through the oral-oral route (by kissing) [Ohri et al., 1999]; this especially occurs in areas where good

sanitation and hygiene are practiced [Kew et al., 2005]. Between 7-10 days before and after symptoms appear, polio is most infectious. Once the virus remains in the feces and saliva, it can be possibly transmitted [Ohri et al., 1999]. Immune deficiency, malnutrition, tonsillectomy [Chandra, 1975], physical activity immediately following the onset of paralysis [Horstmann, 1950], skeletal muscle injury due to injection of vaccines or therapeutic agents [Gromeier and Wimmer, 1998] and pregnancy [Evans, 1960] are some of the factors that can cause an increase in the likelihood of polio infection or affect the severity of the disease. Polio vaccination or maternal infection does not affect the fetus although the virus can cross the maternal-fetal barrier during pregnancy [Joint Committee on Vaccination and Immunisation, 2006]. In the first few months of infancy, the child is protected through the passive immunity gained from maternal antibodies that cross the placenta [Sauerbrei et al., 2002].

2.6 Diagnosis of polio

Clinically, paralytic poliomyelitis may be suspected in individuals who experience a sudden onset of flaccid paralysis in one or more limbs with decreased or absent tendon reflexes in the affected limbs for which no other apparent cause can be attributed, and without sensory or cognitive loss [CDC/MMR, 1997]. Poliovirus can be recovered from a stool sample or a swab of the pharynx for a laboratory diagnosis to be made.

2. 7 Prevention of polio

Poliomyelitis has no cure. It is currently prevented through the administration of polio vaccines.

2.7.1 Oral polio vaccine

There are two main vaccines that are used currently in the world to protect an individual from polio infection. This is achieved by inhibiting wild poliovirus from being transmitted from person-to-person thereby inducing the commonly phrased "herd immunity" by protecting the individual who received the vaccine as well as the wider community [Fine and Carneiro, 1999]. The first polio vaccine was developed by Koprowski. On 27th February, 1950 this vaccine was given to a boy who was eight years old. The vaccine was named Koprowski's prototype vaccine [Koprowski, 2010]. The vaccination of seven million children in Poland against serotypes PV1 and PV3 between 1958 and 1960 was achieved through Koprowski's continuous work throughout the 1950s on his prototype vaccine. Large-scale trials were also held in the then Belgian Congo against serotypes PV1 and PV3 [Sanofi, 2008].

2.7.2 Inactivated and oral polio vaccine

Many researchers continued to work on the development of vaccines against polio virus. The second inactivated polio virus vaccine was developed by Jonas Salk in 1952 [Spice, 2005]. The Salk vaccine, or inactivated poliovirus vaccine (IPV), was based on poliovirus grown in a type of monkey kidney tissue culture (vero cell line), which is chemically inactivated with formalin [Kew et al., 2005]. IPV is very effective thus 90% or more individuals after receiving two doses of IPV develop effective antibodies to protect them against all three poliovirus serotypes. After receiving three doses, at least 99% become immune to poliovirus [Atkinson et al., 2009]. Subsequently, a live, oral polio vaccine (OPV) was developed by Albert Sabin. At sub physiological temperatures, the vaccine was produced by passing the virus repeatedly through nonhuman cells [Sabin and Boulge,

1973]. The gut is the primary site of wild poliovirus infection and there, the virus strain is able to replicate well, whereas it cannot replicate efficiently within tissues in the nervous system [Sabin et al., 1960]. Sabin's oral polio vaccine can be described as very effective since in about 50% of recipients, a single dose produces immunity to all three serotypes of the poliovirus.

2.8 Treatment of polio

Public health efforts are targeted at polio prevention since the infection is not curable. Protection against poliovirus for life can be achieved by giving a child the polio vaccine multiple times. Symptomatic relief, quick recovery, and prevention of disease complications have been the focus of modern treatment. Many individuals benefit from supportive measures including antibiotics which are given to prevent infection in weakened muscles, as well as analgesics for relief of pain, moderate exercise, and a diet which is nutritious [Daniel et al., 1997]. In most cases, paralytic polio is managed with long-term rehabilitation involving physiotherapy and, in some cases, orthopedic surgery [Professional Guide to Diseases, 2005].

2.9 Poliomyelitis Eradication

The most significant result of vaccines being developed in the 1950s was a drastic fall in the incidence of poliomyelitis in the countries that were industrialised. Czechoslovakia took this a step further by entirely eliminating polio in 1960, thus becoming the first acclaimed polio-free country. The Global Polio Eradication Initiative was passed by the World Health Organization (WHO) and development partners such as Rotary International,

the United Nations Children's Fund (UNICEF), as well as the U.S. Centers for Disease Control and Prevention (CDC) in the year 1988. This initiative was aimed at eradicating polio in the next 12 years (by the year 2000). This initiative could not however be achieved by that year; it was therefore updated to include plans for interrupting the transmission of poliovirus, thereby eradicating polio globally. This strategic plan spanned the years 2004-2008 and involved using routine immunization, supplementary campaigns on immunization, and surveillance of possible outbreaks as strategies to achieve its goal. It is estimated by the WHO that eradication of polio can save the world an excess of one billion U.S. dollars per year. This cost-saving would have been due to the reduction of treatment of affected individuals, as well as a reduction in costs associated with disability that can be caused by polio [WHO, 2003].

A declaration of being polio-free is currently held by these regions of the world:

- The Americas (1994)
- Indo-West Pacific Region (1997)
- Europe (1998)
- Western Pacific Region, including China (2000)

Incidences of reported polio cases worldwide continue to decrease with the least ever annual incidence of 37 cases recorded in 2016 [WHO, 2016].

A decade ago, this was not the trend. From 2003-2004, polio vaccination was interrupted in Nigeria while as previously from 2001-2002, immunization had been reduced in India. This brought about resurgence in the transmission of polio with 483 cases reported in 2001. From the year 2002 to 2010 however, the reported cases were higher and ranged from 750 to 2000 per year. For example, the year 2010 recorded 1,349 cases of polio. The fight

against polio has been difficult due to the likelihood of importing the poliovirus to countries that are able to interrupt polio transmission at a point in time. Thirty-one previously polio-free countries therefore had new importations and recorded some of these polio cases. In spite of efforts and success of various countries to interrupt polio transmission in the face of the possibility of such importations, two countries—Pakistan and Afghanistan—have not been able to interrupt poliovirus transmission [WHO, 2003].

2.10 Seroprevalence of polio antibodies and distribution by place and person

Seroprevalence is the number of persons in a population who test positive for a specific disease based on serology (blood serum) specimens; often presented as a percentage of the total specimens tested or as a proportion per 100,000 persons tested.

Important data on how immunization programmes are performing, groups which are susceptible to polio infection and populations facing the risk of future outbreaks can be obtained from seroprevalence studies.

In 2001, 2005 and 2010, Reinheimer et al. conducted a study among patients who were admitted to the University Hospital of Frankfurt Main, Germany. Of 1,632 patients that had their serum samples collected, the observed level of immunity to PV1 ranged between 84.2% (95%CI: 80.3-87.5), 90.4% (88.3-92.3) and 87.5% (85.4-88.8) in 2001, 2005 and 2010 respectively. For PV2, they found 90.8% (87.5-90.6), 91.3% (89.3-93.1) and 89.8% (88.7-90.9), in the same period. Seroprevalence to PV3 was 76.6% (72.2-80.6), 69.8% (66.6-72.8) and 72.9% (67.8-77.5) in 2001 and 2005 and 2010, respectively. Comparing PV3 levels of immunity to that of PV1 and 2 showed significant lower levels of PV3 immunity in 2005 and 2010 [Reinheimer et al., 2012]. These levels of seroprevalence to

the polio antibodies depicted a high level of protection against the general public in Germany on any wild polio virus importation at the time of the study.

From 1984 to 1986, in England and Wales, screening of serum taken from 995 subjects was done to detect poliovirus neutralizing antibodies at a one-eighth dilution. The ages of the subjects ranged from six months to 99 years. The results were that 975 representing 98% were found to contain at least one serotype whiles 763 representing 77% were found to contain antibodies to all the three serotypes. Among the children aged eight to 15 years, there was a low prevalence of poliovirus type 3 antibodies. Specifically, of the children aged 12, four of them (40%) only were protected. It must then be emphasized that it is important to continually find means of giving a booster vaccine dose to children leaving school, because the type 3 component of the oral poliovirus vaccine has a reducing effect on antibodies. This may possibly account for the finding [Philippa and Jonathan, 1984].

In another study, blood specimens taken from 323 young immigrants (European and extra-European) between January 2001 and December 2003 were screened to determine their levels of anti-polio antibodies. The results showed 98.1% prevalence of anti-polio 1 antibodies (titre 1:2), 99.1% of anti-polio 2, and 98.8% prevalence of anti-polio 3 antibodies. The seronegativity against only one or two serotypes (antibody titre <1:2) was found in nine subjects (2.8%) while no subject was found totally seronegative against all three serotypes [Paola et al., 2005]. The herd immunity of the target population at the study period could not be guaranteed.

Furthermore, the prevalence of antibodies was tested in a representative sample of 1,064 residents of northern Greece to assess their immunity to poliomyelitis. Seroprevalence results indicated 91.1% prevalence for type 1, 92.1% for type 2 and 83.1% for poliovirus

type 3. There was also a gap in immunity for poliovirus type three in 10 to 29 year old individuals [Frantzidou, 2004]. The study therefore suggested that in order to ensure there was herd immunity and also to reduce the risk of importing wild poliovirus from countries that are endemic, adolescents who live in northern Greece should be re-vaccinated.

In Spain, a sero-epidemiological study which involved 3,932 people was undertaken in 1996. Across all the study age groups, the prevalence of antibodies against all three types of poliovirus exceeded 94%. No significant differences were observed when the analysis was broken down by environment (urban–rural setting) or by sex [Pacho et al., 2002]. Therefore, a higher immunity against wild polio virus was achieved.

In the Yogyakarta Province-Indonesia among 420 children surveyed, seroprevalence against polioviruses (defined as ≥1:8) were 98.6% against poliovirus type 1, 99.3% against poliovirus type 2 and 98.2% against poliovirus type 3 [WHO, 2008]. After the switch to inactivated polio vaccine (IPV), this data served as a basis for comparison with similar data that were to be collected.

A polio antibody seroprevalence study was done in the Kyunggi province of Korea. It involved children from eight primary schools from whom a total of 500 sera was collected using the WHO recommended cell culture neutralization method. It was found that 82.2% of these children were positive for all the three types of poliovirus. Antibody-positive rates for type 1 was 94.4%, 96.6% for type 2, and 86.8% for type 3; an indication that seropositive rates for types 1 and 2 were remarkably higher than the seropositive rate for type 3 ($P<0.0001$) [Jee et al., 2004].

In 2010, invitation to participate in a study was given out to individuals less than 15 years old who paid visits to prefecture level hospitals or above and for a reason or the other had

to have their blood drawn. This study which was done in three border provinces of China was hospital-based and cross-sectional. An assay of neutralizing antibody titres to polio serotype 1 (P1), serotype 2 (P2) and serotype 3 (P3) was done in accordance with the manual of the World Health Organization for the virological investigation of polio. Out of the 1,360 enrolled subjects, 1,051 (77.3%) subjects were seropositive to all three serotypes (P1, P2 and P3) whiles 1,220 (89.7%) were seropositive to P1, 1,259 (92.6%) were seropositive to P2, and 1,112 (81.8%) were seropositive to P3. The classification for positivity was antibody titers of ≥8 [Wang et al., 2013]. With these levels of seroprevalence to polio antibodies, until there is a worldwide interruption of wild poliovirus transmission, there may continue to be a risk of wild poliovirus importation.

In a micro neutralization test among 129 children aged zero to five years in Maiduguri, Nigeria, 99 (76.8%), 95 (73.6%), and 95 (73.6%) had neutralizing antibodies with the geometric mean titre of 42.7, 31.3, and 33.2 for the poliovirus type 1, 2, and 3, respectively. Combination of poliovirus types 1 and 2, 1 and 3, and 2 and 3 were neutralized by 62.8, 58.9, and 61.2% of the children studied, respectively. Only poliovirus type 1 induced antibody titres greater or equal to 1:1,024 [Baba et al., 2012]. Even when an individual is partially immune to one serotype or a combination of two serotypes, that person is still exposed to infection which may come from the missing serotype(s).

In December 2004, surveys on seroprevalence were done in the Greater Cairo and Upper Egypt regions of Egypt which were known to be “polio-endemic”. A third region, Lower Egypt was used as a control group. Using neutralization assay, testing for poliovirus antibodies was done on sera taken from 973 children aged 6-11 months. Among those tested, seroprevalence to poliovirus type 1 (PV1) was 99%, with 99% for poliovirus type

2 (PV2) and 91% for poliovirus type 3 (PV3). A significant variation in seroprevalence to PV3 with a range of 76-100% was also found. Across board, the study suggested the need for high levels of immunity (greater than 96%) in order to interrupt the transmission of PV1 in the remaining few polio-endemic areas (by mid-January of 2005, the last PV1 was isolated in Egypt). The study also revealed that subjects with low social economic status as well as those in rural areas achieved the lowest PV3 seroprevalence, indicating substantial regional differences in OPV immunogenicity [Nasr El-Sayed et al., 2007].

Polio outbreaks, however, are normally associated with low prevalence of polio antibodies among the population. In a survey of poliovirus antibodies in 327 subjects in Kano-Northern Nigeria, noted for persistent polio outbreaks, seroprevalence was 81% to poliovirus type 1, 76% to poliovirus type 2, and 73% to poliovirus type 3 among subjects aged 6-9 months. Among subjects aged 36-47 months, the seroprevalence was 91% to poliovirus type 1, 87% for poliovirus type 2, and 86% to poliovirus type 3. Association was found between seroprevalence and maternal education, gender and history of OPV doses. It must be noted also that in Nigeria, suboptimal prevalence was as a result of failure-to-vaccinate but not failure of the vaccine [Zubairu et al., 2013].

Two hundred and sixty-four (264) children aged 1–10 years were subjects of poliovirus seroprevalence studies between 2008 and 2009 in Zaria, North West Nigeria. This community based study was descriptive and cross-sectional. There was protection for 55% of the children against the three polio serotypes whereas 86.4% of the children had neutralizing antibodies to P1, 76.1% to P2 and 77.3% had neutralizing antibodies to the P3 polio serotype. Five (1.9%), on the other hand, had no antibodies to any of the three polio serotypes. Polio antibody seropositivity was significantly associated with higher socio-

economic status and immunization was the single most important determinant of seropositivity to poliovirus serotypes [Giwa, 2012].

To evaluate the efficacy of the schedule currently recommended for immunization with trivalent oral poliovirus vaccine (TOPV) (i.e., at birth, 6 weeks, 10 weeks, and 14 weeks after birth), 452 infants were randomly assigned into intervention (231 infants) and control (221 infants) groups. For the intervention group, the final seroconversion rates against poliovirus were 83.5% for poliovirus type 1, 91% for type 2 and 83% for type 3. The control group had seroconversion rates of 75%, 83.2% and 79% against poliovirus types 1, 2 and 3 respectively. This was therefore an indication that there were better results for the schedule of TOPV immunization which started at birth. Infants with low maternal antibodies had the highest antibody levels and seroconversion rates, indicating that maternal antibodies have an effect on the production of polio antibodies [Osei-Kwasi et al., 1995].

2.11 Risk Factors for Low Seroprevalence

In a survey of poliovirus antibodies in 327 subjects in Kano-Northern Nigeria [Zubairu et al., 2013] in terms of risk factors other than low vaccination histories, lower seroprevalence was associated with female gender, lower maternal education, and having fewer number of children in the household. Maternal education as a proxy for socioeconomic status appears to be a good predictor of the immunization status of their children. In contrast to other surveys [El-Sayed et al., 2007; Estívariz, 2012] in Egypt and India, the nutritional status did not seem to affect the seroprevalence levels in Nigeria, for reasons which are not immediately apparent. In fact, wasting or stunting was not associated with seroprevalence

levels to any serotype in the 6-9-month age group. In the 36-37-month age group, severe wasting, although the numbers were small, and severe stunting were associated with lower seroprevalence levels (but this was not statistically significantly).

Total OPV doses (including those given during campaigns) and age of the child had an association with higher seroprevalence in a study done in Pakistan which evaluated poliovirus antibodies as well as the risk factors associated with polio seropositivity. This was in areas with low socioeconomic ratings. Significant risk factors for failure to sero-convert were educational status of the respondent, stunting, and diarrhea in the past six months [Habib et al., 2013].

Testing for the presence of polio-specific IgG antibodies was done on 182 blood samples taken from children in the Emergency Peadiatric Unit of the Jos University Teaching Hospital from March to April 2007. On the lines of gender, age and religion, no significant association was found between IgG detection in children and number of doses. A statistically significant relationship was found between the educational status of fathers and the detection of the antibodies, although there were appreciable levels of protection against poliovirus in the study population [Dashe et al., 2010]. Some of the findings from this study were contrary to similar studies in Nigeria and other places [Dashe et al., 2010]. Therefore, there is a need for further studies to elicit the specific reasons for the conflicting findings. The working protocols and environment may need to be subjected to a critical analysis.

2.12 Seroprevalence assessment and polio outbreaks

The presence of antibody titres of ≥1: 8 has generally been regarded as adequate immunity to poliovirus infection. Individuals who have lower or undetectable levels of antibody may however be protected from poliomyelitis when they have an immune memory that is able to provide rapid immune response should there be an infection. With regards to wild poliovirus importation, there also appears to be a greater risk of being infected among older age groups [Pires de Miranda et al., 2007].

Low seroprevalence to polio antibodies in a population could contribute to an outbreak of polio in a community.

In an outbreak of wild polio virus in the Xinjiang Uygur Autonomous Region of China in 2011, 77.3% of the subjects exhibited seropositivity to all the three poliovirus serotypes whiles 4.0% did not have antibodies to any of the three poliovirus serotypes. There were 89.7% seropositives to P1, 92.6% to P2 and 81.8% to P3 [HaiBo et al., 2013].

Similarly, there was an outbreak of polio in Finland between 1984 and 1985 which involved nine cases due to wild poliovirus type 3. Prior to that observation, only 30% of children aged three years who participated in a seroprevalence survey in Finland in the year 1982 had poliovirus type 3 neutralizing antibodies at a 1:4 dilution [Lapinleimu et al., 1984].

In a similar outbreak of wild poliovirus type 1 among persons aged more than 15years old in the Democratic Republic of Congo, 2010-2011, the seroprevalence assessment indicated that antibodies against polio viruses 1&3 were lower (<80%) in women aged 15-28 years old [Alleman et al., 2014].

Evidence of high vaccination rates reducing the risk of poliomyelitis outbreak also abound. In order to prevent paralytic poliomyelitis outbreaks from occurring, there must be a continuation of high vaccination rates among children especially, those in preschool. This will enable the global elimination of all poliovirus threats which may be from any source (vaccine-derived poliovirus, laboratory strains, or imported wild poliovirus).

Portugal had a declaration of being polio-free in 2002. Before this, the last poliomyelitis case caused by indigenous WPV was recorded in 1986. In a study of residents in mainland Portugal who were above two years of age, results from 1,333 individuals who made up a representative sample indicated that the antibody prevalence and the geometric mean antibody concentration (GMAC) was 91.6% (GMAC: 2.96 IU/ml) for poliovirus type 1, 94.2% (GMAC: 5.03 IU/ml) for poliovirus type 2 and 75% (GMAC: 0.53 IU/ml) for poliovirus type 3. The study showed good protection among Portuguese against poliovirus type 1 as well as poliovirus type 2. To minimize the risk of importing wild poliovirus, there was a need for a booster dose for poliovirus type 3 due to the observation of suboptimal antibody levels in teenagers and young adults [Pires de Miranda et al., 2007].

In Puerto Rico community of Dominican, in 2002, a seroprevalence study of polio antibodies among children in San Juan community had neutralizing antibodies to all three PV serotypes and were considered protected against polio. This observation was supported by data from the Puerto Rico 2002 Immunization Survey, which reported 99% coverage levels with 3 doses of poliovirus vaccine among children aged 24 months [CDC, 2002].

In assessing the immunity status of migrant workers in Israel, seropositivity rates and geometric mean titers (GMTs) for the Mahoney (type 1), MEF (type 2), and Saukett (type 3) poliovirus strains and the wild poliovirus type 1 strain were 99.3% (GMT--233.8),

98.6% (GMT--268.5), 99.3% (GMT--89.4), and 99.3% (GMT--139.5), respectively. These results indicated high levels of immunity among foreign workers and this explained the low risk of polio among these groups [Calderon-Margalit et al., 2005].

In assessing the status of immunity against poliomyelitis in some parts of Europe, Germany had a detection of neutralizing antibodies in 96.2% of the samples against poliovirus type 1, with 96.8% and 89.6% against poliovirus types 2 and 3, respectively. This seroprevalence of the German population is an indication of immunity level, which is very high. There was close to 90% prevalence of antibodies against poliovirus although generally, it was somewhat lower for type 3 than it was for types 1 and 2. On this basis Europe was declared polio free in 1998 [Diedrich et al., 2002].

2.13 Lameness

Lameness is a physical handicap that prevents a person from walking normally. According to the Medical Dictionary, lameness is defined as a condition of diminished function, particularly because of a foot or leg injury. The Oxford dictionary also defines lameness to be applied to a stiff or painful back that makes walking difficult. Lameness of a person or animal occurs when there is inability to walk with ease as the result of an illness or injury affecting the foot or entire leg. Prominent among the medical conditions that may cause lameness includes: infection from human enteroviruses, transverse myelitis, Guillain-Barré syndrome, paralytic poliomyelitis, cerebral palsy, osteomyelitis, traumatic neuritis and road traffic accidents.

2.13.1 Human Enteroviruses

Human enteroviruses (HEV) are among the most common viruses infecting humans worldwide with acute flaccid paralysis (AFP) as one of the clinical manifestations [Pallansch et al., 2001]. The human enteroviruses belong to the genus *Enterovirus*, family Picornaviridae. Human enteroviruses include polioviruses, coxsackieviruses, enterocytopathic human orphan (ECHO) viruses and enteroviruses 68 - 71.

Studies have shown that about 20 to 54% of non-polio enteroviruses have been isolated from acute flaccid paralysis (AFP) in India [Kapoor et al., 2001]. Similarly in America, most of the AFP cases in the post-vaccination era contained non-polio enteroviruses. In Scotland, several cases of paralysis were associated with enteroviruses, especially coxsackie viruses [Grist & Bell, 1984].

The fecal-oral route is the main mode of transmission of HEVs, which mostly spread within families. In stool specimens, it is usually isolated in the longest time and highest titer, and it can be isolated as well from respiratory secretions. Infection transmission normally occurs between siblings and where the living accommodation is crowded, as there is an increased risk of spreading the virus [Chan et al., 2000]. The risk of HEV symptom manifestation is a risk for people of all ages. Infection may occur during early infancy for children in less developed areas, and this is due to their immunity status, hygiene and exposure, which result in a higher rate of infection. In more socio-economically advanced areas, the infection may not occur until adolescence. Clinically-recognizable diseases more often develop in males than females [Theoklis & Klein, 1998].

Most enterovirus infections are self-limited and do not require specific therapy. Simple hygienic measures, such as hand washing, adequate disposal of infected secretions and

appropriate vaccinations are important to prevent the spread of enteroviruses [Ruan et al, 2011].

2.13.2 Guillain-Barré syndrome

Guillain-Barré syndrome is a condition where part of the peripheral nervous system is attacked by the body's immune system. It is normally preceded by an infection (bacteria or viral), administration of a vaccine or surgical operation. The outcome of this syndrome may affect the nerves that control muscle movement as well as those that transmit pain, temperature and touch sensations. This condition may results in muscle weakness and eventually loss of sensation in the legs and/or arms. In a systematic review of Guillain-Barre syndrome, an incidence of 1/100,000 cases was found among studies in several countries. It has also been noted that the number of cases increase with age and males are more affected than females [Sejvar et al., 2011].

The onset of Guillain-Barré syndrome is normally characterized by weakness or tingling sensation which starts from the legs and spreads to the arms and face. Respiratory distress normally occurs suddenly and may require intubation and ventilation at a level of one litre [Lawn et al., 2001].

The diagnosis of Guillain-Barré syndrome includes the observation of loss of deep-tendon reflexes. Management of this syndrome usually includes supportive care and some immunological therapies. Active immune modulation with IvIg or plasma exchange is the common course of treatment [Raphael et al., 2001; Hughes et al., 2006].

2.13.3 Paralytic Poliomyelitis

Flaccid paralysis clinically occurs in about 1% of poliomyelitis infections and is associated with both the wild virus and vaccine-associated polio virus. Both affect the anterior horn cells of all ages. Five to ten percent of paralytic polio cases may die as a result of paralysis of the respiratory muscles [Roberts, 2010]. The paralysis is almost always irreversible, and the legs rather than the muscles of the upper limbs are more often affected. However, the polio virus may invade the brain stem, potentially leading to breathing difficulty and even death. Even among the affected victims of polio who recover, a few are likely to experience an increased intensity of existing weakness, recurrence of muscle pain or a completely new weakness as well as paralysis. This condition may degenerate into 'post-polio syndrome' some 15-40 years later. An estimated 20 million people live with the consequences of polio today because majority of polio victims survive the acute illness [WHO, 2016].

Clinically, paralytic polio presents in diverse ways which include: fever at onset, rapid progression to paralysis within 24-48 hours, asymmetric, proximal more than distal limb paralysis, presence of pain, and residual paralysis [Melnick, 1996]. Respiratory failure and bulbar paralysis are major complications that may lead to death in children. Management consists of mainly supportive therapy [Melnick, 1996].

2.13.4 Cerebral Palsy

Cerebral Palsy (CP) is a group of permanent movement disorders (motor disabilities) found in the early stages of life [Accardo et al., 2007]. This condition is associated with disturbances of sensation, perception, cognition, communication and behavior, epilepsy, and secondary musculoskeletal problems. The etiology of CP is mainly attributed to several

anomalies in the developing fetal brain, alteration in fetal development and pathologic intrauterine processes [Rosenbaumet al., 2007].

The prevalence of cerebral palsy at birth is approximately 2 per 1,000 live births, however, in several population based studies an estimation of 1.5 to more than 4 per 1,000 live births had been documented [Odding et al., 2006; Paneth et al., 2006].

One of the leading conditions in the development of cerebral palsy is prematurity especially before 28 weeks [O'Shea et al., 2009]. Although this observation has been a leading condition for cerebral palsy a recent study has noted post-term pregnancy at 42 weeks or later is also closely associated with cerebral palsy [Moster et al., 2010].

There are several methods of managing cerebral palsy and the most prominent among them is the prevention of factors contributing to preterm delivery; administration of magnesium sulfate to patients who are about to deliver at early preterm and effective management of asphyxia in neonates.

2.13.5 Osteomyelitis

Osteomyelitis is simply an infection and inflammation of the bone [Kumar, 2007]. The various forms of the disease are sub-grouped by the causative agent, route of infection, duration and location of the infection within the patient. Osteomyelitis is usually classified as an acute and chronic infection [Tamparo, 2007]. Osteomyelitis affects the long bones in children, while it affects the vertebrae and pelvis bones in adults.

Patients with pulmonary tuberculosis have about 1-3% chance of infection with osteomyelitis. The infectious organism (bacteria) passes into the circulatory system prior to infecting the bones. The long bones and the vertebrae are usually affected in patients with tuberculosis [Kumar, 2007].

Osteomyelitis is diagnosed through clinical and laboratory assessment. The presence of fever and an elevation of white blood cell count are helpful in arriving at the diagnosis. Confirmation of diagnosis is done by MRI [Howe et al., 2013]. The treatment of osteomyelitis requires a long period of antibiotic therapy. Debridement and amputation may be necessary in the course of managing the condition.

2.13.6 Transverse myelitis

Transverse myelitis is a neurological condition involving the inflammation of the spinal cord.

Incidence of transverse myelitis is about 4.6 in 1 million, affecting men and women equally at any age [Mumenthaler, 2011]. As the inflammation damages the nerve fibres, the myelin coating is lost and this interrupts the electrical conductivity in the brain. When the inflammation extends to the entire width of the spinal cord, it is termed as transverse myelitis and if part of the width, then it is called partial myelitis [West, 2013]. Although not proven yet, it is believed that transverse myelitis occurs as a result of previous infection or vaccination [Frohman, 2010].

Signs and symptoms of transverse myelitis depend on the location of the lesion. When the cervical part of the upper spinal cord is affected, there is a risk of respiratory distress and paralysis of all the four limbs. A lesion at the level of the thoracic spine produces spastic diplegia and the individuals have weaknesses in the lower limb [Alexander, 2015]. A lesion in the lower part of the spinal cord induces weakness and numbness of the limbs [Frohman, 2010].

Management of transverse myelitis mainly includes immunosuppression and supportive care (maintenance of airway, breathing and circulation with bladders catheterization). Corticosteroids are needed in high doses in managing such patients [Frohman, 2010].

13.7 Traumatic neuritis

Traumatic neuritis is a condition that occurs as a result of an unsafe injection practice in the muscle. When the sciatic and radial nerves are involved it presents as an acute peripheral neuropathy with flaccid paralysis of the affected limb within 24 hours. The World Health Organization estimates that of the about 12 billion injections given annually, almost half are given under unsafe conditions and 75% could be averted [Halsey, 2003]. In Pakistan it had been estimated that each person receives up to 15 injections per year whilst children less than five years receive 21 injections annually [Zafar, 2003].

Patients with traumatic neuritis experience an intense pain at the site of injection and hypothermia of the affected limb [Agha, 2001]. It is difficult to distinguish this condition from polio but sensory deficits and lack of CSF pleocytosis favor the diagnosis of traumatic neuritis.

Management of traumatic neuritis is mainly supportive.

2.13.8 Road traffic accident

Road traffic accident (RTA) occurs when a moving vehicle collides with another vehicle, a pedestrian, animal or geographical object. In some instances RTAs occur when vehicles burst a tyre and overturn, without necessarily colliding with something. This event may cause injuries leading to lameness or death, as well as damage to property. There has been a rise in road traffic accidents that involve children and this has become a social problem. Survivors may develop temporary or permanent disabilities which may result in lameness

[Hatamabadi et al., 2012]. The state of lameness demands a lot of care and has a negative impact on the psychosocial and health aspects of life. It also has a negative effect on household financial support [Molcho, 2015].

The major risk factors that contribute to road traffic accidents include drunk driving, excessive speeding, wrong overtaking and poor road network. Some even attribute accidents to supernatural powers [Smart, 2002]. The male sex, non-use of seat belts, driver age (teenage drivers and elderly drivers) are among other recognised risk factors that contribute to road traffic accidents [Bjerre, 2006].

Worldwide, as many as 1.4 million people die as a result of road traffic accidents and 20-50 million people are injured or disabled. If this trend does not decrease, by the year 2020 RTA's may attain the third position of the causes of morbidity and mortality globally [WHO, 2014]. Currently it is the ninth leading cause of death across all age groups and also predicted to be the seventh leading cause of death by 2030 [WHO, 2014]. In Ghana, about six people die from road traffic accidents every day [Coleman, 2014].

Generally, road traffic accidents are preventable and could be prevented. Notwithstanding the global public health concerns on road traffic accidents, there exist proven interventions which can reduce the public health burden [WHO, 2004 & 2008]. Enforcing local laws on avoidance of excessive speed in driving, prohibiting drunk driving, promoting seatbelt usage, and the safer designs of vehicles and roads have contributed to a drastic reduction of road traffic accidents [Peden et al., 2004].

2.14 Lameness among Ghanaian school children

The sequelae of poliomyelitis are distinctive, and surveys of lame children can help to estimate the prevalence of the disease. Lameness carries a high social cost and an understanding of the correct picture in the country is important to plan and execute immunization programmes against poliomyelitis. Studies have shown that paralysis affecting the legs occurs in 75-90% of polio cases [Pendey et al., 1979; Sancheti et al., 1981]. In a study conducted in Ghana in the Danfa field project area, village health examinations showed that the prevalence of lame children aged from six to 15 was 4.6 per thousand [Nicholas et al, 1977]. However, in a specially conducted school survey in the same area the prevalence was 6.3 to 7.2 per thousand [Nicholas et al, 1977]. A lameness prevalence of 5.8 per 1000 school-aged children was estimated from a postal survey of schools throughout Ghana. This lameness attributable to poliomyelitis mean annual incidence of paralytic poliomyelitis was estimated at 23 per 100,000 population. However, official reported incidence rates ranging from 0.1 to 2.1 (mean 1.0) per 100 000 population were reported for paralytic poliomyelitis within the same period in Ghana [Ofosu-Amaah et al., 1977].

2.15 Expanded Programme on Immunization, Ghana.

The Expanded Programme on Immunization (EPI) was developed in 1974 by the World Health Organization (WHO) and the United Nations Children's Fund (UNICEF) to combat six vaccine-preventable diseases of childhood: tuberculosis; poliomyelitis, neonatal tetanus, diphtheria, measles, and pertussis. The aim was to achieve 'universal' childhood immunisation by 1990, with at least 80% of infants fully immunised by their first birthday.

In Ghana, the EPI was introduced in 1978 and the programme has been operational in all regions since 1985 [GHS, 2016]. The EPI Programme aims at reducing morbidity and mortality by controlling, eliminating or eradicating vaccine preventable diseases (VPDs) through immunization; an essential component of Primary Health Care. The Programme currently vaccinates against 13 VPDs in routine immunization.

Ghana launched the polio eradication campaign in 1996 in all ten regions, in response to the World Health General Assembly resolution of 1988 to eradicate poliomyelitis by 2005. Since then, the EPI programme has made remarkable progress in all regions in Ghana, which includes the improvement of vaccination coverage among women and infants leading to a massive reduction in illness and death from vaccine-preventable diseases. Routine polio immunization programme for children includes four doses of live attenuated oral polio vaccine (OPV). Children receive their first vaccination (OPV0) at birth. The remaining three doses (OPV1, OPV2 and OPV3) are at six weeks, ten weeks and 14 weeks after birth respectively. Children less than five years of age are given two doses or more of OPV during the annual national immunization campaigns until the age of five. Generally, the administration coverage of the oral polio vaccine has attained higher than expected target of 90% in all the study sites and especially in the Northern Region of Ghana in the past four years [Figure 3.2].

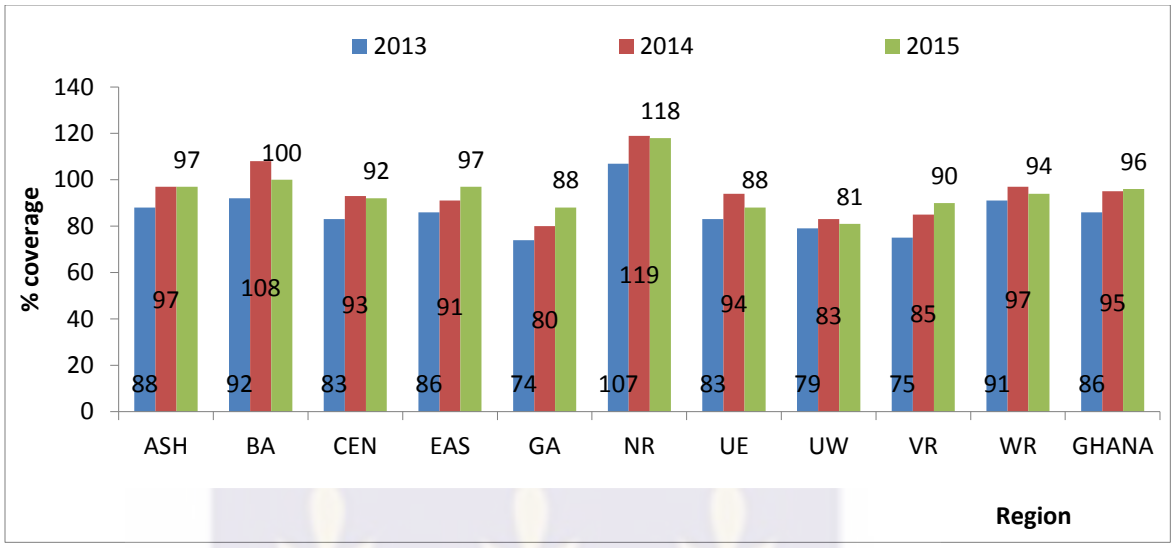

Figure 2.3: Trends of oral Polio vaccine Coverage (OPV3) per Region, Ghana, 2013-2015.*Source: Annual Report Expanded Programme on Immunization, Ghana-2015*

2.16 Acute flaccid paralysis surveillance system

The Acute Flaccid Paralysis (AFP) Surveillance System is integrated within the general framework of the Integrated Disease Surveillance and Response (IDSR) system, which operates within the Ghana Health Service in all regions of Ghana. All surveillance activities including AFP surveillance, is implemented at all levels of the health care delivery system, i.e. community, health facility/sub-district, district, regional and national levels.

At the national level, the Disease Surveillance Department has the overall responsibility and coordinates all surveillance activities including policy formulation, resource mobilization and provision of technical support to the lower levels. The region and district levels are the focus of health service delivery including AFP surveillance implementation. The district level ensures that active AFP surveillance in the health facilities and in the communities is carried out. All the levels have AFP focal persons to lead surveillance at each level of the health system.

When a case of AFP is identified by a clinician at the health facility or by a community-based surveillance volunteer, the clinician or the volunteer notifies sub-district or district level surveillance focal person who then conducts a detailed investigation of the case. Sometimes Regional Focal Persons, Stop Transmission of Polio (STOP) Team Members from the national level, carry out investigation of cases identified during active case searches at the facility and district levels. The person conducting the investigation fills an AFP case investigation form in triplicate. This is followed by initiation of the process of collection of two stool specimens 24 hours apart and transport of the specimen to the polio laboratory, or a referral laboratory, example, Noguchi Memorial Institute for Medical Research (NMIMR) located in the country's capital, Accra. Stool samples are transported to the polio laboratory under a reverse cold chain in a surveillance vehicle or through expedited mail delivery within three days accompanied by one copy of the completed AFP case investigation form.

The condition of the stool samples is assessed in the laboratory for adequacy in terms of quantity, whether they were stored under appropriate temperature, and whether there was any leakage. The stools are analyzed for the presence of any polio virus, as well as the type and sequencing of the virus. The laboratory also assesses for non-polio entero-viruses. The results of AFP stools are communicated to the district through the National Disease Surveillance Department.

The detailed information is entered into a database, which is then analyzed to determine whether the surveillance indicators are being met. For all AFP cases, a 60-day follow-up examination is carried out to find out if the case has residual paralysis. AFP cases with inadequate stools are specially prioritized for 60-day follow up since the result of 60 day

follow- up is necessary for classification. The National Polio Expert Committee meets quarterly to classify all AFP cases and advise on surveillance gaps that need to be addressed.

2.17 Polio outbreaks in Ghana

The total number of confirmed cases of polio continued to decrease till the last confirmed indigenous case in Bole District of Northern Region in October 2000 [Figure 3.3]. However, Ghana suffered a setback when between February and September 2003, 8 cases were detected in 8 districts in 6 regions. In 2008, 8 imported cases were recorded in Northern Region of Ghana. These were imported cases related to strains circulating in Nigeria. To interrupt the transmission of the polio virus, many rounds of supplementary immunization activities (SIA's) and 'mop-up" campaigns were organized in the country. "Mop up" campaigns are immunizations campaigns that are carried out from house to house in localized areas in which the polio virus is suspected to still be circulating at a particular time. These campaigns are carried out in areas where the virus was last recorded and where access to health care services is difficult or in areas which are densely populated with poor sanitation and low routine immunization levels.

The incidence of poliomyelitis has decreased drastically. No polio case has been recorded in Ghana since 2009 [GHS, 2009].

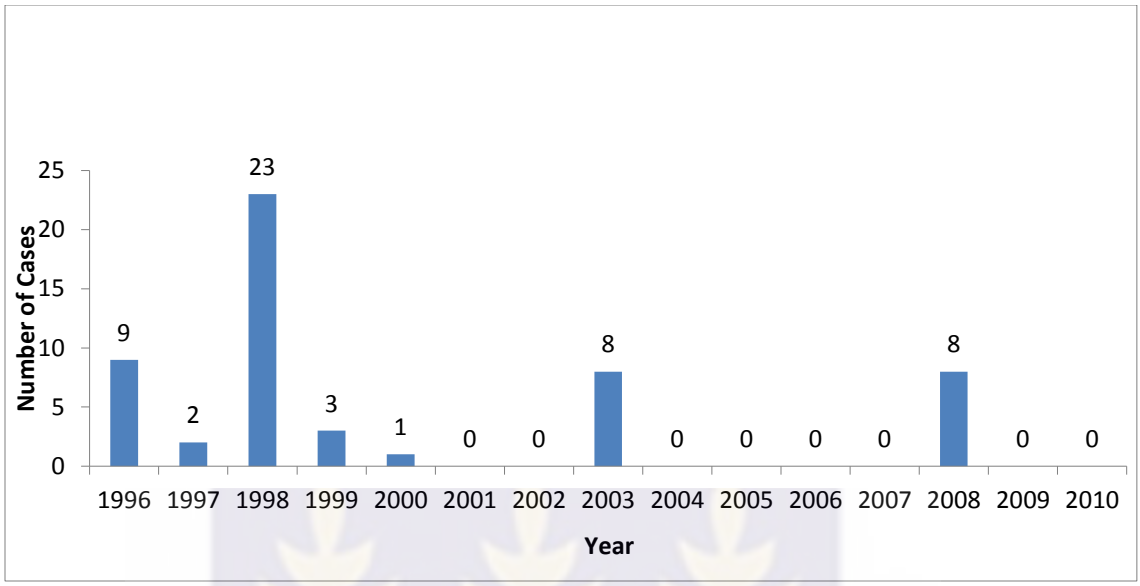

Figure 2.4: Distribution of wild polioviruses between 1996 and 2010.

Source: GHS, National Surveillance Division, 2015

Following the National Surveillance System review in 2000, acute flaccid paralysis (AFP) surveillance was revived as part of the overall disease surveillance system in the country, which led to improvement in the AFP surveillance indicators.

However, surveillance gaps were identified in certain regions, but especially in Western, Volta and Greater Accra. As part of corrective actions, review and planning meetings were held with the Regional Teams followed by technical support visits, which has led to improvement in the AFP surveillance indicators for Western and Volta regions. However, Greater Accra still remains a challenge.

Although there has been no importation of wild polio since 2009, the country is fully aware that the risk of importation still exists. Consequently, Ghana has developed and maintained an active national preparedness and response plan.

The goal of this plan is to maintain the polio free status through the prevention and early detection of possible importation of wild polio into the country. The objective is to achieve high quality surveillance as well as high general population immunity with special focus on high risk population and areas.

The WHO Regional Polio Laboratory, at the Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, is the National Polio Laboratory. An inventory of all national laboratories has been conducted. A plan of action for containment has been elaborated. A national report of containment of polio virus and potentially infectious materials has been prepared.

CHAPTER THREE

3.0 METHODS

3.0 Introduction

This chapter describes the following: study design, site and population; sample size and sampling; data collection techniques and tool; laboratory investigations; data management; statistical analysis and ethical considerations.

3.1 Study design

The study was made up of two components: hospital-based seroprevalence and school lameness studies.

3.1.1 The hospital based seroprevalence study

A cross-sectional analytical hospital-based study was conducted in three regions of Ghana. In this seroprevalence study, selected individuals referred to the laboratory for heamatology at three health facilities namely; Tamale Teaching Hospital, Tamale; Komfo Anokye Teaching Hospital, Kumasi and Korle-Bu Teaching Hospital, Accra partook in the study**.** The respondents were interviewed with a semi-structured questionnaire extracting data on demographic and polio immunization history. Subsequently, their weight and height were measured. Approximately 2-5 mls of blood was taken from the respondents (children and adults) for micro neutralization test. Antibody titers of ≥1:8 were considered positive. Seroprevalence was descriptively analysed by person and place. A simple correlation analysis determined the association between age and the mean titres of neutralizing antibodies among respondents and binary logistic regression models were used

to assess the association of risk factors (gender/sex, education level, age, etc.) on seroprevalence.

3.1.2 The school lameness survey

The prevalence of residual paralysis from poliomyelitis was determined for the infant to 15 year-old children at school in a cross sectional study. Demographic data and history of paralysis were retrieved from respondents by an interviewer administered questionnaire and this was followed by a physical examination. The prevalence of lameness (residual paralysis) was determined by the proportion of children with flaccid paralysis and intact sensation among the total number of children screened.

3.2 Study area

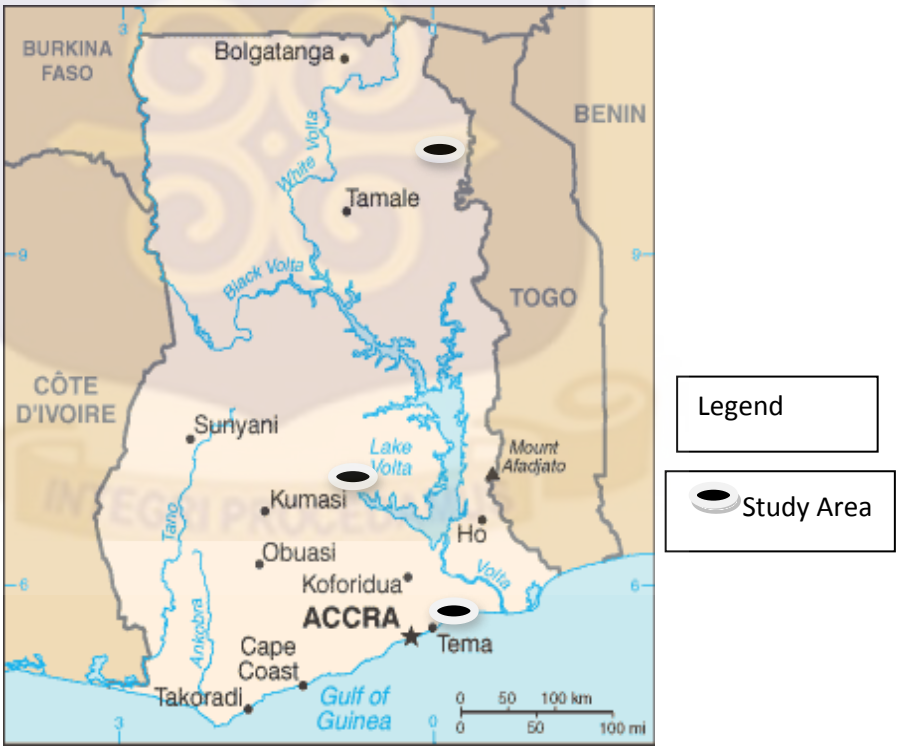

Figure 3.1: Map of Ghana showing study sites

The study was conducted in three sites in Ghana namely, Tamale (Northern region), Kumasi (Ashanti region) and Accra (Greater Accra region) located in the three ecological zones of Ghana [Figure 3.1]. These regions are the most populated and have the biggest referral and teaching hospitals in Ghana. The study was conducted in these referral and teaching hospitals namely: Tamale, Okomfo Anokye and the Korle-Bu Teaching Hospitals. Patients attending these hospitals come from a wide catchment area with mixed socioeconomic backgrounds.

3.2.1 Northern region

The Northern Region is located in the northern part of Ghana and has the largest land surface area in Ghana. The region consists of 26 administrative districts. The Tamale Teaching Hospital is located in Tamale town-ship which is the capital of the Northern Region. The region has an estimated population of 2,479,461 which represents 10.1% of the entire population of Ghana with a land area of 70,384 km^2 [GSS, 2010]. Due to its closeness to the Sahel and the Sahara, the Northern Region experiences much drier weather conditions than southern areas of Ghana. [GSS, 2010]. The temperatures can fluctuate between 14°C (59°F) at night and 40°C (104°F) during the day. There are 345 health facilities, a teaching hospital, and over 5,000 government health professionals [GHS, 2015]. There are 2,489 primary schools (government & private), with an enrollment of 532,018 pupils and 13,488 teachers [GES, 2016]. The level of education is relatively low in the Northern region. Among the female population, six years and older, about 62.5% have never attended school in all the districts. Muslims constitute about 60% of the entire population in the region and Christians 21%. Among the Christians, the Catholics have the highest proportion (7.6%). Traditionalists constitute 16% of the population. Regarding

impairment which restricts people from the performance of specific tasks, sight is the most common disability type, followed by physical, emotional and other forms of disabilities [GSS, 2010].

3.2.2 Ashanti region

The Ashanti Region is located in the middle belt of Ghana and is the third largest of the 10 administrative regions of the country. The region covers a total land area of 24, 389 km^2. This estimate represents about 10.2% of the land coverage of Ghana. In terms of population, however, it is the most populated region with a population of 4,780,380 in 2010, accounting for 19.4% of Ghana's total population and also harbors the capital city of Kumasi [GSS, 2010]. It is located in the middle belt, forest zone of Ghana with relatively higher rainfall pattern. There are 548 health facilities, a teaching hospital and 8,200 government health professionals [GHS, 2015]. There are 3,940 primary schools (government & private), with an enrollment of 790,603 pupils and 28,646 professional [GES, 2016]. Among those six years and older, the percentage of those who have never attended school is 15.2% (10.9% of males and 19.2% of females) of the entire population in the region. The national average of the inhabitants in this category is 23.4%. Christians constitute about 77.8% of the population and Muslims 15.2%. Traditionalists comprise of 0.7% of the population. However, 5.4% of the population has no affiliation to any religion. Out of the 124,504 persons with disabilities in the region, sight is the most common disability type, followed by physical (25.9), emotional (16.1%) and other forms of disabilities [GSS, 2010].

3.2.3 Greater Accra region

Greater Accra Region is situated in the southern part of Ghana. It is the least in terms of size in land area in Ghana. The total land surface area is 3,245km^2 which forms 1.4% of the total land area in Ghana. It has a population of 4,010,054 per the census in 2010. This forms 16.3% of Ghana's total population and this makes it the second most populated region, after the Ashanti Region [GSS, 2010]. The region lies in the Savannah zone. There are two rainy seasons: May-June and September-October. The average annual rainfall is about 730mm, which falls primarily during the two rainy seasons. There are about 500 health facilities, a teaching hospital and over 9,000 government health professionals [GHS, 2015]. There are 3,154 primary schools (government & private), with an enrollment of 489,060 pupils and 17,226 teachers [GES, 2016]. Among the female population, three years and older, about 13.4% have never attended school in all the districts. About 84% of the population in the region are Christians and 11.8% Muslims. Traditionalists constitute 0.5% of the population. However, 3.4% of the population have no affiliation to any religion. Regarding impairment which restricts people from the performance of specific tasks, sight is the most common disability type, followed by physical (23.3), emotional (21.3%) and other forms of disabilities [GSS, 2010].

Majority of the people in the region seek health care from the health facilities of the Ghana Health Service which has the Ministry of Health as its policy making body. The healthcare system has five levels of providers: Community–Based Health Planning and Services (CHPS) (the first level of care), health centers, district hospitals, regional hospitals and tertiary hospitals. There are 172 hospitals in Ghana and, within each region, there is a

regional hospital. The regional hospitals at Tamale, Kumasi and Greater Accra are also referral and teaching hospitals.

3.3 Study population

All children less than five years old and adults referred to the laboratories of the three major referral hospitals (Tamale, Komfo Anokye and Korle-Bu Teaching hospitals) in the Northern, Ashanti and Greater Accra regions of Ghana from 1st of April to July 30th, 2016 were screened for participation in this survey.

The lameness school survey involved primary school children from one urban and one rural district in the three study sites.

3.4 Inclusion and exclusion criteria

All children of consenting parents and adults resident in the three selected regions for the past six months were eligible to participate, except those (a) born or residing outside of Ghana; (b) those with serious acute illnesses requiring hospitalization; (c) those diagnosed or suspected of congenital immunodeficiency disorder or an immediate family member, and (d) those with contraindication to venipuncture.

The lameness survey involved the total enrollment of the selected primary schools in the selected districts. This included pupils of all ages and all classes in the selected primary schools. Pupils in the non-selected primary schools were excluded.

3.5 The Study variables

Table 3.1 provides description and operational definition of the study variables as pertains to this study.

Table 3.1: Study Variables

Variable	Operational definitions	Scale of measurements
Independent Variables		
Age	Age of child less than five years (in complete individual age,) Age of the Mother/caregiver responding and age of adult respondent (in complete individual age)	Numeric
Gender	Sex of child, mother/caregiver/adult respondent	Nominal
Mothers/caregivers occupation	Main work of mother/caregiver	Nominal
Mothers/caregivers educational status	Highest educational level of mother/caregiver	Ordinal
Father's occupation	Main income generation job the of father	Numeric
Father's educational status	Highest educational level of father	ordinal
Children<5yrs in household	Number of children < 5years in the house	Numeric
Weight	Weight of child, adult respondent in kilograms	Numeric
Length/height	Length/height of child, adult respondent in cm	Numeric continuous
Routine Oral Polio Vaccine (OPV) doses	Number of routine OPV doses given to child	Numeric, discrete
Supplementation Immunization Activity (SIA) OPV doses	Number of SIA OPV doses given to child	Numeric, discrete

Number of school children	Number of school children in household attending school	Numeric, discrete
List of lame children	List of lame children attending and not attending school	Numeric, discrete
Dependent variables		
Seroprevalence to :		
Neutralizing Polio virus 1 (PV1) antibodies	Number of children and adults with PVI antibodies in blood serum	Numeric, discrete
Neutralizing Poliovirus 2 (PV2) antibodies	Number of children and adults with PV2 antibodies in blood serum	Numeric, discrete
Neutralizing Polio virus 3 (PV3) antibodies	Number of children and adults with PV3 antibodies in blood serum	Numeric , discrete
Seroprevalence rate	Rate of occurrence of seropositives among children and adults population	Numeric
Geometric mean titre	Titre of in-house and international sera. It is the mean of n titres of in-house and international sera expressed as n√ of their product	Numeric, discrete
Lameness	Weakness or paralysis in the extremities	Nominal

3.6 Sample size estimation – hospital based seroprevalence study

The estimated minimum sample size was 274, however, 307 respondents attending or using the laboratories in the three referral hospitals were recruited into the study. This was based on the Fishers formula for calculation of sample size for populations >10,000 [Cochran, 1997].

$$n = \frac{\left(Z_{\alpha/2} + Z_{1-\beta}\right)^2 p(1-p)}{d^2}$$

where

p = 90%, assuming a seroprevalence rate (p) of 90% [Zubairu, 2013]

d = 95% is the desired level of precision (=0.05)

$Z_{\alpha/2}$ is the critical value for the standard Normal Distribution at α =5% (1.96)

$Z_{1-\beta}$ is the power of the study (=0.8), the sample size of 274 respondents was obtained.

The sample size which was aimed at estimating the prevalence of serotypes, *p*, was computed assuming that *p* has a normal distribution. If a maximum absolute error of *d*=0.05 was tolerated with 95% probability ($Z_{alpha/2}$=1.96), assuming a seroprevalence rate (p) of 90% [Zubairu, 2013] in the population and applying a power of 0.80 (beta) for each poliovirus serotype leads to a minimum sample, n=274. Adjusting for a non-response rate of 10% (blood provision and availability of mothers or caregivers for interview): n= 307. Sample size was then distributed across the three regions by probability proportional to size of the population of the study regions. The sample size for each region was spread across the age groups almost equally (Table 3. 2).

Table 3.2: Enrollment of respondents per region- hospital based seroprevalence study

Region	< 1yr	1-4yrs	5- 14yrs	15-70yrs	Total
Northern	21	21	21	22	85
Ashanti	31	31	30	31	123
G, Accra	25	25	25	24	99
Total	**77**	**77**	**76**	**77**	**307**

The study population was stratified into 4 (four) age groups: <1, 1-4, 5-14 and 15-70 years old.

3.7 Screening for the hospital based seroprevalence survey

The selection of participants for the hospital survey required an initial screening for age (<1, 1-4, 5-14, 15-70 years old) at the respective hospitals in each region. After obtaining informed consent and assent from the participants and the mothers or caregivers who had been sent for laboratory investigations, the study procedure commenced. Screening continued till the sample size of each age group was achieved. Figure 3.2 presents the schematic diagram of the hospital based seroprevalence survey:

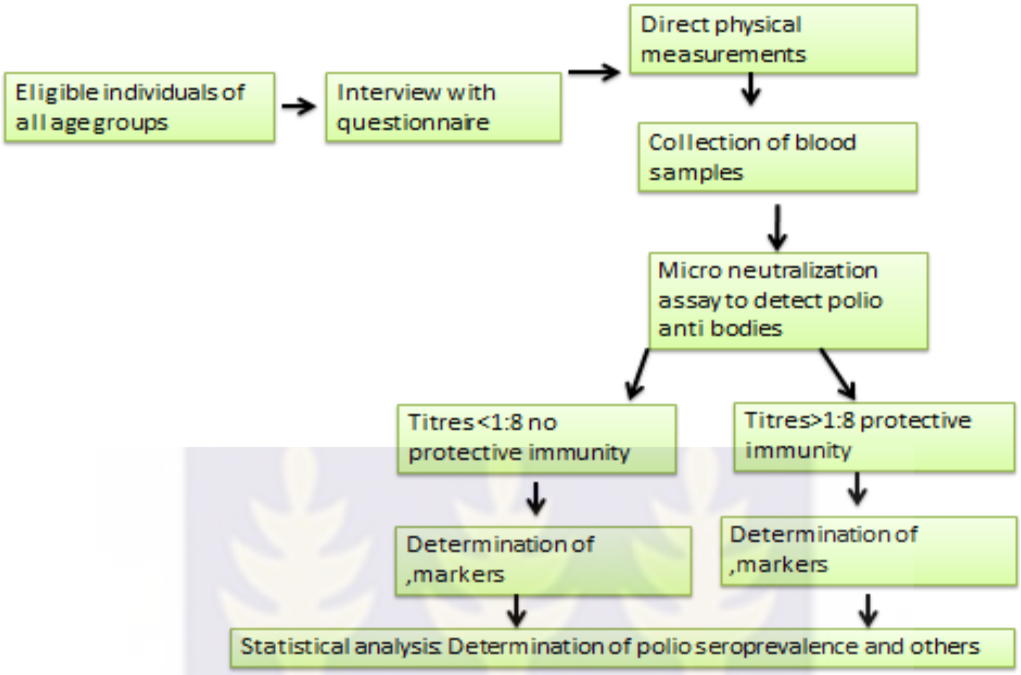

Figure 3.2: Hospital based seroprevalence survey

The Mothers or Care givers and adults selected into the study were interviewed using a semi- structured questionnaire. This was followed by direct physical measurements of (weight and height) the children and adult respondents. Subsequently, blood samples were taken from the participants. Blood samples that tested positive or negative for polio neutralizing antibodies were further analysed. Approximately 2-5 mls of venous blood was taken from the respondents (children and adults) for micro neutralization test to determine the presence of polio antibodies and the titre levels of the three serotypes by WHO-standards [WHO, 1997] for virological investigation of polio.

3.8 Sample size and sampling approach for school lameness survey

The estimated minimum sample size used for this study was 32,588 for school children below 15 years. However, 34,217 school children, zero to 15 years, in the three regions were screened for cases of paralytic poliomyelitis in the school survey. The sample size calculation was done based on the Fishers formula for calculating sample size for populations [Cochran, 1997]. Based on previous studies [Ofosu-Amaah, 1977] the prevalence of lameness was estimated at 4/1000 children ($p=0.004$). Given that the prevalence is below 1%, a conservative precision (d) in the case of the very small prevalence is estimated to be one-fourth of the prevalence i.e. plus or minus 1/1000 ($d=0.001$) (Pourhoseingholi et al., 2013).

Given the Type I and Type II errors as α and β respectively, the minimum required sample size n is given as:

$$n = \frac{\left(Z_{\alpha/2} + Z_{1-\beta}\right)^2 p(1-p)}{d^2}$$

where,

$p = 0.4\%$, the assumed prevalence of lameness in Ghana (p=0.004)

$d =$ the desired level of precision (=0.001)

$Z_{\alpha/2}$, the critical value for the standard Normal Distribution at α =5%; ($Z_{\alpha/2} =$ 1.96)

$Z_{1-\beta}$, the power of the study ($Z_{1-\beta} = 0.9$)

The estimated sample size, $n =$ 32,588

Therefore a minimum sample size of 32588 school children below 15years was required.

Adjusting for a 5% non-response (children out of school), the adjusted sample size n_{adj} = 34,217.

Applying probability proportional to size, the following distribution of the sample size across the three regions and their urban/rural was applied (Table 3.3).

Table 3.3: Distribution of sample size across Regions and Metro/ Districts

Region	Urban/rural	Rural/urban sample size	Regional sample size
Ashanti	Kumasi Metro	13848	14592
	Bosomtwe	744	
Northern	Tamale Metro	4695	7498
	Savelugu-Nanton	2803	
Greater Accra	Accra Metro	11760	12127
	Shai Osu Doku	367	
Total			**34217**

3.9 Sampling approach for school lameness survey

The same regions in which the hospital data were gathered were purposively selected for the school based survey. A two-stage stratified random sampling design was used, where one region was selected from the northern, middle and southern ecological zones of Ghana. Thereafter, in each selected region, one urban and one rural district was selected. In each selected district, the district sampling frame was used to allocate the sample size proportionate to the population size and rural and urban difference for each zone (Northern region: 7498, Ashanti region: 14592 and Greater Accra region 12127 [GES, 2016]. A total

sample size of 34,217 was arrived at for all three study sites. Upon obtaining the desired sample size of school pupils per urban/rural setting, the schools were sampled by simple random sampling. The schools in each urban/rural setting were numbered. The sample elements were selected by rural/urban setting using the Random Number Generator (Calculator). The first randomly selected school was visited and the entire primary school population was screened. This was repeated in the subsequent randomly selected school until the sample size was obtained. Where the school population was more than the desired sample size, the remaining pupils were still screened. In all, 112 schools were visited to obtain the desired sample size.

3.10 Data collection technique and tools for hospital based seroprevalence survey

The technique for data collection was an interview (face to face) using a semi-structured questionnaire as the tool.

The study team was comprised of a physician, nurses, laboratory scientists and field assistants. The study physician explained the purpose of the study to the parents or caregivers, and the adult respondents. After obtaining informed consent from all respondents a standardized questionnaire was administered to them through face-to-face interview. For the children less than five years, vaccination history on routine immunization were extracted from their child health records books, clinic records. Parents or caregivers' recall of doses of vaccines the child had received was acceptable if the parent or caregiver could specify the dose given. Supplemental vaccinations (vaccinations given during national or sub-national immunization days) were obtained through oral histories given by the parent or caregiver. The child's weight and length/height were measured with

a digital weighing scale and an infantometer respectively. For all the adult respondents, weight and height were also measured. Body weight was measured on subjects in light clothing and without shoes to the nearest 0.1 kg using with a heavy-duty Seca 770 floor digital scale (Seca, Hamburg, Germany). Height was measured to the nearest 0.1 cm with a commercial stadiometer in standing position with closed feet, holding their breath in full inspiration and Frankfurt line of vision. The vertical and horizontal placement of the stadiometer and infantometer were checked by using the carpenter's level and standardized rod in regular intervals. The digital weighing scales were checked using the standardized weights in regular intervals [Cogill, 2003].

3.11 Blood collection procedure for hospital based seroprevalence survey

Two-five (2-5) ml of venous blood was collected through venepunture into a vacutainer tube by a phlebotomist. Blood sera were separated within six hours at the hospitals and stored at −20 ^{o}C in a deep freezer. After the data collection, blood samples were transported to the Noguchi Memorial Institute for Medical Research for laboratory analysis in a reverse cold chain at a temperature of +2 to +8 ^{o}C. Sera were tested in triplicate for levels of neutralizing antibody titers against poliovirus types 1, 2 and 3, respectively, using modified micro-neutralization assays.

3.12 Data collection technique and tools- school lameness survey

The survey method was adapted from LA Force's method of school lameness survey with some modifications [LA Force et al., 1980].

On reaching each class in a selected primary school, the teachers were primarily asked if there was any child with walking disability or any kind of weaknesses in the limbs in the class present or absent from school on that day. After that, all children in each class were asked to walk pass the survey team, and children with walking disabilities or lamed were identified. The team then sought permission from the class teachers and the headmaster of the school to invite all such pupils to come to the school with their parents the following day. This included all children who were lamed and absent from school on the day of visit. These children were also made to walk pass the survey team for assessment. Clinical and epidemiological data of the children were obtained from the parents using a semi-structured questionnaire by trained medical officers and research assistants. The questionnaire elicited information on gender, date and place of birth, date of onset of paralysis, residence or place of onset of paralysis, character of paralysis, history of onset of paralysis and sensation.

The child was further examined clinically in a sitting position. The muscle tone was determined in both legs by passive range of motion. The muscle mass was determined by physical examination and palpation. With an aid, knee jerks deep tendon reflexes were observed and subsequently, sensation, by the ability to distinguish sharp and blunt ends of a pin. Finally, the degree of disability was estimated. Based on the information from the parents and children and the necessary physical examinations, the child's lameness was attributed to one of these etiological factors: residual paralysis from poliomyelitis, congenital defects, upper motor neuron disorders (e.g., cerebral palsy), trauma due to road traffic accident, post-infectious complications such as osteomyelitis or a septic joint, Guillain-Barré syndrome and traumatic neuritis.

3.13 Quality control measures – hospital-based seroprevalence survey

Quality control measures consisted of training research assistants, pretesting the questionnaire and procedures, as well as quality checks of data.

3.14 Training of research assistants

The research assistants comprised of national service persons and trained biomedical scientists who were recruited from three teaching hospitals. The training of the research assistants was done a day before the pre-test of the survey tools. They were oriented on the questionnaire and they also translated the questionnaires into the local language. This enabled them provide the same interpretations to the questions, ensure questions were posed in similar manner to avoid inter-interviewer bias. They also practiced how to administer the questionnaire, how to collect information and examine completed questionnaires for any inconsistencies and completeness. A re-training session of one day after a week of start of data collection was done to ensure that the research assistants translated the questionnaire in the same manner and addressed all challenges that were encountered on the field from the onset of survey.

3.15 Pretesting of questionnaire/procedures

The pretesting of the questionnaire was done at the Tetteh Quarshie Memorial Hospital, which was not part of the selected referral hospitals. Some of the questions were dropped or revised after pre-testing. The pretesting assessed the following: relevance and acceptability of the questions, willingness of the respondent to answer the questions, the acceptability of the methods used to establish contact with the study population, whether

the questionnaire would be a reliable tool for collecting the information needed, the time needed to administer the questionnaire and whether there was any need to revise the format of the questionnaires. The sequence and wording of questions were also assessed. Some answers were pre-coded and closed questions changed to open-ended questions.

3.16 Quality checks of data

Before and during data processing, the completed questionnaires were checked again for completeness and internal consistency. Inconsistency was checked for in the field so that where a mistake was detected correction was made before the questionnaires were taken away. There was a daily calibration of instruments to ensure accuracy in the data collected.

3.17 Blood quality control measures for hospital based seroprevalence survey

An adequate blood sample (2-5mls) was collected by a phlebotomist, the serum separation was done within six hours and stored in a deep freezer of -20^0C. The blood samples (sera) were stored in air tight tubes to ensure they arrived at the Noguchi Memorial Institute for Medical Research (NMIMR) without any leakage in a reverse cold chain maintaining a temperature of +2 to +8 ^{o}C. The blood samples were collected from the three study sites and transported to the NMIMR which is located in the Greater Accra Region.

3.18 Quality Control Measures – school lameness survey

Training of research assistants and pretesting of questionnaire and procedures were performed to ensure that quality and relevant data were collected from the field.

3.19 Training of Research Assistants-school lameness survey

The screening team for the school lameness survey comprised of a medical officer, the district or municipal disease control officer and a school health coordinator of the respective municipals or districts. There was an initial orientation on the survey on the translation of each question on the questionnaire into the local dialect to avoid inter-interviewer bias. The team practiced how to administer the questionnaire and observe all the protocols, as the survey touched on sensitive personal issues. The research team was oriented on how to check for data inconsistencies and completeness.

3.20 Pretesting of questionnaire/procedures for school lameness survey

The research team pretested the questionnaire in a primary school in the Ashanti region (Asawasi L/A primary school). Some of the questions were revised after the pretest. The following were assessed during the pretest: relevance and acceptability of the questions, willingness of the respondent to answer the questions, sequencing of questions, wording of the questions for clarity and modifying closed questions into open-ended questions**.**

3.21 Laboratory investigations for hospital based seroprevalence survey

This section outlined the protocol to detect anti-polio antibodies against serotypes 1, 2 and 3.

3.21.1 Cell culture/ Passage of cells

The flask of monolayer of Hep-2C cells was examined for confluency and absence of contamination. The cell layer was washed twice with 3 ml of PBS and 0.5ml of 25% trypsin solution in EDTA was added. The flask was placed in a 36 ^{o}C incubator until the cells detached from the surface. The trypsin was poured off and the flask was tapped a few times vigorously against the sides and the cells re-suspended in 10 ml of growth medium to halt the action of the trypsin. The suspension was diluted with growth medium into an arbitrary split ratio of 1:3 in 3 flasks and incubated at 36 ^{o}C.

3.21.2 Preparation of virus stock

Authenticated Sabin 1, 2 and 3 strains obtained from National Institute of Biological Standards and Control (NIBSC) on January 9, 2016 (Sabin 1-01/520. Sabin 2-01/530 and Sabin 3-01/532) were used to prepare virus working stock for the neutralisation assay. Before preparing the stocks of prototype polio virus, the virus serotype, its source and cell culture passage history was noted. The growth medium on a flask (75cm^3) of monolayer Hep-2C cells was decanted and rinsed gently with PBS. 1ml of the authenticated Sabin prototype poliovirus strain was inoculated and allowed it to adsorb to cell mono layer for an hour. 20 mls of maintenance medium was added and incubated at 36 ^{o}C until a cytopathic effect (CPE) was developed. The stock viruses (type 1, 2 and 3) were aliquoted into 1.5 ml freezing vials for storage at -70 ^{o}C.

3.21.3 Titration of authenticated Sabin poliovirus stock

A 10 fold serial dilution of the virus was prepared. A maintenance medium (9ml of diluent) was added to test tubes labelled 10^{-1} up to 10^{-8}. Into the test tube labelled 10^{-1}, one ml of P1 (polio virus serotype 1) was added and vortexed. One ml of the solution was picked from the first test tube labelled 10^{-1} and transferred into the test tube labelled 10^{-2}. This solution was vortexed and 1 ml was picked into test tube labelled 10^{-3}. This procedure continued till test tube labelled 10^{-8} was reached. The procedure was repeated for the other two polio serotypes (P2 &P3) [Figure 3.5].

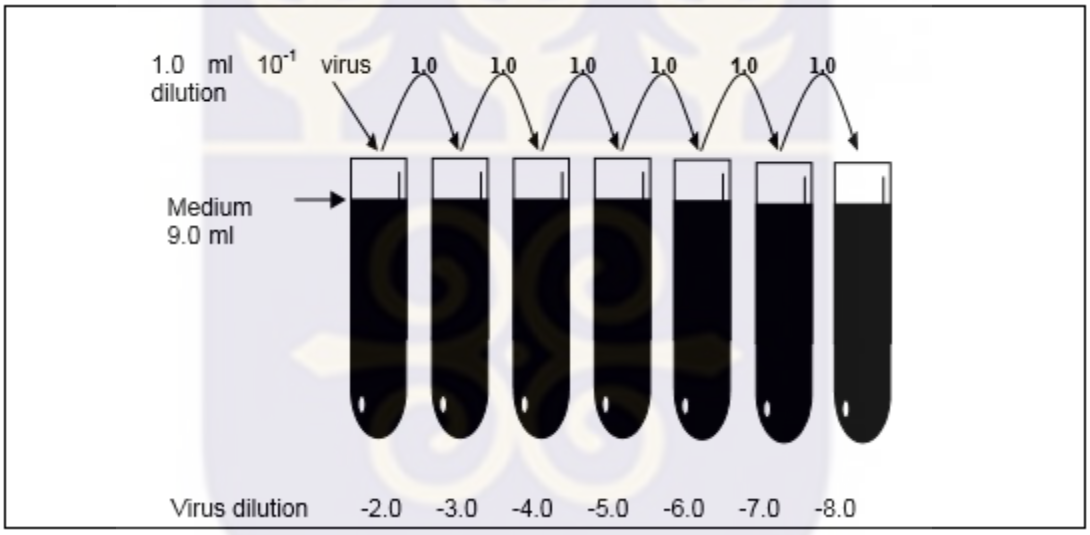

Figure 3.3: Preparation of virus dilution of Sabin poliovirus serotypes 1, 2 & 3 reference strain

The three stock viruses were titrated according to WHO guidelines [WHO, 1997]. A 96-well plates was labelled as shown in Figure 3.4. A 50µl of diluent (maintenance medium) into all wells except for the cell control wells where 100 µl of diluent was added. From the test tube labelled10^{-5}, 50 µl of the polio virus, P1, was taken and added to all the wells on the row labelled A 1-10 and B1-10. From well 10^{-6} 50 µl of P1 was added to the wells

labelled C 1-10 and D 1-10 (Figure 3.4). Similarly, this was done for wells 10^{-7} and 10^{-8} respectively changing microtips at each point. The procedure was repeated for polio serotypes P2 and P3. A flask of Hep-2C cells was observed for confluency, washed twice with PBS and trypsinized. The cells were suspended with 10mls of growth medium and counted to obtain a concentration of $2x10^4$. A 100 µl of Hep-2C cell was added to all the wells. The plates were incubated at 36°C and observed daily for cytopathic effect (CPE). The titre of the virus is calculated using the Karber formula: $TCID_{50}$ =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).

Figure 3.4: Plate layout for titration of laboratory quality control standard

3.21.4 Determination of challenge dose

The challenge dose was prepared using the titres of the stock virus (P1, P2, and P3). To calculate the challenge dose of P1, with the titre of the virus being $10^{6.9}$, the assumption was that, at that titre level the solution contained one viral infectious particle. With the

challenge dose 100 infectious particles were needed. With the viral titre (P1) being the $10^{6.9}$, the challenge dose was $10^{4.9}$. In order to prepare a dilution of $10^{4.9}$, a dilution of 10^{-4} was prepared initially. Antilog of 0.9 equated to 7.9, which was approximated to 8. This was translated to 1 part of 10^{-4} diluted virus solution needing 7 parts of the diluent.

Similarly, to calculate the challenge dose of P2;

P2 titre =$10^{7.1}$, Challenge dose =$10^{5.1}$, Antilog of 0.1=1.3, diluted viral solution to 10^{-5}

One part of virus was taken and added 0.3 ml of the diluent (maintenance medium).

In order to calculate the challenge dose of P3:

The titre of P3 = $10^{6.5}$, Challenge dose= $10^{4.5}$, Anti log of 0.5=3, diluted viral solution to $10^{-4.}$ One part of virus was taken and added 2 parts of diluent (maintenance medium).

3.21.5 Preparation of in-house serum

Twenty volunteers were vaccinated with the trivalent OPV booster obtained from the Expanded Programme of Immunization (EPI) office in Accra, and 5-10 ml of blood was drawn from each person after two weeks and the sera was pooled together. A 1 ml aliquots was prepared and stored at -20 ^{o}C.

3.21.6 Calibration of in-house polio antiserum against international reference serum

The international reference serum (IRS) was received from NIBSC, UK for the calibration of the in-house reference serum (IHRS). The vial of IRS was reconstituted with 1.0 ml sterile distilled water and aliquoted and stored at -70^{o}C for use. The IRS containing 11 IU for poliovirus 1, 32 IU for poliovirus 2 and 3 IU for poliovirus 3 was used to calibrate the potency of the IHRS. The IRS and IHRS were titrated in parallel on six separate occasions using eight replicates per serum dilution in a 96-well microtitre plate as recommended by WHO (WHO, 1997) and the geometric mean titre (GMT) determined for both IRS and

IHRS. The sera were heat inactivated at 56°C for 30 minutes. Ten-fold dilution of the inactivated sera was made from 1:8 to 1:1024 and then incubated in duplicate for three hours at 36°C with 100 x 50% tissue culture infectivity dose ($TCID_{50}$) of poliovirus antigen. A cell suspension containing 2 x 10^4 Hep-2C cells/0.1 ml was added. Cell controls and an in-house reference serum of known neutralizing activity were included in each batch. Plates were incubated at 36°C and observed daily for five days and the highest dilution that protected 50% of the cultures was recorded. The GMT of the IHRS was divided by the GMT of the IRS and the result multiplied by the assigned potency of the IRS in international unit for all three serotypes.

3.21.7 Micro-neutralization Test for polio antibodies

This was a test to measure neutralising antibody titers to poliovirus types 1, 2, and 3 using microneutralisation assay with authenticated Sabin strains according to WHO protocol (WHO, 1997). This measured the ability of a human serum sample to neutralize the infectivity and cytopathic effect of each of the three types of poliovirus on cell cultures in vitro.

This test was limited as it was unable to differentiate between antibodies to wild or vaccine strains. Immunity to poliovirus was measured by determining the ability of serum to neutralize the infectivity of each of the three types of poliovirus for cell cultures. A standard dose of virus was incubated with dilutions of serum.

Procedure All serum samples from respondents were initially inactivated to get rid of complements that may distort the identification of the neutralizing polio antibodies that were supposed to be measured. Three parts of each serum sample were diluted with one part of diluent (maintenance medium) and placed in a water bath at 56°C for 30 minutes.

Two-fold serial dilution: Serum samples were diluted in diluent into 1: 4 and activated at 56^0C for 30 minutes. A 96-well plate was labelled for each plate to carry four test samples. A 25 µl of diluent was added to all the wells except columns 11 and 12 (A11and 12 – H 11and 12). A 25 µl of the inactivated test sample was added into the wells labelled A1 and B1. Similarly, the second test sample, 25 µl was also added to well C1 and D1, then third sample was added to E1 and F1 and the fourth sample to G1 and H1. This resulted in the dilution of the first well to 1:8 from the 1^{st}-9^{th} column, two serial dilutions were made using multichannel pipettes. Column 10 was labelled as viral control and column 11 and 12 as cell control. This procedure was repeated in plate 1 for P1, plate 2 for P2 and plate 3 for P3 test serum. The challenge dose, 0.25 µl, was added to all the wells except the cell control. The plates were covered with plate sealers and incubated without CO2 at 36^0C and observed daily for CPE. This procedure was repeated for P2 and P3.

3.21.8 Expressing results in international units (IU)

The in-house reference serum was tested in each assay using eight replicates per serum dilution. For the assay to be valid, the titre of the in-house reference was ensured to be within one two-fold dilution of the established GMT for the in-house reference serum. The titre of the test serum was divided by the established GMT for the in-house reference serum and multiplied by the potency value in IU of the in-house reference serum, thus giving the potency of the test serum in IU.

3.22 Data management

Questionnaires were numbered before the research team left for the field each day, to indicate the sample units. Questionnaires were stored in well-labeled envelopes, and then

in polythene bags since field work was during the rainy season. A random selection and review of completed questionnaires and measurements were done. Raw data was coded using an already developed coding scheme, then edited and entered into a computer before analysis. The data entry template had a consistency and range checks embedded in it to ensure that the exact data was entered into the computer. Access to the data was limited to the researcher and the supervisor at the initial stage of the research till completion. All data were entered twice to ensure accuracy and data quality using STATA version 13. The results were presented using appropriate charts and tables..

3.23 Statistical methods and data analysis

The statistical procedure employed for the data analyses were determined by the study objectives:

Objective One: To determine the level of antibodies against poliovirus types 1, 2, and 3 with specific micro neutralization assay in three regions of Ghana. To meet this objective, the levels of antibodies against polio virus serotypes were obtained from the laboratory diagnosis. A serum sample was considered positive for a particular polio virus serotype, if antibodies were present at a dilution ≥1:8. The estimated prevalence (p) was defined as the number of individuals immune to poliomyelitis for each poliovirus type (presence of anti-polio neutralizing antibodies to serotypes 1, 2 or 3) divided by the total sample size (n). This variable was classified according to the three regions.

Objective two: To determine the distribution of antibodies that neutralizes the three polioviruses with relation to person (sex, age) and place. Seroprevalence was analysed descriptively by person and place. Wilcoxon rank sum test was used to compare differences

in median titres by sex and Kruskal–Wallis test was used to compare differences in median titres by age and residence.

Objective three: To determine the association between age and the mean titres of neutralizing antibodies among respondents. Given that the two variables (mean titres of neutralizing and age) were captured as continuous variables, a simple correlation analysis was done and a non-parametric test (Spearman Rank correlation) was used to test for statistical significance.

Objective four: To determine the risk factors for seroprevalence against polio virus antibodies among Ghanaians. Chi-square tests were used to determine factors that were significantly associated with seroprevalence in univariate analysis. Binary logistic regression models were used to obtain adjusted odds ratios and to assess the association of other risk factors (sex, education level, age, etc.) on seroprevalence. *P* values of less than 0.05 were considered as significant.

Objective five: To estimate the prevalence of lameness among school children in Ghana. The prevalence of lameness was calculated by dividing the number of children with flaccid paralysis and intact sensation by the total number of children screened.

3.24 Ethical considerations

Ethical clearance was obtained from the Ghana Health Service Ethics Committee and the Institutional Review Board of Noguchi Memorial Institute for Medical Research

(NMIMR). Informed consent, child assent and permission from the Ghana Education Service, relevant participating schools, hospitals authorities and participants prior to the study were sought.

Children, caregivers and adults were given the permission to leave the study at any time. The parents and pupils in the school survey were also informed that participation was completely voluntary and they were free to withdraw their participation in this study without losing any benefits from the health services or benefits that may accrue from the findings of this study. All the collected samples in the hospital based survey were not linked to names. All records were stored securely and only accessed by researchers working on this study.

Findings from the study were reported in the aggregate to guarantee confidentiality. Thus the confidentiality of the respondents was protected through the use of de-identified and coded data. The blood samples collected from children less than five years old and above and adults were treated according to routine laboratory work procedures, hence names were replaced with number codes. Data were stored in a cabinet with restricted access, only available to the research team. The blood specimen were used only for the purpose of this study and discarded afterwards. The aims and objectives of the study were explained to adults and caregivers recruited. Their consent was sought before the collection of blood samples. All the blood samples were collected by health personnel and followed the WHO regulations on seroprevalence (WHO, 1997).

Risks and benefits:

Risks: The risk directly associated with the sample collection in the hospital based seroprevalence survey described in this protocol was a slight discomfort while pricking, which was minimized by using trained health personnel.

Anticipated benefits: Potential direct benefits were limited. The respondents' participation in this research may help the University of Ghana and the Ghana Health Service identify any immunity gaps for system improvement towards polio eradication in Ghana.

CHAPTER FOUR

4.0 RESULTS

4.0 Introduction

The sections in this chapter describe the findings of the hospital based seroprevalence and lameness surveys in the three study regions. There were descriptive and analytical analyses. Data was presented in the form of tables, charts and graphs.

4.1 Attributes of respondents

Between April 1st and July 31st, 2016 a total of 307 respondents were enrolled in the hospital based seroprevalence survey and 307 serum samples were taken for the analysis. Of the total number of respondents, 153 (49.8%) were females (Table 4.1).

Table 4.1: Demographic and other attributes of respondents, (Northern, Ashanti and Greater Accra regions of Ghana) - Hospital based survey

Attribute	Prevalence N (%)			
	Age <1 year n=77	Age 1-4years n=77	Age 5-14years N=76	Age 15-77years n=77
Gender				
Male	44/77 (57.1)	45/77 (58.5)	32/76 (42.1)	33/77 (42.9)
Female	33/77 (42.9)	32/77 (41.6)	44/76 (57.9)	44/77 (57.1)
Mothers Education				
Primary	2/77 (2.59)	25/77 (32.5)	20/76 (26.0)	10/77 (13.0)
Post-secondary	6/77 (7.8)	1/77 (8.3)	/76 (0.0)	5/77 (41.6)
Routine OPV doses				
0	21/76 (27.6)	13/76 (17.0)	7/68 (10.0)	1/16 (6.3)
1	12/76 (15.8)	2/76 (2.6)	0/68 (0.0)	0/16 (0.0)
2	6/76 (7.9)	2/76 (2.6)	1/68 (1.5)	0/16 (0.0)
3	18/76 (23.7)	9/76 (11.8)	8/68 (11.8)	0/16 (0.0)
4	19/76 (25.0)	50/76 (66.0)	52/68 (76.5)	15/16 (94.0)
SIAs OPV doses				
0	30/76 (39.0)	6/76 (7.8)	1/68 (1.5)	0/16 (0.0)
1-3	46/76 (61.0)	43/76 (57.0)	14/68 (20.6)	2/16 (13.0)
4-6	0/76 (0.0)	26/76 (34.0)	19/68 (28.0)	0/16 (0.0)
≥7	0/76 (0.0)	1/76 (1.3)	26/68 (38.2)	6/16 (38.0)

On enrollment, the respondents were stratified into four age groups: 77 (25. 1%) in the less than one age group; 77 (25.1%) in the 1-4 years age group; 76 (24.8%) in the 5-14 years age group and 77 (25.1%) 15-70 years age group. The median and intra-quartile range age of respondents was 4 (1-14) years old. The geographical distribution of the respondents with serum samples were 32.2% (99/307) for Greater Accra, 40.1% (123/307) for Ashanti and 27.7.0% (85/307) for Northern region.

4.1.1Titration of authenticated poliovirus stock

The titre values of authenticated polio stock virus were $10^{6.9}$ for polio serotype 1, $10^{7.1}$ for polio serotype 2 and $10^{6.5}$ for polio serotypes 3 [Appendix 1].

4.1.2 Geometric mean titre of International and In-House Reference Serum

A titre of $\geq$ 1:8 was defined as indicative of adequate protection (immunity/neutralizing antibodies) [de Miranda, et al., 2007] for that specific poliovirus serotype. The Geometric Mean Titre (GMT) determined for International Reference Serum (IRS) were 128 for polio virus type 1, 512 for poliovirus type 2 and 64 for poliovirus type 3 while the GMT for In-House Reference Serum (IHRS) were 512 for poliovirus type 1, 1024 for polio virus type 2 and 512 for poliovirus 3. The corresponding titres in international unit for IRS were 11 IU/ml for polio virus type 1, 32 IU/ml for polio virus type 2 and 3 IU/ml for poliovirus type 3, and that of IHRS were 44 IU/m for polio virus type 1, 64 IU/ml for type 2 and 24 IU/ml for poliovirus type 3.

4.2 Seroprevalence of polio neutralizing antibodies

Neutralising polio antibodies against poliovirus types 1, 2 and 3 poliovirus were detected in 86.0% (264/307) [95% confidence intervals CI: 82-90%] in polio virus type1, 84% (258/307) [95% CI 79.4-87.9%] for type 2 and 75% (230/307) [95% CI 70-80%] for poliovirus type 3 of samples (Figure 4.1). The confidence interval estimates of the respective antibody seropositivity for PV1, PV2 and PV3 were significant. Neutralizing polio antibody seropositive rates of sera from respondents for polio virus types 1 and 2 were considerably higher than for type 3.

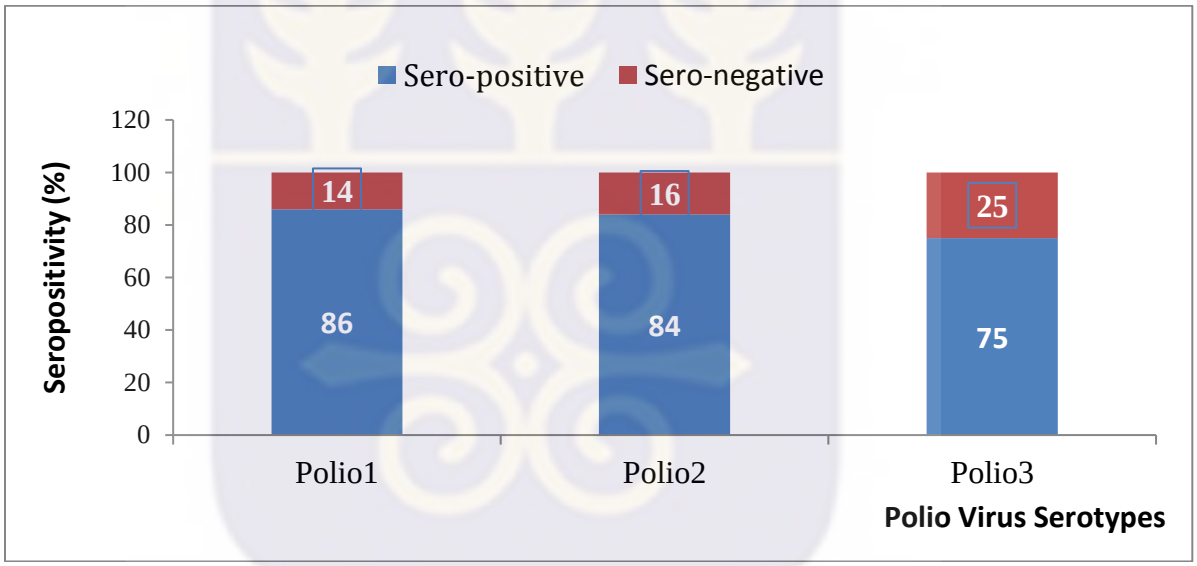

Figure 4.1: Seroprevalence of polio antibodies among respondents in the three regions of Ghana, 2016

Approximately, 60.1% (185/307) of the sera of respondents were seropositive for the three polio serotypes and nine (2.9%) sera had no antibodies at all to the three poliovirus serotypes [Table 4.2].

Table 4.2: The Percent of subjects without neutralizing antibodies to one or more poliovirus (PV) serotypes types or a combination of PV1, PV2, and PV3.

Polio Serotypes	All (n=307)	Northern region (n=85)	Ashanti region (n=123)	Greater Accra Region (n=99)
PV1, PV2, PV3	9 (2.9%)	0 (0.0%)	3 (2.4%)	6 (6.0%)
PV1&PV2	17 (5.5%)	3 (3.5%)	6 (4.9%)	8 (8.0%)
PV1& PV3	24 (7.8%)	2 (2.4%)	9 (7.3%)	12(21.1%)

Among the sera from the three regions, neutralizing polio antibodies for PV1 was highest [91.8% (95% CI: 83.6-96.1%)] in the Northern Region and lowest [83% (95% CI: 75.2-88.6%)] in Ashanti region (Figure 4.2 and Figure 4.3)

In the Northern Region, seroprevalence among the neutralizing polio antibodies was highest (91.8%) among PV1 serotype. However, there was a relatively lower coverage in PV3 (77.6%). Seronegativity was highest (22.4%) among polio neutralizing antibodies in PV3 [Figure 4.2].

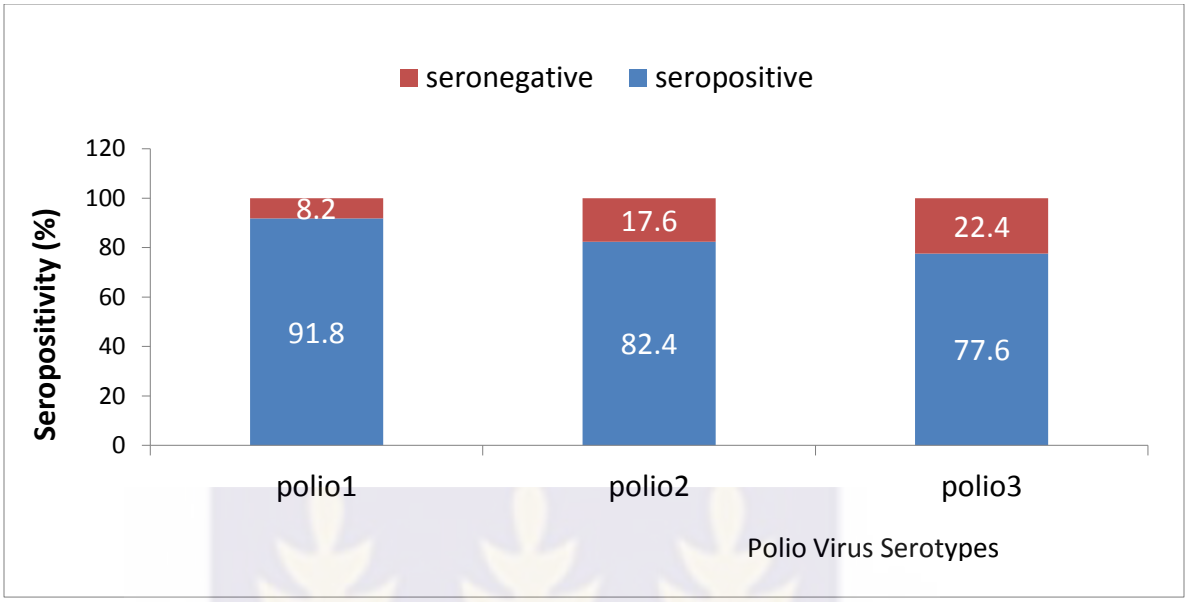

Figure 4.2: Seroprevalence of polio antibodies among respondents from the Northern region of Ghana, 2016

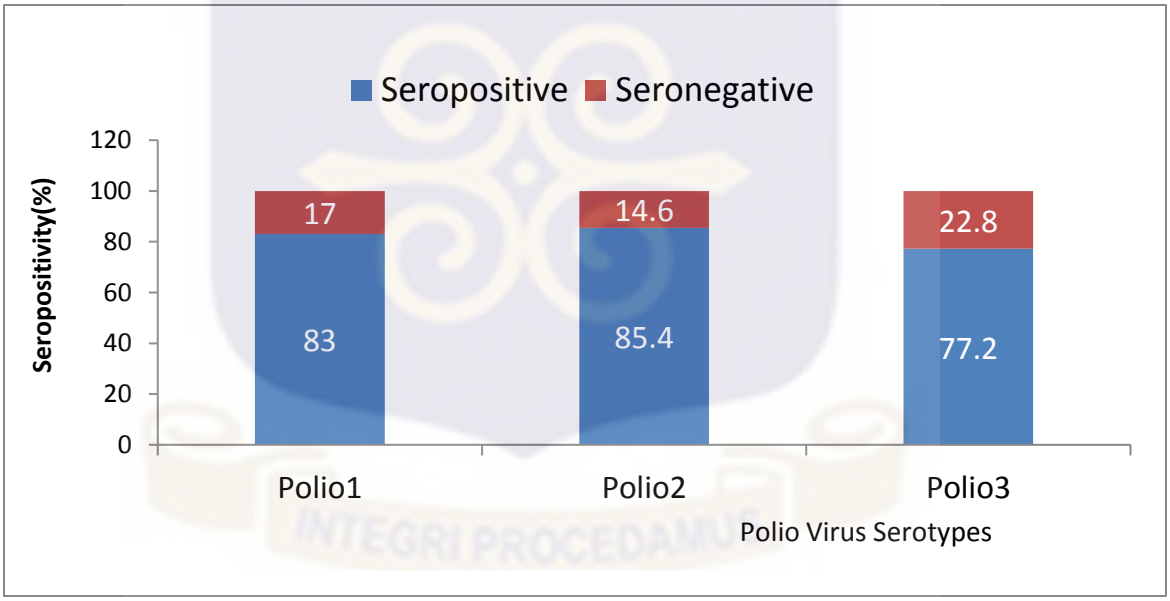

Figure 4.3: Seroprevalence of polio antibodies among respondents from the Ashanti region of Ghana, 2016

On the other hand, neutralizing polio antibodies for PV2 was highest in Ashanti Region [85.4% (95% CI: 77.9-90.6%] and lowest [82.4% (95% CI: 75.1-89.9%)] in Northern

region (Figure 4.2, 4.3 and 4.4). Seropositivity among neutralizing polio antibodies (in Ashanti region) was highest (85.4%) in PV2 whilst seronegativity was highest (30.3%) in PV3 and least (17%) in PV1 (Figure 4.3].

Neutralizing polio antibodies for PV3 was lowest [69.7% (95% CI: 59.9- 78.0%)] from the sera of respondents in the Greater Accra Region [4.4].

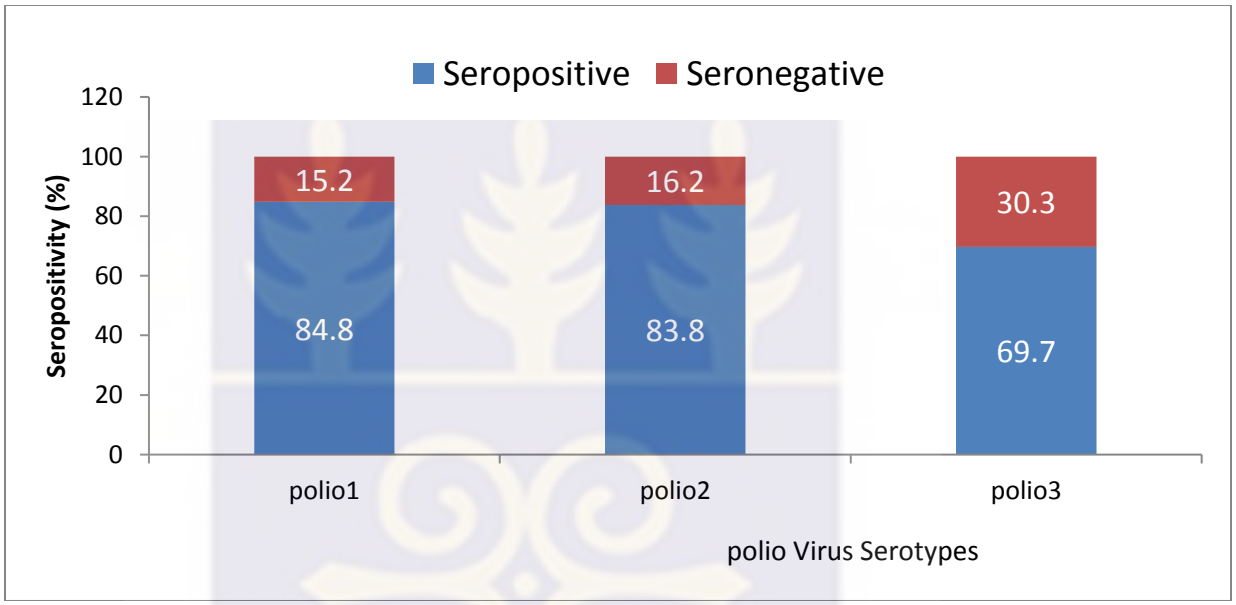

Figure 4.4: Seroprevalence of polio antibodies among respondents from the Greater Accra region of Ghana, 2016

In the sera from respondents in Greater Accra, there was a marginal difference between neutralizing antibodies of PV1 and PV2 (84.8% vs. 83.8). Seronegativity was 30% in PV3, the highest among the three polio serotypes [Figure 4.4].

4.3 Distribution of poliovirus serotypes neutralizing antibodies by sex, age and place

4.3.1 Distribution of polio serotypes neutralizing antibodies by sex

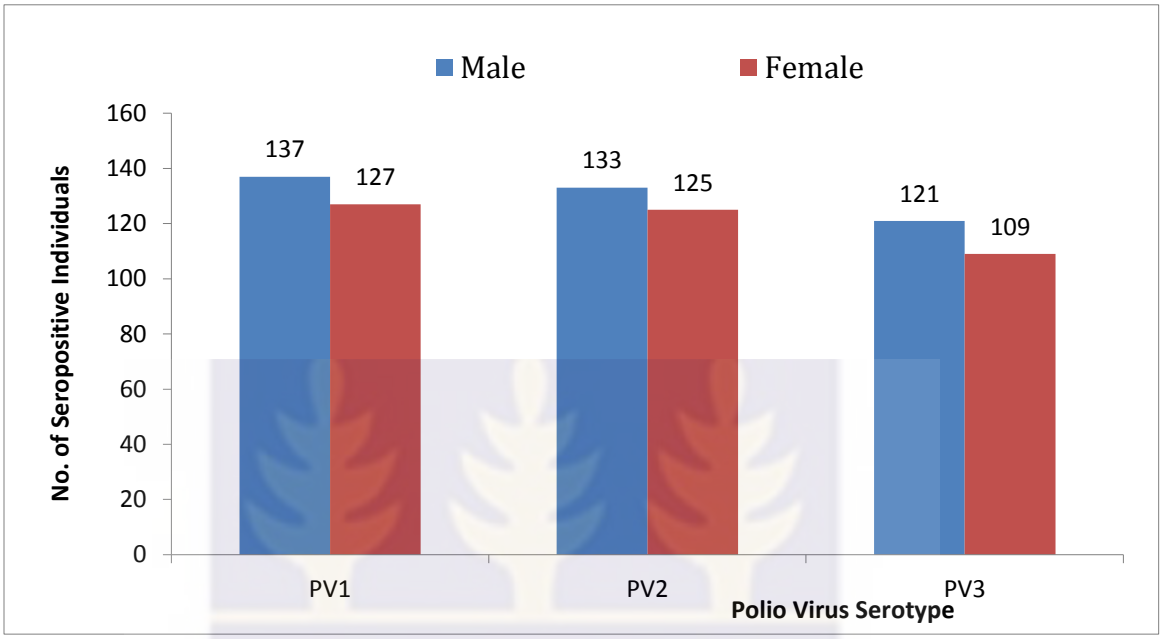

Figure 4.5: Sex distribution of respondents with neutralizing antibodies to poliovirus serotypes in the three regions, 2016

The proportion of males compared to females was greater among the seropositives in all the age groups in the three polio antibody serotypes 1(137/264=51.9%,) 2(133/258=51.6%), 3 (121/230=52.6%) in all three regions [Figure: 4.5].

4.3.2 **Distribution of polio serotypes neutralizing antibodies by Sex and Place**

Neutralising polio antibodies of polio serotype 1 (OPV1) was highest at 93.88% (CI: 82.42-98.04) among males in the Greater Accra region but lowest at 83.88 % (CI: 72.39-91.16) among the surveyed male population in the Ashanti region. Among the females, Northern region had the highest neutralising polio antibodies of polio serotype 1 (OPV1) at 92.86% (79.77- 97.72) [Table 4.3].

Table 4.3: Distribution of polio antibodies that neutralized the three polio viruses with respect to sex and place

Polio serotypes	Female			Male		
	Northern Region n=42 x % CI (%)	Ashanti Region n=61 x % CI (%)	Greater Accra Region n=50 x % CI (%)	Northern Region n=43 x % CI (%)	Ashanti Region n=62 x % CI (%)	Greater Accra Region n=49 x % CI (%)
OPV1	39 **92.86** (79.77-97.72)	50 **81.97** (70.13-89.80)	38 **76.00** (62.12-85.95)	39 **90.70** (77.42-96.52)	52 **83.88** (72.39-91.16)	46 **93.88** (82.42-98.04)
OPV2	35 **83.33** (68.67-91.94)	52 **85.25** (73.84-92.20)	38 **76.00** (62.12-85.95)	35 **81.40** (66.72-90.52)	53 **85.48** (74.23-92.33)	45 **91.83** (79.95-96.95)
OPV3	32 **76.19** (60.86-86.81)	43 **70.49** (57.78-80.66)	54 **68.00** (53.77-79.52)	34 **79.07** (64.17-88.85)	52 **83.87** (72.39-91.16)	35 **71.42** (57.14-82.42)

X=numerator

A similar trend was observed for neutralising polio antibodies of polio serotype 2 (OPV2), which was highest 91.83% (CI: 79.95-96.95) among males in the Greater Accra region but lowest among males in the Northern region, at 81.40% (CI: 66.72-90.52). Neutralising polio antibodies of polio serotype 2 (OPV2) was highest at 85.25% (CI: 73. 84-92.20) neutralising among the females in Ashanti region. However, neutralising polio antibodies of polio serotype 3 (OPV3) were highest among males in the Ashanti region at 83.87% (CI: 72.39- 91.16) and lowest at 71.42% (CI: 57.14-82.42) in the Greater Accra region. Among the females Northern region recorded the highest at 76.19% (60.86- 86.81) [Table 4.3].

4.3.3 Distribution neutralising polio antibodies by age and place

Out of the 264 sera from respondents in the three regions that were positive with neutralizing polio antibodies type 1 (PV1), 29.2% as highest, was in age group 1-4 years and the lowest was (22.7%) in age group 15-70. There were 258 sera positive for PV2 and the lowest (21.3%) was recorded in age group 15-70. Age group 1-4 years recorded the highest (PV1=29.2%; PV2=27.9%; PV3=28.7%) seropositivity in all polio serotypes [Figure 4.6].

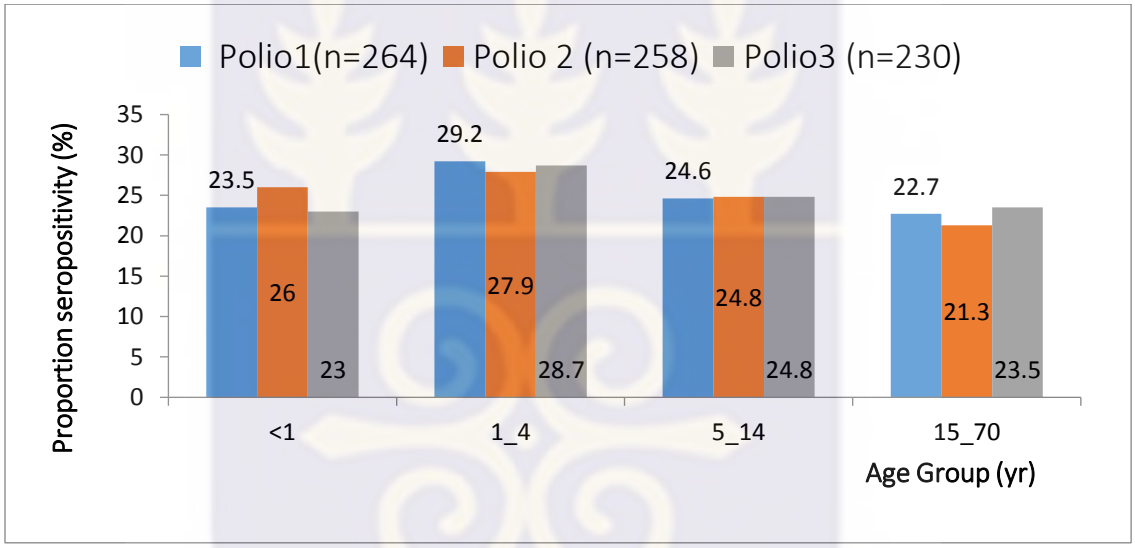

Figure 4.6: Seroprevalence of poliovirus antibodies among respondents in the three regions, by age group, 2016

4.3.3.1 Seroprevalence of poliovirus type 1, 2, 3 neutralizing antibodies for Greater Accra region

In the Greater Accra region, there were 84 sera from respondents positive for PV1 neutralizing antibodies. Out of this number, the highest (29.8%) was in age group 1-4 years old and the lowest was 21.4% in age group 15-70 [Figure 4.7].

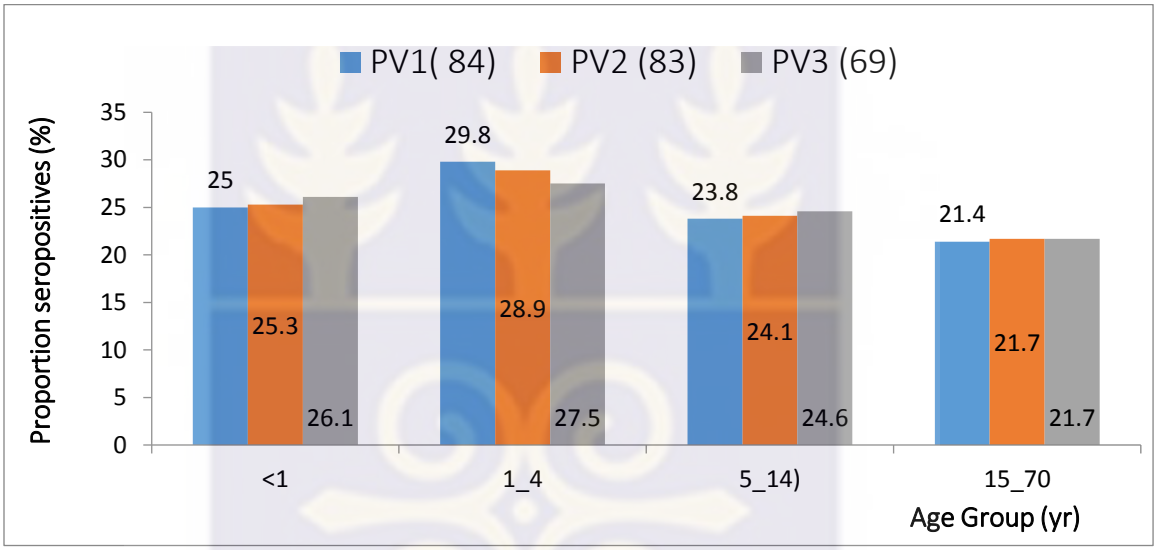

Figure 4.7: Seroprevalence of polio antibodies among respondents in the Greater Accra region, by age group, 2016

For seroprevalence of poliovirus type 2 neutralizing antibodies, there were 83 sera from respondents positive in the Greater Accra region. Out of this number, the highest (28.9%) was in age group 1-4 year and the lowest 21.4% in age group 15-70 [Figure 4.7]. There were 69 sera from respondents positive for PV3 neutralizing antibodies for Greater Accra and out of this number, the highest (27.5%) was in age group 1-4 year and the lowest 21.7% in age group 15-70 [Figure 4.7]. Thus for Greater Accra PV neutralizing bodies were consistently lower for the age group 15-70.

4.3.3.2 Seroprevalence of poliovirus type 1, 2, 3 neutralizing antibodies for Northern region

Similarly, for PV1 neutralizing antibodies, 78 sera from respondents were positive for PV1 in the Northern region. The highest 29.5% was in age group 1-4 and the lowest 21.8% in age group 15-70 [Figure 4.8]. Seventy sera from respondents were positive for PV2 in the Northern region. The highest 32.9% was in age group 1-4 and the lowest 17.1% in age group 15-70 [Figure 4.8].

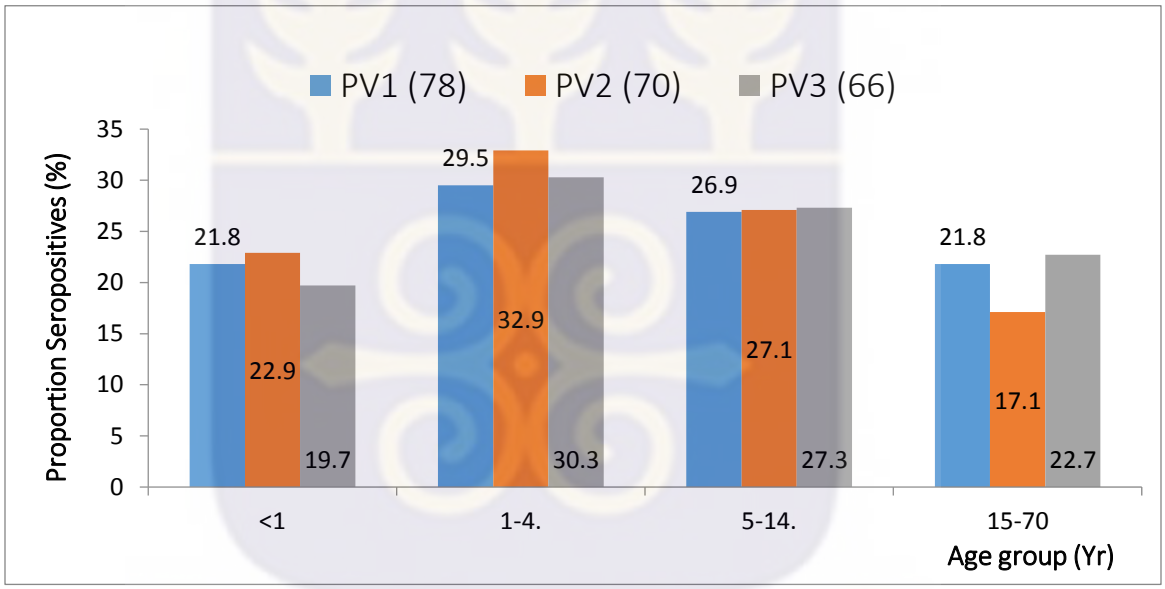

Figure 4.8: Seroprevalence of polio virus antibodies among respondents in the Northern region, by age group, 2016

Also 66 sera from respondents were positive for PV3 in the Northern region. The highest 30.3% was in age group 1-4 and the least 19.7% in age group less than one year [Figure 4.8].

Unlike in the Greater Accra region where the age group 15-70 had the lowest neutralizing antibodies for PV1, 2 and 3 (Figure 4.7) in the Northern Region, this consistency was not

observed as neutralizing antibodies for PV1 and PV2 were lowest for the age group 15-70 but lowest for age group less than one year neutralizing antibodies for PV3. Similarly, in the Northern region neutralizing polio antibodies were found in all the age groups.

4.3.3.3 Seroprevalence of poliovirus type 1, 2, 3 neutralizing antibodies for Ashanti region

Neutralizing polio antibodies for the PV1, PV2 and PV3 in Ashanti region were highest 30.8% (66/214) in age group 1-4 years. However, the lowest 20.6% (44/214) was found in age group 15-70 years. In the Ashanti region, PVI neutralizing antibodies was highest (28.4%) in 1-4 and lowest 23.5% in age groups less than one year and 5-14 years. However for PV2, neutralizing antibodies were highest (28.6%) in age groups <1 year in the Ashanti region [Figure 4.9].

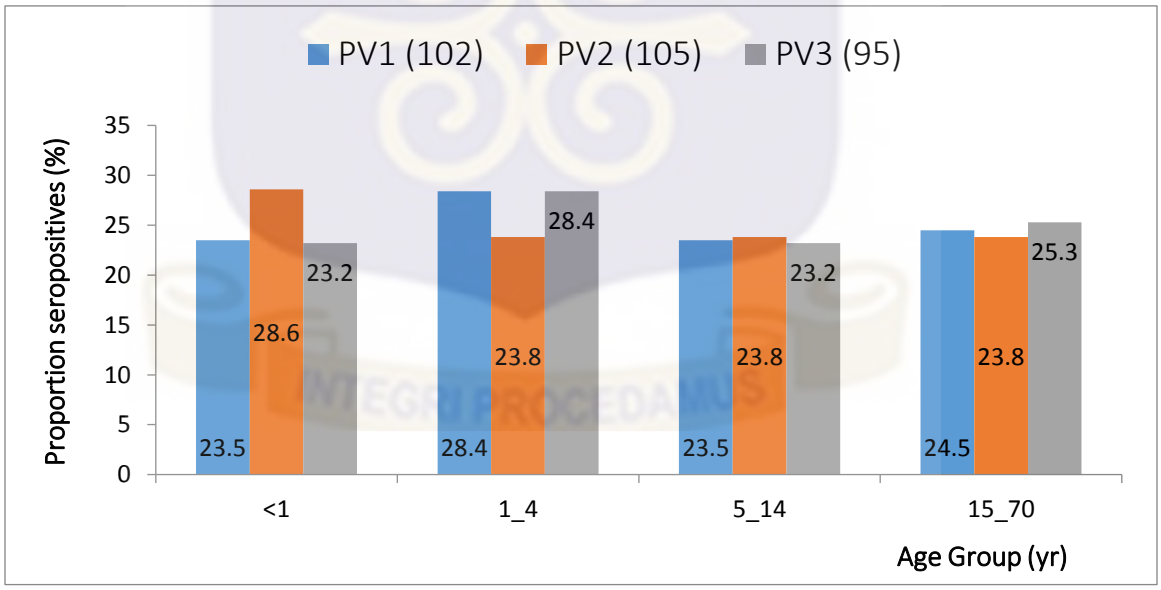

Figure 4.9: Seroprevalence of polio virus antibodies among respondents in the Ashanti region, by age group, 2016

However, PV3 neutralizing antibodies was highest (28.4%) in age groups 1-4 and lowest 23.2% in age group less than one year [Figure 4.9]. The Ashanti region therefore showed consistently highest neutralizing antibodies for PV1and PV3 for age group 1-4. In the Ashanti region majority 53.6% (162/302) of the polio neutralizing antibodies were therefore found in the age group 1-4. However, the lowest 23.5% (71/302) of the polio neutralizing antibodies was in the age groups <1 and 5-14 years [Figure 4.9].

There were no significant differences in the seroprevalence for all polio type 1 (p= 0.133) , 2 (p=0.263) and 3 (p=0.139) neutralizing antibodies by sex.

4.3.3.4 Seroprevalence of poliovirus type 1, 2, 3 neutralizing antibodies geographical location

Seroprevalence rate of polio neutralizing antibodies of the three polio serotypes (1, 2 & 3) were highest in the Northern Region: PV1=91.8% (78/85); PV2= 82.4% (70/85) and PV3=77.4% (66/85) [Figure 4.10]. There was however, an ascending trend of seronegativity in the entire region from PV1-PV3.

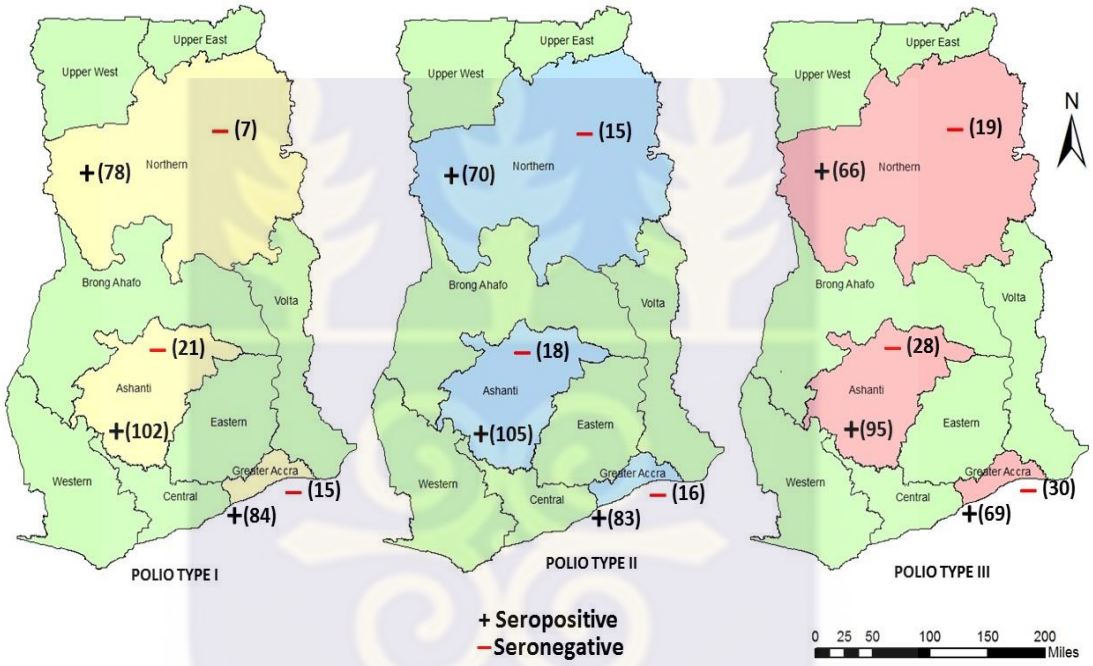

Figure 4.10: Seroprevalence of polio virus antibodies among respondents in the three regions, by place, 2016

4.4 Distribution of polio virus antibodies titres that neutralized the three polioviruses with relation to person (sex, age) and place.

Seroprevalence was analysed descriptively by person and place. There was a significant difference in the median PV1 and PV3 (p-value=0.0514 and 0.0254 respectively) poliovirus neutralizing antibody titre values between males and females. Similarly, there was a significant difference in the median PV1 and PV2 (p-value=0.0001) titre values

among the age groups. There was also a significant difference in the median poliovirus neutralizing antibody titre values of PV2 in the three study sites (p-value =0.0046) [Table 4.4]. However PV1 (p=0.2823) and PV3 (p=0.4151) were not significant.

Table 4.4: Distribution of respondents' median neutralizing poliovirus antibody titres

	PV1		PV2		PV3	
	Titre (95% CI)	p-value	Titre (95%CI)	p-value	Titre (95% CI)	p-value
Gender						
Male	4.2871 (3.341-5.499)		2.8367 (2.221-3.622)		1.3493 (1.098-1.657)	
Female	2.864 (2.337-3.510)	**0.0514**ǂ**	2.265 (1.828-2.807)	0.2937	0.9582 (0.809-1.134)	**0.0254****
Age group						
< 1 year	4.629 (3.199-6.698)		4.7911 (3.282-6.994)		1.6055 (1.515-2.2379)	
1-4 years	6.796 (4.847-9.52)		3.243 (2.34-4.493)		1.1300 (0.8664-1.4738)	
5-14 years	2.504 (1.958-3.201)	0.0001**ǂ	2.1734 (1.651-2.860)	0.0001**	0.993 (0.772-1.279)	0.1995
15-70 years	1.910 (1.471-2.479)		1.222 (0.962-1.552)		0.927 (0.754-1.140)	
Residence						
Northern	3.275 (2.485-4.317)		1.605 (1.270-2.027)		1.226 (0.957-1.571)	
Ashanti	3.118 (2.411-4.031)	0.2823	3.366 (2.539-4.462)	**0.0046**ǂ**	1.009 (0.831-1.226)	0.4151
Greater Accra	4.301 (3.155-5.865)		2.642 (1.975-3.534)		1.237 (0.947-1.616)	

Median neutralizing polio antibody titre; ǂ is p-value estimate from Wilcoxon rank sum test; ǂ is p-value estimate from Kruskal Wallis

4.5 Association between age and mean titres of neutralizing antibodies of the three polio serotypes of respondents

The age of the respondents had a negative linear relationship on the mean titres of the neutralizing antibodies against the three polio serotypes [Table 4.5 & Figures 4.11, 4.12 & 4.13]. The presence of neutralizing polio antibodies in the sera of respondents decreased with age. This implies that, generally, as one grows older, the presence of neutralizing polio antibodies decreases, and the opposite trend occurs as participant ages decrease. The PV2 gives the highest negative significant (rho=-0.31, $p<0.001$) correlation followed by PV1 (rho=-0.2617, $p<0.001$) and PV3 (rho=-0.1099, $p=0.0545$).

Table 4.5: Association between age and mean titres of the neutralizing polio antibodies of the three polio serotypes

Polio serotype	Spearman Rank correlation **(rho)**	**p-Value**
PV1	-0.2617	< 0.001
PV2	-0.3100	< 0.001
PV3	-0.1099	0.0545

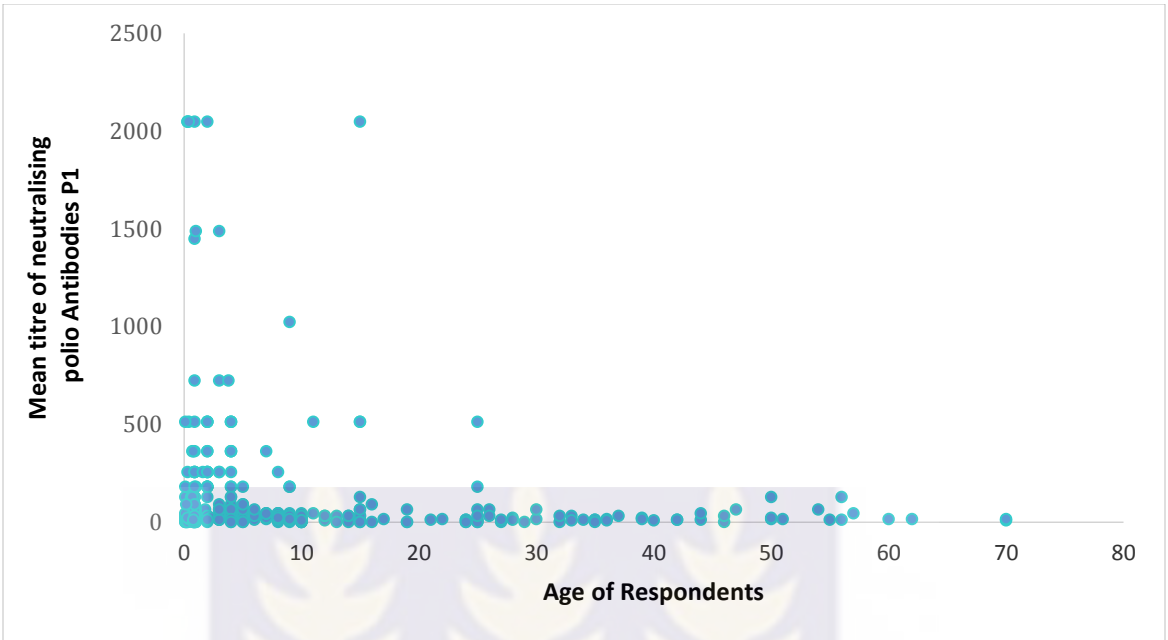

Fig. 4.11: Association between age and mean titre of neutralising polio antibodies P1

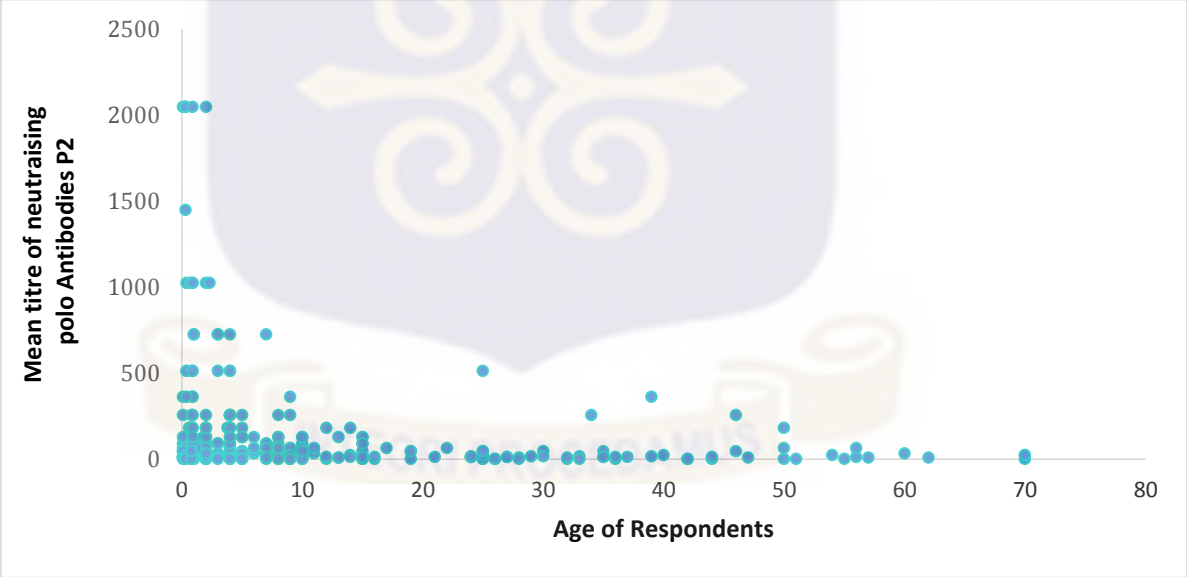

Fig. 4.12: Association between age and mean titre of neutralising polio antibodies P2

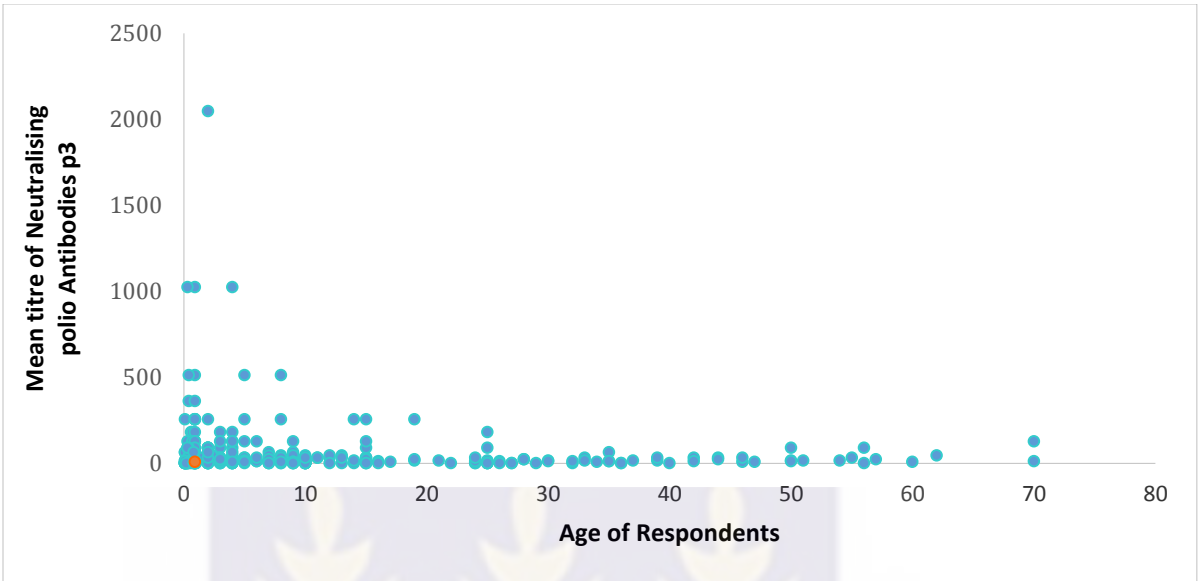

Fig. 4.13: Association between age and mean titre of neutralising polio antibodies P3

Table 4.6: Association of Age and mean titres of the neutralizing antibodies of the three polio serotypes in the three study regions

Region	Polio serotype	Spearman Rank correlation (rho)	p-Value
Northern region	PV1	-0.0495	0.6529
	PV2	-0.2533	0.0193*
	PV3	-0.0625	0.5532
Ashanti Region	PV1	-0.2873	0.0013*
	PV2	-0.4351	<0.0001*
	PV3	-0.0186	0.8378
Greater Accra Region	PV1	-0.3536	0.0003*
	PV2	-0.245	0.0145*
	PV3	-0.2501	0.0125*

***Statistically significant correlation (p<0.05)**

Although age of the respondents had a negative linear relationship with mean titres of the neutralizing antibodies against the three polio serotypes in all the study regions, in the Northern and Ashanti regions the relationship was not statistically significant with PV3 [(p=0.5532- Northern Region, 0.8378-Ashanti region)]. In the Greater Accra Region, the

mean titre levels of neutralizing polio antibodies for all the three polio serotypes generally significantly decreased with age (PV1=0.0003: PV2= 0.0145: PV3=0.0125)[Refer Table 4.6].

4.6 Risk factors for low seroprevalence of Polio virus antibodies among respondents

The educational status of mothers played a significant role in the presence of antibodies against poliovirus serotype type 1 & 2 among the respondents. With poliovirus serotype 1, the odds of being seronegative among respondents whose mothers have never attended school was 3.9 times ($p<0.003$) the odds of being seronegative among respondents whose mothers have attended school. A similar picture was found for poliovirus serotype 2 ($p<0.001$) [Tables 4.7, 4.8 & 4.9]. This implies that mother's education is a significant determinant of whether a child will have neutralizing polio antibodies for polio serotypes 1 & 2.

Table 4.7: **Risk factors for low seroprevalence of PV1 antibodies among respondents**

Variable	Seronegative (%) n=**43**	Seropositive (%) n=**264**	cOR (95% CI)	p-value	aOR (95% CI)	p-value
Mothers schooling status						
Attendant	21(48.8)	182(68.9)	1.0		1.0	
Non attendant	**22(51.2)**	**82(31.1)**	**2.3(1.2-4.5)**	**<0.011***	**3.9(1.59-9.48)**	**<0.003***
Age group						
<1	14(32.6)	63(23.9)	1.0		1.0	
1-4	3(6.9)	74(28.0)	0.18 (0.05 - 0.67)		0.29 (0.07-1.08)	
5-14	11(25.6)	65(65.6)	0.76 (0.32-1.80)		1.8 (0.6-5.3)	
15-70	15(34.9)	62(23.5)	1.1 (0.49-2.44)	**0.0466***	2.6 (0.93-7.6)	**0.0148***
Sex						
Female	26(60.5)	127(48.1)	1.0		1.0	
Male	17(39.5)	137(51.9)	1.5(0.9-2.4)	0.14	1.2(0.6-2.6)	0.48

Non Attendant mother= A mothers who has no formal education

Table 4.8: **Risk factors for low seroprevalence of PV2 antibodies among respondents regions**

Variable	Seronegative (%) n=**43**	Seropositive (%) n=**264**	cOR (95% CI)	p-valve	aOR (95% CI)	p-value
Mothers schooling status						
Attendant	25(50.0)	178(69.0)	1.0		1.0	
Non attendant	**24(49.0)**	**80(31.0)**	**2.1 (1.2-3.97)**	**0.016**	**5.9 (2.4-12.6)**	**<0.001***
Age group						
<1	9(18.4)	68(26.4)	1.0		1.0	
1-4	9(18.4)	68(26.4)	1.0 (0.37-2.7)		1.84 (0.65-5.16)	0.24
5-14	12(24.5)	64(24.8)	1.42 (0.56-3.59)		4.5 (1.4613.75)	0.01
15-70	19(38.7)	58(22.4)	2.48 (1.04-5.89)	**0.10**	8.3 (2.76-24.80	**<0.001***
Sex						
Female	28(57.1)	125(48.5)	1.0		1.0	
Male	21(42.9)	133(51.5)	1.4 (0.8-2.6)	0.27	0.9(0.5-1.5)	0.82

Table 4.9: **Risk factors for low seroprevalence of PV3 antibodies among respondents**

Variable	Seronegative (%) n=**43**	Seropositive (%) n=**264**	cOR (95% CI)	p-valve	aOR (95% CI)	p-value
Mothers schooling status						
Attendant	46 (59.7)	157 (68.3)	1.0		1.0	
Non attendant	31(40.3)	73(31.7)	1.45(0.85-2.5)	0.173	1.5(0.76-2.9)	0.24
Age group						
<1	22 (28.6)	55(23.9)	1.0		1.0	
1-4	15 (19.5)	62(26.9)	1.6 (0.26-1.28)		0.71(0.33-1.6)	
5-14	19 (24.9)	57(24.8)	0.83 (0.41-1.7)		1.03(0.442.50)	
15-70	21(27.3)	56 (24.4)	0.94(0.4.6-1.9)	0.58	1.20 (0.5-2.7)	0.65
Sex						
Female	44 (57.1)	109 (47.4)	1.0		1.0	
Male	33 (42.7)	121(52.6)	1.5(0.9-2.5)	0.14	0.7(0.4-1.2)	0.24

4.7 Lameness survey

The total number of schools visited was 112 out of which 34217 pupils from zero to 15 years at the primary school level were screened. Pupils found with walking disabilities were 108. Paralytic polio, defined as flaccid weakness, muscle atrophy, decreased bone growth in the affected limb, diminished deep tendon reflexes, normal sensation and a history compatible with acute poliomyelitis accounted for 18.5% (20/108) and upper motor neuron disorders (cerebral palsy, Encephalitis) accounted for 25.9% (28/108) of walking disability among the school children screened [Table 4.10].

Table 4.10: Diagnosis of reported paralysis or inability to walk estimated by school lameness surveys in Northern, Ashanti and Greater Accra regions of Ghana, 2016

Cause	Number of cases	% of total cases
Poliomyelitis	20	18.5
Congenital (including clubfeet, tibia torsion)	10	9.3
Upper motor neuron disorders (cerebral palsy, Encephalitis)	28	25.9
Trauma	25	23.1
Post infection complications (including osteomyelitis, septic joint)	25	23.1
Total	108	100

The prevalence of residual paralysis of poliomyelitis estimated from the lameness survey was 0.58/1,000 (5.8/10000) children aged 0-15 years old [Table 4.11]. The prevalence rates of residual paralysis in urban and rural districts of the studied regions were not the same. The Bosomtwe district (rural) in Ashanti region recorded the highest (4/1000) prevalence rate of paralytic polio and the lowest (0.08/1000) was recorded in the Accra metro, which is urban.

Table 4.11: Prevalence of paralytic poliomyelitis with residual paralysis estimated by school lameness surveys in Northern, Ashanti and Greater Accra regions of Ghana, 2016

Region	No of children	Residual Paralysis	
		Number of cases	Prevalence /1000 children
Northern			
Urban (Tamale Metro)	4695	7	1.40
Rural (Savelugu-Nanton)	2803	5	1.78
Ashanti			
Urban (Kumasi metro)	13848	3	0.22
Rural (Bosomtwe)	744	3	4.0
Greater Accra			
Urban(Accra Metro)	11760	1	0.08
Rural (Shai Osu Doku)	367	1	2.72
Total	**34217**	**20**	**0.58**

Among the study population, 85% (17/20) of children with residual paralysis from poliomyelitis were in the 10-15 year old group [Figure4.14]. The mean age of the children was 10.65± 2.23 years.

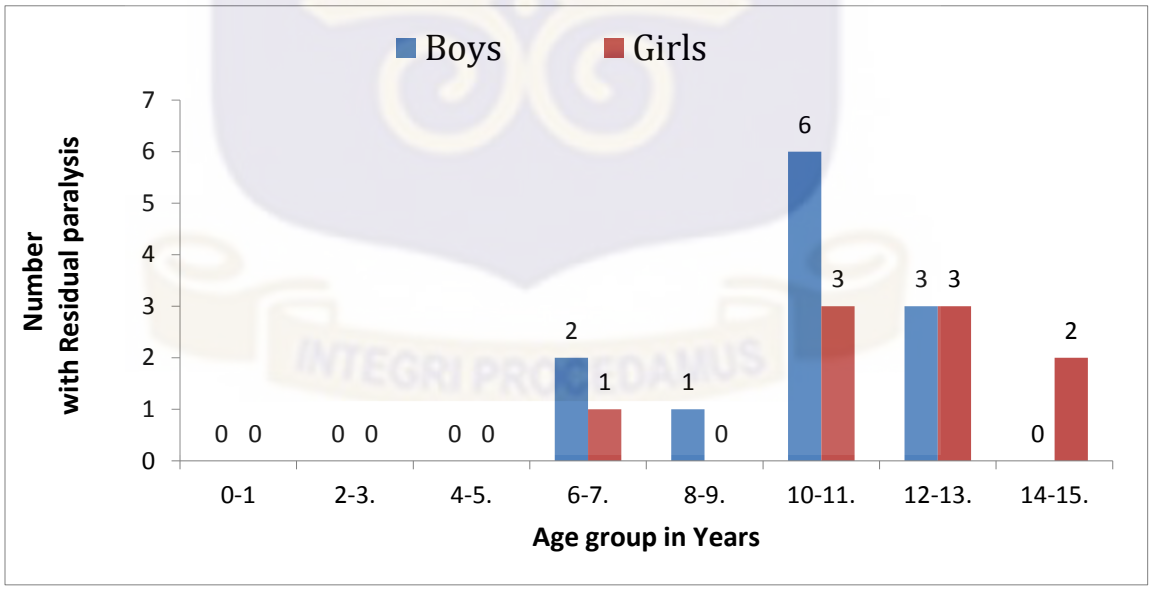

Figure 4.14: Age and sex distribution of paralytic poliomyelitis with residual paralysis estimated by school lameness surveys in Northern, Ashanti and Greater Accra regions of Ghana, 2016

The majority 60% (12/20) of those with residual paralysis were males. All the parents of the 20 children with residual paralysis were interviewed and they indicated an acute onset of weakness in a previously healthy child occurring before the age of five years. The majority (50%; 10/20) of the affected children had the onset of illness between the ages of 2-2.5 years [Figure 4.15].

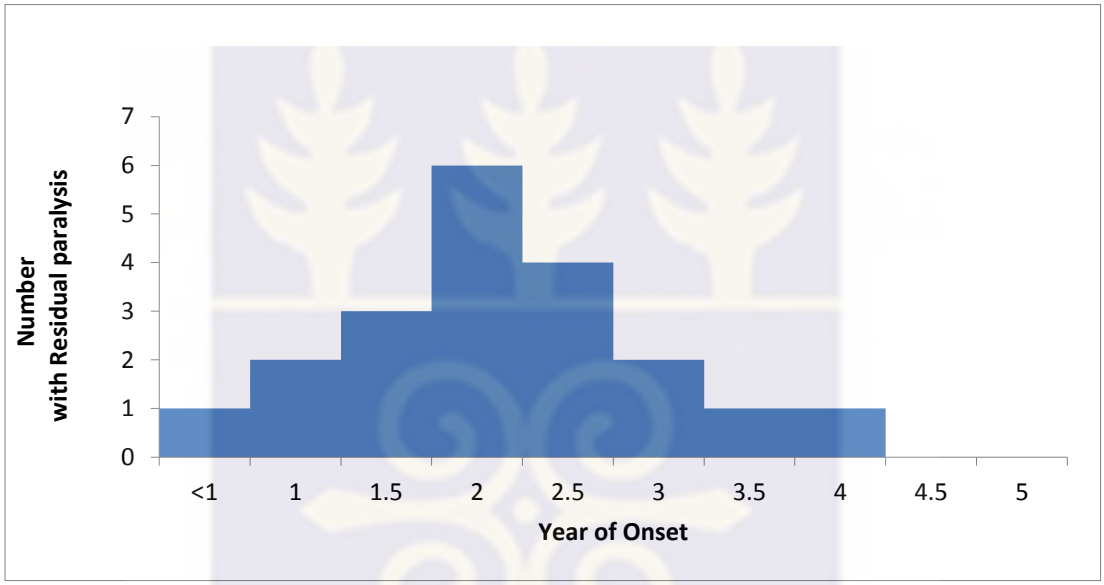

Figure 4.15: Poliomyelitis with residual paralysis cases estimated by school lameness surveys by date of onset of symptoms in Northern, Ashanti and Greater Accra regions of Ghana, 2016

All the extremities of both upper and lower limbs including the waist were affected. The right leg was the most affected limb (30%; 6/20). The sex of respondents with residual paralysis were more in males (55%; 11/20) than in females [Table 4.12].

Table 4.12: Site and sex distribution of poliomyelitis with residual paralysis cases estimated by school lameness surveys in Northern, Ashanti and Greater Accra Regions of Ghana, 2016

Sex	Right Hand	Left Hand	Right Leg	Left Leg	Both legs	Both hands	Right hand, Right leg	Left hand Left leg	Waist
Boys	2	2	2	2	1	0	2	0	0
Girls	1	0	4	1	1	0	1	0	1
Total	**3**	**2**	**6**	**3**	**2**	**0**	**3**	**0**	**1**

4.8 Hypotheses testing

Hypothesis 1:

For the hypothesis that "The seroprevalence of the three serotypes of polio virus antibodies in three regions of Ghana is less than 90%" and at 95% confidence level for difference in prevalence (or difference in proportion), the seroprevalence of the three regions being concurrently less than 90% [86.0% (264/307) [95% confidence intervals CI: 82-90%], 84% (258/307) [95% CI: 79.4-87.9%] and 75% (230/307) [95% CI: 70-80%]] implies that the hypothesis that the seroprevalence of the three serotypes of polio virus antibodies in three regions of Ghana is less than 90% was confirmed.

Hypothesis 2:

The hypothesis that there is no association between Age and Mean Titres of the neutralizing antibodies of PV1 in the three study regions is not accepted [ρ=-0.2617, $p<0.001$]. Hence

there is a significant association between Age and Mean Titres of the neutralizing antibodies of PV1 in the three study regions.

The hypothesis that there is no association between Age and Mean Titres of the neutralizing antibodies of PV2 in the three study regions is also not accepted [ρ=-0.3100, $p<0.001$]. This also implied that there was a significant association between Age and Mean Titres of the neutralizing antibodies of PV2 in the three study regions.

However, the hypothesis that there is no association between Age and Mean titres of the neutralizing antibodies of PV3 in the three study regions is accepted [ρ=-0.1099, $p=0.0545$], implying that for PV3 there is no association between Age and Mean Titres of the neutralizing antibodies of PV3 in the three study regions.

CHAPTER FIVE

5.0 DISCUSSION OF STUDY FINDINGS

From epidemiological point of view, eradication of polio is a matter of urgency. The spread of wild polioviruses from endemic areas to polio-free countries remains a potential risk, as vaccination coverage rates can decrease, and vaccine-induced immunity can wane. For these reasons, vaccination campaigns and epidemiological surveillance are absolutely necessary to maintain and verify polio-absence in polio-free countries.

This research is a maiden attempt to document the seroprevalence of antibodies against the three polio serotypes since the introduction of oral polio vaccine into the Ghana Expanded Programme of Immunization in 1978.

The study found some regional differences in level of seroprevalence of neutralizing antibodies to PV1, PV2 &PV3. Neutralizing polio antibodies to PV3 was low compared to that of PV1 and PV2. The Neutralizing antibodies to polio virus generally decreased with age. Mother's education was found crucial to seropositivity and the prevalence of paralytic poliomyelitis was low.

It is noted in this study that polio virus serotypes neutralizing antibodies are 75%-86% of the sera of the respondents. This study however, shows a seroprevalence of 1 > 2 > 3 for the polio serotypes amongst the study population in the three study regions (Ashanti, Greater Accra and Northern). These findings from the study confirm that substantial immunity gaps (differences in the levels of polio neutralizing antibodies) to all three poliovirus serotypes exist in the three (3) regions of Ghana. Despite intensive efforts to increase immunity levels against polio in Ghana since the introduction of routine immunization in 1978, the prevalence of antibodies against the three poliovirus serotypes

remains at a moderately lower level. Many developing countries have recorded through similar seroprevalence studies, relatively moderate immunogenicity of OPV [Grassly, 2013]. With the administration of the trivalent vaccine and OPV immunogenicity approaching 100% in industrialized countries, only 73% (range 36–99%) and 70% (range 40–99%) of children in developing countries had detectable antibody to PV1 and PV3 respectively after three doses [Patriarca et al.,1991].

In studies conducted in developed countries, neutralising antibodies in the sera of respondents had shown a higher seropositivity compared to those in developing countries [In Germany, Reinheimer et al, 2012; England and Wales, Philippa and Jonathan, 1984; Northern Greece Frantzidou, 2004; In Spain, Pacho et al., 2002; Yogyakarta Province-Indonesia WHO, 2008; Korea, Jee et al., 2004; China, Wang et al., 2013; in the USA, Wallace et al., 2016]. In Maiduguri Nigeria, Baba et al. (2012); North Kano, Nigeria Zubairu et al. (2013); Zaria Giwa (2012) recorded lower seroprevalence rates compared to the findings of this study. In order to evaluate the efficacy of the schedule currently recommended for immunization with trivalent oral poliovirus vaccine (TOPV), Osei-Kwasi et al. (1995) found a slightly lower seropositivity rate (75%, 83.2%, and 79.1% for PV1, PV2, PV3 respectively), among the test group. In Egypt, seroprevalence surveys were conducted in "polio-endemic" regions (Greater Cairo and Upper Egypt) and in one control region (Lower Egypt) in December 2004. Nasr El-Sayed, et al. (2007) found a significantly higher seroprevalence to PV type 1 (PV1), PV type 2 (PV2) and PV type 3 (PV3) as 99%, 99% and 91%, respectively.

Previous polio vaccinations and or infections of wild polio virus (WPV) together with the polio outbreak in 2008, in the Northern region, may account for the levels of polio

population immunity or the antibody levels in the three regions of Ghana. These gaps in immunity levels raise concerns of either primary vaccine failure, that is, lack of initial antibody responses where potent vaccines are used or, failure of the cold chain and the subsequent use of non-potent vaccines in the field [Grassly, 2006; 2007; CDC, 2000]. Even though the seropositive rate required for maintaining population immunity to polio has not been universally determined by WHO, studies have demonstrated that, the critical vaccination coverage most likely needed to stop any transmission of poliovirus has been determined to be 80–85% of the population [Anderson, 1992].

The herd immunity threshold above which one can guarantee the prevention of an outbreak is unclear in Africa and typically for Ghana. However, it has been documented by Sutter et al. [2004] that with population immunity levels of 66%–80%, polio outbreaks in developed countries can be prevented. In developing countries with suboptimal sanitation and hygiene leading to the potential for increased PV transmission and greater force of infection, wild polio virus outbreaks could however, occur with population immunity levels as high as 94%–97% [Sutter et al., 2004]. Moreover, polio outbreaks including sustained PV transmission among fully immunized children have been recorded in developing countries [Sutter et al., 1991]. Despite the high performing routine immunization system (OPV3 coverage >90%) and excellent reported performance on national immunization days and mop-ups (>90 coverage) wild polio transmission continued in Egypt until January 2005. Therefore, until polio is eradicated globally, Ghana remains at risk for a polio virus outbreak [Nasr El-Sayed, et al., 2007].

In an outbreak of wild polio virus in the Xinjiang Uygur Autonomous Region of China in 2011, a survey indicated that 4.0% of the sample population had no antibodies at all to the three poliovirus serotypes [HaiBo Wang et al., 2013]. In the wild polio outbreak in Finland in 1984 and 1985, wild poliovirus type 3 was implicated. Prior to that outbreak in 1982, a seroprevalance survey reviewed that only 30% had neutralizing antibodies to type 3 poliovirus [Lapinleimu et al., 1984]. Similarly, most of the infected children during an outbreak of polio in the Netherlands from 1992-1993, had not been vaccinated and had no neutralizing antibodies in their sera. There was an outbreak of polio in 2000-2001 reported from the Caribbean island of Hispaniola, a country divided between Haiti and the Dominican Republic. This outbreak occurred in areas of very low OPV coverage [CDC, 2000; 2001].

Studies have indicated that persons with low serum neutralizing antibody titre post immunization can be re-infected by wild virus or when challenged, with vaccine virus [Nishio et al., 1984; Magrath et al., 1981]. Serological studies have shown that the outbreak of polio in Kinshasa and Bandundu in the Democratic Republic of Congo in 2010-2011 was likely due to the immunity gap in PV1 [Alleman et al., 2014]. In the outbreak of polio in Zaria, Nigeria, from 2005 through 2010, Giwa et al. noticed that the population with neutralizing antibodies was low [Giwa et al., 2012]. In a series of polio outbreak in Northern Nigeria between 2012 and 2013, seroprevalence studies indicated that neutralizing antibody levels among children aged 36–47 months in the study population was lower than the required levels for poliovirus interruption [Zubairu et al., 2013]. As long as poliovirus circulation continues anywhere in the world, importations remain a risk

and consequently, there remains a limited risk of possible outbreaks among unvaccinated subpopulations.

Evidence exist suggest that high neutralizing polio antibodies in a population most likely interrupts wild polio transmission. The last WPV imported case in the U.S. occurred in 1993. Recent results from the National Health and Nutrition Examination Survey during 2009–2010 in the USA, indicated that neutralizing polio antibodies was high (83.1 %–97.0 %) for all three types of poliovirus. Immunity gaps to poliovirus were not large, suggesting an imminent substantial population risk from a poliovirus importation at a population level [Wallace et al, 2016].

In Germany, the last indigenous case of poliomyelitis was diagnosed in 1990. The last imported wild viruses were detected in patients with travel history to India and Egypt in 1992. In a seroprevalence survey in Germany, neutralizing antibodies against poliovirus type 1, 2 and 3 were detected in 96.2%, 96.8% and 89.6% of samples, respectively. This seroprevalence indicates a very high level of immunity of the German population [Sabine Diedrich et al., 2002].

Portugal was declared polio-free in 2002 prior to the last indigenous wild poliovirus case in 1986. A follow up study after the declaration revealed a high seroprevalence rate of PV 1 and 2 above 90% [Pires de Miranda et al., 2007]. A similar observation has been documented in Puerto Rico community of Dominican, in 2002 where 99% of neutralizing polio antibodies was found among children aged 24 months [CDC, 2002]. In assessing the immunity status of migrant workers in Israel, seropositivity rates of PV1, PV2 and PV3 were above 95%. These results indicated high levels of immunity among foreign workers,

which highlighted the low risk of polio among these groups [Calderon-Margalit et al., 2005].

It is observed in this study that the level of neutralizing polio antibodies of PV3 was the lowest in the sera of respondents. These results are similar to findings in studies in European countries such as Greece [Frantzidou et al., 2005], Germany [Diedrich et al., 2002], the Netherlands and Italy [Conyn-Van et al., 2001]. In similar studies in South Africa (Natal/KwaZulu) and other developing countries (Abijan, Bombay-India and Cuba) low levels of neutralizing antibodies to PV3 had been documented [Schoub et al., 1992; Akoua-Koffi et al., 1995; Deshpande et al., 1995; Mas Lago et al., 1994]. These observations may be explained by a lower potency of poliovirus type 3 antigens in the vaccine. However, a study in South Africa has demonstrated that, although there was an increase in the component of PV3 in the production of the vaccine, antibodies to PV3 were still lower than the other two types during evaluation. It has been reported from a study in Oman that the injection of the fourth dose of IPV, after three doses of OPV, was effective in enhancing the seropositive rate from 87.8 to 97.1% [Sutter, 1997].

The low level of neutralizing antibodies of PV3 has also been explained by the fact that PV1 antibodies are due to both vaccination and natural immunity [Sutter, 2004], whereas PV2 and PV3 antibodies are mainly due to vaccine induced immunity [Bassioni, 2003]. There is therefore, the need for a strategy to boost the immunogenicity of PV3 in the polio eradication programme to avoid any future outbreak of polio involving WPV3. In the second quarter of 2016, the Expanded Programme on Immunization (EPI) in Ghana resolved to switch from the administration of trivalent oral polio vaccine to bi-valent. This

was in conformity to the WHO strategic plan on polio eradication [WHO, 2012]. This policy direction is in the right path to boost the population immunity levels on PV3 [EPI, 2016].

This study did not find any significant association between sex and seroprevalence. In south-western Nigeria, studies on seroprevalence did not show any association in connection with sex even though there were more females with immunity than males [Williams et al., 1990; Adewumi et al., 2006]. However, in a Population-Based Survey in the City of São Paulo, Brazil, Carlos and his colleagues found a significant association between sex and seroprevalence [Carlos et al., 2002]. Although a statistically significant association was found, the real meaning is ambiguous. The likely explanation could be that both sexes have equal chances of exposure to either natural infection or the vaccine.

This study finds that neutralizing antibodies to polio viruses generally decrease with age, however, differences for each age group varies by serotype. Prior to the first birthday, neutralizing antibodies are low but subsequently there was an observed rise as the age increased till about 14 years old, then a decline. Before the first birth day the child normally receives four polio doses from the routine immunization session then one or more additional doses from the national immunization days vaccination yearly. There is an additional back-up of neutralizing antibodies from the mother if she is immune. The Expanded Programme on Immunization was initiated 38 years ago and national immunization days about 15 years ago in Ghana. All respondents who were born before the onset of the programme are expected to have fewer neutralizing polio antibodies except

those who suffered symptomatic or asymptomatic infection naturally from the environment and their babies.

The above finding is consistent with what had been detected in Uruguay [Maria et al., 2009], United states [Bass, 1978], Greece [Frantzidou et al., 2005], and South Africa [Schoub et al., 2001] indicating that there is a decline in seroprevalence with age. Contrary to this observation, according to [Williams et al., 1990] other studies, percentage detection of neutralizing antibodies increased with age. Studies have shown that intestinal immunity to poliovirus wanes over time, therefore individuals could become re-infected and shed poliovirus [Grassly, 2012].

As vaccine induced immunity declines with age, the priority of the Ghana Health Service, parents and caregivers is to sustain high routine immunization coverage and to emphasize a timely completion of primary immunization in accordance with the vaccine schedule.

The older age groups may contribute to wild polio transmission without clinical symptoms, and the World Health Organization has therefore recommended that older individuals get vaccinated as part of the outbreak response [WHO, 2013]. Lack of, or partial immunity to poliovirus observed in this study group poses a serious threat to the success of polio eradication programme in Ghana. There may be the need to include adults in the polio eradication program to avoid outbreaks, as seen in other countries.

It is also noted from this study that neutralizing polio antibodies against the polio serotypes are highest in the Northern Region. After the last outbreak that involved eight case-patients in the Northern Region, there has been series of “mop-up” immunization sessions especially in the Northern Region. This might explain why the respondents in the Northern

Region have higher seropositivity of neutralizing polio antibodies compared to the other regions in this study population. The frequencies of national immunization days and "mop-ups" have recently reduced drastically. There was no polio immunization campaign last year, 2016, and there was only one campaign in some selected regions in 2015 compared to the four or two nation-wide polio campaigns after the last outbreak of polio in 2008 [EPI, 2016]. There might be a lurking danger ahead as the study has shown that in some populations in the study regions, neutralizing polio antibodies are at a lower level.

Maternal formal education appears to be a good predictor of the immunization status of their off-springs. This study underscores the importance of maternal education on seroprevalence. It is noted that maternal education has a statistically significant effect on the presence of neutralizing antibodies for the polio serotypes. A lower seroprevalence of neutralizing antibodies depicts a lower maternal education. This argument of lower maternal education supporting lower seroprevalence has been supported by similar findings from a study in the Northern Nigeria [Zubairu et al., 2013]. However, studies in Egypt and India [El-Sayed et al., 2007] found severe wasting and stunting as associated with lower seroprevalence, but these findings were not statistically significant.

One of the key players in reducing infant and child mortality is women's education. The higher a woman's level of education, the more likely it is that she will marry later, play a greater role in decision making and exercise her reproductive rights. Her children will tend to be better nourished and enjoy better health (Hobcraft et al., 1984).

Investing in the health of children and their mothers is not only human rights imperative, it is a sound economic decision and one of the surest ways for a country to set its course

towards a better future (UNICEF, 2008). Simple, reliable and affordable interventions with the potential to save and improve the lives of millions of children are readily available in Ghana. The challenge, particularly in developing countries such as Ghana, has been how to ensure that these remedies reach the children and families, and how to embark on a better health seeking behavior on behalf of one's children. Immunization service is free and no matter the educational status of parents, awareness must be created, through health education and promotion for the child to receive vaccines against all the 13 vaccines preventable diseases.

This study also finds that the prevalence of residual paralysis cases estimated by the school lameness survey is less than one in a thousand (<1/1000) population, this value is quite low in this study compared to the estimate in similar studies in Ghana (Nicholas et al., 1977; Ofosu Amaah et al., 1997), Philippines (WHO, 1978) and Thailand (WHO, 1979). Oral polio vaccine coverage (OPV3) has increased from as low as 76% in 2003 and 2004 to 92% in 2015 in Ghana. In 2004, Greater Accra, Ashanti and Northern regions of Ghana recorded 56%, 66% and 93% OPV3 coverage respectively whilst in 2014, 80%, 97% and 119% were recorded [EPI, 2015]. The increase in the oral polio vaccine coverage in these populations in the regions and Ghana as a whole may account for this drastic drop in the estimated number of residual paralysis in the study population. Studies have indicated that neutralizing polio antibodies of levels higher than 66-80% is a contributory factor for preventing polio infection [Sutter et al., 2004]. In this study population, the levels of neutralizing polio antibodies of polio serotypes P1, P2 & P3 in the sera of respondents are within 75%-85%. These results may provide some biological explanation for the reduction in the observed number of paralytic polio cases in the study population. This finding also

corroborates the importance of routine and mass polio vaccinations in developing countries such as Ghana, which is yet to eradicate polio.

Over 50% of all poliomyelitis with residual paralysis occurred among children less than three years [Boche et al., 1973; Guyer et al., 1976]. This study also supports similar finding in Cameroon (Heymann et al, 1983). The age of onset in this health condition is clearly different from what is usually encountered in temperate countries, where the disease tends to occur in older age groups and may inflict even elderly people [Collingham et al, 1978]. This pattern may indicate individual variation in susceptibility to disease for different populations, or may reflect recall bias in the interviewees.

Study Limitations

These findings of the hospital-based seroprevalence study should be considered in the light of limitations. First, there was no immunization history available for adult participants, so it is unclear whether polio sero-immunity was due to past OPV receipt and/or natural immunity. Secondly, this study is hospital based, but this limitation may have resulted in an overestimation of seroprevalence of antibody against poliovirus, as the children who are not reached by immunization activities may be less likely to visit hospitals. The results, although not generalizable will give the Ghana Expanded Programme on Immunization a fair idea as to the status of immunity in the study population, facilitate innovative strategies to reach the unreached and acquire the needed herd immunity to interrupt the transmission of any future importation of wild polio virus into the country.

The most accurate technique to measure the prevalence of poliomyelitis is a house-to-house survey. However, such surveys are time-consuming and are costly if carried out independently for one particular disease. Since the demographic and school enrollment data suggested that school attendance was very high (greater than 90%) most of the information needed to complete the prevalence survey could be obtained from the schools [Laforce, 1980]. These study findings provide baseline data for a periodic evaluation of efforts to control paralytic poliomyelitis.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

Considering the objectives of the study, vis-a-vis the study results, the following conclusions are reached:

6.1 Conclusions

The findings of this study suggest a moderate level of seroprevalence of neutralizing antibodies to the three polio serotypes with regional differences. Identification of some respondents without detectable antibodies to one or more poliovirus serotypes, in their sera, show that pockets of susceptible individuals are still present within the population.

Although sex had no significant association with seroprevalence, seropositivity is generally low with increasing age. Neutralizing antibodies to polio virus serotype three is relatively low. Mother's education is crucial to seropositivity.

Despite relatively moderate levels of PV seroprevalence, Ghana might remain at risk of a PV outbreak, particularly in lower-seroprevalence populations and in adults due to the persistent circulation of wild polio in neighboring countries which is yet to finally interrupt the transmission of PV.

Immunity to PV3 is insufficient in this cohort because as immunity reduced with increasing age, a booster dose with bivalent type 1 and 3 oral poliovirus vaccine can be considered for teenagers in Ghana and adults travelling to polio endemic countries. It is crucial to note that further studies on antibody profile to polioviruses in adults at a wider scale would support or reject the idea of adult inclusion in polio immunization in Ghana.

This survey provides some evidence for determining target age groups for Supplementary Immunization Activities (SIA), outbreak response, and prioritizing the new recommendations for introduction of inactivated polio vaccine and concurrent use of bivalent OPV.

It is clear that, the seroprevalence of neutralizing antibodies to polio serotypes in the study population are not as high as previously thought as a result of the numerous national polio immunization vaccination days conducted. However, there persists a lurking danger.

Ghana has reduced the number of polio immunization days since 2014 compared to the practice a few years back. Until the total interruption of polio transmission in Nigeria, Ghana remains at risk of importation of wild polio virus.

The switch of trivalent oral polio vaccine to bi-valent is in the right direction to avoid any circulating vaccine-derived poliovirus (cVDPV) in the country and could strengthen the immunity status of the population through the administration of additional doses of OPV1 & OPV3 vaccines.

The drastic reduction of paralytic poliomyelitis in the study population can be attributed to the subsequent increase in the oral polio vaccination coverage and the levels of neutralizing polio antibodies. Since Ghana is at the verge of polio eradication, there should be conscientious effort to sustain the gains made in the Expanded Programme on Immunization in the country.

6.2 Implications of study results on the Expanded Programme on Immunization, Ghana

The major study findings are a moderate level of seroprevalence of neutralizing antibodies to the three polio serotypes with some regional differences prior to the withdrawal of OPV2 in the routine immunization programme of Ghana. Seropositivity is generally low with increasing age. Neutralizing antibodies to polio virus serotype three is relatively low. Mother's education is crucial to seronegativity. The drastic reduction of paralytic poliomyelitis in the study population is attributed to the subsequent increase in the oral polio vaccination coverage.

The seroprevalence of neutralizing polio antibodies ranged between 75-86%. This implies that the immunity against polio among respondents in the study regions has not reached the optimum status. Ghana conducted on yearly basis, a minimum of four mass polio immunization campaigns after the last polio outbreak in 2008. Unfortunately there has been a drastic reduction of the number of mass polio campaigns due to inadequate provision of funds for procurement of vaccines, logistics and motivation of the work force. This situation, if it continues, may prevent the target population for mass immunization from attaining the status of herd immunity against poliomyelitis if there should be any importation of wild polio virus from neighboring countries. The initial policy of more than one mass polio campaign per year in Ghana should be reviewed and if possible continued till the eradication of polio from Ghana and the entire Africa. Mass polio campaigns are supplements for increase in polio neutralizing antibodies in a given population especially among those who hitherto the campaign had not received any polio dose. This strategy has

been the key Global Polio Eradication Initiative (GPEI) which administers additional doses of oral poliovirus vaccine (OPV) to each child aged less than five years old, regardless of their immunization history. Andrianarivelo and other colleague researchers found in a study in Madagascar that, neutralizing polio antibodies increased among children who had not used routine immunization services or had missed routine OPV doses after a mass polio campaign exercise [Andrianarivelo, 2001].

The Global Polio Eradication Initiative (GPEI) has an endgame plan that includes a transition from using oral poliovirus vaccine (OPV) to inactivated poliovirus vaccine (IPV). Subsequently, the World Health Organization has recommended the introduction of IPV into the routine immunization programmes by 2015 [Global Polio Eradication Initiative, 2013]. The initiative has not commenced yet in Ghana, although a lot of preparatory work has taken place. It is envisaged that the effective mucosal and humoral immunity induced by IPV may contribute to an upsurge of neutralizing polio antibodies and a reduction of vaccine activated paralytic poliomyelitis [Onorato et al., 1991]. In the study population were respondents with inadequate protection against polio infection. There is therefore an urgent need for policy makers to prioritize the introduction of IPV in the Ghanaian population.

Seroprevalence to polio neutralizing antibodies generally decreased with age in respect to our study population. The Expanded Programme on Immunization in Ghana vaccinates children less than five years against polio and not the adult age group. There had been outbreaks of polio in several countries including: Angola, Namibia, Cape Verde, Albania and the Democratic Republic of Congo that involved the adolescent and the adult

population [Brown, 1999; Alleman et al., 2014; MMWR, 2006; MMWR, 2000]. The case fatality ratios were rather high ranging from 12% to 41% in Albania in 1994 and Democratic Republic of Congo in 2000 respectively. There is therefore the need to seek other supporting evidence to advocate for the vaccination against poliomyelitis in the adult population in Ghana.

Lameness among the primary school children reduced drastically compared to the findings from previous studies in Ghana. The seroprevalence rate of 80% and more noticed from the study population may have contributed to this finding. Periodic nationwide lameness and seroprevalence studies should be encouraged to gauge the performance of the Expanded Programme on Immunization.

6.3 Recommendations

On the basis of existing literature on seroprevalence of polio neutralizing antibodies as well as on observations made during the field work, the following are recommended for consideration by:

Ministry of Health

1. To further strengthen the gains made in polio eradication efforts and to weaken the barriers to immunization, there is the need for more funding from the Government's budgetary allocation and from stakeholders to increase awareness of polio eradication.

2. Intensify the final efforts through mass immunizations and mop ups. For Ghana to maintain its polio-free status, adequate population immunity must be maintained through routine immunization and continued mass immunization campaigns. Program performance measures and representative serologic surveys may help assess population protection.

Specific Recommendations:

Health programming

3. The EPI programme may urgently implement the planned introduction of IPV to supplement the oral polio vaccine in the Ghana target population for immunization service. Regional differences in seroprevalence were noted in the study. Nigeria has recently recorded an outbreak of polio after two years of silence.
4. **Policy on Advocacy**

   In consideration of the fact that seropositivity was generally low with increasing age in this study, and given that a number of outbreaks of polio had involved transmission among adults (Democratic Republic of Congo), consideration should be given for a booster dose of OPV among teenagers by EPI.
5. The EPI may consider replicating this study in other parts of Ghana to have a broader idea about the neutralizing antibodies against polio serotypes to gauge the status of herd immunity against polio in the Ghanaian population. Likewise, EPI may consider a nationwide population based study on lameness for a better insight into the status of lameness in the country for programmatic consideration.

6. **Local Government**

   Female child education should be intensively encouraged by all District Assemblies, and career counseling intensified, for JSS pupils and upward, as well as by the churches. Many of the mothers had a lower educational status and that had a likely effect on seroprevalence based on our study population.

6.4 Contribution to knowledge

1. Neutralising antibodies against poliovirus serotypes types 1, 2 and 3 were detected in 86.0% (PV1), 84% (PV2) and 75% (PV3) of study samples. About 80% of the study population in the three regions (Greater Accra, Ashanti and Northern regions) have adequate protection against polio infection. This implies that not all the respondents are protected from polio infection. There persists an immunity gap on polio infection in the study regions.

2. Seroprevalence rate of polio antibodies of the three polio serotypes (1, 2 & 3) are generally lower in the Ashanti and Greater Accra regions compared to the rate in the Northern region. The sera of respondents that had no polio neutralizing antibodies were lowest in the Northern region

3. Nine (2.9%) of those tested in the study population were sero-negative for the three serotypes. PV1, PV2, PV3: Northern region 0 (0.0%), Ashanti region 3 (2.4%), Greater Accra 6 (6.0%). Based on this study population, there are still some people

with inadequate protection against polio infection. The highest is found in Greater Accra region.

4. The prevalence of residual paralysis of poliomyelitis estimated from the lameness survey data in the three study regions (Greater Accra, Ashanti and Northern regions) is 0.58/1,000 children aged 0-15years old.

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Appendix 1:

Results of Titration of authenticated Sabin polio virus stock, material for microneutralisation assay and preparation of reagents.

1. **Results of Titration of authenticated Sabin polio virus stock**

Polio serotype 1

Dilution	No of CPE	Ratio
10^{-5}	20	1
10^{-6}	20	1
10^{-7}	8	0.4
10^{-8}	0	0

Using log $TCID_{50}$=L-d(S-0.5)

=-5-1(2.4-0.5)

=-5-1(1.9)

=-5-1.9

=-6.9

Virus titre =6.9($TCID_{50}$)

Virus titre =$\mathbf{10^{6.9}}$

Polio serotype Type 2

Dilution	No of CPE	Ratio
10^{-5}	20	1
10^{-6}	20	1
10^{-7}	12	0.6
10^{-8}	0	0

Using log $TCID_{50}$=L-d(S-0.5)

=-5-1(2.6-0.5)

=-5-1(2.1)

=-5-2.1

=-7.1

Virus titre =7.1($TCID_{50}$)

Virus titre =$\mathbf{10^{7.1}}$

Polio serotype Type 3

Dilution	No of CPE	Ratio
10^{-5}	20	1
10^{-6}	16	0.8
10^{-7}	4	0.2
10^{-8}	0	0

Using log $TCID_{50}$=L-d(S-0.5)

=-5-1(2.0-0.5)

=-5-1(1.5)

=-5-1.5

=-6.5

Virus titre =6.5($TCID_{50}$)

Virus titre =$\mathbf{10^{6.5}}$

2. Materials and equipment: Micro-neutralization test for polio antibodies

The titre of neutralizing antibodies against poliovirus types 1, 2 and 3 was determined by micro neutralization assay, using the materials and equipment below: HEp-2C cells (Human Caucasian larynx carcinoma epitheliod cells); Cell culture flasks ; 96-well cell culture plates; Microcentrifuge tubes (1.5ml); Pipettes; 10ml, 25ml; Pipettors: P20-200, 12-channel P20-200, repeater pipette P20-200; Pipette tips; Cell culture medium (MEM); Fetal Bovine Serum; In-House Reference Sera; Sabin virus stocks grown in HEp-2(C) cells; CO_2 incubator; Phosphate buffered saline PBS (without calcium and magnesium ions); Cell culture tubes; Culture flasks; MEM (Minimum Essential Medium); Trypsin (6% trypsin in saline A; PBS (0.13M sodium chloride, 2Mm potassium chloride, 0.9Mm calcium chloride, 0.5nM magnesium chloride, 11mM sodium phosphate, 0.9mM potassium phosphate); Penicillin/Streptomycin (20,000 unit/ml of each); FCS (Foetal calf serum); Growth medium and Maintenance medium.

3. Media preparation for Micro-neutralization test for polio antibodies

1. Phosphate Buffered Saline (PBS)

In the preparation of phosphate buffered saline (PBS), the following materials were used: PBS tablets and Double Distilled or Deionized Water (DDW).

Procedure: One tablet of PBS was put into 500ml of DDW and stirred. This was sterilized by autoclaving.

2. Minimum Essential Medium (MEM) – Eagle's

The Materials needed for the preparation of MEM were MEM (Eagle's) powder and DDW (double distilled water).

Procedure: For 1 litre preparation, 9.6g of MEM was dissolved in 900ml of DDW; 6 litres, (9.6gx 6) MEM was dissolved in (900ml x 6) of DDW. In preparation of a litre of growth medium, 853ml of prepared MEM is used, and for Maintenance Medium, 923ml of prepared MEM. This was then sterilized by autoclaving.

1. $NaHCO_3$ (Sodium Bicarbonate) Solution – 7.5 %(w/v)

$NaHCO_{3\ powder}$ and Deionized Water (DDW) were used in the preparation of $NaHCO_3$ (Sodium Bicarbonate) Solution.

Procedure: Calculated the mass of $NaHCO_3$ powder needed to make 7.5% (w/v) $NaHCO_3$ solution. For the 200ml $NaHCO_3$ solution,

Mass, m ($NaHCO_3$) = 7.5/100 × 200 (w/v)

= 15g

This calculated amount was weighed and DDW was added up to the required volume. This was stirred and sterilized by autoclaving.

4. L-Glutamine (200mM)

The materials needed in the preparation of L-Glutamine (200mM) were L-Glutamine powder and Sterile PBS solution.

Procedure: Calculated the mass of L-Glutamine needed to make 200mM of solution in a required volume. For the 1 litre solution needed:

Concentration, C (L-Glutamine) =no. of moles, n (L-Glutamine)/volume, V (solution)

$$C = n/v$$

& n = m/ Molar mass, M (L-Glutamine)

$\Rightarrow m = C \times V \times M$

$C = 200mM = 0.2M = 0.2mol/dm^3$

$V = 1litre = 1\ dm^3$

$M = 146.15g/mol$

Therefore, mass of (L-Glutamine) needed for a 1 litre solution,

$= 0.2mol/dm^3 \times 1\ dm^3 \times 146.15g/mol$

$= 29.23g$

Calculated amount L-Glutamine was weighed and made it up to 1litre with already prepared, sterile PBS solution. This was stirred and sterilized by filtration.

5. Pen/Strep (Penicillin/Streptomycin) solution

Already prepared Penicillin-Streptomycin solution stabilized was used. For 1 ml, it contained 10,000 units of penicillin and 10mg streptomycin.

The materials that were involved in the preparation were: Penicillin G powder, Streptomycin sulphate powder (761 streptomycin units/mg) and powder/tablets, or solution.

6. HEPES buffer (1M)

HEPES powder and Double Distilled or Deionized Water were used in the preparation

Procedure: The mass of HEPES powder needed was calculated to make 1M HEPES solution in a required volume. For a 1litre HEPES solution,

$C = n/V \quad \text{and} \quad n = m/M$

$\Rightarrow C = m/V \times M$

Therefore, m = C ×V× M

C = 1M = 1mol/dm^3

V = 1litre = 1dm^3

M (HEPES) = 238.3g/mol

Mass, m = 1mol/dm^3 ×1dm^3×238.3g/mol

= 238.3g

Calculated amount HEPES powder was weighed and dissolved in 1litre DDW. This was then sterilized by autoclaving

8. FBS (Fetal Bovine Serum), liquid

NB: Heat inactivation at 56 ºC for 30 minutes

Procedure: A clean water bath was filled with distilled water. Water bath was turned on and the temperature set to 56ºC. This was then allowed to attain the desired temperature. The FBS was set to be heat inactivated in the bath, making sure that the water did not reach the neck of the bottle, and the time was set to 30 mins. After the 30 minutes the FBS was removed and the water bath turned off. The serum was observed for debris as a result of recrystallization. When debris occurred, the serum was sterilized by filtration.

8. Trypsin in EDTA (Ethylene Diamine Tetraacetic Acid) in PBS (Phosphate Buffered Saline) solution 1:5000(w/v)

The materials used were: Trypsin, EDTA, PBS and DDW

Procedure: EDTA in PBS, 1 : 5000 (w/v). PBS was prepared in DDW as described above of volume x ml, and the needed amount was weighed in EDTA and dissolved in DDW to make a volume y ml, such that x ml+ y ml = 1litre (1000ml)

EDTA in DDW = 500ml

Plus (+) PBS in DDW = 500ml

Using the amounts of EDTA and PBS powder for preparing a 1 litre solution, EDTA and PBS were autoclaved separately.

For a 1 litre solution, this was the amount of EDTA that was used;

1: 5000 (w/v)

1l = 1000ml

$$\Rightarrow \frac{1}{5000} \times 1000 \text{ ml} \quad (w/v)$$

$$= 0.2\text{g}$$

In a Biosafety cabinet (BSC), EDTA and the PBS solution were added up and autoclaved.

TRYPSIN

2.5% Trypsin was prepared in PBS

TRYPSIN in EDTA in PBS (0.25%) solution

- For a 100 ml solution, 0.25% implied

$$\frac{0.25}{2.5} \times 100 \text{ ml}$$

$$= 10 \text{ ml}$$

Therefore, 10 ml of 2.5% trypsin was needed to make a 100ml of 0.25% trypsin in EDTA in PBS.

90ml (100ml – 10ml) of EDTA in PBS was added to10ml 2.5% trypsin to make 100ml of 0.25% trypsin.

9. Trypan Blue stain (0.1% w/v) solution

Already prepared trypan blue from source was used. This had been prepared from the laboratory with the following: Trypan blue powder and PBS solution.

10. Growth and maintenance media

The following materials were used for the preparation of the Growth and maintenance media:

Eagle's Minimum Essential Medium; Sodium Bicarbonate ($NaHCO_3$) solution (7.5%) ; L-Glutamine (200mM); Penicillin-Streptomycin solution; HEPES Buffer (1M) and Fetal Bovine Serum (heat inactivated)

Procedure: In a Biosafety Cabinet, for a **1000ml** (1l) medium preparation, the following amounts of sterile reagents (mls) were aliquoted into a sterile bottle:

	Growth medium	maintenance medium
Eagle's Minimum Essential Medium	855ml	925ml
Sodium Bicarbonate ($NaHCO_3$) solution (7.5%)	15ml	25ml
L-Glutamine (200mM)	10ml	10ml
Penicillin-Streptomycin solution	10ml	10ml
HEPES Buffer (1M)	10ml	10ml
Fetal Bovine Serum	(10%) 100ml	(2%) 20ml

Stirred by swirling was done and tested for sterility of the reconstituted medium by:

1. Dispensing 5ml of prepared Thioglycollate broth into a sterile cell culture flask with screw cap,

2. Incubating it at (36+-0.5) ^{o}C for a period of 10 days, and
3. Checked for cloudiness over the period.

Observation made visually was recorded each day, whether medium looked clear or cloudy.

Appendix 2:

Consent form (adults)-hospital based seroprevalence study

Title of study: **Poliovirus antibody levels and lameness among individuals in three regions of Ghana, 2016**

Investigator: Dr. Joseph K L. Opare

Address: School of Public Health, University of Ghana, P. O. Box LG13, Legon, Ghana.

Tel; 0208112634, email: oparej@yahoo.com

General information about study

School of Public Health, University of Ghana, Legon, the Ghana Health Service and the Virology Department, Noguchi Memorial Institute for Medical Research, University of Ghana, Legon are doing a research to elicit how protected the Ghanaian individual is against the polio virus in case there is any importation (transfer-in) of the polio virus into the country. This will assist the stake holders to identify any immunity gaps for system improvement. We are asking you to be part of the study because of your presence at the hospital laboratory. As part of the study, you will be asked to answer questions about yourself. An additional blood of about a tablespoonful will be taken from you to assist in the research after the interview. There will be direct physical measurements on you. These will include weight and height. You may decline to answer any question or resist any additional blood to be taken from you. After we have collected the sample from you, your participation in the study will be complete. Answering the questions and taking the additional blood sample from you will take just about 5 minutes.

Possible Risks and Discomforts

If you participate in the study, an additional tablespoonful of blood will be taken from you through the normal routine procedure. This will involve some few seconds and a little pain while pricking.

Possible Benefits

Your participation in this research may help the University of Ghana and the Ghana Health Service identify any immunity gaps for system improvement against polio eradication in Ghana.

Confidentiality

Information about you will be kept confidential. All records will be stored securely and will only be accessed by researchers working on this study. Findings from the study may be published but will not use your name or identification information.

Voluntary Participation and Right to Leave the Research

Your participation in this study is completely voluntary. The health care provider will treat you with the same quality of care even if you decide not to participate. You are free to withdraw your participation in this study with no explanation at any time.

Contacts for Additional Information

If you have questions about the study, feel free to contact the Principal Investigator, Dr Joseph K. L Opare (School of public Health, Ghana Health Service), through mobile number 0208112634.

VOLUNTEER AGREEMENT

The above document describing the benefits, risks and procedures for the research titled "**Poliovirus antibody levels and lameness among individuals in three regions of Ghana, 2016**" has been read and explained to me. I have been given an opportunity to have any questions about the research answered to my satisfaction. I agree to participate as a volunteer.

_____

Date Name and signature or thumbprint of volunteer

If volunteers cannot read the form themselves, a witness must sign here:

I was present while the benefits, risks and procedures were read to the volunteer. All questions were answered and the volunteer has agreed to take part in the research.

Date Name and signature of witness

I certify that the nature and purpose, the potential benefits, and possible risks associated with participating in this research have been explained to the above individual.

_____ _____

Date Name and signature of person who obtained Consent

Appendix 3:

Child Assent Form- Hospital based seroprevalence study

Introduction

My name is **Dr Joseph K L Opare** and I am from the School of Public Health, University of Ghana, Legon. I am conducting a study entitled **"Poliovirus antibody levels and lameness among individuals in three regions of Ghana, 2016".**This will assist the stake holders to identify any immunity gaps for system improvement i.e. addresses issues of maximum protection. We are asking you to be part of the study because of your presence at the hospital.

General Information about Research

School of Public Health, University of Ghana, Legon, the Ghana Health Service and the Virology Department, Noguchi Memorial Institute for Medical Research, University of Ghana, Legon are doing a research to elicit how protected the Ghanaian individual is against the polio virus in case there is any importation (transfer-in) of the polio virus into the country. This will assist the stake holders to identify any immunity gaps for system improvement. We are asking you to be part of the study because of your presence at the hospital laboratory. As part of the study, you will be asked to answers questions about yourself and child. An additional blood of about a tablespoonful will be taken from your child to assist in the research after the interview. There will be direct physical measurements on your child. These will include weight and height. You may decline to answer any question or resist any additional blood to be taken from your child. After we have collected the sample from your child, your participation in the study will be complete.

Answering the questions and taking the additional blood sample from your child will take just about 5 minutes

Possible Risks and Discomforts

If you participate in the study, an additional tablespoonful of blood will be taken from your child through the normal routine procedure. This will involve some few seconds and a little pain.

Possible Benefits

Your participation in this research may help the University of Ghana and the Ghana Health Service identify any immunity gaps for system improvement against polio eradication in Ghana.

Confidentiality

Information about you and your child will be kept confidential. All records will be stored securely and will only be accessed by researchers working on this study. Findings from the study may be published but will not use your name or identification information.

Voluntary Participation and Right to Leave the Research

Your participation in this study is completely voluntary. The health care provider will treat you with the same quality of care even if you decide not to participate. You are free to withdraw your participation in this study with no explanation at any time.

Contacts for Additional Information

If you have questions about the study, feel free to contact the Principal Investigator, Dr Joseph K L Opare (School of public Health, Ghana Health Service), through mobile number 0208112634.

VOLUNTARY AGREEMENT

By making a mark or thumb printing below, it means that you understand and know the issues concerning this research study. If you do not want to participate in this study, please do not sign this assent form. You and your parents will be given a copy of this form after you have signed it.

This assent form which describes the benefits, risks and procedures for the research entitiled "**Poliovirus antibody levels and lameness among individuals in three regions of Ghana, 2016"**has been read and or explained to me. I have been given an opportunity to have any questions about the research answered to my satisfaction. We agree to participate.

Child's Name:……..

Researcher's Name:……

Child's Mark/Thumbprint…… **Researcher's Signature……**

Date:…… **Date:** ……

Appendix 4:

Child assent form- lameness survey

Introduction

My name is **Dr Joseph K L Opare** and I am from the School of Public Health, University of Ghana, Legon. I am conducting a research study entitled **"Poliovirus antibody levels and lameness among individuals in three regions of Ghana, 2016".**This will assist the stake holders to identify any immunity gaps for system improvement i.e. addresses issues of maximum protection. **In addition to the study, there will be a survey to determine the proportion of poliomyelitis associated lameness among school children**. We are asking you to be part of the study because of your presence at the school.

General information

As part of the study, you will be asked to answers questions and you will be medically examined. Answering the questions and the medical examination will take about 5 minutes.

Possible benefits

Your participation in this research may help the University of Ghana and the Ghana Health Service have an idea of polio associated paralysis among primary school children and identify any immunity gaps for system improvement against polio eradication in Ghana.

Possible Risk/ Discomfort

We do not expect any harm to happen to you with regards to the information that will be provided and the medical examination

Confidentiality

Your information will be kept confidential. No one will be able to know how you responded to the questions and your information will be anonymous.

Voluntary Participation

Your participation in this study is completely voluntary. You are free to withdraw your participation in this study with no explanation at any time.

Contacts

If you have questions about the study, feel free to contact the Principal Investigator, Dr Joseph K L Opare (School of public Health, Ghana Health Service), through mobile number 0208112634.

Please talk about this study with your parents before you decide whether or not to participate. I will also ask permission from your parents before you are enrolled into the study. Even if your parents say "yes" you can still decide not to participate.

Your rights as a Participant

This research has been reviewed and approved by the Institutional Review Board of Noguchi Memorial Institute for Medical Research (NMIMR-IRB). If you have any questions about your rights as a research participant you can contact the IRB Office between the hours of 8am-5pm through the landline 0302916438 or email addresses: nirb@noguchi.mimcom.org

VOLUNTARY AGREEMENT

By making a mark or thumb printing below, it means that you understand and know the issues concerning this research study. If you do not want to participate in this study, please do not sign this assent form. You and your parents will be given a copy of this form after you have signed it.

This assent form which describes the benefits, risks and procedures for the research **"Poliovirus antibody levels and lameness among individuals in three regions of Ghana, 2016", with an aspect to determine the proportion of poliomyelitis associated lameness among school children"** has been read and or explained to me. I have been given an opportunity to have any questions about the research answered to my satisfaction. We agree to participate.

Child's Name:…… Researcher's Name:……

Child's Thumbprint…… Researcher's Signature:……

Date……**Date:** ……

Appendix 5:

Consent form- lameness survey

Introduction

My name is **Dr Joseph K L Opare** and I am from the School of Public Health, University of Ghana, Legon. I am conducting a research study entitled" **Poliovirus antibody levels and lameness among individuals in three regions of Ghana, 2016".** This will assist the stake holders to identify any immunity gaps for system improvement i.e. addresses issues of maximum protection. **In addition to the study, there will be a survey to determine the proportion of poliomyelitis associated lameness among school children**. We are asking you and your ward to be part of the study because of your wards presence at the school.

General information

As part of the study, you will be asked to answer questions about your ward and your ward will be medically examined. Answering the questions and the medical examination will take about 5 minutes.

Possible benefits

Your participation in this research may help the University of Ghana and the Ghana Health Service have an idea of polio associated paralysis among primary school children and identify any immunity gaps for system improvement against polio eradication in Ghana.

Possible Risk/ Discomfort

We do not expect any harm to happen to you and your ward with regards to the information that will be provided and the medical examination.

Confidentiality

Your information will be kept confidential. No one will be able to know how you responded to the questions and your information will be anonymous.

Voluntary Participation

Your participation in this study is completely voluntary. You are free to withdraw your participation in this study with no explanation at any time.

Contacts

If you have questions about the study, feel free to contact the Principal Investigator, Dr Joseph K L Opare (School of public Health, Ghana Health Service), through mobile number 0208112634.

Your rights as a Participant

This research has been reviewed and approved by the Institutional Review Board of Noguchi Memorial Institute for Medical Research (NMIMR-IRB). If you have any questions about your rights as a research participant you can contact the IRB Office

between the hours of 8am-5pm through the landline 0302916438 or email addresses: nirb@noguchi.mimcom.org

VOLUNTARY AGREEMENT

By making a mark or thumb printing below, it means that you understand and know the issues concerning this research study. If you do not want to participate in this study, please do not sign this form.

This consent form which describes the benefits, risks and procedures for the research titled **"Poliovirus antibody levels and lameness among individuals in three regions of Ghana, 2016" with an aspect to determine the proportion of poliomyelitis associated lameness among school children"** has been read and or explained to us. I have been given an opportunity to have any questions about the research answered to my satisfaction. I do agree to participate.

_____ _____

Date Name and signature or thumb print of volunteer

If volunteers cannot read the form themselves, a witness must sign here:

I was present while the benefits, risks and procedures were read to the volunteer. All questions were answered and the volunteer has agreed to take part in the research.

_____ _____

Date Name and signature of witness

I certify that the nature and purpose, the potential benefits, and possible risks associated with participating in this research have been explained to the above individual.

_____ _____

Date Name signature of person who obtained consent

Appendix 6:

A QUESTIONNAIRE

Date of interview…….identification number of Respondents……

Name of Zone ……

Information collected in this survey will be used to develop programmes and services, which may benefit you. Some of the questions are personal, but necessary in order to get to the useful information. Please make every effort to answer each question as honestly as possible. All information you will give be kept strictly confidential. Do you have any question(s)?

Part 1: Social-economic and demographic characteristics of Mother/care givers

1. Name of the respondent:_____ 2. Sex Male 1 Female 2

3. DOB: ____ \ _____ \ _____ 4.Place of residence.....

5. How old are you? (Age in completed years)……

6. Have you ever attended school? Yes (1) No (0)

If yes, how many years of schooling did you complete (circle answer)

(a) Primary school 1 2 3 4 5 6

(b) Middle school 1 2 3 4

(c) Voc/Tech /Comm. school 1 2 3 4

(d) Secondary /SSS School 1 2 3

(e) Post secondary 1 2 3 4

(f) Higher (specify) 1 2 3 4 5 6

7.Has the father of the child ever attended school? Yes (1) No (0)

If yes, how many years of schooling did you complete (circle answer)

(g) Primary school 1 2 3 4 5 6

(h) Middle school 1 2 3 4

(i) Voc/Tech /Comm. school 1 2 3 4

(j) Secondary /SSS School 1 2 3

(k) Post secondary 1 2 3 4

(l) Higher (specify) 1 2 3 4 5 6

8. What is your marital status?

Never married	1
Currently married	2
Separated	3
Divorced	4
Widowed	5
Cohabitating	6
Refused	88

8. Which of the following best describes your main work status?

Government employee	1
Non-government employee	2
Self employed	3
Non-paid	4
Student	5

Homemaker	6
Retired	7
Unemployed (able to work)	8
Unemployed (unable to work)	9
Farmer	10
Refused	88

9. Which of the following best describes the main work status of Father?

Government employee	1
Non-government employee	2
Self-employed	3
Non-paid	4
Student	5
Homemaker	6
Retired	7
Unemployed (able to work)	8
Unemployed (unable to work)	9
Farmer	10
Refused	88

Immunization History of child:

10. DPT Hib, Hep. dates: 1st dose _____ 2nd dose _____ 3rd dose _____

11. Number of routine OPV doses: Unknown / birth dose /1 / 2 / 3 / 4

12. Source of information: Immunization Card (1) Recall (2)

13. Number of SIA doses: Unknown / 1 / 2 / 3 / 4 / 5 / 6 / 7-10 / >10

14. Source of information: Any written proof (1) Recall (2)

15. Date of last OPV: ___ / ____ / ____

Physical exam of child

16. Weight (g) _____ Length (cm) _____ Temp (ºC) _____

17. Finally meets Eligibility Criteria Yes / No (Blood sample to be taken only if Yes)

18. Blood collected: Yes / No If not collected, specify reason:

_____

19. Time of collection:_____ (hh:mm) Quantity sufficient (≥ 1 ml): Yes / No

20. Any untoward incidents during observation: Yes / No

21. Referral to immunization clinic if required? Yes / No

Data collection sheet for blood sample (Adult and child)

Date of blood collection……

Patients Identification number……

Age ……..

Sex……

Weight…….. Height/Length……

Location……

Physical exam

16. Weight (g) _____ 22. Length (cm) _____ 23. Temp (ºC) _____

17. Finally meets Eligibility Criteria Yes / No

(Blood sample to be taken only if Yes)

B Lameness Survey Tool

(*Parents or care givers to answer questions on behalf of lamed children****)***

1. Presence of fever at onset of paralysis? Yes/ No /Don't know
2. Did the child experience any form of injury of the limbs prior to the onset of paralysis?

   Yes/ No /Don't know
3. Did the child receive any injection at buttocks prior to the paralysis?

   Yes/ No/ Don't know
4. Did the child suffer an acute onset of paralysis of the affected limb?

   Yes/ No /Don't know
5. Was the affected limb initially painful?

   Yes/ No /Don't know
6. Was the child sent to the hospital at the onset of paralysis?

   Yes /No

7. If yes, what was the diagnosis if you were told and you remember?

   ---

8. Age of child at onset of paralysis---------

9. Did the child have any congenital limb deformities at birth?

   Yes / no

Name of the school…… Current enrolment……

Name of lamed Child	Current age	sex	Age of onset of lameness	Residence of onset	Affected leg(L/R/b oth)	Character of paralysis

Appendix 7: Ethical Approval

GHANA HEALTH SERVICE ETHICS REVIEW COMMITTEE

In case of reply the number and date of this Letter should be quoted.

My Ref. GHS/RDD/ERC/Admin/App/
Your Ref. No.

Research & Development Division
Ghana Health Service
P. O. Box MB 190
Accra
Tel: +233-302-681109
Fax + 233-302-685424
Email: ghserc@gmail.com

Opare Joseph Kwadwo Larbi
School of Public Health
University of Ghana
P. O. Box 13
Legon

The Ghana Health Service Ethics Review Committee has reviewed and given approval for the implementation of your Study Protocol.

GHS-ERC Number	**GHS-ERC 14/11/15**
Project Title	"Seroprevalence of Antibodies to Polio Virus among Individuals in Three Regions of Ghana"
Approval Date	31st May, 2016
Expiry Date	30th May, 2017
GHS-ERC Decision	**Approved**

This approval requires the following from the Principal Investigator

- Submission of yearly progress report of the study to the Ethics Review Committee (ERC)
- Renewal of ethical approval if the study lasts for more than 12 months,
- Reporting of all serious adverse events related to this study to the ERC within three days verbally and seven days in writing.
- Submission of a final report **after completion** of the study
- Informing ERC if study cannot be implemented or is discontinued and reasons why
- Informing the ERC and your sponsor (where applicable) before any publication of the research findings.

Please note that any modification of the study without ERC approval of the amendment is invalid.

The ERC may observe or cause to be observed procedures and records of the study during and after implementation.

Kindly quote the protocol identification number in all future correspondence in relation to this approved protocol

SIGNED.....
DR. CYNTHIA BANNERMAN
(GHS-ERC CHAIRPERSON)

Cc: The Director, Research & Development Division, Ghana Health Service, Accra

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Phone: +233-302-916438 (Direct)
+233-289-522574
Fax: +233-302-502182/513202
E-mail: nirb@noguchi.mimcom.org
Telex No: 2556 UGL GH

Post Office Box LG 581
Legon, Accra
Ghana

My Ref. No: DF.22
Your Ref. No:

6th January, 2016

ETHICAL CLEARANCE

FEDERALWIDE ASSURANCE FWA 00001824 **IRB 00001276**

NMIMR-IRB CPN 011/14-15 *revd. 2016* **IORG 0000908**

On 6th January, 2016, the Noguchi Memorial Institute for Medical Research (NMIMR) Institutional Review Board (IRB) at a full board meeting conducted continuing review and renewed your protocol titled:

TITLE OF PROTOCOL	:	**Immunity against poliomyelitis among individuals in the three ecological zones of Ghana**
PRINCIPAL INVESTIGATOR	:	**Dr. Joseph K. L.Opare**
CO –INVESTIGATORS	:	**Dr. Patricia Akweongo, Prof. Edwin Afari & Dr. John Odoom**

Please note that a final review report must be submitted to the Board at the completion of the study. Your research records may be audited at any time during or after the implementation.

Any modification of this research project must be submitted to the IRB for review and approval prior to implementation.

Please report all serious adverse events related to this study to NMIMR-IRB within seven days verbally and fourteen days in writing.

This certificate is valid till 5th January, 2017. You are to submit annual reports for continuing review.

Signature of Chair: .....
Mrs. Chris Dadzie
(NMIMR – IRB, Chair)

cc: Professor Kwadwo Koram
Director, Noguchi Memorial Institute
for Medical Research, University of Ghana, Legon