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ROTAVIRUS DISEASE EPIDEMIOLOGY AND MOLECULAR CHARACTERISTICS
OF CIRCULATING STRAINS BEFORE AND AFTER VACCINE INTRODUCTION IN
KENYA

By

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“A Dissertation Submitted in Partial Fulfilment of the Requirements for the Award of the
Degree of

Doctor of Philosophy”

Program for Nurturing Global Leaders in Tropical and Emerging Communicable Diseases

Graduate School of Biomedical Sciences

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ABSTRACT

Background: Group A rotavirus (RVA) gastroenteritis is an important cause of childhood morbidity and mortality worldwide. Safe and effective RVA vaccines are considered to be a high-impact and cost-effective public health intervention tool to greatly reduce the burden of rotavirus disease. RVA virion is composed of three layers enclosing a genome of 11 segments of double-stranded RNA. The two outer capsid proteins, VP7 and VP4 define the G and P genotypes, respectively. Pre-vaccine introduction data on RVA disease burden and genotype features is critical to support an informed and evidence-based decision about necessity of introducing rotavirus vaccines into a country and to provide baseline data for monitoring the impact of the introduced vaccines. In July 2014, Kenya introduced rotavirus vaccine into her national immunization program. This national rollout of rotavirus vaccine provided an opportunity to assess the real-world impact of rotavirus vaccination on rotavirus disease epidemiology and strain distribution in the country. Thus, in the course of my PhD program, I endeavored to determine the disease epidemiology and molecular characteristics of RVA strains among children aged <5 years in Central Kenya before and after the introduction of rotavirus vaccine. Furthermore, since immunization coverage will have an influence on the overall impact of rotavirus vaccination in the country, I estimated the rotavirus immunization coverage in a sub-county within my study area.

Methods: Between July 2009 and June 2016, a total of 2204 (1546 in pre-vaccine and 658 in post-vaccine periods) fecal specimens were collected from children <5 years of age hospitalized with acute gastroenteritis (AGE) at Kiambu County Hospital, Central Kenya. The specimens were screened for RVA antigen using ELISA. Multiplex semi-nested RT-PCR was used to determine the G and P genotypes. Phylogenetic analysis of nucleotide sequences of the VP7 genes and VP4 genes of the unusual G8 and P[6] strains was carried out using the Neighbor-Joining method. Rotavirus immunization coverage in Kiambu sub-county was estimated using the administrative coverage data on rotavirus vaccinations.

Results: Of the 2,204 fecal specimens collected between July 2009 and June 2016, 520 were found to be positive for RVA, representing an overall prevalence rate of 23.6%. The proportion of children aged <5 years with AGE who tested positive for rotavirus significantly declined from
27.5% (95% CI: 25.5-30.1) in pre-vaccine period to 13.8% (95% CI: 11.3-16.6) in post-vaccine period. This represents a reduction of 49.8% (95% CI: 34.6-63.7; P= 0.007). The rate of reduction doubled from 30.2% in the first year of vaccine introduction to 64.4% in the second year, concurrent with the increasing national and sub-county rotavirus vaccination coverage. Kiambu sub-county recorded a high percentage coverage for rotavirus vaccination (≥80%), demonstrating good access to rotavirus vaccine in this sub-county. Reductions in RVA cases were most pronounced for children <1 year of age (vaccine-eligible group), among whom RVA prevalence fell by 38.9% from 64.8% in the pre-vaccine era to 39.6% in the post-vaccine period. Following vaccine introduction, the monthly hospitalizations for all-cause AGE among children <5 years of age decreased by 44.1% (95% CI: 34.7-53.9). Remarkable genotypic diversity of rotavirus strains, including unusual, emerging and mixed strains and temporal strain fluctuations, was observed before and after vaccine introduction. Overall, G1 was the most frequently detected genotype in both the pre-vaccine and post-vaccine periods. G3 which had been detected in the pre-vaccine era at only 1% became the second most common strain in the post-vaccine period at 21%. The proportion of G2 increased from 5% to 17% during the post-vaccine. There was a decline in the G8 frequency whereas no G9 and G12 were detected after the vaccine introduction. There were no notable variations in the distribution of the P genotypes between the pre- and post-vaccine periods. On G-P combinations, G1P[8] was the most common genotype in the pre-vaccine and the first year of post-vaccine introduction. There was increased detection of G3P[8] and G3P[6] in the first year of post-vaccine introduction, with substantial declines in the second year. G2P[4] replaced G1P[8] as the most dominant strain in the second year of vaccine introduction. Thus, there was observed change in distribution of RVA strains such as the re-emergence of G2P[4] and G3P[8] coinciding temporally with the timing of vaccine introduction. RVAs with unusual G-P combinations tended to be detected more frequently in the younger age groups. Phylogenetic analysis of nucleotide sequences of the unusual G8 and P[6] strains showed that the VP7 nucleotide sequences of G8 clustered in lineage 6 which includes most African G8 strains and that there are at least two distinct VP4 nucleotide sequence clusters of the Kenyan P[6] strains.

Conclusions: Findings in this thesis highlight the importance of RVA gastroenteritis in Kenya and reveal a remarkable genotypic diversity of rotavirus strains circulating in the country.
Phylogenetic analysis of the Kenyan G8 and P[6] strains provide evidence for the geographic segregation of G8 RVAs in Africa and point to at least two groups of P[6] RVAs circulating in Kenya. The rapid and marked reductions in rotavirus and all-cause AGE hospitalizations after rotavirus vaccine introduction are suggestive of a significant public health impact of the vaccine in Kenya, and thus provides early evidence for health administrators and policy makers in Kenya to support the sustained use of the vaccine in the routine national immunization program. However, given the limited period of post-vaccine observation, the changes in RVA strain distribution may not this far be conclusively attributed to the effect of vaccination. Therefore, continued surveillance is necessary to document the long-term impact and effectiveness of rotavirus vaccine on the rotavirus disease burden and strain epidemiology in Kenya.
CHAPTER ONE

INTRODUCTION

1.1 Background

Rotavirus is the most common cause of severe diarrhea among children <5 years of age globally and is estimated to cause 215,000 deaths annually. This accounts for 37% of the 578,000 diarrheal deaths in this age group [Tate et al., 2016a]. Majority of these deaths occur in low-income countries, particularly in sub-Saharan Africa and South East Asia due to a lack of timely and appropriate treatment for dehydration [Parashar et al., 2006]. In sub-Saharan Africa, the number of rotavirus deaths is estimated at 121,000 annually, representing 56.3% of all global rotavirus deaths [Tate et al., 2016a].

In Kenya, the estimated median incidence of rotavirus disease is 3015 outpatient visits and 279 hospitalizations per 100,000 children under five years of age per year [van Hoek et al., 2012]. In addition, rotavirus is estimated to cause more than 3,908 deaths in Kenya annually, which is 2% of all global rotavirus deaths. This high mortality rate places Kenya among the 10 countries (India, Nigeria, Pakistan, Democratic Republic of Congo, Angola, Ethiopia, Afghanistan, Chad and Niger) accounting for almost two-thirds (65%) of all global annual deaths due to rotavirus [Tate et al., 2016a]. Furthermore, before vaccine introduction, rotavirus disease put a considerable burden on the Kenya’s healthcare system at an estimated cost of US$10.8 million annually [Tate et al., 2009].

The burden of rotavirus disease can be reduced by improving sanitation, providing rehydration therapy to prevent child mortality and morbidity from dehydration and vaccinating the children to prevent the disease [O'Ryan et al., 2009]. Improving sanitation is complicated by lack of proper infrastructure and poor funding in many developing countries where the disease impact of rotavirus is particularly devastating. Notably, standard sanitary measures that kill most bacteria and parasites are ineffective in controlling rotavirus, and because low numbers (10-100 particles) of viruses can cause infection, transmission is common even with good hygiene practices [Parashar et al., 2003]. This is demonstrated by the fact that rotavirus incidence is similar in countries with both high and low sanitation standards [Tate et al., 2008]. Intravenous treatments which are effective against severe dehydration are largely unavailable to the children...
under age five in the developing world. While the alternative treatment of oral rehydration therapy (ORT) is more available, there are still significant setbacks in its distribution or instructions for its production in the developing world [Parashar et al., 2003].

Preventing rotavirus gastroenteritis through vaccination is therefore a much more high-impact and cost-effective public health intervention tool to greatly reduce the number of deaths due to diarrheal diseases, greatly reduce the burden on the health system, thereby contributing to the achievement of the Sustainable Development Goal 3 [Tessa et al., 2010; Rodrigo et al., 2010]. For instance, cumulated over the first five years of life, rotavirus vaccination is predicted to prevent 34% of the outpatient visits, 31% of the hospitalizations and 42% of the deaths in Kenya [van Hoek et al., 2012]. It is further estimated that between the years 2014 and 2033, rotavirus vaccination will avert 60,935 undiscounted deaths and 216,454 hospital admissions in Kenya’s under-fives. Over the 20-year period, the discounted government health service costs avoided will be US$ 30 million. In addition, the cost per disability-adjusted life-year (DALY) averted from a government perspective will be US$ 38 million [Sigei et al., 2015].

In 2009, two rotavirus vaccines: RotaTeq® (Merck & Co. Inc., USA) composed of five bovine-human reassortant strains including G types G1–G4 and P type P[8] and Rotarix® (GlaxoSmithKline Biologicals, Belgium) including one human attenuated G1P[8] strain were recommended by the World Health Organization (WHO) for inclusion in the global program for childhood immunization, particularly where diarrheal deaths account for ≥10% of child mortality [GlaxoSmithKline, 2011; Merck & Co., 2011; WHO, 2009]. Whereas clinical trials of these vaccines demonstrated high efficacy against severe rotavirus gastroenteritis in high- and middle-income countries in the Americas and Europe (85%–98%) [Ruiz-Palacios et al., 2006; Vesikari et al., 2006], trials in low- and middle-income countries in Africa and Asia demonstrated lower efficacy ranging from 40% to 70% with an average of 50% to 60% [Armah et al., 2010; Madhi et al., 2010; Zaman et al., 2010]. In the Kenyan arm of the RotaTeq® vaccine trial, the overall vaccine efficacy was 63.9% (95% CI -5.9-89.8) [Armah et al., 2010]. However, given the high baseline burden of severe rotavirus disease in these low resource settings, the benefits of vaccination could still be substantial in these countries even with the lower efficacy. As of May 1, 2016, these vaccines had been implemented in the national immunization programmes of 81
countries, including 38 low-income countries that are eligible for support from GAVI Alliance Accelerated Vaccine Initiative [PATH, 2016].

In July 2014, Kenya introduced the two-dose Rotarix® into her Expanded Programme on Immunization (EPI) with an aim of protecting over 1.5 million children from severe diarrhoea through co-financing of the vaccine cost with GAVI Alliance. Prior to the vaccine introduction, rotavirus strain characterization along with data on rotavirus disease burden was critical to support an informed and evidence-based decision about the necessity of introducing rotavirus vaccines in the country and suitability of a particular vaccine with regard to the genotypes circulating in the country [Hoshino et al., 2004; O'Ryan et al., 2009; Rodrigo et al., 2010]. Pre-vaccine surveillance was also necessary to provide baseline data for monitoring the impact of rotavirus vaccination on the diarrheal disease burden and for evaluation of any possible changes in the G and P genotype distribution following vaccine introduction [WHO, 2008; Bányaí et al., 2012]. In this regard, I conducted a hospital-based surveillance between July 2009 and June 2014 to determine the prevalence and molecular epidemiology of rotavirus gastroenteritis in Kenya before the introduction of rotavirus vaccine into the country’s national immunization programme.

The global rollout of rotavirus vaccines has offered an opportunity to assess the real-world impact of rotavirus vaccination in preventing and reducing the health burden of severe childhood diarrhoea. Impressive declines in rotavirus and all-cause diarrhea hospitalizations have been observed in many high and middle-income countries in the Americas and Europe following the introduction of rotavirus vaccination [Richardson et al., 2010; do Carmo et al., 2011; Patel et al., 2011]. Similarly, there is increasing evidence to suggest that the vaccines will have a significant effect on childhood morbidity and mortality in developing countries in Africa, despite the vaccines’ lower efficacy in clinical trials in these settings [Parashar et al., 2016]. However, the variation in vaccine efficacy by national gross domestic product (GDP) [Nelson and Glass, 2010] highlights the necessity of assessing the impact of rotavirus vaccination in these low-income, high-mortality settings during routine programmatic use as vaccine performance may differ from the ideal conditions of clinical trials. Furthermore, the implications of the increasing rotavirus strain diversity on vaccine effectiveness are not fully understood, although available
data provided by pre- and post-licensure studies have shown that both vaccines induce cross-protection against the prevalent strains encountered [Cherian et al., 2012; Dóró et al., 2014].

Thus, following the introduction of rotavirus vaccination, there is a need to assess the vaccine impact on rotavirus disease epidemiology (disease burden, age distribution and seasonality) [WHO, 2008]. In addition, it is necessary to monitor G and P genotype prevalence to aid in the assessment of vaccine effectiveness against various rotavirus strains [WHO, 2008]. Post-licensure surveillance of G and P genotypes will also allow monitoring of G and P genotype changes that may alter vaccine effectiveness or that may be a result of vaccination, such as possible breakthrough events under vaccine immune selective pressure [Dóró et al., 2014]. In view of this, I endeavoured to assess the impact of rotavirus vaccination on the disease epidemiology and strain distribution in Kenya two years after the vaccine introduction into the country.

A high vaccination coverage is essential to maximize the impact of the rotavirus vaccine. In countries with high levels of immunization coverage, the impact of rotavirus vaccines in reducing the burden of severe childhood diarrhea has been remarkable [Patel et al., 2011; 2012; Parashar et al., 2016]. Conversely, in low income settings like Kenya, vaccine coverage rates are likely to be lower due to programmatic, geographical and social challenges [Cherian et al, 2012]. According to the WHO/UNICEF estimates of national infant immunization coverage [2015], rotavirus vaccine achieved only 38% coverage in 50% of the national target Kenyan population in 2014 and 66% in 2015. There was a countrywide dropout rate of 5.8% with 8 counties reporting >10%. However, given the fact that Kenya is a socio-economically diverse country [KNBS, 2010], variability in rotavirus vaccine coverage across the sub-counties would not be surprising. In view of this, I estimated rotavirus vaccine coverage at Kiambu, a peri-urban sub-county which is the study area for the pre- and post-vaccine surveillance reported in this thesis, and examined the factors associated with the vaccine coverage in this sub-county.

The rotavirus virion is a triple-layered, non-enveloped icosahedron enclosing an 11-segment genome of double-stranded (ds)RNA. Due to the segmented nature of the genome, reassortment between and within human and animal strains is common leading to genetic evolution of rotavirus strains (Estes and Greenberg, 2013). Rotavirus has two outer capsid
proteins, VP7 and VP4, which define the G and P types, respectively. Rotaviruses have been classified into at least 27 G types and 37 P types so far [Matthijnssens et al., 2011; Trojnar et al., 2013]. The major G-P types in rotaviruses are G1P[8], G2P[4], G3P[8], G4P[8], and G9P[8], which comprise more than 80% of rotaviruses worldwide [Santos and Hoshino, 2005; Bánya et al., 2012]. Over the last decade, there has been a worldwide emergence of G8 and G12 strains [Gentsch et al., 2005; Rahman et al., 2007; Matthijnssens et al., 2009, 2010]. More recently, G8, G12 and P[6] strains have been increasingly identified in diarrheic children in several African countries [Page et al., 2009, 2014; Cunliffe et al., 2002; 2009; Mwenda et al., 2010; Nakagomi et al., 2012; Oluwatoyin et al., 2012; Enweronu-Laryea et al., 2013; Ndze et al., 2013; Pukuta et al., 2014; Seheri et al., 2014], indicating the ongoing expansion of these strains on the continent. Recent studies have reported significant rates of detection of these strains, G8, G12 and P[6], in Kenya [Mwenda et al., 2010; Nokes et al., 2010; Nyangao et al., 2010; Kiulia et al., 2014].

The G8 genotype was first recovered from a young child with gastroenteritis in Indonesia [Hasegawa et al., 1984]. Thereafter, G8 strains have been recovered sporadically from humans and animals like pigs and cows worldwide [Esona et al., 2009; Midgley et al., 2012], leading to a postulation of an interspecies transmission of the strain especially in Africa due to a close proximity of humans to livestock [Jere et al., 2012; Adah et al., 2003; Cunliffe et al., 2000]. Globally, the human G8 strains have been found associated with a wide range of P genotypes (P[1], P[2], P[4], P[6], P[8], P[10], P[11] or P[14]) [Santos and Hoshino, 2005; Pietsch et al., 2009; Banyai et al., 2009, 2010; Esona et al., 2009, 2010; Nokes et al., 2010; Nyangao et al., 2010]. In Kenya, G8 strains have increasingly been reported in association with P[6] besides other P genotypes such as P[1], P[4] and P[8] [Ghosh et al., 2011; Mwenda et al., 2010; Nokes et al., 2010; Nyangao et al., 2010; Kiulia et al., 2014].

The P[6] genotypes was reported in early epidemiological studies exclusively from children with asymptomatic rotavirus infection, suggesting that the P[6] specificity is associated with virus attenuation [Flores et al., 1986]. These observations led to the consideration of the P[6] strains as probable components of future vaccine candidates [Vesikari et al., 1991]. However, subsequent studies have recognized P[6] strains, in association with a wide variety of G genotypes, as important human pathogens causing both asymptomatic and symptomatic infections [Cunliffe et al., 2002; Griffin et al., 2002; Rahman et al., 2003; Dennis et al., 2014].
The P[6] genotype has recently become epidemiologically important in Kenya and has been detected quite commonly in association with a wide variety of G genotypes [Mwenda et al., 2010; Nokes et al., 2010; Nyangao et al., 2010; Kiulia et al., 2014].

The recently proposed classification scheme by Rotavirus Classification Working Group (RCWG) which is based on full genome analyses of rotavirus strains, has provided an excellent means of deciphering the true origin and evolutionary patterns of rotavirus strains, especially those derived from interspecies reassortment events [Matthijnssens et al., 2008; Ghosh and Kobayashi, 2011]. Applying this classification scheme, the full-length or nearly full-length nucleotide sequences of the 11 gene segments of several human G8 strains (G8P[1], G8P[4], G8P[6], G8P[8], G8P[10] and G8P[14]) have so far been analyzed, thus yielding vital information on the origin of these strains [Komoto et al., 2016; Ghosh et al., 2011; Nakagomi et al., 2013; Heylen et al., 2015; Dennis et al., 2014; Jere et al., 2011; Esona et al., 2009; Matthijnssens et al., 2006; Pietsch et al., 2009; Banyai et al., 2010; Heiman et al., 2008]. In Kenya, the whole or partial genomic sequences of a number of G8 strains have been characterized, with their VP7 gene sequences demonstrating phylogenetic clustering based on the year of isolation [Page et al., 2010] and country of origin in relation to other African strains [Heylen et al., 2015]; and clustering with bovine G8 strains [Ghosh et al., 2011]. Similarly, the whole or partial genomes of a few Kenya P[6] strains have been analyzed, providing evidence of their VP4 gene clustering together with the southern African strains from Malawi and South Africa [Page et al., 2010; Komoto et al., 2014] but being distinct from the West African strains [Heylen et al., 2015]. Further, the Kenyan P[6] strains were found to be more closely related to human P[6] strains, rather than to porcine P[6] strains [Heylen et al., 2015].

Thus, in order to gain further insights into the genetic variability within the Kenyan genotypes G8 and P[6], the VP7 gene of five G8 strains (G8P[4] and G8P[6]) and the VP4 gene of three P[6] strains (G1P[6] and G8P[6]) identified during the rotavirus surveillance in Kenya in 2010 and 2011 were sequenced and analyzed in this thesis. The whole genomic analysis of the G12 rotavirus strains during this surveillance was carried out previously [Komoto et al., 2014].
1.4 Research Questions

I. What is the epidemiology of rotavirus gastroenteritis and genetic characteristics of the strains circulating in Kenya before the introduction of rotavirus vaccine into the country’s national immunization programme?

II. What is the impact of rotavirus vaccination on rotavirus disease epidemiology and strain distribution in Kenya after the introduction of rotavirus vaccine into the country?

1.5 Objectives

1.5.1 General Objective
To determine the rotavirus disease epidemiology and molecular features of circulating strains among children aged <5 years in Kenya before and after the introduction of rotavirus vaccine into Kenya’s national immunization programme.

1.5.2 Specific Objectives

I. To determine the prevalence, seasonality and age distribution of rotavirus gastroenteritis among children aged <5 years in Kenya before and after vaccine introduction.

II. To determine the genotypic diversity of rotavirus strains detected in children aged <5 years in Kenya before and after vaccine introduction.

III. To determine the phylogeny of some unusual rotavirus strains detected in children aged <5 years in Kenya before vaccine introduction.

IV. To estimate rotavirus vaccine coverage at the sub-county level using the vaccine administrative data and examine the factors relating to the coverage.
2.1 Virology of Rotavirus

2.1.1 Discovery of Rotavirus

Rotavirus was discovered in 1972 by an Australian research group led by Dr. Ruth Bishop [Bishop et al., 1973]. The virus was recognized by direct electron microscopy visualization in the duodenal biopsies of a child with acute diarrhea and named duovirus. The virus was subsequently observed in large numbers in faeces as demonstrated by direct thin layer electron microscopy and significant antibody titre was shown between acute and convalescent sera from the children by immune electron microscopy [Bishop et al., 1974]. The virus was renamed rotavirus because of its characteristic wheel-shaped (rota is Latin for wheel) morphology when viewed under an electron microscope [Prasad and Chiu, 1994].

2.1.2 Structure and Taxonomy

Rotavirus is a non-enveloped virus of the family Reoviridae (Anderson and Weber, 2004). It has a wheel-like appearance on electron microscopy [Prasad and Chiu, 1994]. The virus has a triple-layered icosahedral capsid 76.5 nm in diameter and has a buoyant density of 1.36 g/ml in CsCl [Maldonado and Yolken, 1990; Pesavento et al., 2006].

2.1.3 Genome

Rotavirus genome is made up of 11 segments of double stranded RNA (dsRNA) held in the inner core of the three-layered virus [Varani and Allain, 2002]. The genome consists of 18,555 nucleotides in total. Each segment is a gene, numbered 1 to 11 by decreasing size. The segmented genome can be separated by polyacrylamide gel electrophoresis (PAGE) to reveal an RNA migration pattern or electropherotype. The RNA pattern is both constant and characteristic for a particular strain and has been widely used in epidemiological studies for monitoring the transmission and spread of rotavirus [Steele et al., 1993]. Each of the 11 segments of dsRNA
codes for one of six structural and six nonstructural proteins with only segment 11 being bicistronic (encoding two proteins) [Anderson and Weber, 2004].

2.1.4 Proteins

The six viral proteins (VP1, 2, 3, 4, 6 and 7) form the virus particle (virion). VP1 is the RNA-Dependent, RNA Polymerase for rotavirus [Varani and Allain, 2002; Rodrigo et al., 2006]. VP2 is a replication intermediate and binds the RNA genome while VP3 acts as the mRNA capping enzyme called guanylyl transferase [Fresco and Buratowski, 1994]. VP4 determines the rotavirus P serotype as well as host specificity, virulence and protective immunity [Maunula and Von Bonsdorff, 2002]. VP7 is a glycoprotein that determines the G serotype. VP6 determines the A-G groupings, and I, II sub-groupings of rotavirus [Laird et al., 2003].

The six non-structural proteins (NSP1, 2, 3, 4, 5 and 6) are only produced in cells infected by rotavirus [Graff et al., 2002; Anderson and Weber, 2004]. NSP1 binds Interferon Regulatory Factor 3 and may inhibit interferon response during rotavirus infection [Graff et al., 2002]. In conjunction with NSP5, NSP2 is involved in the synthesis and packaging of viral RNA, creation of viroplasms and is required for genome replication. NSP3 binds viral mRNA at the 3’ end, promotes viral protein synthesis and is responsible for the shutdown of host cell protein synthesis. NSP4 is a viral enterotoxin and induces diarrhea during infection [Dong et al., 1997]. NSP6 is an RNA binding protein encoded by gene 11 from an out of phase open reading frame Rainsford and McCrae, 2007).

2.1.5 Replication

Rotavirus entry into enterocytes is accompanied by the loss of the VP4 and VP7 outer layer, thereby converting the triple-layered particles (TLPs) to double-layered particles (DLPs). The RNA-dependent RNA polymerase (RdRp) VP1 of the DLP functions as a transcriptase to synthesize the 11 viral plus-strand RNAs [Lawton et al., 1997]. The plus-strand RNAs are extruded from DLPs through channels at the vertices that extend through both the VP2 and VP6 protein layers. The plus-strand RNAs contain 5’ caps but lack 3’ poly(A) tails and are translated to give rise to six structural proteins and six nonstructural proteins. The plus-strand RNAs also
function as templates for the synthesis of the dsRNA genome segments. RNA replication occurs concurrently with the packaging of the genome segments into newly formed cores and is coordinated such that the 11 segments are produced at equimolar levels [Patton and Gallegos, 1990; Patton, 1990].

Rotavirus infection leads to the formation of perinuclear, non-membrane-bound cytoplasmic inclusions (viroplasms). NSP2 and NSP5 have critical roles in viroplasm formation [Lawton et al., 1997]. Viroplasms are the putative sites of RNA replication (minus-strand synthesis) and core and DLP assembly. The DLPs migrate to the endoplasmic reticulum where they obtain their third, outer layer (formed by VP7 and VP4). The progeny viruses are released from the cell by lysis [Jayaram et al., 2004].

**Figure 1.** Rotavirus structure showing protein coding assignments of 11 genome RNA segments separated on polyacrylamide gel (left). Schematic diagram (middle) and cryoelectron microscopic reproduction of a virion (right) show the location of major structural proteins (VP). Outer capsid proteins VP4 and VP7 are neutralization antigens, which induce neutralizing antibody; protein that makes up intermediate protein shell, VP6, is the subgroup antigen. NSP, nonstructural protein [Gentsch et al., 2005].
2.1.6 Classification

Based on the antigenic properties of VP6, rotaviruses have been subdivided into seven serological species (A-H) and one additional tentative species (I) according to the International Committee on Taxonomy of Viruses (ICTV) [Ball, 2005; Ramig et al., 2005]. These rotavirus species are commonly referred to as rotavirus groups. Rotaviruses belonging to group A, B, C and H (RVA, RVB, RVC and RVH, respectively) have been associated with acute gastroenteritis in humans and animals, whereas group D, E, F and G (RVD, RVE, RVF and RVG, respectively) rotaviruses are known to infect only animals, mostly birds [Estes and Greenberg, 2013]. A novel tentative group I was recently described in Hungarian sheltered dogs [Mihalov-Kovács et al., 2015].

Epidemiologically, RVA is the most important for human infection and disease and unless otherwise mentioned, rotavirus usually means RVA [Anderson and Weber, 2004]. RVA is classified further using various approaches. Based on the subgroup antigens that are carried on the VP6 protein, RVA strains are categorized into subgroups I, II, I+II, nonI, and nonII [Greenberg et al., 1983]. The migration pattern of the RNA genome segments when subjected to polyacrylamide gel electrophoresis has been used to classify RVA strains into long, short, supershort or atypical electropherotypes [Fischer and Gentsch, 2004]. Another classification of RVA into genogroups (Wa, DS-1 and AU-1) is defined by RNA-RNA hybridization with probes prepared from the prototype strains [Flores et al., 1982; Nakagomi et al., 1989].

Further classification of RVA strains into serotypes is based on two outer capsid proteins VP7 and VP4 [Martinez-Laso et al., 2009]. VP7 (G protein for ‘glycoprotein’ forming the matrix of the capsid) defines G serotypes. VP4 (P protein for ‘protease-sensitive’ due to its trypsin mediated cleavage required for virus adsorption into cells) determines the P serotypes [Inoue et al., 2003; Laird et al., 2003]. For G types, serotypes (determined by neutralization assay) and genotypes (determined by RT-PCR) are largely identical, thereby allowing the use of the same numbering system. For P types, more genotypes than serotypes have been identified, owing to lack of monospecific P antisera. As a result, P types are identified as serotypes by Arabic numbers and as genotypes by Arabic numbers in square brackets. Thus, the serotype of prototype
human rotavirus strain Wa is described as G1P[8]. To date, at least 27 G types and 37 P types have been found in humans and animals [Matthijnssens et al., 2011; Trojnar et al., 2013].

In addition to the G and P genotyping of rotavirus, a whole genome-based genotyping system was recently proposed based on the assignment of genotypes to all the 11 gene segments (i.e., G/P and non-G/P genes) [Matthijnssens et al., 2008]. In the new genotyping system, the acronym Gx-P[x]-Ix-Rx-CxMx-Ax-Nx-Tx-Ex-Hx, where x is an integer, defines the genotype of the VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5 genes of a given rotavirus strain. The Wa-like strains are characterized by non-G/P genotypes (I1-R1-C1-M1-A1-N1-T1-E1-H1), and tend to have G/P genotypes G1P[8], G3P[8], G4P[8], or G9P[8] [Dennis et al., 2014]. In contrast, the DS-1-like strains are characterized by non-G/P genotypes (I2-R2-C2-M2-A2-N2-T2-E2-H2), and tend to have G/P genotype G2P[4]. The third minor AU-1-like strains are characterized by non-G/P genotypes (I3-R3-C3-M3-A3-N3-T3E3-H3) and tend to have G/P genotype G3P[9]. Whole genome-based analysis is a reliable method for obtaining conclusive data on the origin of an RVA strain and for tracing its evolutionary pattern [Matthijnssens et al., 2008; Ghosh and Kobayashi, 2011].

2.2 Epidemiology of Rotavirus

Rotavirus is distributed evenly across the globe. Regardless of hygiene practices or access to clean water, nearly every child in the world will be infected with rotavirus before age five [Parashar et al., 2003]. However, the consequences of infection are markedly severe depending on where the child lives and the majority of deaths due to rotavirus diarrhea occur in the developing countries of the Indian subcontinent and sub-Saharan Africa due to limited access to medical intervention [Parashar et al., 2006; Cunliffe et al., 2005].

Humans of all ages are susceptible to rotavirus infection, but children aged 6 months to 2 years, premature infants, and the elderly and immuno-compromised individuals are particularly prone to more severe symptoms. Children become most susceptible after 6 months of age when the protection conferred by maternal antibodies wanes [Patel et al., 2009]. The median age of children hospitalized with rotavirus diarrhea in many African and Asian countries is 6-9 months, and up to 80% are less than 1 year old [Cunliffe et al., 1998]. In contrast, the median age in developed countries is 13-16 months and the highest proportion of cases occurs in the second year of life [Nakagomi et al., 2005]. By 15 months of age many have developed some protection
after primary infection [O’Ryan et al., 2009]. Nevertheless, in both developing and developed countries, rotavirus is the major cause of severe gastroenteritis and is associated with approximately 40% of hospitalizations among children aged <5 years worldwide [CDC, 2008].

High transmission rates of rotavirus have been associated with the dual condition of extremely high virus concentration in faeces of symptomatic and asymptomatic individuals (more than $10^9$ virus particles/g) and the low inoculums required for infection (10–100 virus particles). Widespread viral contamination of different water bodies (with the possible exception of seawater) and prolonged persistence of infective virus in ground and surface water may be contributing to the high prevalence rates of rotavirus infection worldwide [Grassi et al., 2009; Espinosa et al., 2008]. In temperate countries, rotavirus infections peak in the winter and early spring, with fewer cases at other times. In tropical countries, infections occur throughout the year, although more cases are observed in the cooler and drier months [Nakagomi et al., 2005].

Molecular epidemiological studies of rotavirus have identified 5 common serotypes, including G1, G2, G3, G4, and G9, which tend to predominate globally (Desselberger et al., 2001; Santos and Hoshino, 2005). G1 is the most prevalent strain worldwide whereas G9 is the fastest emerging worldwide [Page et al., 2010; Nyangao et al., 2010; Kirkwood et al., 2003]. However, in developing countries, additional serotypes may circulate and even predominate in some setting (eg, G5, G8, G10, and G12). Of the 27 VP4 genotypes identified, genotypes P[8], P[4] and P[6] are detected most frequently in children [Hoshino et al., 2000; Santos and Hoshino, 2005]. Analogously to VP7 epidemiology, supplementary P genotypes, including P[9] and P[10] may also predominate or circulate at lower levels in developing countries [Santos and Hoshino, 2005].

### 2.3 Pathogenesis of Rotavirus

The primary mode of person-to-person transmission of rotavirus is faecal-oral, although some studies have reported low titers of virus in respiratory tract secretions and other body fluids, indicating the possibilities for air-borne and water-borne transmissions of rotavirus (Dennehy, 2000). After ingestion, the rotavirus particles exclusively infect the mature differentiated enterocytes in the mid and upper part of the villi of the small intestine leading to structural changes in the intestinal epithelium [Lundgren and Svensson, 2001]. Unlike the
parvovirus, rotavirus can infect neither the immature villous crypt cells nor the colonic enterocytes. Rotavirus attaches to its cellular receptors (sialoglyco-protein and integrins) via the VP4 protein. The virus is thought to invade target cells in two possible ways; by direct entry or fusion with the enterocytes, and through Ca²⁺-dependent endocytosis [Pérez et al., 1998; Jayaram et al., 2004].

Three mechanisms have been described by which rotavirus might cause diarrhoea. First, within 12-24 hours post-infection, enterocytes are intact but the levels of the brush-border disaccharidases (sucrase, maltase, lactase) are greatly reduced. As a result, disaccharides in the diet cannot be hydrolysed to monosaccharides and thus cannot be absorbed, leading to osmotic diarrhoea [Anderson and Weber, 2004]. Second, NSP4 has an effect in opening calcium channels in the enterocytes. This causes an efflux of sodium and water, producing secretory diarrhea [Dong et al., 1997]. Finally, the raised intra-enterocyte calcium concentration causes enterocytes to die by oncrosis. The rate of death of the mature villous tip enterocytes exceeds the rate of growth of immature enterocytes that are regenerated from the stem cells in the crypt, causing villous blunting and thus malabsorption [Leung et al., 2005]. Infection resolves both as the virus runs out of susceptible mature enterocytes and an immune response is generated [Lundgren and Svensson, 2001].

### 2.4 Immune Response to Rotavirus

Primary rotavirus infections induce production of rotavirus-specific memory B and T cells [Velazquez et al., 2000]. However, in humans, high titers of IgG do not seem to be as protective as IgA against moderate to severe illness, so serum IgA is seen as the primary indicator of protective immunity to rotavirus. One reason these antibody responses do not confer full protection is that they are serotype specific. Given the diversity of the various rotavirus serotypes, this prevents these antibodies from mediating full protection against infection by a different serotype. However, each additional infection expands the population of B cells producing cross-reactive antibodies that can recognize multiple serotypes and explains why repeat infections are less severe. Any vaccine effort would need to generate these cross-reactive antibodies to generate effective protection [Rodrigo et al., 2010].
CD4+ helper T (T_H) cells also play a vital role in the successful clearance of a rotaviral infection [VanCott et al., 2001]. Thus the correlates of immunity to rotavirus include both the presence of high amounts of cross reactive secretory IgA, and serotype specific serum IgA and IgG, which requires a rotavirus-specific T_H cell response as well as a rotavirus-specific CTL response [Anderson and Weber, 2004]. Protection of neonates against rotavirus infection appears to be conferred by both transplacentally acquired maternal antibodies and by antibodies and other factors in breast milk. Interestingly, rotavirus infection in neonates often results in asymptomatic infection unless novel serotypes emerge, and rotavirus can circulate silently in neonatal units [Patel et al., 2009].

2.5 Clinical Features of Rotavirus Infection

The outcome of rotavirus infection varies from asymptomatic through mild short-lived watery diarrhea, to an overwhelming gastroenteritis with dehydration leading to death. The onset of symptoms is abrupt after a short incubation period of 1-3 days. The disease is characterized by fever, frequent abdominal pain and vomiting for 2-3 days, followed by pale watery or loose non-bloody diarrhea for 3-8 days. Diarrhea can be profuse, with patients commonly having 10-20 bowel movements each day. Such severe diarrhea without fluid and electrolyte replacement may result in death. Temporary lactose intolerance may also occur. Respiratory signs are often found during rotavirus gastroenteritis but its aetiological association with rotavirus infection is not clear. It has been recently shown that rotavirus gastroenteritis may lead to extra-intestinal manifestations such as viraemia. Patients continue to excrete virus for extended periods of time and may thus be a reservoir for infecting others [Maldonado and Yolken, 1990].

It is not possible to distinguish rotavirus gastroenteritis from other viral causes of non-inflammatory diarrhea solely on clinical grounds [Lundgren and Svensson, 2001]. However, rotavirus diarrhea tends to be more severe than that due to other enteropathogens. Co-infection with another pathogen does not increase the severity of disease due to rotavirus infection [Leung et al., 2005].
2.6 Diagnosis of Rotavirus

Diagnosis of rotavirus can be done by identifying the virus in the patient's stool using techniques such as antigen detection assays, electron microscopy (EM), polyacrylamide gel electrophoresis (PAGE), reverse transcription-polymerase chain reaction (RT-PCR) and virus isolation [Cunliffe et al., 2002]. Antigen detection tests are the most widely used in diagnostic laboratories and include enzyme-linked immunosorbent assay (ELISA), latex particle agglutination assay (LA) and immunochromatography [Smith et al., 1993]. Though the sensitivity and specificity of these tests are generally high, they are only designed to detect group A rotavirus. Furthermore, ELISA is not a reasonable method after day 10 post-infection when antigen levels in the stool drop [Greenberg et al., 1983]. Antibody detection may also be employed for rotavirus diagnosis but is not commonly used. Successful cultivation of some animal and human rotaviruses has been achieved through the use of primary and transformed monkey kidney cells and by proteolytic activation of the virus with trypsin prior to infection [Sato et al., 1981; Arnold et al., 2009]. However, different rotavirus strains vary in their capacity to grow in culture and the viral culture is limited to research purposes.

Electron microscopy is relatively quick and can be used to identify non-group A rotaviruses [Bishop et al., 1974]. However, access to electron microscopes is not usually available in developing nations. PAGE is convenient for the detection of rotavirus RNA extracted directly from the stool specimens. The assay also allows detection of non-group A rotaviruses. The technique is relatively cheap and simple with good specificity and sensitivity. In addition, this assay provides epidemiological information based on the electrophoretic migration pattern of the 11 segments of the dsRNA [Nakagomi et al., 1988]. RT-PCR is generally considered the standard tool in virus detection for research purposes [Gouvea et al., 1990]. The technique provides information on the G and P genotypes of the circulating rotavirus strains and the duration of viral shedding in the stool [Fischer and Gentsch, 2004].
2.7 Management of Rotavirus Gastroenteritis and Vaccination

2.7.1 Management of Rotavirus Gastroenteritis

There is no cure for rotavirus infection. Therefore, the mainstay of management involves replacement of lost fluid by oral rehydration with fluids of specified electrolyte and glucose composition [Anderson and Weber, 2004]. Intravenous rehydration therapy is indicated for patients with severe dehydration, shock or reduced levels of consciousness. Human or bovine colostrum and hyperimmune human serum immunoglobulin may be used to manage chronic rotavirus infection in immunocompromised children. Administration of probiotics such as Lactobacillus casei GG may also be beneficial. Anti-diarrheal medicines are not recommended because they may prolong the infection [Leung et al., 2005]. Following the magnitude of disease associated with rotavirus infections and because public health interventions to improve sanitation are unlikely to decrease the incidence and burden of this disease, vaccines are thought to be the first line strategy for prevention of rotavirus gastroenteritis [O'Ryan et al., 2009; Rodrigo et al., 2010].

2.7.2 History of Rotavirus Vaccination

Several clinical studies in children including well designed cohort studies have conclusively demonstrated that a natural rotavirus infection protects against reinfection but protection is incomplete [Velasquez et al., 1996; Fischer et al., 2002]. Children (and probably adults) can be re-infected many times throughout the years but the great majority will suffer at most one moderate to severe clinical episode during the first encounter with the virus. This clinical observation was the basis for the concept of ‘infection induced protective immunity’ leading to the concept that ‘vaccine induced protective immunity’ could be obtained [Rodrigo et al., 2010]. Proof of the concept of ‘infection induced protective immunity’ was provided in the early 1990s with the first generation of rotavirus vaccines using initial Jennerian approach. The vaccines were naturally attenuated monovalent animal strains. Clinical trials of three of these vaccines-G6 bovine serotype (RIT 4237 and WC3) and G3 rhesus serotype (rhesus rotavirus vaccine [RRV])-yielded high efficacy (82%–100%) against severe rotavirus disease in high-income countries. However, the first generation vaccines produced suboptimal protection in low-
income settings in the Gambia, Peru, Rwanda, and Central African Republic and at American Indian Reservations [O'Ryan et al., 2009; Patel et al., 2009]. This observation provided early indication of potential for differences in efficacy between developing and developed regions.

The second generation of rotavirus vaccines used the modified Jennerian approach, which includes reassortant viruses with the backbone of an animal strain that incorporate human VP7 or VP4 genes. In August 1998, Rotashield (Wyeth-Lederle, USA), an oral formulation of a simian-human tetravalent reassortant vaccine, was licensed for use in children at 2, 4, and 6 months of age. However, the product was withdrawn from the market a year later due to an increased risk of intestinal intussusception with an estimated attributable risk of 1:10000 [ACIP, 1999; CDC, 1999].

In 2006, two vaccine candidates proved to be well tolerated and effective in large phase III trials: RotaTeq® (Merck & Co. Inc., USA) composed of five bovine-human reassortant strains including G types G1–G4 and P type P1A[8] and Rotarix® (GlaxoSmithKline Biologicals, Belgium) including one human attenuated P1A[8]G1 strain. Both vaccines are administered orally; Rotarix® in a two-dose schedule and RotaTeq® in a three-dose schedule [GlaxoSmithKline, 2011; Merck & Co., 2011]. Having demonstrated good efficacy (85%–98%) against severe rotavirus diarrhea in clinical trials conducted in the Americas and Europe [Ruiz-Palacios et al., 2006; Vesikari et al., 2006], these vaccines have already been introduced in the routine immunization schedule of several countries in these regions. Following the vaccine introduction, impressive declines in rotavirus and all-cause diarrhoea hospitalizations were observed in many countries [Patel et al., 2011]. In Mexico and Brazil 35% and 22% reductions in diarrhea-related mortality, respectively, were observed in children under 5 years, following the introduction of rotavirus vaccine [Richardson et al., 2010; do Carmo et al., 2011].

Nevertheless, the World Health Organization (WHO) required that the efficacy of these vaccines be demonstrated specifically in low-income countries of Africa and Asia before it recommends its inclusion in the global program for childhood immunization [WHO, 2007]. This was based on the fact that the efficacy of other live oral vaccines has varied between different population groups, with efficacy being lower in poor resource settings. In addition, the GAVI
Alliance will assist the developing countries in financing introduction of rotavirus vaccine only if its efficacy is demonstrated in the region [Patel et al., 2009].

Consequently, various clinical trials evaluating the efficacy of both Rotarix® and RotaTeq® in developing countries in Africa and Asia have been carried out. Results of these trials indicated that efficacy is substantially lower in these more challenging settings with rates of efficacy ranging from 40% to 70% with an average of 50% to 60% [Armah et al., 2010; Madhi et al., 2010; Zaman et al., 2010]. A study of Rotarix® in South Africa and Malawi found an efficacy of 61% [95% confidence interval (CI): 44–73%] against severe rotavirus gastroenteritis in the first year of life [Madhi et al., 2010]. RotaTeq® had an overall efficacy of 64% (95% CI: 40–79%) against severe rotavirus gastroenteritis in the first year of life in three study sites (Kenya, Mali and Ghana) [Armah et al., 2010]. Kenya had the highest point estimate of overall efficacy at 63.9% (95% CI -5.9-89.8), with an efficacy of 83.4% (95% CI 25.5-98.2) in the first year of life and a drastic decline to -54.7% in the second year of life. Assessment of the clinical efficacy of RotaTeq® for prevention of severe rotavirus gastroenteritis in infants in Bangladesh and Vietnam resulted in a vaccine efficacy of 48.3% (95% CI 22.3-66.1) against severe disease during nearly two years of follow-up [Zaman et al., 2010].

The reason for this lower efficacy has not been fully explained but mimics the lower efficacy of other live oral vaccines such as OPV and oral cholera and typhoid vaccines in similar settings [Patel et al., 2009]. Possible explanations include the inhibitory effect of high titers of maternal antibody transferred to the infant either through the placenta or breast milk each of which could decrease the titer of the vaccine and diminish its effect; environmental enteropathy; differences between the gut microbiome or intestinal villae of infants living in these resource poor settings and those in wealthier ones or interference from other viruses in the gut, including oral polio vaccine viruses; and differences in the rotavirus epidemiology and the serotype distribution of candidate strains among different settings.

Nonetheless, even with lower efficacy than in high and middle-income settings, a much greater reduction in absolute numbers of cases of severe gastroenteritis and related deaths would be expected with use of these vaccines in the developing countries in Africa and Asia due to the higher incidence of severe disease and associated mortality in these settings. Subsequently, in
2009, WHO recommended that rotavirus vaccines be introduced into all national immunization programs, particularly where diarrheal deaths account for ≥10% of child mortality [WHO, 2009]. This recommendation was made on the basis that despite the lower efficacy, the vaccines would still prevent a large amount of severe disease and deaths in the high mortality developing countries in Africa and Asia. As of May 1, 2016, these vaccines had been introduced into the EPI of 81 countries, including Kenya in July 2014 (Fig. 2) [PATH, 2016].

Figure 2. National rotavirus vaccine introduction, by geographic region, as of May 1, 2016. Source: PATH rotavirus vaccine country introduction maps available at http://sites.path.org/rotavirusvaccine/country-introduction-maps-and-spreadsheet/. Abbreviation: UAE, United Arab Emirates.
2.7.3 Impact and Effectiveness of Rotavirus Vaccination

Having demonstrated good efficacy in preventing severe rotavirus gastroenteritis in pre-licensure clinical trials in the Americas and Europe [Ruiz-Palacios et al., 2006; Vesikari et al., 2006], rotavirus vaccines were soon adopted in many countries in these regions as part of their routine childhood immunization programmes. Following the introduction of rotavirus vaccinations, remarkable declines in rotavirus diseases and all-cause diarrhoea hospitalizations have been observed in many of these countries [Richardson et al., 2010; do Carmo et al., 2011; Patel et al., 2011]. A systematic review of data from eight countries reported a 49%-89% decline in rotavirus hospitalizations and 17%-55% decline in all-cause gastroenteritis hospitalizations among children aged <5 years within two years of vaccine introduction [Patel et al., 2012]. Interestingly, in some countries, rotavirus vaccination of young infants has also resulted in the declines in rotavirus disease among children who missed vaccination and among older children and even adults who were not vaccine eligible [Gastanaduy et al., 2013; Clarke et al., 2011]. This phenomenon, referred to as herd immunity, has been hypothesized to be due to reduction in community transmission of rotavirus because vaccination limits the number of children susceptible to rotavirus disease. Most notably, studies from Mexico, Brazil, and Panama showed a reduction in childhood deaths from all-cause diarrhea following vaccine implementation, a key outcome that was not evaluated in clinical trials [Richardson et al., 2010; do Carmo et al., 2011; Bayard et al., 2012].

Impressive impact of rotavirus vaccination in reducing morbidity and mortality from severe gastroenteritis has been noted in many developing countries in Africa, Eastern Europe/Central Asia and Latin America that were early adopters of vaccination [Parashar et al., 2016]. Studies in a number of African countries (Malawi, Botswana, South Africa, Ghana, Togo, Zambia and Rwanda) have provided evidence of rapid and substantial declines in severe diarrhea and/or rotavirus disease following vaccine introduction [Enane et al., 2016; Groome et al., 2016; Armah et al., 2016; Tsolenyana et al., 2016; Mpabalwani et al., 2016; Bar-Zeev et al., 2016; 2015; Ngabo et al., 2016; Tate et al., 2016b]. In these evaluations, the evidence of vaccine impact on rotavirus and/or all-cause gastroenteritis was provided by sharp declines coinciding temporally with the timing of vaccine introduction; greater declines during the months of the
year with seasonal peaks of rotavirus disease; and greater initial declines in younger age groups that receive vaccination in the initial years of the vaccination, followed by a progressive decline in older age groups in later years after introduction. Reductions in all-cause gastroenteritis ranged between 18% and 65% [Enane et al., 2016; Groome et al., 2016; Tsolenyanu et al., 2016; Mpabalwani et al., 2016; Bar-Zeev et al., 2016; Ngabo et al., 2016]. Proportions in rotavirus-associated hospitalizations declined at rates ranging from 24% to 56%, with most reductions being pronounced in children aged <1 year [Tsolenyanu et al., 2016; Mpabalwani et al., 2016; Armah et al., 2016; Bar-Zeev et al., 2016; 2015; Msimang et al., 2013]. Of noteworthy, data from Botswana and Zambia showed a decline of 27%-48% in in-hospital mortality from gastroenteritis at various sentinel hospitals [Enane et al., 2016; Mpabalwani et al., 2016].

Furthermore, observational studies have been conducted in a number of developing countries in Africa to measure the field effectiveness of rotavirus vaccination in routine programmatic use [Beres et al., 2016; Armah et al., 2016; Bar-Zeev et al., 2016; 2015; Tate et al., 2016b]. Some of these studies have also included in their evaluations groups that may have been excluded from clinical trials such as the malnourished or immunocompromised children [Armah et al., 2016; Bar-Zeev et al., 2016; Groome et al., 2016]. In general, reports from these studies provide evidence that the real-world effectiveness of rotavirus vaccination in developing countries is similar to the vaccine efficacy in pre-licensure trials. Vaccine effectiveness against severe rotavirus gastroenteritis was well sustained over the first two years of life in some studies. In other studies, a decline in effectiveness in the second year of life compared with the first year was observed. There was also some evidence of protection from incomplete series of rotavirus vaccine. One of the studies found no statistically significant difference in the vaccine effectiveness by HIV status or nutrition status of the children [Bar-Zeev et al., 2016]. Finally, the monovalent vaccine provided protection against a range of circulating rotavirus strains. However, higher point estimates of the vaccine effectiveness were seen against fully homotypic G1P[8] strain and lowest for totally heterotypic strains such as G2P[4] [Armah et al., 2016; Bar-Zeev et al., 2016].
2.8 Rotavirus Vaccination in Kenya: National Coverage and Challenges

In July 2014, Kenya introduced the two-dose Rotarix® into her Expanded Programme on Immunization (EPI) with an aim of protecting over 1.5 million children from severe diarrhoea through co-financing of the vaccine cost with GAVI Alliance. The WHO/UNICEF estimates that the rotavirus vaccine achieved 38% coverage in 50% of the national target Kenyan population in 2014 and 66% in 2015 [WHO/UNICEF, 2015]. A dropout rate of 5.8% was reported across the country with eight counties reporting >10% dropout. The GAVI Joint Appraisal Report for Kenya 2015 [GAVI, 2015] attributes the low coverage of rotavirus vaccination to challenges like phased introduction of the rotavirus vaccine as late as October 2014 in some counties as a result of delayed disbursement of the rotavirus Vaccine Introduction Grant (VIG); stock-outs due to the vaccination of children out of the target age range; low reporting and adherence to age restrictions even after removal since some reporting tools (Mother-Child Cards) still age restrictions; cold-chain capacity challenges in some counties and the facility level with the introduction of the rotavirus vaccine; frequent industrial strikes by health workers; and reduced funding for the immunization program at both the national and county level due to devolution of health funds.

Some of these challenges have been addressed by including an adequate buffer in the vaccine supply to mitigate vaccine stock-outs; acquiring 42 cold-chain equipment to increase the number of health facilities providing immunization services and frequent pickups to help ease the pressure on the cold-chain system; and issuance of a policy statement to correct the application of age restrictions [GAVI, 2015]. These challenges could be further addressed by accurate vaccine forecasting to eliminate vaccine shortages and wastage. With 50% of the country having no access to electricity, the government should consider procuring solar driven cold-chain equipment to supplement the existing electric ones. Refresher training of all health workers on the appropriate age for administration of the rotavirus vaccine should be conducted and immunization cards revised to account for this. The roles and responsibilities of both the national and county governments should be clarified and coordination regarding immunization services improved.
2.9 Rotavirus Research in Kenya

Several epidemiological studies on rotavirus gastroenteritis in children <5 years old have been conducted in Kenya since the year 1975. A review of rotavirus research in children with diarrhea conducted in Kenya between 1975 and 2005 revealed rotavirus prevalence ranging from 11% to 56.2% in children <5 years of age and 6% in neonates [Kiulia et al., 2008]. Early studies established the importance of rotavirus infections in the overall burden of diarrheal diseases in infants and young children and established the general peak age of infection (between 6 and 23 months) [Leeuwenburg et al., 1978; Metselaar et al., 1978; Mutanda et al., 1979; 1980; 1990]. In addition, the studies determined the seasonality of rotavirus gastroenteritis: rotavirus diarrhoea occurs throughout the year with seasonal peaks during the dry seasons (January–March and June–September) [Mutanda et al., 1984; 1985; 1986].

The earliest study demonstrating the diversity of rotavirus strains in Kenya was conducted by Urasawa and colleagues in Kilifi and Mombasa between 1982 and 1983 using ELISA and serotype-specific monoclonal antibodies [Urasawa et al., 1987]. In this study, serotype G1 was identified as the predominant strain, and mixed infections were also detected. In a similar study conducted in Nairobi, Nanyuki, and Narok between 1989 and 1991, serotype G1 strains was observed in the majority of specimens analyzed [Gatheru et al., 1993].

The first large-scale study in Kenya was conducted in Nairobi, Kitui and Nanyuki between 1991 and 1994 [Nakata et al., 1999]. The overall frequency of detection of rotavirus antigen in the specimens analyzed was 19.7%, although the prevalence differed between the areas of study. In the study, Reverse Transcription Polymerase Chain Reaction (RT-PCR) was employed for the first time to genotype rotavirus strains in Kenya. Serotype G4 was found to be the most prevalent strain, followed by G1 and G2. G8 strains were identified for the first time in Africa, whereas G3 were rarely isolated.

A further study conducted in Nairobi between 1999 and 2000 among HIV infected children revealed that G3 were the predominant strains, with G4, G8, and G9 circulating at lower levels [Kiulia et al., 2009]. In a study conducted between 2000 and 2002 in two hospitals in Nairobi and one clinic in Kisumu, serotype G1P[8] strains were pre-dominant, followed by G2P[4], G8, and G9 strains [Nyangao et al., 2010]. These results indicated that the serotype G3
strains circulating in 1999 and 2000 were replaced by G1 strains during 2000 to 2002. G4 strain was detected in very low frequency.

In a study conducted in Kilifi, Coastal Kenya between 2002 and 2004, rotavirus was detected in 29% of children aged <13 years hospitalized for diarrhea [Nokes et al., 2008]. Molecular investigation of the rotavirus positives cases found P[8]G1 as the predominant genotype, followed by P[8]G9, P[4]G8, P[6]G8, and P[8]G8, with 10% mixed strains [Nokes et al., 2010]. The study revealed no evidence of an association between genotype and age, sex, or disease severity. An additional study in Maua, Eastern Kenya between 2004 and 2005 indicated G9 strains as the most prevalent strain, followed by G8 and G1 strains [Kiulia et al., 2006].

With the imminent availability of rotavirus vaccines, a few baseline surveillance studies have been conducted in Kenya. One of these studies was carried out from January 2010 to December 2011 in the inpatient facility of Siaya District Hospital and in the outpatient facilities of Ting‘wang’i Health Center and Njejra Health Center in Western Kenya [Khagayi et al., 2014]. In this study, rotavirus was detected in 27% and 20% specimens obtained from inpatient and outpatient children aged <5 years, respectively. Rotavirus AGE accounted for 501 hospitalizations per 100,000 person-years of observation among children aged <5 years. Rotavirus-associated mortality was 136 deaths per 100,000 person-years of observation children aged <5 years. In a study conducted between September 2009 and August 2011 among children <5 years of age admitted for acute diarrhea in hospitals in the Eastern region of Kenya, rotavirus was detected in 38% of the samples analyzed [Kiulia et al., 2014]. A long-term surveillance study was conducted in Central Kenya between July 2009 and June 2014 and whose findings ae reported in this thesis.

Overall, the data collected from the rotavirus studies in Kenya have contributed to the national baseline estimates of disease burden and virological features of rotavirus gastroenteritis in the country. Such data was useful for policy-makers seeking to determine the need for rotavirus vaccination in Kenya and would provide the baseline data for evaluating the impact of rotavirus vaccination in the country. However, to my knowledge, there is no report to date of the real-world impact and effectiveness of rotavirus vaccination in Kenya since the introduction of the vaccine more than two years ago.
CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area and Population

The study on disease epidemiology and molecular characteristics of rotavirus strains among children aged before and after the introduction of rotavirus vaccine was conducted in Kiambu County in the Central region of Kenya. This is a peri-urban county located on the northern border of Nairobi, the capital city of Kenya. The county has an estimated population of 1,623,282 according to the 2009 Kenya Population and Housing Census [KNBS, 2010]. The major economic activities in the county include agriculture and industries. Besides the small-scale farming of tea and coffee as cash crops; maize, beans, assorted vegetables and sweet potatoes as food crops, there are several large-scale coffee and tea farms that are serviced by local industries. According to the 2014 KDHS, 82.8% of children aged 12-23 months in this area had received all basic vaccinations; 82.8% had been fully vaccinated; whereas none of the children had not received any vaccines [KDHS, 2015]. According to the Unit of Vaccines and Immunization (UVIS), Kiambu sub-county, the population <1 year being targeted for rotavirus vaccination in this sub-county was 1425, 3420 and 3534 for 2014, 2015 and 2016, respectively. The monthly target for rotavirus vaccination was 285 for 2014 and 2015 and 295 for 2016.

During the study, stool samples were collected from Kiambu County Hospital which is run by the Government of Kenya. The hospital is one of the few main referral healthcare facilities in the central region of Kenya. Kiambu County Hospital has 316-bed general wards and 67 cots and generally serves populations from Kiambu County and its environs. Study subjects were infants and young children below 5 years of age hospitalized at Kiambu County Hospital with severe diarrhea. The children either came directly from the community or were referred from community health centres and dispensaries. Decisions regarding hospitalization, investigations and treatment were at the discretion of the attending clinicians.
3.2 Sampling

3.2.1 Sampling Method

At Kiambu County Hospital, convenience sampling technique was used for all diarrheic children aged less than five years and meeting all the inclusion and none of the exclusion criteria.

3.2.2 Inclusion Criteria

Only children under 5 years of age who presented with acute diarrhea for not more than 7 days and having experienced an episode of 3 looser than normal or watery stools in a 24-hour period with or without episodes of vomiting were enrolled in this study [WHO, 2009].

3.2.3 Exclusion Criteria

Children more than 5 years of age and with diarrhea lasting for more than seven days and having bloody diarrhea were excluded from the study [WHO, 2009].

3.2.4 Ethical Considerations

This study was approved by KEMRI/National Ethical Review Committee (SSC No. 2483). Patient names were not used; instead, unique identification codes were used in order to ensure confidentiality. Written consent was sought from parents/guardians of the participants prior to sample collection. Information obtained from the patients was strictly confined to academic use only, unless otherwise there was clinical indication necessary to allow a shared confidentiality in good faith of the patient concerned.

3.2.5 Sample Collection

Between July 2009 and June 2016, a total of 2204 fecal specimens were collected from children <5 years of age hospitalized for acute gastroenteritis (AGE) at Kiambu County Hospital, Central Kenya. Demographic and clinical data were collected from the children who met all the inclusion and none of the exclusion criteria using a pathological investigation form adapted from the WHO generic protocol for rotavirus surveillance [WHO, 2009]. After written parental consent was granted, stool samples were collected in clean sterile containers within 48 hours of
admission. Each sample was labeled according to the date of collection and the sample number. The samples were kept at 4°C at the hospital before being transported to the Nagasaki University, Institute of Tropical Medicine, Kenya Research Station where they were stored at -80°C until use.

### 3.3. Enzyme Linked Immuno-Sorbent Assay (ELISA) for Detection of RVA

About 1 ml of 10% fecal suspension was prepared for Enzyme Linked Immuno-Sorbent Assay (ELISA) and RNA extraction. Briefly, about 1g of stool sample or 100µl of rectal swab suspension was added to 1ml of 0.01M phosphate-buffered saline (PBS) (pH 7.2). The mixture was vortexed vigorously for 40 seconds followed by centrifugation at 10,000 rpm for 5 minutes. All the supernatant was transferred to new tubes and stored at -30°C until use.

Enzyme Linked Immuno-sorbent Assay (ELISA) was performed to screen for the presence of RVA antigen in the 10% sample suspension as described previously [Taniguchi et al., 1987]. Briefly, 100µl of non-neutralizing monoclonal anti-human rotavirus antibody (Yo-156) directed against VP6, the group-specific antigen for all RVAs, was coated on each plastic microtiter well as the capture antibody by an overnight incubation at 4°C. Unbound antibodies were washed away with 10mM PBS and each well blocked with 250µl of 1% bovine serum albumin (BSA) in 10mM PBS containing 0.05% Tween 20 (PBST). Fifty microliters of 10% sample suspension was added to each well, and the analyte in the sample would bind to the capture antibody on the solid phase during an overnight incubation at 4°C. Unbound components were washed away with 10mM PBST. Fifty microliters of anti-human rotavirus hyperimmune rabbit serum diluted 1:5000 with PBST containing 2.5% skim milk was then added to each well as the detector antibody followed by 1 hour incubation at 37°C.

Unbound detecting antibody was washed away with 10mM PBST. Fifty microliters of peroxidase-conjugated donkey anti rabbit IgG (H+L chains) diluted 1:5000 with PBST (an enzyme-labeled antibody binding specifically to the detection antibody) was added to each well followed by 1 hour incubation at 37°C. Unbound antibody was washed away with 10mM PBST. Finally, 100µl of O-phenylenediamine, a non-coloured substrate was added to each well, and the
substrate would be converted to a coloured product by the enzyme bound to the antigen-antibody complex following 10-30 minute incubation at room temperature.

Results were read spectrophotometrically at 490 nm with reference to 620 nm using a microplate reader (Model 680, Bio-Rad Laboratories, Inc., CA). Specimens with absorbance $\geq 0.3$ were considered positive for group A human rotavirus whereas those with absorbance $<0.3$ were considered negative. KU strain (kind donation by Fujita Health University, Department of Virology and Parasitology) was used as the positive control while phosphate buffered saline (PBS) was used as the negative control for this procedure.

3.4 Viral RNA Extraction and Polyacrylamide Gel Electrophoresis (PAGE)

In order to determine the RNA migration patterns (electrophoretype) of the segmented rotaviral genome and for confirmation of rotavirus ELISA results, polyacrylamide gel electrophoresis (PAGE) was carried out. Rotavirus double-stranded RNA was extracted from the ELISA positive 10% sample suspensions with ISOGEN-LS (Nippon Gene Co., Ltd., Toyama, Japan) according to the manufacturer’s protocol. In brief, 250µl of each of the 10% sample suspensions was homogenized in 750µl of ISOGEN-LS for 5 minutes at room temperature. The homogenate was separated into the aqueous and organic phases by the addition of 200µl chloroform and subsequent centrifugation at 12,000 rpm for 15 minutes at 4°C. RNA would remain exclusively in the aqueous phase. RNA was then precipitated from the aqueous phase by the addition of 500µl of isopropanol, followed by centrifugation at 12,000 rpm for 10 minutes at 4°C. The resultant pellet was washed with 1ml of 75% ethanol, briefly air-dried and finally solubilized in 50µl of nuclease-free water.

The total RNA solution was electrophoresed on either 10% polyacrylamide gel, 4.2 mm wide with 14 preformed wells for 1 hour 20 minutes at 35mA, 300V and 100W at room temperature or on 10% polyacrylamide gels (size: 138 (W) x 130 (H) mm; thickness: 2 mm) for 16 hours at 20mA at room temperature. RNA segments were visualized by silver staining using EzStain Silver kit (ATTO Corporation, Japan) according to the manufacturer’s protocol.
3.5 Reverse Transcription-Polymerase Chain Reaction (RT-PCR) for VP4 and VP7 Genotyping

To determine the G and P genotypes of rotavirus strains present in the specimens and to confirm rotavirus ELISA results, a multiplexed semi-nested reverse transcription-polymerase chain reaction (RT-PCR) was carried out as described previously with some modifications [Gouvea et al., 1990; Taniguchi et al., 1990; Taniguchi et al., 1992]. In brief, 2μl of the total RNA solution from each sample was reverse transcribed into the complementary DNA (cDNA) on a thermocycler with a Ready-To-Go ReverTra Ace® qPCR RT Kit (Toyobo Co., Ltd., Japan) at the following temperatures for the following times: incubation at 42°C for 30 minutes; incubation at 99°C for 5 minutes; holding at 4°C for 5 minutes; and chilling on ice to primary PCR. The cDNA was then amplified in two steps, that is, primary PCR followed by nested PCR.

In the first amplification, cDNAs corresponding to the full-length VP7 and VP4 genes were each amplified with a pair of primers for the 3’ and 5’ ends of each of the genes. 25μl of PCR reactions contained 2.5μl of KOD DNA polymerase reaction buffer, 2mM of each deoxynucleoside triphosphate, 0.4μM of each primer, 0.5μl of KOD-Plus-Ver.2 high fidelity DNA polymerase (TOYOBO Biotechnology Co. Ltd.) and 2μl of cDNA. PCR was performed on a thermocycler under the following conditions: 2 minutes at 95°C; 25 cycles of 30 seconds at 94°C, 30 seconds at 48°C and 1 minute at 72°C, and a final step of 7 minutes at 72°C followed by holding at 4°C. The PCR product was then subjected to 1.2% agarose gel electrophoresis at 100v for 35 minutes. Visualization of the PCR product bands was achieved by staining the gels with ethidium bromide.

The second amplification of the primary PCR product of the VP7 gene was carried out using two sets of a mixture of primers, each paired separately with a primer for the 3’ end of the VP7 gene. The first set included primers that are specific to each of six variable regions of the VP7 genes of G1–4, G8, and G9. The second set contained primers that are specific to each of five variable regions of the VP7 genes of G5, G6, G10, G11 and G12. Similarly, the primary PCR product of the VP4 gene was amplified simultaneously using a mixture of primers that are specific to each of four variable regions of the VP4 genes of P[4], P[6], P[8] and P[9], paired with a primer for the 5’ end of the VP4 gene. PCR reactions contained 2.5μl of KOD DNA
polymerase reaction buffer, 2mM of each deoxynucleoside triphosphate, 0.4μM of each primer, 0.5μl of KOD-Plus-Ver.2 high fidelity DNA polymerase (TOYOBO Biotechnology Co. Ltd.) and 2μl of primary PCR product. PCR was performed on a thermocycler under the same conditions as in the primary amplification except that the number of cycles was increased to 35. The PCR product was then subjected to 1.2% agarose gel electrophoresis and bands visualized by ethidium bromide staining.

3.6 Nucleotide Sequence Analysis of Rotavirus G8 and P[6] Strains

In this study, the VP7 gene sequences of five G8 strains: KDH861 (G8P[6]), KDH897 (G8P[4]), KDH908 (G8P[4]), KDH1073 (G8P[4]), and KDH1075 (G8P[4]), and the VP4 gene sequences of three P[6] strains: KDH783 (G1P[6]), KDH789 (G1P[6]), and KDH861 (G8P[6]) were analyzed. Briefly, the full-length cDNA of the VP4 and VP7 genes of seven RVAs with G8 or P[6] was prepared by RT-PCR. The PCR products were sequenced with ABI PRISM BigDye terminator cycle sequencing ready reaction kits (PE Biosystems, Chiba, Japan), and an automated sequencer, ABI PRISM 3100 genetic analyzer (PE Applied Biosystems, Foster City, Calif.). Nucleotide sequences were analyzed for construction of a phylogenetic tree by the Neighbor-Joining method using MEGA6.06. Nucleotide sequences of the VP4 and VP7 genes obtained in this study were deposited in the DDBJ/EMBL/GenBank database under the following accession numbers: LC177385-LC177387 (VP4 gene) and LC177388-LC177392 (VP7 gene).

3.7 Estimation of Rotavirus Vaccine Coverage in Kiambu Sub-County

I estimated the percentage of rotavirus immunization coverage and dropout rate in Kiambu sub-county between August 2014 and April 2016 and reviewed factors related to the coverage. Coverage was calculated by dividing the number of individuals vaccinated with rotavirus dose 1 or 2 (numerator) by the number of individuals targeted for vaccination (denominator) within the same period. This proportion was then multiplied by 100 to obtain the percentage coverage. Dropout rate was calculated by subtracting the number of individuals who received the last dose of rotavirus vaccine from the number of individuals who received the first dose of the vaccine (numerator) and dividing the difference by the number of individuals who received the first dose of the vaccine (denominator). This proportion was then multiplied by 100 to obtain the percentage dropout. Access to rotavirus vaccination was measured using percent
coverage and could either be describe as good (≥80%) or poor (<80%). Utilization of rotavirus vaccine (the ability to retain the children accessed by the vaccine until they receive the last dose on the schedule) was measured using the dropout rate and described as either high (when the dropout rate was <10%) or low (when the dropout rate was ≥10%) [MOH, 2013].

3.8 Data Extraction

I reviewed pediatric ward admission logbooks at Kiambu County Hospital from July 2009 to June 2016. I recorded daily gastroenteritis-related admission among children <5 years of age to the pediatric wards, noting the patient age and outcome of admission (discharged home, transferred, or deceased). Admissions were considered related to gastroenteritis of any cause if the diagnosis listed in the register was gastroenteritis, acute gastroenteritis, acute diarrheal disease, gastroenteritis and dehydration, diarrhoea, vomiting or dysentery. Cases for which there were additional diagnoses were included. Cases presenting with chronic gastroenteritis, chronic enteritis, chronic diarrhea, vomiting secondary to toxic ingestion were excluded. For the days in which admission registers had gaps or were not available, numbers of gastroenteritis admissions were obtained through review of medical records data. The administrative coverage data on rotavirus vaccinations in Kiambu sub-county were obtained from the Unit of Vaccines and Immunization Services (UVIS) of the sub-county. The administrative coverage data included the monthly doses (1 and 2) of rotavirus vaccine routinely administered to the target population at each of the health facilities providing EPI services within the sub-county.

3.9 Data Analysis

The prevalence, age and seasonal distribution of rotavirus gastroenteritis before and after vaccine introduction were calculated using EPI Info version 3.5.3.2 (USD, Inc., Stone Mountain, GA, USA). I analyzed AGE hospitalizations due to rotavirus before and after vaccine introduction and calculated the percentage decline in the overall prevalence in rotavirus AGE and among the vaccine-eligible age category of children. I also examined total hospital admissions for all-cause AGE pre- and post-vaccine introduction and calculated the percentage decline in the all-cause AGE hospitalizations. Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS for IBM-PC, release 18.0; SPSS Inc., Chicago, IL, USA). Differences in proportions were tested using the chi-square (χ²) test. A p-value of <0.05 was considered significant.
CHAPTER FOUR

RESULTS

4.1 Epidemiology of RVA Gastroenteritis before and after Vaccine Introduction

4.1.1 Demographic Characteristics of the Samples

A total of 2,204 fecal specimens were collected from children <5 years of age hospitalized at Kiambu County Hospital over the July 2009-June 2016 surveillance period. Among these samples, 1,546 were collected in the pre-vaccine period (July 2009 to June 2014) whereas 658 were collected in post-vaccine period (July 2014 to June 2016). The average number of gastroenteritis cases analyzed in each year of rotavirus surveillance (that’s a 12 month period starting from July and ending in June the following year) was 315. Of the 2,204 fecal samples, 1,223 (55.5%) were collected from males whereas 981 (44.5%) were from females. Majority (67.4%) of the samples were obtained from children aged between 6 and 17 months.

4.1.2 Prevalence of RVA Gastroenteritis

Of the 2,204 fecal specimens collected between July 2009 and June 2016, 520 were found to be positive for RVA, representing an overall prevalence rate of 23.6%. During the pre-vaccine introduction period (July 2009-June 2014), a total of 1,546 children <5 years of age with AGE were enrolled, of whom 429 were positive for RVA, providing a baseline prevalence rate of 27.5% (95% CI: 25.5-30.1) (Table 1). The proportion of RVA-associated hospitalizations ranged from 15.9% to 40.4% before introduction of the vaccine. During the post-vaccine introduction period (July 2014-June 2016), a total of 658 children <5 years of age with AGE were enrolled, of whom 91 were positive for RVA, representing a prevalence rate of 13.8% (95% CI: 11.3-16.6). The proportion of RVA-associated hospitalizations declined from 19.2% (95% CI: 14.9-24.1) in the first year of the vaccine introduction to 9.8% (95% CI: 7.1-13.1) in the second year of introduction (Table 1). Thus, comparing the rate of RVA-associated hospitalizations during the pre-vaccine period to two years after rotavirus vaccine introduction reveals a significant overall decline of 49.8% (95% CI: 34.6-63.7; p= 0.007) in the proportion of children aged <5 years with AGE who tested positive for RVA. The rate of reduction in RVA-associated hospitalizations
increased >2-fold from 30.2% (95% CI: 19.0-45.6) in the first year of vaccine introduction to 64.4% (95% CI: 48.7-76.6) in the second year.

### Table 1: Prevalence of Rotavirus Gastroenteritis in Kenya between 2009 and 2016

<table>
<thead>
<tr>
<th>Period</th>
<th>Year</th>
<th>Samples tested</th>
<th>Rotavirus positive</th>
<th>% Positive (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-vaccine</td>
<td>Jul2009-Jun2010</td>
<td>356</td>
<td>76</td>
<td>21.3 (17.3-25.8)</td>
</tr>
<tr>
<td></td>
<td>Jul2010-Jun2011</td>
<td>300</td>
<td>104</td>
<td>34.7 (29.4-40.2)</td>
</tr>
<tr>
<td></td>
<td>Jul2011-Jun2012</td>
<td>319</td>
<td>129</td>
<td>40.4 (35.1-45.9)</td>
</tr>
<tr>
<td></td>
<td>Jul2012-Jun2013</td>
<td>288</td>
<td>75</td>
<td>26.0 (21.2-31.3)</td>
</tr>
<tr>
<td></td>
<td>Jul2013-Jun2014</td>
<td>283</td>
<td>45</td>
<td>15.9 (11.9-21.5)</td>
</tr>
<tr>
<td></td>
<td>Subtotal</td>
<td>1546</td>
<td>429</td>
<td>27.5 (25.5-30.1)</td>
</tr>
<tr>
<td></td>
<td>Subtotal</td>
<td>658</td>
<td>91</td>
<td>13.8 (11.3-16.6)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>2204</td>
<td>520</td>
<td>23.6 (21.9-25.4)</td>
</tr>
</tbody>
</table>

#### 4.1.3 Age Distribution of RVA Gastroenteritis

There was a marked difference in the distribution of RVA gastroenteritis among the different age groups of children between the pre-vaccine and post-vaccine periods (Fig. 3). Before vaccine introduction, RVA was detected most frequently in infants and young children aged <18 months (87.6%) with a peak at 6-8 months and a drastic reduction in the number of cases after 18 months of age (12.4%) (Fig. 3). The proportion of RVA cases in children <1 year of age (vaccine-eligible) constituted 64.8% of all RVA cases in this pre-vaccine era (Fig. 4). However, following vaccine introduction, more RVA cases occurred in age groups >11 months than in the pre-vaccine period, with a peak at 12-17 months (Fig. 3 & 4). The proportion of RVA cases in children <1 year of age (vaccine-eligible group) decreased from 64.8% in the pre-vaccine era to 39.6% of all RVA cases in the post-vaccine era, representing an overall reduction rate of 38.9% (Fig. 4). Reductions in RVA-associated gastroenteritis were sustained in this age group in both the first and the second year of vaccine introduction (Fig. 4). Compared with the first year, the second year recorded a decline in RVA infections among the children aged 12-23 months and an increase in cases among those aged >2 years (Fig. 4).
Figure 3: Variation by age of the study population (0-59 months) in the frequencies of RVA gastroenteritis among children admitted for AGE at Kiambu County Hospital, Central Kenya in pre-vaccine (Jul 2009-Jun 2014) and post-vaccine (July 2014-June 2016) periods of study.

Figure 4: Age distribution of RVA gastroenteritis in Central Kenya during the pre-vaccine period (Jul 2009-Jun 2014) and post-vaccine period (July 2014-June 2016) and variations in age distribution of RVA cases between and within the first year (July 2014-June 2015) and the second year (July 2015-June 2016) of vaccine introduction.
4.1.4 Seasonality of Rotavirus Gastroenteritis

The monthly counts of RVA hospitalizations among children <5 years of age substantially decreased in the period between July 2014 and June 2016 (post-vaccine) compared to the period between July 2009 and June 2014 (pre-vaccine) (Fig. 5). Whereas rotavirus infection occurred year-round and peaked mainly in February-April and September-November during the pre-vaccine period, a defined peak of rotavirus infection was observed in July in the post-vaccine period (Fig. 6). Cross-correlation analyses of the relationships between the weekly number of rotavirus diarrhea cases and temperature, relative humidity, precipitation and wind speed showed no significant association between the potential risk of rotavirus gastroenteritis and the climatic variables over lags of 0-20 weeks (data not shown). However, in the crude relationship, the potential risk of RVA gastroenteritis seemed to increase as the temperature and wind speed increased while the precipitation and relative humidity decreased (Fig. 7).

Figure 5: Seasonal variation in the frequencies of RVA gastroenteritis among children admitted for AGE at Kiambu County Hospital, Central Kenya before and after rotavirus vaccination.
Figure 6: Monthly pattern of hospital admissions for RVA gastroenteritis at Kiambu County Hospital, Central Kenya before and after rotavirus vaccination.
b) Relationship of Rotavirus Gastroenteritis with Relative Humidity

![Graph showing relationship between Relative Humidity and Rotavirus cases]

c) Relationship of Rotavirus Gastroenteritis with Precipitation

![Graph showing relationship between Precipitation and Rotavirus cases]
4.1.5 Trends in All-Cause Gastroenteritis

Prior to rotavirus vaccine introduction (July 2009- June 2014), there was a monthly median of 102 admissions to Kiambu County Hospital due to AGE among children <5 years of age. Following the vaccine introduction (July 2014-June 2016), the monthly median fell to 57 cases, representing a reduction of 44.1% (95% CI: 34.7-53.9). The rate of reduction in all-cause hospitalizations increased more than two-fold from 28.4% (95% CI: 20.3-37.8) in the first year of vaccine introduction to 60.8% (95% CI: 51.1-69.9) in the second year. Before vaccine introduction, all-cause AGE exhibited a distinct seasonality, usually with two obvious peaks of during the months of February-April and September-November. Following rotavirus vaccine introduction, the seasonal peaks of all-cause AGE were notably dwarfed (Fig. 8).
Figure 8: Trends in hospital admissions for all-cause AGE at Kiambu County Hospital, Central Kenya in pre- and post–rotavirus vaccine periods.

4.2 Molecular Epidemiology of RVA Strains before and after Vaccine Introduction

4.2.1 Distribution of G and P genotypes

The 429 and 91 samples positive for RVA by ELISA in the pre-vaccine and post-vaccine periods, respectively, were subjected to G and P genotyping by multiplexed semi-nested RT-PCR. A total of seven different G genotypes and three different P genotypes were detected either in single or in mixed infection. Overall, among the G genotypes, G1 most dominant (43%), followed by G9 (15%), G8 (8%), G2 (7%), G3 (5%), G12 (4%) and G4 (3%). Mixed G genotypes constituted 4% of the G genotypes detected (Fig. 9). The G genotypes of the remaining samples (11%) could not be determined. G1 was the most frequently detected genotype in both the pre-vaccine and post-vaccine periods. G3 which had been detected in the pre-vaccine era at only 1% markedly increased in frequency to replace G9 as the second most common strain in the post-vaccine period at 21%. There was a substantial increase in the proportion of G2 strains during the post-vaccine from 5% to 17%. There was a slight decline in the G8 frequency while no G9 was detected after the vaccine introduction (Fig. 9).
Of the P genotypes, P[8] predominated (54%), followed by P[4] (25%), P[6] (10%) and mixed infection, P[4][8] (3%) (Fig. 10). The P genotypes of 40 samples (8%) could not be determined. There were no notable fluctuations in the distribution of the P genotypes between the pre-vaccine and post-vaccine periods (Fig. 9).

Figure 9: Distribution of RVA G genotypes detected in Central Kenya before and after vaccine introduction. GNT refers to non-typeable G genotypes using the existing primer set.

Figure 10: Distribution of RVA P genotypes detected in Central Kenya before and after vaccine introduction. P[NT] refers to non-typeable P genotypes using the existing primer set.
4.2.2 Distribution of G-P Combinations

Based on the global distribution of G-P combinations, various G-P combinations were classified into two groups: usual combinations (G1P[8], G2P[4], G3P[8], G4P[8], and G9P[8]) and unusual combinations (any combinations other than the usual ones). Six usual and fourteen unusual combinations of G and P genotypes were detected during the entire study period (Fig. 11). Of the usual combinations, G1P[8] was the most common genotype in both the pre-vaccine (28%) and post-vaccine (42%) periods. G9P[8] which was the second most dominant strain before vaccine introduction (12%) was replaced by G2P[4] (15%) after vaccine introduction and the strain was not detected during this period. The G3P[8] strain that had never been detected in this area during the pre-vaccine period became the third most common genotype at 14% during the post-vaccine period. Of the unusual G-P combinations, G8P[4] was detected at a lower frequency (2%) after vaccine introduction compared to the 7% before vaccine introduction. On the other hand, G3P[6] which circulated in negligible frequencies in the pre-vaccine period was detected in a considerably higher proportion (6%) in the post-vaccine period (Fig. 11).

4.2.3 Temporal Distribution of G-P Genotypes

Some temporal fluctuations in the G-P genotype distribution were observed year by year. The most dominant G-P combinations in the pre-vaccine era were G1P[8] followed by G9P[4] in July 2009-June 2010; G1P[8] followed by G9P[8] in July 2010-June 2011; G9[8] followed by G8P[4] in July 2011-June 2012; and G1P[8] in July 2012-June 2014 (Table 2). The prevalence of unusual genotypes G8 and G12 decreased significantly after 2013. G8 genotype which was the third and second most dominant G genotype in July 2011-June 2012 and July 2012-June 2013 seasons, respectively, was detected in negligible proportions in the subsequent years. Similarly, genotype G12 having circulated in considerably high frequencies during the July 2012-June 2013 season markedly declined in proportion after this season. Interestingly, G1P[8] predominated only in the first year of vaccine introduction and this strain was replaced by G2P[4] during the second year. G3P[8] and G3P[6] which had circulated in relatively high frequencies in the first year of vaccine introduction declined in proportions in the second year. Thus, the major G-P combination shifted from G1P[8] to G9P[8] and G8P[4] and vise-versa in the pre-vaccine period and from G1P[8] to G2P[4] in the second year of the post-vaccine period (Table 2).
Figure 11: Distribution of G-P genotypes of RVAs detected in Central Kenya before and vaccine introduction. Partially untypeable refers to those strains whose either G or P genotype could not be detected using the existing primer set. Fully untypeable refers to those strains whose both G and P genotypes could not be detected using the existing primer set.
Table 2: Yearly Distributions of G-P Genotypes of RVAs between 2009 and 2016 in Kenya

<table>
<thead>
<tr>
<th>G-P Combination</th>
<th>Number of RVA Strains in a Year</th>
<th>Pre-vaccine</th>
<th>Post-vaccine</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Usual strains</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1P[8]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G3P[8]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G4P[8]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G9P[8]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unusual strains</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1P[4]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1P[6]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G2P[8]</td>
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<td></td>
<td></td>
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<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>G3P[6]</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>G4P[4]</td>
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</tr>
<tr>
<td>G8P[4]</td>
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</tr>
<tr>
<td>G8P[6]</td>
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<td></td>
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<tr>
<td>G8P[8]</td>
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<td></td>
</tr>
<tr>
<td>G9P[4]</td>
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<td></td>
</tr>
<tr>
<td>G9P[6]</td>
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<td></td>
</tr>
<tr>
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<tr>
<td>G12P[6]</td>
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<tr>
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</tr>
<tr>
<td>Partially</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>untypeable</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fully untypeable</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Total</td>
<td>76</td>
<td>104</td>
<td>129</td>
<td>75</td>
</tr>
</tbody>
</table>

4.2.4 Association of G-P Genotypes with Rotavirus Peak Seasons

Although RVA-associated hospitalizations occurred all through the year during the surveillance period, distinct peaks were observed in various months of each year (Fig. 5). A look at the rotavirus strain prevalence in the most pronounced peaks revealed varied strain predominance associated with each peak (Fig. 12). Whereas G1P[8] dominated in most of the peak seasons (June 2010; February-May 2011; November 2012; June 2013; July 2014; and September 2015), G9P[4] (30%) and G9P[8] (31%) were the most commonly detected strains in the August-September and September-October 2011 seasons, respectively. The highest RVA
peak in the entire surveillance period was observed in February 2012. During this peak season, the unusual G8 genotype in association with P[4] (34%) and P[8] (11%) was the predominant strain. The strain was also found in mixed infections which constituted 29% of all the strains detected in this peak season (Fig. 12). Following vaccine introduction, three rotavirus seasons were observed. Despite G1P[8] dominating in all these seasons, there were substantial proportions of G2P[4] (20%) and G3P[8] (20%) in the July 2014 season; G3P[8] (40%) in almost equal measures with G1P[8] (53%) in the February-March 2015 season; and a considerable prevalence of G3P[6] (17%) in the September 2015 season (Fig. 12).

Figure 12: Variation in the prevalence of RVA strains during the peak seasons of RVA gastroenteritis among children admitted to Kiambu County Hospital, Central Kenya in the surveillance period (July 2009-June 2016). Red, blue and green bars refer to the most dominant, second most dominant and the third most dominant rotavirus strain in each peak season.

4.2.5 Association of G-P Genotypes with Age

The age specific distribution of G-P genotypes is shown in Table 3. There was apparent variation in the G-P genotype distribution among children of different age groups. I observed a tendency of the RVAs with unusual G-P combinations being detected more frequently in the younger age groups. The ratios of unusual G-P combination: usual G-P combination were as follows: 35:38 (0.92) in 73 RVA strains in 0-5 months, 130:121 (1.07) in 251 RVA strains in 6-
11 months, 66:79 (0.84) in 145 RVA strains in 12-23 months and 20:31 (0.65) in 51 in RVA strains in 24-59 months.

Table 3: Association of G-P Genotypes with Age in Kenya during the period between 2009 and 2016

<table>
<thead>
<tr>
<th>Strain</th>
<th>0-5</th>
<th>6-11</th>
<th>12-23</th>
<th>24-59</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Usual</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1P[8]</td>
<td>19</td>
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<td>23</td>
<td>163</td>
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<td>G2P[4]</td>
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<td>15</td>
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<td>23</td>
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<td>G3P[8]</td>
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<td>14</td>
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<td>8</td>
<td>35</td>
<td>10</td>
<td>4</td>
<td>57</td>
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<tr>
<td>Unusual</td>
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<td></td>
</tr>
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</tr>
<tr>
<td>G1P[6]</td>
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<td>5</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
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<td>3</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>G3P[4]</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>G3P[6]</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>G4P[4]</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>G8P[4]</td>
<td>8</td>
<td>17</td>
<td>7</td>
<td>2</td>
<td>34</td>
</tr>
<tr>
<td>G8P[6]</td>
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<td>0</td>
<td>0</td>
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<td>G8P[8]</td>
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<td>0</td>
<td>7</td>
</tr>
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<td>G9P[4]</td>
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<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>G12P[4]</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>G12P[6]</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>G12P[8]</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Mixed</td>
<td>6</td>
<td>17</td>
<td>9</td>
<td>6</td>
<td>38</td>
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<tr>
<td>Partially untypeable</td>
<td>9</td>
<td>36</td>
<td>19</td>
<td>7</td>
<td>71</td>
</tr>
<tr>
<td>Fully untypeable</td>
<td>0</td>
<td>9</td>
<td>1</td>
<td>1</td>
<td>11</td>
</tr>
</tbody>
</table>

| Total    | 73  | 251 | 145  | 51   | 520  |

Following rotavirus vaccine introduction, there was a rising prevalence of G2P[4], G3P[8] and G3P[6]. In view of this observation, I examined the age distribution of these strains in an effort to shed some light on whether their increased prevalence could be attributed to vaccine pressure. Although G2P[4] was more commonly detected among the children aged <1 year during the pre-vaccine period, the strain was almost equally distributed across all age groups in the post-vaccine period, with slightly more cases among the children aged >2 years.
G3P[8] circulated in negligible proportions among children <2 years old in the pre-vaccine period whereas in the post-vaccine period, the strain was detected in higher proportions and across all age groups. As for G3P[6], the strain was found only in children <1 year old and in all age groups before and after vaccine introduction, respectively (Fig. 13).

![Figure 13: Age distribution of rotavirus G2P[4], G3P[8] and G3P[6] strains in Kenya before (July 2009–June 2014) and after (July 2014–June 2016) rotavirus vaccine introduction.](image)

**4.3 Phylogenetic Analysis of the Kenyan G8 and P[6] RVA Strains**

Among the unusual G or P genotypes, G8 and G12 of the G types, and P[6] of the P types have been the characteristic genotypes in Kenya. Since the whole genome sequences of the G12 RVAs detected in this surveillance had been analyzed in the past [Komoto et al., 2014], in this thesis, the VP7 gene sequences of five G8 strains: KDH861 (G8P[6]), KDH897 (G8P[4]), KDH908 (G8P[4]), KDH1073 (G8P[4]), and KDH1075 (G8P[4]), and the VP4 gene sequences of three P[6] strains: KDH783 (G1P[6]), KDH789 (G1P[6]), and KDH861 (G8P[6]) were described. On phylogenetic analysis, all the five Kenyan G8 VP7 gene sequences clustered in lineage 6, which includes most African G8 strains (Fig. 14). The identities of the VP7 gene sequences among the G8 strains in lineage 6 were very high (98.5-99.6%). In relation to some other Kenyan human G8 strains for which VP7 gene sequences are available in the GenBank database, the VP7 gene sequences of all the Kenyan G8 strains analyzed in this study were more
closely related to strain 1290 than both KY6914/02 and KY6950/02, although they all clustered in lineage 6. On the other hand, all the Kenyan strains were distantly related to strain B12 which clustered in lineage 2 (Fig. 14). On phylogenetic analysis of the VP4 gene sequences of three Kenyan P[6] strains, the VP4 gene sequences of KDH783 (G1P[6]) and KDH789 (G1P[6]) were found to be very similar to that of Kenyan G12P[6] strain (KDH633), but were distinct from that of KDH861 (G8P[6]) (Fig. 15).

**Figure 14:** Phylogenetic tree constructed from the nucleotide sequences of the VP7 genes of G8 RVAs in Kenya. The strains labeled with filled circles represent the G8 RVAs in Kenya analyzed in this study. The scale bar indicates genetic distance.
**Figure 15:** Phylogenetic tree constructed from the nucleotide sequences of the VP4 genes of P[6] RVAs in Kenya. The strains labeled with filled circles represent the P[6] RVAs in Kenya analyzed in this study. The scale bar indicates genetic distance.
4.5 Rotavirus Vaccination Coverage in Kiambu Sub-County

In this thesis, I estimated the percentage of rotavirus immunization coverage and dropout rates in Kiambu sub-county for the period between August 2014 and April 2016 using administrative coverage data on rotavirus vaccinations. Overall, rotavirus immunization coverage for dose 1 and 2 in Kiambu sub-county was 124.3% and 109.7%, respectively, demonstrating a good access to rotavirus vaccine in this sub-county. The overall vaccine dropout rate was 11.9%, indicates a slightly poor utilization of the vaccine in this area. The vaccination coverage for the first year of the post-vaccine surveillance period (August 2014-June 2015) was 107.4% for dose 1 and 96.6% for dose 2 representing a dropout rate of 16.5%. During the second year of vaccine introduction (July 2015-April 2016), the coverage for dose 1 and 2 increased to 133.8% and 124.5%, respectively, with a low dropout rate of 7.2% (Fig. 16).

![Rotavirus vaccination coverage graph](image)

Figure 16: Rotavirus vaccination coverage in Kiambu sub-county as estimated from vaccine administrative data for the period between August 2014 and April 2016. The vaccine administrative data was used with permission from the Kiambu sub-county Unit of Vaccines and Immunization Services (UVIS).
CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Rotavirus Disease Epidemiology and Genotypic Diversity of Strains before and after Vaccine Introduction

5.1.1 Prevalence of Rotavirus and All-Cause Gastroenteritis

With the imminent availability of rotavirus vaccines in Kenya, I conducted a hospital-based surveillance in Central Kenya to determine the disease burden and molecular epidemiology of rotavirus gastroenteritis before the vaccine introduction and to assess the early impact of the vaccine on rotavirus disease epidemiology and strain distribution during the first two years of routine programmatic use of the vaccine.

Before vaccine introduction, RVA was detected in 27.5% of children <5 years of age hospitalized with acute gastroenteritis in this study. This finding is consistent with those from the recent pre-vaccine introduction studies carried out in different regions of Kenya that have indicated an overall high prevalence of RVA infection ranging from 27% to 38% [Khagayi et al., 2014; Kiulia et al., 2014; van Hoek et al., 2012; Nokes et al., 2008]. The high infection rate of rotavirus may be associated with the dual condition of extremely high virus concentration in faeces (more than $10^9$ virus particles/g) of symptomatic and asymptomatic individuals and the low inoculums (10–100 virus particles) required for infection [Grassi et al., 2009]. Similarly, several authors have suggested that the ability of rotavirus to remain viable on inanimate surfaces for several days when dried from a fecal suspension provide indirect evidence to show that fomites and environmental surfaces possess a strong potential for spreading rotavirus gastroenteritis [Ansari et al., 1991; Haffejee, 1995; Torok et al., 1997]. In addition, widespread viral contamination of water bodies and prolonged persistence of infective virus in ground and surface water [Espinosa et al., 2008] may also act to contribute to the high prevalence rate of rotavirus infection observed in this study.

Following the nationwide introduction of rotavirus vaccine, there was about 50% reduction in the rate of rotavirus gastroenteritis among children aged <5 years during the post-vaccine period compared with the pre-vaccine period. Furthermore, comparing the incidence of
hospitalizations for all-cause AGE at Kiambu County Hospital before and after rotavirus vaccine implementation, I observed a downward trend amounting to a 44% reduction in the number of all-cause AGE hospitalizations coinciding temporally with the timing of rotavirus vaccine introduction. The marked reductions in rotavirus AGE and all-cause hospitalizations observed in this study following rotavirus vaccine implementation in Kenya are consistent with what has been observed in several middle-income countries in the Americas and early vaccine introducing low-income countries of Africa. Several middle-income countries in the Americas have reported a 49%–89% decline in rotavirus hospitalizations and 17%–55% decline in all-cause gastroenteritis hospitalizations among children aged <5 years within two years of vaccine introduction [Patel et al., 2012; 2011; Richardson et al., 2010; do Carmo et al., 2011]. Among the early introducing African countries, reductions in all-cause gastroenteritis have been reported to range from 18% and 65% following the routine rotavirus immunization [Enane et al., 2016; Groome et al., 2016; Tsolenyanu et al., 2016; Mpabalwani et al., 2016; Bar-Zeev et al., 2016; Ngabo et al., 2016; Parashar et al., 2016]. In addition, declines in rotavirus-associated hospitalizations have been shown to range between 24% and 49% following the nationwide introduction of rotavirus vaccination in these countries [Tsolenyanu et al., 2016; Mpabalwani et al., 2016; Armah et al., 2016; Bar-Zeev et al., 2016; 2015].

Although I did not determine the rotavirus vaccine effectiveness (VE) due to challenges with record keeping regarding vaccination coverage among the study population, it is interesting to note that the vaccine impact on rotavirus gastroenteritis observed in this study is consistent with estimates from the limited pre-licensure clinical trials and observational studies in Kenya and elsewhere in Africa. Clinical trials of the two-dose Rotarix® in Malawi and South Africa found an efficacy of 49.4% and 76.9% against severe rotavirus gastroenteritis, respectively [Madhi et al., 2010]. The efficacy of the three-dose RotaTeq® vaccine against severe rotavirus gastroenteritis was 17.6% in Mali and 55.5% in Ghana, with Kenya recording the highest point estimate at 63.9% [Armah et al., 2010]. In post-vaccine introduction evaluations of routine Rotarix® use in Africa, VE has been estimated at 58%–64% in Malawi [Bar-Zeev et al., 2016; 2015]; 54% (95% CI, 32%–68%) in South Africa [Groome et al., 2014]; and 60% (95% CI, −2% to 84%) in Ghana [Armah et al., 2016]. Following vaccine introduction, reductions in rotavirus hospitalizations of 54% and 58% in the first and second years (2010 and 2011), respectively, were observed in South Africa [Msimang et al., 2013]; 43% in Malawi [Bar-Zeev et al., 2015];
and 49% in Ghana [Armah et al., 2016]. My observed 49.8% reduction in rotavirus hospitalizations in Kenya is quite similar to that of South Africa, Malawi and Ghana, all of which are using Rotarix® in their expanded programmes on immunization. Taken together, these data may be extrapolated to speculate the rotavirus vaccine effectiveness in Kenya to being within the range of that which has been observed in these African countries (54%-64%).

Since rotavirus vaccine introduction, I have observed successive reductions in rotavirus and all-cause diarrhoea hospitalizations each year concurrent with increasing vaccine coverage. The countrywide rotavirus vaccine coverage increased from 38% in 50% of the national target Kenyan population in 2014 and 66% in 2015 [WHO/UNICEF, 2015]. In this thesis, a high vaccine coverage in Kiambu sub-county is demonstrated and which seems to increase with subsequent years of the vaccine introduction. The high rotavirus vaccine coverage in Kiambu sub-county indicates good access to the vaccine in this area and may be attributed to the enhanced social mobilization and communication activities by the sub-county UVIS. Several Community Health Officers (CHOs) and Community Health Volunteers (CHVs) are also engaged in conducting health talks in the community and tracing vaccine defaulters. In a recent national survey which did not cover rotavirus vaccine, full immunization coverage was high among children from households in the highest wealth quintile (71%) or whose mothers have attended a secondary school (74%) [KDHS, 2015]. This association was most distinct for the series vaccinations. Kiambu is among the sub-counties in the highest wealth quintile and with high literacy level [KNBS, 2010; KDHS, 2015]. Rotavirus vaccine is given alongside the series vaccines. These two factors might also help explain the high coverage of this vaccine in Kiambu sub-county. However, since disparities in vaccination coverage in Kenya have been reported [KDHS, 2015; Calhoun et al., 2014; Mutua et al., 2011; Maina et al., 2013], efforts should be made to strengthen the immunization system to facilitate equitable delivery of vaccination services across the country in order to maximize the benefits of the rotavirus vaccination.

Taken together, these data suggest notable reductions in rotavirus and all-cause AGE hospitalizations commensurate with high vaccine coverage following the introduction of rotavirus vaccine into the routine immunization program in Kenya. As the nationwide coverage rates of the two doses of rotavirus vaccine rise to reach the overall coverage of 97% of first two doses of the pentavalent vaccine [KDHS, 2015], I anticipate even greater declines in the burden of severe AGE in Kenyan children.
5.1.2 Age Distribution of Rotavirus Gastroenteritis

Before vaccine introduction, RVA infections occurred in all age groups from 0-59 months, with an early peak at 6-8 months of age. Interestingly, RVA could be detected in seven neonates (1.6%). A similar phenomenon of RVA infecting infants early in life is common in developing countries [Cunliffe et al., 1998; Mulholland et al., 2008; Glass et al., 2013], and is noteworthy in the contextual definition of an optimal RVA immunization schedule. Ideally, an optimally effective vaccination schedule should aim to induce immunity before a sizeable proportion of the target population acquires natural infection. Thus, completion of the rotavirus immunization schedule early in infancy is desirable for such a low-income setting as Kenya in order to maximize the vaccine impact. In addition, there was a drastic reduction in the number of cases of RVA infection among children aged >18 months (12.4%). This finding concurs with the WHO Strategic Advisory Group of Expert (SAGE)’s observation that providing RVA vaccine to children >24 months of age will be of little benefit [WHO, 2012].

Following the introduction of rotavirus vaccine, the most pronounced reductions in rotavirus positivity were noted in infants <12 months of age, who constitute the vaccine-eligible population. These reductions were sustained in this age group in both the first and the second year of vaccine introduction, with the second year recording a greater decline in RVA-associated gastroenteritis among the children aged 12-23 months than the first year. These observations are consistent with what has been reported by several African countries that were among the early adopters of rotavirus vaccine describing greater initial declines in the proportion of RVA cases among younger age groups that receive vaccination in the initial years of the vaccination, followed by a progressive decline in older age groups in later years after introduction, when the vaccine coverage increases among these older children [Enane et al., 2016; Groome et al., 2016; Armah et al., 2016; Tsolenyanu et al., 2016; Mpabalwani et al., 2016].

Consequently, the age distribution of rotavirus cases tended to shift toward older age groups, with an evident steady increase in disease incidence in children >2 years old over the two-year post-vaccine period. Similar observations have been reported in other African countries [Enane et al., 2016; Groome et al., 2016] and are suggestive of waning immunity conferred by the vaccines and a lack of indirect protection (i.e., herd immunity) from these vaccines to the unvaccinated individuals in these settings. Although indirect protection from rotavirus
vaccination has been well documented in Australia and developed countries in the Americas and Europe [Gastanaduy et al., 2013; Clarke et al., 2011], it is unclear if these observations will extend to developing countries, given differences in population age-group structure and intensity of viral transmission. If herd immunity is not achieved with these vaccines, waning immunity is likely to manifest as resurgence in disease in older groups. Thus, further evidence is required to understand the extent of herd protection across a range of geographic and socio-economically diverse settings.

5.1.3 Seasonality of Rotavirus and All-Cause Gastroenteritis

Before vaccine introduction, RVA was found year-round with seasonal peaks occurring in most years in February-April and September-November. This observation is consistent with recent country-level assessment of RVA epidemiology conducted in various tropical countries in anticipation of RVA vaccination programs [Levy et al., 2009]. The assessment revealed that seasonal peaks occur year-round in different tropical countries and can vary over time in the same country. However, the effect of such seasonal changes on RVA infection is not as extreme as is seen in temperate areas of the world [Cook et al., 1990]. One possible explanation for this is that less climatic variability exists in tropical zones, therefore, the variations in climatological variables are not large enough to cause an effect similar to that in temperate areas [Atchison et al., 2010; D’Souza et al., 2008].

Attempts to relate the seasonal patterns of RVA infections in this study to climatic variables such as temperature, rainfall, relative humidity and wind speed by time-series analysis established no statistically significant association (data not shown). However, in the crude relationship, the potential risk of RVA gastroenteritis seemed to increase as the temperature and wind speed increased while the rainfall and relative humidity decreased. This finding concurs with the earlier observations that a relative drop in humidity and rainfall combined with drying of soils in higher temperatures might increase the aerial transport of dried, contaminated fecal material (in the form of droplet nuclei), and might also lead to increased formation of dust, which could provide a substrate for the virus particles [Ansari et al., 1991; Hashizume et al., 2008]. Airborne particles could settle out and infect water supplies or environmental surfaces, or could be ingested [Haffejee, 1995]. Some mechanical force would likely be required for aerosolization to occur, and wind might play this role, as well as help disperse the particles once formed. In a
four-year study of rotavirus in Pune, India, a tight correlation was seen between number of days with easterly wind and the number of rotavirus diarrhea cases as functions of time [Purohit et al., 1998]. Therefore, the observation in this study of the crude relationship between rotavirus gastroenteritis and climatic variables, alongside the fact that RVA responds to climatic changes in many different climatic zones throughout the world suggests that paying close attention to local climatic conditions may help improve our understanding of the transmission and epidemiology of RVA disease.

Similar to rotavirus-associated gastroenteritis, the all-cause AGE exhibited a distinct seasonality, mainly with two pronounced peaks during the months of February-April and September-November, before vaccine introduction. However, following the introduction of rotavirus vaccination, I observed greater declines in rotavirus and all-cause AGE hospitalizations among children aged <5 years at Kiambu County Hospital, with the great majority of the reductions occurring during the months of the year with seasonal peaks of rotavirus disease and all-cause gastroenteritis, further providing evidence that the declines could be attributed to the effect of rotavirus vaccination. Similar observations of declines in hospitalizations from rotavirus and all-cause gastroenteritis have been reported elsewhere in Africa following rotavirus vaccine introduction [Enane et al., 2016; Groome et al., 2016; Tsolenyanu et al., 2016; Mpabalwani et al., 2016] and will require continued monitoring to assess if the observed changes can be sustained over a long term.

5.1.4 Genotypic Diversity of Rotavirus Strains

Remarkable genetic diversity of RVA strains, characterized by substantial frequencies of unusual, emerging, mixed and untypeable genotypes, was observed during the pre- and post-vaccine introduction periods of the surveillance. Of the seven G genotypes detected, G1 was the most dominant genotype detected in the entire study period, followed by G9, G8, G2, G3, G12 and G4. Three different P genotypes were detected throughout the surveillance with P[8] being the most commonly detected, followed by P[4] and P[6]. The prevalence of G1 has remained predominantly high in various settings in Kenya over the last three decades [Kiulia et al., 2008; Nyangao et al., 2010; Nokes et al., 2010]. In this study, the unusual association of G1 with P[4] and P[6] is noteworthy. The G9 strain was found mainly associated with P[4] and P[8]. This strain was detected in Kenya for the first time in the late 1990s, and has displayed a marked
fluctuating prevalence in different settings in the country from time to time [Steele and Ivanoff, 2003; Kiulia et al., 2008]. The G9 strain was not detected in this area from the year 2013 all through the post-vaccine period of this study.

The globally uncommon G8 strains has become increasingly prevalent in many parts of Africa [Cunliffe et al., 1998; Mwenda et al., 2010], and have been recovered sporadically from humans and animals like pigs and cows worldwide [Esona et al., 2009]. The G8 strains were identified for the first time in Kenya in the early 1990’s [Nakata et al., 1999], and various studies have described their prevalence in the country to range from 4.7% to 16.6% [Nyangao et al., 2010; Nokes et al., 2010; Kiulia et al., 2014]. In this study, G8 strains, in association with P[4], P[8] and P[6], were detected at an overall prevalence rate of 8% throughout the study period, with considerably high detection rates of 11% in 2011 and 16% in 2012. G8 was the most dominant genotype in February 2012, which was the highest rotavirus peak recorded in this entire surveillance. The genotype in association with P[4], P[6] and P[8] constituted 48% of all the single strains detected in this peak. The strain was also the most commonly detected one in mixed infections that formed 29% of all the strains detected during this peak. However, since 2013, the proportion of G8 strains has relatively declined and the strain was detected in low frequencies in this study during the post-vaccine period. The partial genome sequence analysis of some of the G8 strains detected in this study is discussed below.

The G12 strains, first identified in 1987 [Taniguchi et al., 1990; Urasawa et al., 1990], have increasingly been identified in several African countries in the recent past [Page et al., 2009; Cunliffe et al., 2009; Oluwatoyin et al., 2012; Ndze et al., 2013]. From Kenya, there were no reports of the detection of human G12 strains until 2014, when three papers independently reported the identification of G12 strains from diarrheic children (Kiulia et al., 2014; Seheri et al., 2014; Komoto et al., 2014). In this study, I observed a substantial detection of G12 strains (15%), in combination with P[6], P[4] and P[8] in 2012, thereby confirming the growing transmission and increased medical importance of these strains in Kenya. However, similar to the G9 strain, the prevalence of G12 strain in this study area has notably diminished since 2013.

The globally usual G genotypes G2, G3 and G4 circulated at remarkably low frequencies during the pre-vaccine period. Notably, G3 which predominantly circulated in Kenya in the late 1990s and early 2000s, was found to be notably absent in the recent studies conducted in the country before vaccine introduction [Kiulia et al., 2008], and this strain had seemed to be
vanishing from circulation in Kenya. A similar temporal decline of G2, G3 and G4 in Africa prior to rotavirus vaccine introduction have been reported [Bányai et al., 2012]. However, during the first two years after rotavirus vaccine introduction in Kenya, I observed an upsurge of G2P[4] (15%), G3P[8] (14%) and G3P[6] (6%). Of note, G3P[8] strain which was never detected in this area during the pre-vaccine period became the second most common genotype in first year of the post-vaccine period. Interestingly, G2P[4] which circulated in negligible frequencies in the pre-vaccine period became the most dominant strain in the second year of the post-vaccine period.

There is still lack of a broad scientific consensus regarding the long-term effects of vaccination on the circulating RVA strain distribution. Although changes in RVA genotype distribution have been observed following mass vaccination with Rotarix and/or RotaTeq in Brazil, the United States and some Australian states [Carvalho-Costa et al., 2011; Hull et al., 2011; Kirkwood et al., 2011], it remains unclear if these changes can be attributed to the vaccines. In Belgium, where Rotarix is mainly used, a substantial long-term increase in the proportion of G2P[4] genotype was noted following vaccine introduction [Zeller et al., 2010], with G2P[4] being detected more frequently in vaccinated children, thereby suggesting that Rotarix may exert selective pressures on the RVA genotype distribution [Matthijnssens et al., 2014]. Recently, mathematical models have estimated that natural- and vaccine-derived immunity was strongest against completely homotypic strains and weakest against fully heterotypic strains, with an intermediate immunity amongst partially heterotypic strains [Pitzer et al., 2015]. Applying these models, the authors could postulate that the predominance of G2P[4] infections in Belgium after vaccine introduction was due to a combination of natural genotype fluctuations and weaker natural and vaccine-induced immunity against infection with strains heterotypic to the vaccine, in the absence of significant variation in strain-specific vaccine effectiveness against disease [Pitzer et al., 2015].

In Africa, a few studies have reported changing dominance in rotavirus strain distribution following the vaccine introduction. In Ghana, G1P[8] which had predominated in the pre-vaccine years was replaced by G12P8] as the most common strain, followed by G12P[6], G2P[4] and G3P[6] [Armah et al., 2016]. In Malawi, the dominance of G2 genotype in the season following vaccine introduction in Malawi [Bar-Zeev et al., 2015]. However, a follow-up study found that many of the G2 cases had occurred in children age-ineligible for vaccination and the detection rates of the strain decreased in subsequent seasons with increasing vaccine coverage data,
thereby suggesting that the rising G2 incidence at the time of vaccine introduction in Malawi was likely due to temporal oscillation [Bar-Zeev et al., 2016]. The study also estimated the vaccine effectiveness against the major circulating genotypes and found higher point estimates of VE against the G1 genotype; highest of all against fully homotypic G1P[8] genotypes; and lowest for totally heterotypic G2P[4] strains.

Temporal and geographical rotavirus genotype fluctuations have been detected in many countries [Bányai et al., 2012; Iturriza-Gomara et al., 2011; Zeller et al., 2010] including Kenya [Mwenda et al., 2010; Nokes et al., 2010; Nyangao et al., 2010; Kiulia et al., 2014] before the introduction of vaccines. In this study, temporal fluctuations in rotavirus genotypes was observed, with major shifts in G-P predominance involving G1P[8] to G9P[8] and G8P[4] and back to G1P[8] in the pre-vaccine period and G1P[8] to G2P[4] in the second year following vaccine introduction. The G2P[4] strains which were most commonly detected among the children aged <1 year during the pre-vaccine period, became most frequently detected among the children aged >1 years in the post-vaccine period. However, in the absence of data on strain-specific vaccine effectiveness in this setting and the short post-vaccine introduction period that could not allow for monitoring for any sustained predominance of G2P[4] strain, it is difficult to conclusively attribute the changing prevalence of this strain to vaccine-induced selective pressure. It is likely that the rising G2P[4] prevalence following vaccine introduction in Kenya is due to temporal fluctuations as many of the cases occurred in children age-ineligible for vaccination.

In this study, there was a tendency that unusual G-P combinations such as G8P[4], G8P8], G9P[4] and G1P[4] were detected more in the younger age groups. In the older age groups, the cases may have had sufficient immune potency of cross-protection against the various genotypes through multiple infections against human RVAs. Recently, a study on comparative analysis of the RotaTeq vaccine strains, which possess a bovine genetic backbone and G8 rotavirus strains identified during vaccine trials in Ghana, Mali and Kenya has shown that the high vaccine efficacy against the G8 strains might be partially explained by the fact that all these strains contain a complete or partial bovine-like backbone [Heylen et al., 2015]. The study also provided evidence to support the hypothesis that other gene segments besides VP7 and VP4 play a role in vaccine-induced immunity. Nonetheless, it’s worth noting that most of the African countries including Kenya have introduced Rotarix into their national immunization
programmes. Although studies have shown that Rotarix protects against a variety of rotavirus strains, waning immunity to partly or fully heterotypic strains (ie, strains that share either the G or P type components of the vaccine strain, or share neither component, respectively) may be of concern in these poor settings where genotypic diversity is common, characterized by high frequencies of unusual genotypes [Leshem et al., 2014]. Thus, it will be insightful to evaluate the vaccine effectiveness against the unusual genotypes.

During the study, we also detected a substantial amount of mixed infections, including both the unusual and emergent combinations. The epidemiological implications of these mixed infections and unusual strains remain to be elucidated. Nonetheless, the high proportions of these strains point to possible natural reassortment events arising from co-circulating local strains. Complete genome sequencing of the strains would be necessary to help determine any degree of natural reassortment.

5.1.5 Phylogenetic Analysis of G8 and P[6] Strains

Phylogenetic analysis showed the clustering of the VP7 gene sequences of all the five Kenyan G8 in lineage 6, which consist of most of the African G8 strains. Previously, the whole or partial genome characterization of a number of Kenyan G8 strains has been reported [Page et al., 2010; Ghosh et al., 2011; Heylen et al., 2015]. Following the characterization of G8 strains from Malawi, South Africa and Kenya, Page and colleagues [2010] observed that the VP7 gene sequences of the Kenyan G8 strains clustered on the phylogenetic tree based on the year of isolation, with the 2002 strains related to a Brazilian strain from the same year and the 1991 and 1999 strains related to “southern African” strains from Malawi and South Africa. In a recent study on comparative analysis of the RotaTeq vaccine strains and G8 rotavirus strains identified during vaccine trials in Ghana, Mali and Kenya [Heylen et al., 2015], the VP7 gene sequences of all the eight G8 strains were found in three distinct clusters according to their country of origin in relation to other African strains, thereby providing evidence for geographic segregation of G8 RVAs in Africa. The Kenyan G8P[6] strain investigated in this study was distantly related to both the Ghanaian and Malian strains. By phylogenetic analysis, Ghosh et al. [2011], found that strain B12, the first human G8P[1] strain which was identified in an asymptomatic child in Kenya in 1987, clustered with bovine G8 strains, away from the clusters comprising other human G8 strains. In the current study, the VP7 gene sequences of all the five Kenyan G8 clustered
separately from the B12 strain. Taken together, the Kenyan G8 strains have demonstrated their tendency phylogenetically cluster based on the year of isolation and the region/country of origin in relation to other African strains.

Similar to the G8 strains, the whole or partial genomes of a few Kenya P[6] strains that have so far been analyzed have demonstrated regional segregation of their VP4 gene sequences. The Kenyan P[6] strains have been found to cluster together with the southern African strains from Malawi and South Africa [Page et al., 2010; Komoto et al., 2014] but not with the West African strains [Heylen et al., 2015]. In addition, the Kenyan P[6] strains were found to be more closely related to human P[6] strains, rather than to porcine P[6] strains in a study conducted by Heylen et al. [2015]. Interestingly, the two G1P[6] and one G8P[6] strain analyzed in my thesis formed distinct clusters, thereby providing evidence that there are at least two groups of P[6] RVAs circulating in Kenya.

5.1.6 Study Limitations

It is worth pointing out that an ecological observation study such as this has some limitations. First, I had no control on the quality of the hospital administrative data on all-cause gastroenteritis admissions and the administrative data on rotavirus vaccination coverage provided by the sub-county’s Unit of Vaccines and Immunization Services (UVIS). There were gaps in the data available from admission registers. These were supplemented as completely as possible with hospital records that should provide comparable data. Second, the observed reduction in all-cause diarrhea did not take into account seasonal trends in other causes of diarrhea or other interventions, such as improvements in sanitation or changes in hospital referral patterns, which may have impacted diarrhea hospitalizations. The yearly total hospitalizations due to diarrhoea at the study facility were stable all through the pre-vaccine period, suggesting that there was not a pre-existing secular trend before vaccine introduction. To my knowledge, there were no abrupt changes in hospital referral patterns at the study facility and area. Furthermore, one would assume that changes in sanitation would consistently improve over the years, leading to a steadier decline in annual diarrheal hospitalizations, rather than the more accelerated decline that we observed in the post-vaccine era. Thus, the large reductions in hospitalizations observed over a short period of time, which were concentrated among the rotavirus vaccine-eligible children
and during the rotavirus season and sustained over the two years across are unlikely to be explained by such changes if any. Third, the study has not evaluated rotavirus vaccination status of each individual child, which could allow for the estimation of vaccine effectiveness. However, this process is currently in progress. Fourth, vaccine administrative data was used to estimate the rotavirus vaccine coverage in Kiambu sub-county. Administrative coverage data have the advantage of being available at all levels of the health system with very little delays, which enables program managers to do real-time monitoring, investigate potential problems and take remedial action [Cutts et al., 2013]. However, these estimates can be biased due to inaccurate numerators or denominators, errors in recording vaccinations at health facilities and errors in compiling data on vaccinations for reporting to higher levels [WHO, 2015].

5.2 Conclusions

Data from this study on rotavirus strain characterization and disease burden before vaccine introduction highlight the importance of rotavirus infections in the overall burden of diarrheal diseases in children aged <5 years in Kenya, and thus strongly support the recent introduction of rotavirus vaccine into the country’s expanded program on immunization.

The accelerated decline in rotavirus AGE and all-cause diarrhea hospitalizations observed over a short period of time following the rotavirus vaccine introduction and which were most pronounced among the rotavirus vaccine-eligible children and during the rotavirus season and sustained over the two years are suggestive of a significant public health impact of rotavirus vaccination in Kenya. To my knowledge, this is the first report of the real-world impact of rotavirus vaccination in Kenya since the introduction of the vaccine. Thus, this finding has implications for Kenya as the country is set to graduate from the GAVI support and will consequently be responsible for financing her national vaccination programme. In this regard, my data provides early evidence for health administrators and policy makers in Kenya to support the sustained use of rotavirus vaccines in the routine national immunization programme. In addition, these data in conjunction with findings from other early-introducing African countries will serve to encourage other countries in the region considering introducing rotavirus vaccination into their national immunization programs.

Demonstration of the protective effect of rotavirus vaccination in this study is quite encouraging, as it is in populations with remarkable genotypic diversity of rotavirus strains,
including unusual, emerging and mixed strains and temporal strain variation. Although there was observed change in distribution of RVA strains such as the re-emergence of G2P[4] and G3P[8] coinciding temporally with the timing of vaccine introduction, the natural fluctuations of the predominant RVA genotypes from year to year in the absence of vaccination, lack of strain-specific vaccine effectiveness assessment and the limited duration of post-vaccine observation, limits any attempts to conclusively attribute the changes to vaccination. Since many of the cases of these strains occurred in children too old for vaccination, it is possible to attribute the upsurge of the strains at the time of vaccine introduction in Kenya to natural fluctuations.

The clustering of all the five Kenyan G8 VP7 gene sequences in the same lineage 6 which is composed of African G8 strains may suggest the geographic segregation of G8 RVAs in Africa. The Kenyan P[6] strains were grouped into two distinct clusters, indicating that there are at least two groups of P[6] RVAs prevailing in Kenya.

5.3 Recommendations

Since the vaccine impact described in this thesis is limited to the first two years after vaccine implementation in Kenya, there is need for continued monitoring to assess whether over the long term the observed changes in rotavirus disease epidemiology are sustained. Vaccine effectiveness studies should also be conducted in the country in order to support evaluation of the impact of vaccination on RVA strain distribution due to the possible vaccine-driven selection pressure. The increasing age of RVA cases underscores the need for continued assessment in case the likely waning immunity might lead to the resurgence of rotavirus disease.

Further studies are also preferable for establishment of the relationship between climate and rotavirus disease, especially in the tropical settings, which may increase our understanding of the transmission and epidemiology of rotavirus disease before after vaccine introduction.

Given that the immunization coverage is essential to maximize the impact of the rotavirus vaccine and crucial in assessing the real-world impact of this vaccine, efforts should be made to facilitate optimal and equitable coverage of the vaccine across Kenya. In addition, vaccination coverage surveys should be conducted in the country to verify administrative coverage estimates and help identify and respond to factors that are specifically associated with rotavirus vaccine coverage at the national and sub-county levels.


Nota Bene

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