MOLECULAR DETECTION OF ROTAVIRUS INFECTIONS AND RISK FACTOR ANALYSIS IN PIGLETS FROM KIAMBU, KENYA.

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Declaration

I declare that this is my original work and has not been used in any institution for award of any degree

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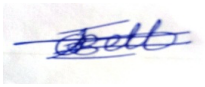
Signature: …  …… Date…7/11/2023……..…

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Dedication

I dedicate this work to my husband Dr. Wyckliff Ng'etich and my sons Aaron, Abel and Adriel who gave me support, encouragement, and motivation throughout the course of this project.

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Abbreviations

RV Rotavirus

AMR Antimicrobial resistance

FAO Food and Agricultural Organization of the United Nations.

KNBS Kenya National Bureau of Statistics

HBGA Histo-blood group antigens

RNA Ribonucleic acids

IDRC International Development Research Centre

CT Threshold cycle

Ig G Immunoglobulin G

Ig A Immunoglobulin A

RVA Rotavirus group A

RVB Rotavirus group B

RVC Rotavirus group C

PCR Polymerase Chain Reaction

RT-PCR Reverse Transcriptase Polymerase Chain Reaction

PBS Phosphate buffered solution

MEM Minimal essential media

PHPT Public Health Pharmacology and Toxicology

VP Viral protein

NSP Nonstructural protein

ELISA Enzyme linked immunosorbent assay

PAGE Polyacrylamide gel electrophoresis

RIG-I Retinoic acid-inducible gene I

DNTP Deoxynucleoside triphosphate

ABSTRACT

Pig production is an important livestock subsector globally contributing immensely to food security and household incomes. Diseases such as rotavirus infections are challenges that hamper its productivity. Rotaviruses are members of the *Reoviridae* family with wheel-shaped appearance when viewed under electron microscopy. There are ten rotavirus groups (A-J) but only five groups (A, B, C, E and H) are known pathogens to pigs. The aim of this study was to detect rotaviruses that circulate in pig farms and to determine the risk factors associated with infections of piglets in Kiambu, Kenya. Two hundred and fifty-five fecal samples from fifty two farms were collected from clinically healthy piglets aged three months and below. Molecular detection of rotavirus geno-groups was done using real time reverse transcriptase polymerase chain reaction (RT-qPCR) and conventional reverse transcriptase PCR. Similarly, semi-structured questionnaires were administered to pig farmers to capture data on their socio-economic characteristics, herd size and pig farm management practices. Out of the 52 farms, 38.5% of them had piglets that were infected with at least one rotavirus group. Rotavirus group A (RVA) was detected in 13.5% of the farms, group C (RVC) in 23.1%, however, group B (RVB) was not detected in any of the farms. One farm had co-infection with RVA and RVC. Animal level prevalence was 16.1% where RVC was more prevalent with 10% followed by RVA with 6% and lastly co-infection with RVA and RVC with a prevalence of 0.4% in the pig farms. Most (61.5%) of the interviewed farmers were males and a high percentage (71.2%) had attained post- primary level of education. More than half (57.8%) of the farmers had kept pigs for less than 6 years and the main reason for keeping them was for sale (84.6%). Half of the farmers kept crossbred pigs and most of them (82.7%) used natural mating as the breeding method and 58.5% produced their own replacement stocks. About sixty five percent reported history of diarrhea in their farms. All the farmers kept pigs in

confinement until they attained market weight. Within most farms, biosecurity measures were less practiced with only 26.9% and 7.7% of the farms practicing disinfection of premises and vaccination of pigs respectively. However, none of the farmers interviewed had vaccinated their pigs against rotavirus. Gender of the pig farmer influenced the occurrence of rotavirus infections in farms; with farms managed by women having a reduced odds of test positivity. Pig houses made of concrete floor and wooden walls, feeding mixed feed and keeping other animals within the farm was shown to reduce the risk of diarrhea in pig farms. In conclusion, porcine rotavirus A and C are circulating in pig farms in Kiambu. Furthermore, there is low levels of biosecurity measures implemented in farms which may support persistence of the virus in pig farms. It is recommended that surveillance systems should prioritize rotavirus infections in pig farms and enhanced farmer education on importance of biosecurity measures to prevent of rotavirus infections to other connected farms.

CHAPTER ONE: INTRODUCTION

1.0 Background information

Rotavirus infection is an important zoonotic viral disease that commonly affects piglets and children (Doro *et al*., 2015). Rotaviruses have a wide host range such as bovine, horses, birds, canines and small rodents (Barros *et al*., 2018). It is caused by an RNA virus from the family *Reoviridae*. The virus is transmitted through the fecal-oral route. It is a frequent cause of viral acute gastroenteritis in weaned and suckling piglets leading to profuse diarrhea (Theuns *et al*., 2016). The diarrhea can persist for up to ten days leading to up to 20% weight loss and eventually stunted growth. This therefore causes significant economic losses to pig farming especially small holder pig farmers. The losses are associated with deaths of piglets, reduced/stunted weight gain and high cost of treatment (Amimo *et al*., 2016). Naïve adult pigs and piglets are most susceptible due their immature immune system and slower rate of enterocyte turn over. Most adult pigs are resistant to infection due to post natural exposure immunity (Amimo *et al*., 2013a).

There are ten Rotavirus genogroups but only five are known to cause infections in pigs. These genogroups are rotavirus A-J but Rotaviruses A, B, C, E and H have been associated with diarrhea in piglets (Vlasova *et al*., 2017). This classification is based on their antigenic relationships with the viral proteins (VP6). These viruses persist in the environment outside the pigs and are resistant to disinfectants. Due to this, the infection is commonly widespread posing a constant threat to pig industry and food security. There are a number of antivirals such as oseltamivir, zanamivir, T-1105 etc to manage viral infections in livestock. However, these products are not effective and therefore antibiotics have been used to prevent secondary bacterial infections. Due to the emergence of antibacterial resistance (AMR) some countries have banned the use of antibiotics and hence the shift to probiotics supplement in pig feeds to prevent diarrhea (Vlasova *et al*., 2016).

Age of the pig, keeping pigs with other animals, source of feeds and water, production system are some of the factors that have been documented to be associated with rotavirus infections in pig farms (Amimo *et al*.,2016; Murao *et al*., 2019; Bwogi *et al*., 2023). These rotaviruses have been shown to have zoonotic potential as some of the isolated porcine rotavirus strains have genetic relatedness with human strains and some human strains have been shown to have animal origin (Doro *et al*., 2015; Amimo *et al*., 2015; Vlasova *et al*., 2017).

1.1 Problem statement

Pig production is an important livestock sub-sector in Kenya. It contributes immensely to food and nutritional security and to the economy (Malik *et al*., 2014). Major challenge to this sector is viral diseases, one of them being Rotavirus. Rotavirus has been reported to be the leading cause of gastro-enteritis in piglets resulting in huge economic and revenue losses and has public health significance as it affects children (Vlasova *et al.*, 2017). Globally, Rotavirus has been reported to be the most prevalent enteropathogens in pig farms (Vlasova *et al*, 2017). A study by Amimo *et al.* (2013b) in USA, identified Rotavirus group C (RVC) and from genetic characterization and phylogenetic analysis revealed that RVC is genetically heterogenic and related to other strains. Amimo *et al.* (2015), detected the presence of Rotavirus group A in asymptomatic pigs from East Africa. They reported genotypes P6, P8 and P13 with P8 being the predominant genotypes from partial sequence of VP4 gene. In Kenya, apart from the study that was done at the border of Kenya and Uganda by Amimo *et al.* (2015), there are no other studies on molecular analysis of porcine rotavirus in the country. Therefore, for the last seven years, rotavirus groups circulating in pig farms in Kenya remains unknown. In 2016, a study was done on risk factors focusing on free range and backyard production system from Western Kenya. In that study it was found out that age and production system influenced the occurrence of rotavirus where piglets less than four months and

pigs kept in free range system had more risk of exposure to rotavirus as compared to older pigs and those tethered/housed respectively (Amimo *et al*., 2016). The risk factors associated with rotavirus infections in intensive/confined production systems in Kenya are unknown. Rotaviruses are extensively and genetically diverse and are very persistent on the environment (Monteagudo *et al*., 2022). These characteristics and the zoonotic nature of the virus necessitates constant surveillance to identify rotavirus groups and the risk factors contributing to infections. Therefore, investigating the types of rotaviruses and the risk factors associated with infections is important in understanding the epidemiology and mitigating measures in pig farms.

1.2 Justification

Due to the persistent nature of the rotavirus in the environment, genetic diversity, the negative economic impact and the zoonotic potential of the disease, there is therefore need for more information on molecular epidemiology of the virus groups that are circulating in Kenyan farms. Determining the types of rotaviruses circulating in pig farms, the possible effects on production and the associated risk factors will help in putting in mitigating measures to control and prevent this viral disease from devastating the farm productivity. The molecular detection of rotavirus will update and expand the available knowledge on circulating types of rotaviruses including new variants that may be in the pig farms. The outcome of this study will contribute immensely to advances on management, control and prevention measures of rotavirus infections in pig production systems therefore increased income and improved human food and nutritional security.

1.3 Research hypothesis

i. Pig farms recruited in the study were not infected with rotaviruses
ii. There are no risk factors associated with occurrence of rotavirus infection in these pig farms

1.4. General objective

To detect rotavirus infections and analyze associated risk factors in piglets from Kiambu, Kenya.

1.5 Specific objectives

i. To detect rotavirus infections in piglets using reverse transcriptase quantitative Polymerase Chain Reaction (RT-qPCR).

ii. To evaluate effectiveness of using conventional reverse transcriptase PCR (cRT-PCR) for detection of rotaviruses as compared to RT-qPCR

iii. To determine the risk factors associated with rotavirus infection in piglets

CHAPTER TWO: LITERATURE REVIEW

2.0 Pig production

Globally, pig production is widely practiced, and pork largely consumed by millions of people as a cheap source of animal protein and its demand is still expected to rise in tandem to the increasing global human population (FAO, 2021). In Africa, pig production is a fast-growing venture and hence posing a potential risk of emergence and spread of infectious diseases to connected systems and farms and a possible spill over to human population with disastrous consequences on health outcomes (Torgerson *et al*., 2015). According to the recent Kenya population and housing census report (KNBS, 2019), there are approximately 442,000 pigs that are kept either in intensive or free-range scavenging systems (Mbuthia *et al*., 2015). The intensive system is mainly practiced within the urban and peri urban areas of the country where land for large scale production is limited. On the other hand, free range pig farming system is practiced mainly in the rural areas where pigs are allowed to scavenge for food (Murungi *et al*., 2021).

Murungi *et al*. (2021), estimated that this subsector produced 25,785 thousand tons of pork in 2020 with a projection of 125% increase in demand by the year 2030. It has been estimated that per capita consumption of pork in Kenya is 0.4kg per year and this is expected to increase (Murungi *et al*., 2021). The increasing demand of pork and the challenges faced by pig farmers has the potential to create food safety risks (FAO, 2017). Pigs have good concentrate feed conversion rate and shorter reproduction cycles making this sector a very important quick source of food and economic opportunities for farmers and the country (FAO, 2012). Despite the economic contribution this sector gives to the country's economy, it is still faced with challenges ranging from lack and or poor quality of feeds, marketing, poor management practices and frequent

outbreaks of diseases (Murungi *et al*., 2021) some of which have zoonotic potential like Rotavirus that causes dehydrating diarrhea in children (Doro *et al*., 2015).

2.1 Structure of rotavirus

Rotaviruses are members of the *Reoviridae* family with wheel-shaped appearance when viewed under the electron microscope (Ferrari *et al*., 2022). This explains the origin of the name rotavirus as "Rota" means wheel in Latin language (Chang and Saif, 2012). These viruses are non-enveloped double stranded RNA with three-layered capsid protein with a diameter of approximately 70nm. The capsid encloses 11 genome segments of double stranded RNA (Vlasova *et al*., 2017). The segments encode for six non-structural (NSP1 – NSP6) proteins and six structural viral proteins (VP1, VP2, VP3, VP4, VP6, VP7) (Figure 1). Viral protein 1-3 makes the inner capsid, VP6 is the middle capsid while VP4 and VP7 are on the outer capsid (Crawford *et al*., 2017). Viral proteins 1, 2 and 3 are involved in transcription and replication while VP6 determines the group of the rotavirus. Viral proteins 4 and 7 play an important role in viral attachment to the host cells inducing humoral immune response (Crawford *et al*., 2017). Similarly, VP4 and VP7 have been used to determine P (proteinase sensitive) and G (glycoprotein) genotypes of the rotavirus respectively (Papp *et al*, 2013). Non-structural protein 1 is involved in host immune evasion for the RV to enter the intestinal cells and replicate (Amimo *et al.*, 2021). Nonstructural protein 4 (NSP4) produces enterotoxins and contributes to the pathogenesis of the infection (Estes and Greenberg, 2013) (Figure 2.1).

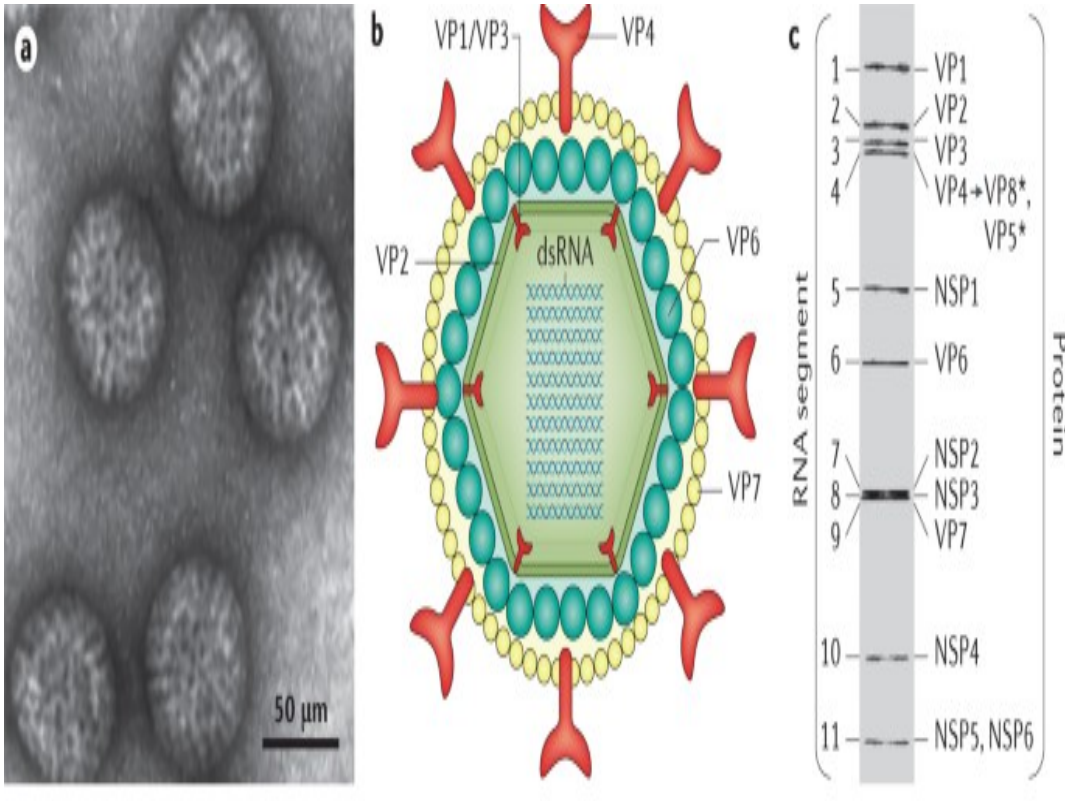

Figure2. 1: Rotavirus structure (Adopted from Crawford *et al*., 2017). *(a) Electron microscopy of Rotavirus structure. It shows the wheel like structure of the rotavirus (b) diagrammatic representation of Rotavirus structure. This illustrates the three capsids: the inner, middle and the outer capsid. The inner capsid is made up of viral protein 1, 2 and 3. The middle has viral protein 6 and viral protein 4 and 7 making the outer capsid. (c) 11 segments of Rotavirus and the respective proteins they code for. VP1, 2 and 3 are involved in ds RNA synthesis, VP4 helps in viral attachment and is the G type neutralization antigen while VP7 is the P type neutralization antigen. NSP1 is Interferon antagonist, NSP2, 5 and 6 are involved in viroplasm synthesis, VP3 helps in mRNA synthesis and NSP4 is an enterotoxin and regulates calcium homeostasis.*

It has been reported that every pig within their lifetime will be exposed to rotavirus infection with the older pigs getting mixed strain infections (Homwong *et al*., 2016). Naïve adult pigs and suckling piglets are more susceptible to rotavirus infection due to their immature immune system. The adult pigs are resistant to infection due to development of post natural exposure immunity (Amimo *et al.,* 2013a). Piglets and young pigs which received inadequate colostrum have been reported to be susceptible to rotavirus infection due to lack of passive immunity (Zimmerman *et al*., 2019). Similarly, piglets in their early life lacks immunoglobulins making them more susceptible to these viral infections as they are unable to launch a quick and robust immune response against the infections (Chepngeno *et al*., 2019).

2.2 Classification of Rotavirus

Presently there are 10 Rotavirus groups (A-J) detected and distributed in both humans and animals with groups A, B and C being the most common (Amimo *et al.,* 2013a). This grouping is based on the gene sequencing of viral protein 6 (VP6) and full genomic sequencing (Ferrari *et al*., 2022). Among these groups, Rotavirus A (RVA) has been reported to have the highest prevalence in both humans and animals worldwide (Bányai *et al.,* 2017). Rotaviruses have been reported globally as the major causes of viral gastroenteritis in pig farms. Five genotype groups (RVA, RVB, RVC, RVE and RVH) have been detected in pigs (Vlasova *et al.*, 2017). They cause significant economic losses to pig farmers due to high morbidity and mortality of piglets, retarded growth of the infected pigs and high cost of treatment (Amimo *et al*., 2015).

Rotavirus A (RVA) has been reported to be the most frequent strain associated with porcine diarrhea (Vlasova *et al*., 2017; Marthaler *et al*., 2014a) causing about 90% of rotavirus infections (Wu *et al*., 2022). Its prevalence is high in nursing and post weaning pigs (Ferrari *et al*., 2022). A study by Ferrari *et al*. (2022) reported a prevalence of 53% in Northern Italy while a study by

Amimo *et al*. (2015) in East Africa (Kenya and Uganda) had RVA prevalence of 26.2%. The number of samples in each study could be the reason behind the difference in prevalence. However, there is limited literature on porcine RVA in other parts of Kenya apart from the previous report by Amimo *et al*. (2015). The G and P genotypes that are common for RVA include G3-5, G9, G11, P6, P7, P13 and P19 (Amimo *et al*., 2015). Rotavirus A strains are diverse, and their infections are widespread and endemic in both clinical and asymptomatic piglets (Amimo *et al*., 2015). For instance, in the United States, the most predominant genotype in 2012 was G9P [13] (Amimo, 2013a) but in 2021 the predominant genotype was G5P [13] (Doerksen *et al*., 2022)

Rotavirus B (RVB) is the most diverse group of rotaviruses and has 20G genotypes (Shepherd *et al*., 2017; Vlasova *et al*., 2017). It co-infect piglets with RVA and RVC suggesting that RVB could be a secondary pathogen (Marthaler *et al*., 2014). A recent study by Miyabe *et al*., (2020) reported RVB as the primary pathogen with a prevalence of 71.1% in new born piglets. Ferrari *et al*., (2022) reported a higher prevalence in fattening pigs (46.42%) compared to suckling (20.75%) and weaning piglets (43.93%) suggesting that exposure increases with age of the pig.

Rotavirus C (RVC) has been reported in different countries worldwide (Tuanthap *et al*., 2018). This group has been detected from all ages of pigs (Marthaler *et al*., 2013). Nine G genotypes and 7 P genotypes have been molecularly characterized (Tuanthap *et al*., 2018). From the sequence analysis of the VP6 gene, 11 I genotype have been identified (Kattoor *et al*., 2017). Chepngeno *et al*. (2019) reported a prevalence of 79.1% of RVC RNA in nursing piglets with more infections in clinical than in asymptomatic piglets.

Rotavirus H (RVH) has been detected in pigs from Brazil, Japan, South Africa and United States of America (Vlasova *et al*., 2017). In the United States of America, a prevalence of 15% was reported (Marthaler *et al*., 2014a) and 9.4% in Brazil (Flores *et al*., 2021). It has been reported that

RVH mostly co infect with RVC (Suzuki and Inoue, 2018). From the sequence of VP4 and VP7 genes, Suzuki and Inoue (2018) identified 6P and 10G genotypes respectively in Japan and from the NSP1 gene, they revealed the presence of 6 genotypes (A1-A6). The first complete genome of RVH was done in South Africa and it showed close relationship with the Brazilian and Japanese strains (Nyaga *et al*., 2015).

2.3 Transmission

Rotavirus is very stable and persistent in the environment and can cause infection even under harsh conditions. Rotaviruses are very diverse due to genetic reassortment events that occur frequently (Vlasova *et al*., 2017). Transmission of these viruses is through fecal oral route (contact with contaminated water, feed and fomites) with several factors exacerbating the transmission process. The virus is shed in feces and it is estimated that one gram of fecal material contains high concentration of the virus of about 10^{10} particles of infectious rotavirus. Furthermore, for infections to occur, less inoculum is required and as the sows farrow, they shed the virus increasing infection to piglets (Zimmerman *et al*., 2019). The prolonged persistence of the virus in the environment is argued to increase the risk of infections and its overall prevalence in pig farms (Zimmerman *et al*., 2019; Fongaro *et al*., 2015).

2.3 Pathogenesis and clinical signs

Viral infection starts with entry and attachment of rotaviruses into the epithelial cells (Cui *et al*., 2019). This virus enters the intestinal epithelial cells through either direct entry or epithelial endocytosis as it is dependent on these cells for their transmission and replication (Amimo *et al.,* 2021). Rotavirus requires certain receptors to attach to the epithelial cells. These receptors include sialic acid, Histo blood group antigens (HBGAs), integrins, Heat shock cognate 70 protein (Hsc70) among other co-receptors (Cui *et al*., 2019, Amimo *et al.,* 2021). Many studies and experiments

have been done and many are still underway to try and understand the role of these receptors in rotaviral infection. In a study done by Guo *et al.* (2021) using porcine crypt-derived 3D intestinal enteroids (PIEs), it was reported that different strains of RVA prefer certain types of HBGA and that sialic acid plays a role in attachment and replication of some strains of rotavirus.

Cell entry involves lysis of the outer proteins, attachment to the cell followed by the digestion of the outer capsid and lastly entry of the rotavirus double layer particles into the cell cytoplasm. The study by Cui *et al*. (2019) used cultured porcine enterocytes to demonstrate the importance of intestinal epithelium integrity in defense against rotavirus infection. The host's immune system counteracts it through different mechanisms such as mucus production, activation of signaling pathways like toll like receptor pathway and RIG-I signaling pathway and chemical production such as cytokines (Amimo *et al.,* 2021). For a successful entry and replication into the epithelial cells, rotavirus has developed ways of evading those host immune mechanisms. For example, by use of NSP1, rotavirus can degrade the production of interferons which are responsible for innate immune response. The NSP1 inhibits production of proinflammatory chemicals responsible for apoptosis making rotavirus persist in infected cells (Arnold and Patton, 2011). Rotaviruses replicate in the mature enterocytes of the small intestines causing atrophy of the microvilli (Chepngeno *et al*., 2020). During infection rotavirus NSP4 causes disruption in regulation of host cell calcium signaling pathways leading to impaired homeostasis and hence secretory diarrhea (Chang *et al*., 2020). Rotaviruses form clusters covered in vesicles and they leave the host cells before lysis therefore avoiding degradation (Santiana *et al*., 2018).

There are several mechanisms that contribute to diarrhea development during infection period. Destruction of enterocytes, ischemic villi and infected epithelial cells releasing vasoactive agents contribute to malabsorption leading to diarrhea (Vlasova *et al.*, 2017). Another mechanism is that

rotaviruses have non- structural protein 4 (NSP4) which acts as a secretory agonist and an enterotoxin (Saurabh *et al*., 2018). This protein induces age and dose dependent diarrhea response by causing efflux of calcium from endoplasmic reticulum. These calcium increases cell permeability and alteration of epithelial barrier integrity causing secretory diarrhea (Estes *et al*., 2001). The NSP4 protein also stimulates the enteric nervous system increasing intestinal motility contributing to diarrhea (Estes *et al*., 2001). Opportunistic enteric pathogens can coexist with rotavirus infection with pathogens such as *Clostridium, Escherichia coli, Salmonella, Astrovirus, Coronavirus, Norovirus* enter due to breakdown of intestinal mucosa and therefore complicating the infections (Chatzopoulos *et al*., 2013).

Rotavirus infections have been reported in both clinical and subclinical pigs (Amimo *et al*., 2015; Theuns *et al*., 2016). The clinical manifestation includes profuse watery diarrhea, dehydration, vomiting and anorexia, weight loss, weakness and death. The severity of these signs depends on age of the pig, immune status, farm herd health, rotavirus strain and the presence of other bacteria (Zimmerman *et al.,* 2019). Amimo *et al*. (2016) reported that piglets of less than four months and those pigs kept under free range production system were at high risk of rotavirus infections. When combined with enteric bacterial infection, the severity of rotavirus infection increases (Theuns *et al*., 2014).

2.4 Diagnosis

Rotavirus infections have similar clinical signs with other enteric pathogenic infections and the fact that these infections have been detected in asymptomatic pigs necessitates laboratory testing for diagnosis. There are several methods that can be used to diagnose rotavirus infection in animals. These methods include Electron microscopy, viral isolation, antigen detection assays such as enzyme linked immunosorbent assay (ELISA), real time reverse transcriptase PCR and

polyacrylamide gel electrophoresis (PAGE) (Zimmerman *et al*., 2019). Fecal material is a commonly used sample to detect the double stranded RNA or the antigens. Reverse transcriptase PCR is commonly used as a diagnostic method to determine the concentration of the virus within a fecal sample (Costantini *et al*., 2007). Multiplex RT PCR has been developed with primers for several enteric viruses making it possible to distinguish the different rotavirus strains (Theuns *et al*., 2016). Real time quantitative (RT qPCR) has high sensitivity, fast turnaround time, risk of contamination is reduced and also high reproducibility. With the threshold cycles (Ct) values nucleic acids can be quantified with standardized curves. These makes this method more advantageous in molecular detection and quantification of nucleic acids (Caffarena *et al*., 2022).

2.5 Management of rotavirus infection in pigs

There is no specific treatment to viral infections including rotavirus infections and therefore antibiotics have been used to prevent secondary bacterial infections. Rehydration and feeding of the infected pigs with high energy diet is important to replace the lost fluids and nutrients. Some probiotics have been shown to reduce the severity of the infections and improve immune response by the intestinal mucosa (Vlasova *et al*., 2016). Improving the host immunity is a way of controlling rotavirus infections (Holloway and Coulson 2013; Amimo *et al*., 2021). A study by Mao *et al.* (2018) showed that dietary supplementation of feed with l-isoleucine improves piglets' immunity and growth. Piglets fed on Isoleucine showed increased immunoglobulins and rotavirus antibody levels, a sign of improved humoral immunity. The study also show that isoleucine decreases NSP4 levels therefore decreasing diarrhea in piglets. Another study by Chepngeno *et al*. (2022) suggested that vitamin A supplementation and rotavirus A inoculation of the sows during pregnancy and lactation elevates immune responses of the sow and will pass the passive immunity to piglets. A recent study on effects of vitamin A deficiency on T cell and innate immune response

in sows concluded that deficiency of vitamin A compromises T cell and innate immune responses. This eventually impairs B cell immune response and passive immunity to piglets (Chepngeno *et al.,* 2023). Tian and colleagues (2016) suggested that Vitamin D3 supplementation reduces rotavirus infection in pigs through cell degradation of infected cells. Vitamin D also activates the RIG-I signaling pathway reducing the negative effects of rotavirus infection though the exact mechanism of action is not known (Lee, 2020).

Maternal immunity is very important in prevention of infection in piglets (Chepngeno *et al*., 2019). Therefore, piglets should receive enough quantities of colostrum to gain antibodies against the rotavirus infection. Sows' colostrum and milk contains Ig G and Ig A. Lack of colostrum and early weaning causes rotavirus diarrhea in piglets showing the significance of maternal antibodies in early age. This passive immunity declines over time requiring active immunity through vaccination (Nguyen *et al*., 2007). Some of the biosecurity measures recommended include practice of All-in All-out system to break the cycle of viruses, high level of sanitation in pig pens including cleaning and disinfection between farrowing to reduce the viral load and exposure to rotaviruses (Iowa State University, 2023).

2.6 Epidemiology

2.6.1 Distribution of rotaviruses

Rotaviruses, especially RVA and RVC have been reported in most parts of the world (Vlasova *et al*., 2017). However, RVB has been reported in United States, Japan, Russia, Switzerland, and South Africa. Rotavirus H strain has been reported in Japan, Brazil, United States of America, and South Africa (Kumar *et al*., 2022). Nyaga and colleagues did a complete genome analysis of the first RVH in South Africa in 2015. However, RVE has been reported only in the UK in 1980s and no other literature on it has been published. Rotaviruses A and C have been extensively researched

on unlike other genotypes. In African countries, there is scarce literature on porcine rotaviruses due to lack of surveillance systems. For instance, in Kenya only one study has been done on porcine rotaviruses since 2015 and that only detected RVA and RVC. With the diversity and the zoonotic nature of rotavirus, more research and surveillance should be put in place especially for the other types (RVB, RVH and RVE).

Rotaviruses have a wide host range. It has been reported in bovine, horses, birds, canines and small rodents (Barros *et al*., 2018). Porcine rotaviruses have been reported worldwide with different strains (Vlasova *et al*., 2017). Rotaviruses are zoonotic and has been reported in humans especially children under five years of age. Most of the human infections are from animal origin such as porcine or bovine (Doro *et al*., 2015, Geletu *et al*., 2021, Kunic *et al*., 2023).

2.6.2 Molecular epidemiology

Molecular tools have played a very important role in understanding the genetic diversity of rotaviruses and the zoonotic potential. This is through identification of different genotypes and their genetic relationships (Zimmerman *et al*., 2019). Primers specific to Viral Protein (VP4 and VP7) gene segments have been used to identify different G and P genotypes (Theuns *et al*., 2016). Twenty-seven different G genotypes and 37 different P genotypes have been identified for Rotavirus A in both animals and humans (Vlasova *et al*., 2017). Twelve G and 16 P genotypes have been identified in pigs (Amimo *et al*., 2015). In a recent study by Monteagudo *et al*. (2022) in Spain revealed the following genotypes for RVA: G4, G9, G3, G5 and G11 for VP7 gene and P7, P23, P6 and P13 for VP4 gene. Amimo *et al*. (2015) detected P6, P8 and P13 genotypes from sequencing of VP4 gene where P6 and P8 had close genetic relationship with the human strains. Whole genome classification of RVB in the United States of America identified 26G and 5P genotypes (Shepherd *et al.*, 2018). In a study in Thailand by Tuanthap *et al*. (2018), Rotavirus C,

genetic analysis of VP7 and VP4 genes revealed presence of nine G and seven P genotypes. Whole genome analysis of RVA strains in samples from sub–Saharan Africa has shown mixed infections with coinfection of porcine and bovine strains being reported (Nyaga *et al*., 2015). Similarly, RVH has been characterized in South Africa which related to the Brazilian and Japanese RVH (Nyaga *et al*., 2015). In East Africa Amimo *et al*. (2015) detected and characterized RVA strains in Kenya and Uganda with P6, P8 and P13 genotypes.

Since the last study on rotavirus infection in Kenya was in 2015 and with the evolutionary pressure and selection for viruses to adapt to the prevailing conditions, there is need to analyze the current genetic profile of circulating genes in pigs.

2.6.3 Risk factors

There are several factors that have been associated with occurrence of rotavirus infections in pig farms. These factors range from animal factors, farm level factors, management factors to seasonal factors. Animal factors includes age, sex, and breed. Piglets under four months have higher risk of exposure to rotavirus infection as compared to the older ones (Amimo *et al*., 2016; Bwogi *et al*., 2023). Murao *et al*. (2019) reported higher incidence of RVA in asymptomatic adult pigs. In a review by Raev *et al*. (2023) reports that intestinal mucus layer is more penetrable in piglets than adults. This suggests that adult pigs are asymptomatic carriers and hence they play a big role in transmission of the virus to the piglets. Herd size, presence of other animals in the farm and husbandry systems are among the farm-level factors. Large herd size, presence of other animals such as goats, cattle and chicken and free ranged pigs have been reported to increase the risk of exposure to rotavirus infections (Murao *et al.,* 2019). Management factors include sanitation and waste disposal, source of feed and water and biosecurity measures in the farm influence the risk

of rotavirus infections in pig farms (Amimo *et al*., 2016; Murao *et al*., 2019). Infections have been reported to increase during the cooler months of the year worldwide (Patel *et al.,* 2013).

A study in India showed that poor ventilation of pig pens, feeding of homemade feed and sourcing of water from shallow wells increases the risk of rotavirus infections (VinodhKumar *et al*., 2020). Frequent change of ingredients in homemade feed leads to improper gut health. Shallow well water is easily contaminated by fecal matter and hence the increase in infections. A study from Nigeria implicated intensive pig farming systems, water sourcing from dams and mixed farming to higher rotavirus infections in pig farms (Delia *et al*., 2019)

In Kenya, there is limited literature on the risk factors associated with rotavirus infection in pigs especially in intensive production systems in urban and peri-urban areas. The recent study was carried out in 2015 among free-range and backyard pigs in western Kenya where age and production system were found to influence exposure to rotavirus infections (Amimo *et al*., 2016).

Farmers do not see the disease but they notice the clinical signs. Diarrhea being the major clinical sign of rotavirus infection most farmers report having seen their pigs/piglets diarrhea. It is therefore important to look at diarrhea and the factors associated with it. Diarrhea has several causes ranging from viruses, parasites, bacterial and other non-pathogenic causes such as stress from changes in management (Jacobson *et al*., 2022). Poor management plays very important role in the development of diarrheal diseases in pig farms (Katsuda *et al.*, 2006). Diarrhea causes a lot of losses to pig farmers due to stunted growth of the pigs and therefore delay to attain market or reproduction age. Diarrhea also causes high mortality especially in piglets. Effects of diarrhea in a pig include, intestinal damage, loss of water and electrolytes, nutrient absorption is poor and reduce immunity (Business Queensland, 2019). Some of the predisposing factors to diarrhea includes: Housing where it should be proper with temperature conditions that support the growth

of pigs. Cold conditions have been found to reduce peristaltic activity of the intestines which therefore promotes bacterial colonization responsible for diarrhea in pigs (Fairbrother and Gyles, 2012). Manure and soiled beddings host pathogenic bacteria. Removal of these wastes reduces bacterial load and eventually diarrhea (Rhouma *et al*., 2017). Early weaning of piglets predisposes them to infections. This is because the intestinal immunity of piglets in early life is immature and they require enough colostrum for their immunity. Early withdrawal of the sows' milk therefore predisposes the piglets to enteric pathogens causing diarrhea (Chepngeno *et al*., 2019).

2.7 Zoonotic aspect of rotaviruses

Rotaviruses have evolved through different mechanisms to form new genotypes which may be of zoonotic importance. These mechanisms of evolution include point mutations, recombination and majorly reassortment (Collins *et al*., 2010). Rotaviruses are prone to reassortments due to the segmented nature of their genome leading to formation of new variants which may be more virulent than the parent gene (Malik *et al*., 2020). There has been development of uncommon/novel rotavirus genotypes in the human population and many of them have been reported to have originated from domestic animals (Cook *et al*., 2004) (Figure 2.2).

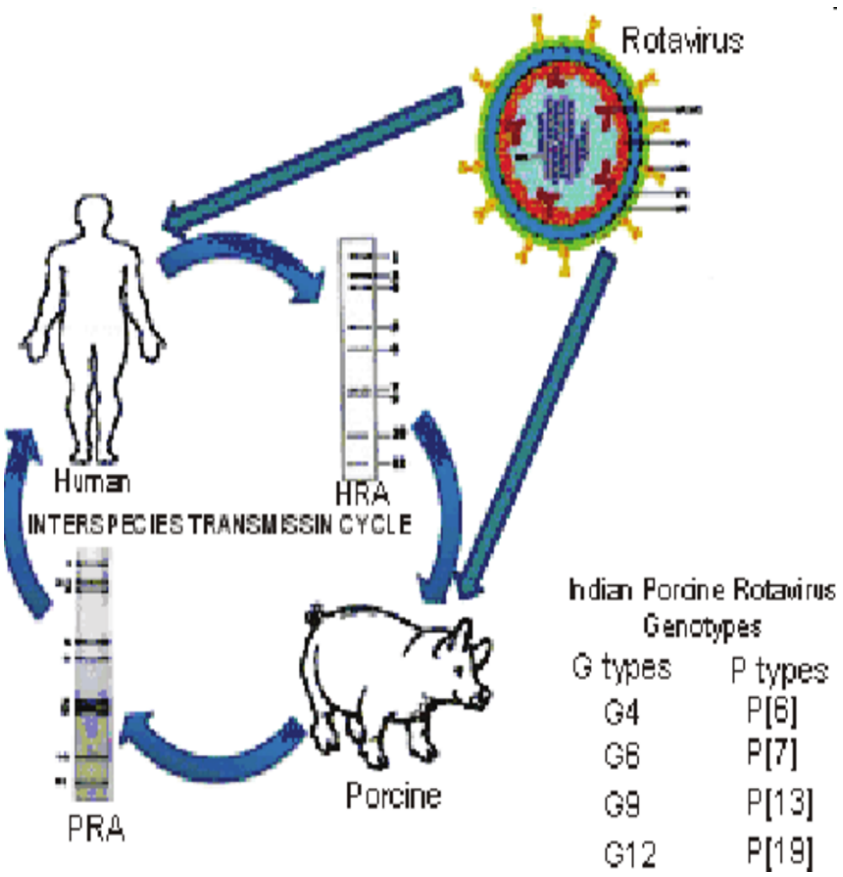

Figure2. 2: Zoonotic potential of porcine rotavirus strains (adopted from Malik *et al*., 2014). *This illustrates the interspecies transmission of rotavirus A between pigs and humans. Through reassortment, the strains from the two species are able to exchange gene segments and form a new strain that could be more virulent than the parent strains.*

Studies have shown that some of the Rotavirus A strains have genetic relatedness with human stains. Amimo *et al*. (2015) reported that P6 and P8 genotypes detected in pigs were genetically closely related to human strains and that there could be a possible interspecies transmission. Human Rotavirus A strain diversity shows susceptibility of humans to rotavirus infections from animal origin (Doro *et al*., 2015). Rotavirus A G9 and G12 human genotypes have similarities with porcine G9 and G12 normally observed in piglets (Vlasova *et al*., 2017). Wu *et al.* (2017) reported RVA strains with high genetic similarity detected in children and pigs.

The suggested animal reservoirs for human rotavirus infections include porcine, bovine, rodents and ovine. Reports have described sporadic cases of human infections coming from different animal origin through interspecies transmission (Vlasova *et al*., 2017; Wakuda *et al*., 2011). Ten G genotypes and 7 P genotypes from porcine origin have been detected in humans (Doro *et al*., 2015). The seasonal pattern for porcine RVA circulation resembles that of human RVA circulation which occurs during the cooler months suggesting that pigs could be the reservoirs for human infections (Patel *et al.,* 2013). Whole genome analysis of rotavirus A from Moroccan nomadic livestock revealed that some livestock strains had similarities with the human ma31 strain suggesting zoonotic transmission between livestock and humans (Alaoui *et al.,* 2020). Rotavirus C has also been shown to be of zoonotic significance. There is evidence of genomic reassortments between human RVC and porcine RVC. Porcine RVC strains carrying human-like NSP4 and NSP5 has been detected (Costa *et al*., 2020). Another study by Kattoor *et al*., (2017) has shown human-like VP6 gene in porcine RVC.

There are shared HBGAs between humans and animals suggesting a possible cross-transmission of rotavirus strains between them (Jiang *et al*., 2017). A recent study in Croatia reported that RVA strain that was detected in children came from porcine to human transmission (Kunic *et al*., 2023).

CHAPTER THREE: MATERIAL AND METHODS

3.1 Study area

The study was conducted in Kiambu County in Central Kenya. This site was chosen because of intensive pig production system practiced and the peri-urban nature of the County. There is no study that has been done on porcine rotavirus in the area. The county has a population of approximately 2.4 million people according to the Kenya population and Housing Census (KNBS, 2019) and it covers an area of 2,449km^2. This County borders Nairobi and Kajiado to the South, Machakos to the East and Nakuru to the West. North West it borders Nyandarua and Muranga on the North and North West. It is subdivided into twelve sub-counties: Lari, Kikuyu, Kiambu, Kiambaa, Kabete, Ruiru, Thika, Githunguri, Gatundu North, Gatundu South, Limuru and Juja. For this study, seven sub-counties were randomly selected that included Kiambu, Kabete, Ruiru, Thika, Githunguri, Gatundu North and Limuru. It is the most populated county in Kenya after Nairobi. Approximately 60% of it is urban majorly because of its proximity to Nairobi city and as Nairobi expands it spills over to Kiambu. This therefore puts a lot of pressure on its resources such as land use (Martin and Odera, 2015). Though the County is metropolitan it is largely dominated by the Kikuyu tribe. This county receives annual rainfall of 1200mm and 26^{o}C as the mean temperature. The highland part of it lies between 1800 – 2550m while the lower part lies between 1200 – 1360m above sea level. It lies on a latitude of 1.0314^o south and longitude of 34.8681^0 east. Main economic activities of this county include livestock farming (dairy, poultry, and pig), agricultural farming (tea, coffee), business activities (real estate development, retail businesses).

The pig population in the county was estimated at 98,725 (KNBS, 2019).

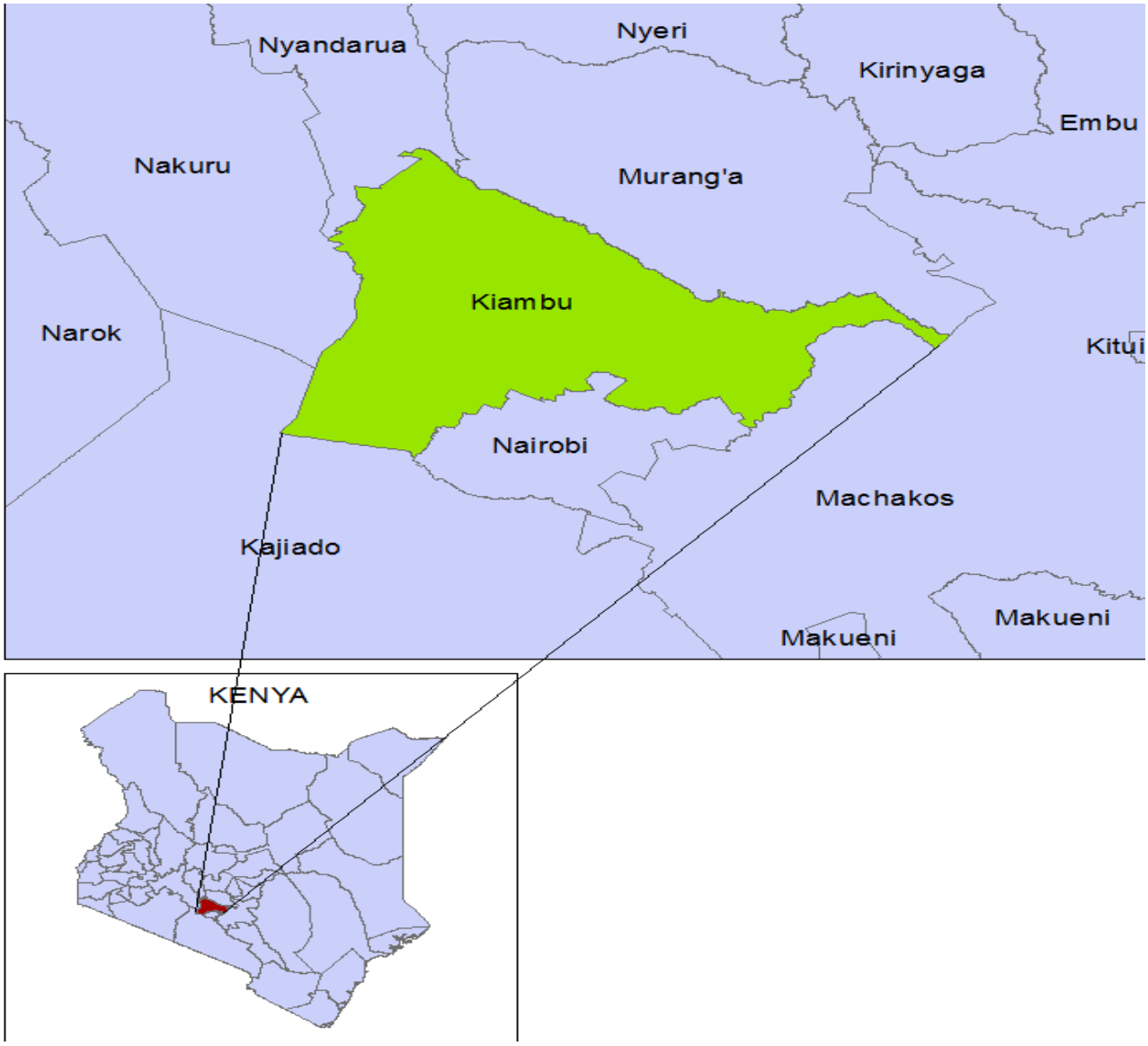

Figure3. 1: Map of Kiambu County (Adopted from Martin and Odera, 2015). *This map shows Kiambu County and its neighboring counties. It borders six counties including Nairobi County which is the capital city of Kenya. This makes Kiambu largely urban and periurban due to spillover from the capital city.*

3.2 Study population

The study involved pig farms with piglets of less than three months of age. Pig farms in Kiambu are majorly small holder and they practice small-scale intensive production system. The pigs in this system are completely confined and are kept for commercial purpose (Dick, 2004). As most of the County is urban, the available land for farming is limited and therefore pigs' production occurs within restricted housing units in the farm compounds. This indicates that there is proximity and continuous interactions between pigs and household members, which complicates the implementation of farm biosecurity measures.

3.3 Study design

This was a cross-sectional study design where biological samples (rectal swabs) were collected from sampled piglets, and semi-structured questionnaires administered at a point in time. This study design was chosen because it is easy to conduct, relatively cheap, takes shorter time and ease in generation of study hypotheses. This design also would allow the study to be used as a baseline study for other future advanced studies of different designs (Wang and Cheng, 2020). This design allowed determination of proportion of samples positive for rotavirus and the associated risk factors.

3.4 Sample size determination

Sample size to detect disease was used in this study to detect presence of rotavirus infection in piglets (Dohoo *et al*., 2003). The formula for sample size calculation is described below.

$$n = [1 - (1 - a)^{1/D}] [N-(D-1)/2]$$

Where n is the required sample size; a is the probability of observing at least one diseased animal in a sample (confidence interval set at 0.95); D is the estimated number of diseased animals in the

group i.e. population size * minimum expected prevalence. N is the population size of the pigs in the herd.

Therefore; In this case the minimum expected prevalence was 50% and the estimated population size was 50 therefore D is 25

$n= [1-(1-0.95)^{1/25}][50-(25-1)/2]$

$= [1-(0.05)^{0.04}][50-(24)/2]$

$= 0.112928145*38$

$=4.29 \approx 5$

The required sample size therefore was 5 piglets/ biological samples of fecal swabs from each piglet per farm.

3.5 Selection of study farms and piglets

Purposive sampling technique was used where samples were taken from farms with piglets of less than three months of age. The sampled farms were through referrals by veterinarians and animal health practitioners in the area. The names and contacts of animal health practitioners in the study area were obtained from the Kenya Veterinary Association forum and referrals by colleagues. The pig farmers in Kiambu were not registered and therefore the need for animal health practitioners from the area to refer to those farmers who they knew were raising pigs. Once the farm had been referred, a phone call was made to the owner and they were introduced to the project by giving them a brief description of the project and its objectives. If the farmer accepted to participate in the study, then the farm would be enrolled in the study, and an appointment was made to visit the farm. Before sampling the farm, the farmer had to give a verbal consent. From each farm, at least five piglets were randomly sampled and fecal swabs obtained.

3.6 Ethical clearance and informed consent

Committee on biosafety, animal use and ethics of the faculty of veterinary medicine, University of Nairobi approved this study (FVM BAUE/2022/401). In addition, all the respondents were briefly informed about the objectives of the study and they gave verbal consent to participate in the study.

3.7 Fecal sample collection

Induction training of the research team on biosafety was done before commencing sample collection to mitigate potential hazards and for consistency and uniformity in data collection. Pre-testing of sample collection was done in a few farms in the area to ensure that data collection tools were appropriate. In the pig farms, lactating sows were restrained using a pig restrainer and the piglets restrained manually by hand to avoid any unnecessary suffering. A sterile swab was inserted into the rectum and rolled severally to collect fecal content, removed, and returned to the casing. The casings containing the sample swabs were labelled with farm details and put into a cool box and transported to Public Health Pharmacology and Toxicology (PHPT) laboratory the same day. In the laboratory, the samples were recorded on a research book and then processed. All the equipment (disposable overalls, used fecal swabs) used for sample collection were put in a disposal waste pin and transported to the Department of Public Health, Pharmacology and Toxicology for safe disposal according to the waste disposal policy.

3.8 Fecal sample processing

Two milliliters of diluted phosphate buffered solution (PBS)/ Minimal essential media (MEM) treated with antifungal and anti-bacterial was put in a plastic centrifuge tube, put the sample swab into PBS and agitate to free the content onto PBS/MEM (Figure 3.2). Discard the swab and cap the centrifuge tube and centrifuge at 3000 rpm for eight minutes. The supernatant was then

transferred into cryo-vials and stored at -20^{0}C awaiting ribonucleic acid (RNA) extraction. This temperature is to keep the samples frozen in order to avoid degradation of RNA before extraction.

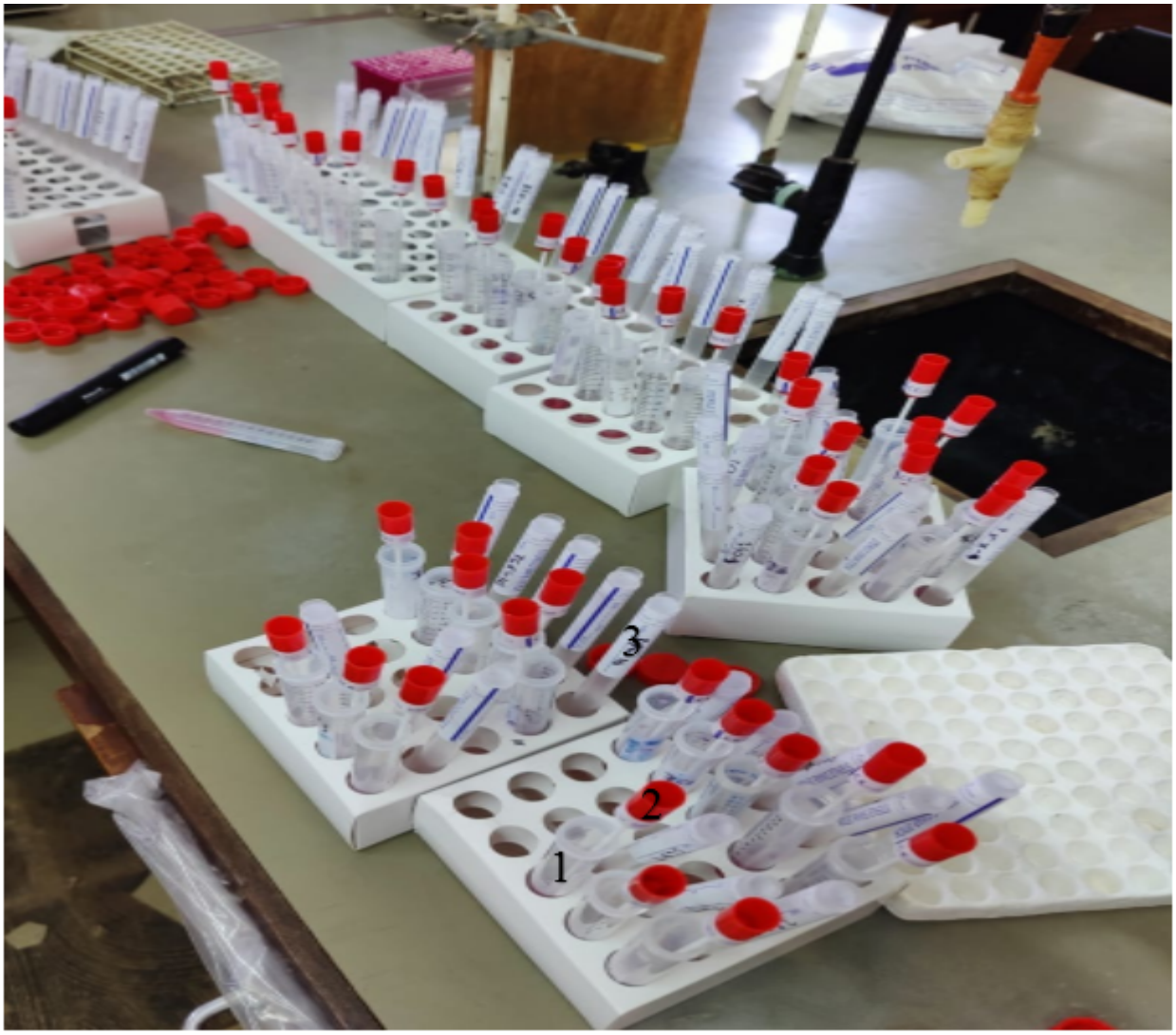

Figure3. 2: Fecal sample preparation and processing in laboratory for RNA extraction: *(1) Is the 15 ml centrifuge tube with 2ml minimal essential media (MEM) where fecal swab containing fecal material is inserted and agitated to release the fecal material into the MEM. (2) Shows the fecal swab inside the centrifuge tube. (3) Shows the fecal swab casing.*

3.9 Rotavirus RNA extraction

The RNA from the phosphate buffered solution suspension was extracted using 5x MagMAX^{TM} 96 Viral Isolation Kit (Applied Biosystems-Thermo Fisher Scientific-REF AMB1836-5, Lithuania) following the manufacturer's instruction. Briefly for one plate, lysis/binding solution was prepared by mixing 845 µl of binding solution concentration, 13µl of carrier RNA and 845 µl of 100% isopropanol in a tube. Bead mix was also prepared by mixing 130 µl of RNA binding beads with 130 µl of lysis/binding enhancer in a separate tube. For plate preparation, 150 µl of wash solution 1 was pipetted into each well in rows B and C with the same amount of wash solution 2 in each well of rows D and E. However, 50 µl of elution buffer was put to each well in row F. For row A, 130 µl of the prepared lysis/binding solution was put to each well, and 50 µl of the samples was added and finally, 20 µl of the bead mix was added to each well. The plate was then put in the MAG MAX machine for extraction of the RNA. The extracted RNA (collected from row F wells) was then transferred to tubes and stored at -20^{0}C awaiting analysis (Figure 3.3).

Figure3. 3: RNA extraction plates: *(a) Empty plate with nine (A-H) rows having twelve wells each (1-12) (b) plate loaded with samples and reagents (c) plate inside MagMax total RNA extraction machine.*

3.10 Detection of rotaviruses using reverse transcriptase quantitative PCR (RT- qPCR)

Reverse transcriptase qPCR was used to detect rotavirus strains present using RT-qPCR kit (Qiagen Onestep RT-qPCR kit Reagent (1000) Cat. No./ID 210215) with forward and reverse primers and probes specific to rotavirus groups A, B and C targeting Viral Protein seven (VP6) gene. The procedure was carried out according to manufacturer's instructions. The premix was prepared and dispensed into the PCR tubes. Briefly, for detection of RVA, the premix consisted of 5µl QIAGEN one-step buffer 5X, 1µl dNTP mix, 0.4µl RVA forward primer (100µm), 0.4µl RVA reverse primer (100 µm), 0.3µl RVA probe (10µm), 1µl Enzyme mix and 11.9µl of Nuclease free water to bring to 20µl of the premix. Then 5µl of the template RNA was added to the PCR tube with the premix bringing the total volume of 25µl for one reaction. For detection of RVB, the premix consisted of 5µl QIAGEN one step buffer 5X, 1µl dNTP mix, 0.4µl RVB forward primer (100µm), 0.4µl RVB reverse primer (100 µm), 0.3µl probe (10µm), 1µl Enzyme mix and 11.9µl of Nuclease free water to bring to 20µl of master mix. Then 5µl of the template RNA (treated with DMSO (1.5µl, at 97^0C for 5 minutes) was added to the PCR tubes with the premix bringing the total volume of 25µl for one reaction. For detection of RVC, the premix consisted of 5µl QIAGEN one step buffer 5X, 1µl dNTP mix, 1.5µl RVC forward primer (10µm), 1.5µl RVC reverse primer (10 µm), 0.25µl RVC probe (10µm), 1µl Enzyme mix and 8.75µl of Nuclease free water to bring to 20µl of the premix mix. Then 5µl of the template RNA (treated with DMSO (1.5µl, at 95^0C for 5 minutes) was added to the PCR tube with the master mix bringing the total volume of 25µl for one reaction. The real time PCR machine was programmed for both reverse transcription and PCR amplification. For detection of rotavirus type A, the PCR conditions were as follows: reverse transcription at 50^0C for 30 minutes, initial PCR activation at 95^0C for 15 minutes, 35 amplification cycles with denaturation at 95^0C for 15 seconds, annealing at 56^0C for 20 seconds and extension

at 72^0C for 1 minute and final extension at 72^0C for 10 minutes. For detection of rotavirus type B, the PCR conditions were as follows: reverse transcription at 45^0C for 10 minutes, initial PCR activation at 95^0C for 15 minutes, 35 amplification cycles with denaturation at 95^0C for 15 seconds, annealing at 50^0C for 45 seconds and extension at 72^0C for 30 seconds and final extension at 72^0C for 10 minutes. For detection of rotavirus type C, the PCR conditions were as follows: reverse transcription at 30^0C for 30 minutes, initial PCR activation at 95^0C for 15 minutes, 35 amplification cycles with denaturation at 94^0C for 1 minute, annealing at 50^0C for 60 seconds and extension at 72^0C for 1 minute and final extension at 72^0C for 10 minutes. The primers and probes used for amplification of RVA, RVB and RVC are outlined in Table 3.1 below (Marthaler *et al.,* 2014b).

Table3. 1: List of RVA, RVB, and RVC primers and hydrolysis probes.

Rotavirus group	Primer/probe	Sequence
RVA	Forward	5'-GCT AGG GAY AAA ATT GTT GAA GGT A-3'
	Reverse	5'-ATT GGC AAA TTT CCT ATT CCT CC-3'
	Probe A-1	5'-FAM-ATG AAT GGA AAT GAY TTT CAA AC-MGB-3'
	Probe A-2	5'-FAM-ATG AAT GGA AAT AAT TTT CAA AC-MGB-3'
RVB	Forward-A	5'-GGT TTA AAT AGC CCA ACC GGT GC-3'
	Forward-B	5'-GGT TTA AAT AGC CCA ACC GAC GC-3'
	Reverse-A	5'-TGC AAT TTR ATG CAT GCG TT-3'
	Reverse-B	5'-GTR TTY AAA TTS GTR TTT GGC GCT A-3'
	Probe	5'-FAM-AGC ATG GAT CTG ATY GAA ACR GT-MGB-3'
RVC	Forward	5'-ATG TAG CAT GAT TCA CGA ATG GG-3'
	Reverse	5'-ACA TTT CAT CCT CCT GGG GAT C-3'
	Probe	5'-VIC-GCG TAG GGG CAA ATG CGC ATG A-TAMRA-3'

3.11 Generation of standard Curves for quantification of detected RNA

The dilution series of known template concentrations were used to establish a standard curve. For this study Plasmid DNA construct for RVA, RVB, and RVC that had been lyophilized were reconstituted in nuclease free water and concentrations of 2018.5ng/µl, 1123.1ng/µl and 1798.5ng/µl was obtained respectively using a Nano-Drop Spectrophotometer. A ten (10) fold serial dilution was done on the samples, RT-qPCR run, and the threshold cycles (CT) values obtained were used to generate standard curves. From the amplification plots of the standard curves, CT was used to quantify RNA for the positive samples.

3.12 Conventional reverse transcriptase PCR amplification of rotaviruses

The positive samples were amplified by conventional reverse transcriptase PCR with primers specific to RVA and RVC (Table 3.2). The master mix preparation for both RVA and RVC briefly were as follows: 5µl of 5X reaction buffer, 1µl of dNTP, 1µl of Enzyme mix, 5µl (10pmoles/ µl) each of forward and reverse primers specific for each rotavirus group and 3µl of nuclease-free water. Twenty microliters of the master mix and 5µl of the RNA samples were dispensed into PCR tubes. PCR conditions were, reverse transcription at 50^{o}C for 30 minutes preheating at 94^{o}C for 15 minutes, denaturation at 95^{o}C for 30 seconds, annealing at 46.5^{o}C for 30 seconds, extension at 72^{o}C for 1 minute and final extension at 72^{o}C for 7 minutes. The conditions were similar with modifications for RVC where denaturation was at 95^{o}C for 10 seconds, annealing at 50^{o}C for 20 seconds and extension at 72^{o}C for 2 minutes. These were for 35 cycles. To detect the amplicons, 1.5% gel agarose was prepared by adding 1.5g of agarose into 100ml of 10x Tris acetate ethylene‑diamine‑tetraacetic acid (TAE) buffer. Seven microlitres of ethidium bromide added and poured into gel holders to solidify. The gel was loaded with 5µl of the amplified RNA samples mixed with 2µl of loading dye. One Kb ladder was also loaded. The agarose gel was then subjected

to electrophoresis at 150V for 55 minutes. The PCR bands and the ladder marker was visualized using Ultraviolet trans illuminator and photo documentation done by a computer program.

Table3. 2: Conventional RT PCR primers for rotavirus A and C.

Rotavirus group	Target gene	Sequence	Amplicon length	Source
RVA	VP7	GGCTTTAAAAGAGAGAATTTC – F GGTCACATCATACAATTCTAA – R	1062bp	Amimo et al., 2013a
RVC	VP6	GCAWTWAAAATCTCATTCACAATGG –F AGCCACATAGTTCACATTTCATCC – R	1352bp	Amimo et al., 2013b

3.13 Farm-level questionnaire survey

A semi-structured questionnaire was administered to every household/farm sampled. Before going to the field, the questions would be discussed among the administrators in order to bring out consistency in administering the questionnaire and reporting. The questionnaire administration was through face to face interviews with the pig farmers once they had consented and agreed to take part in the study. Some of the farmers were not at the farm at the time of the visit, but they provided consent so that the farm managers/ farm man could respond on their behalf. The questionnaire was prepared in Standard English but administered in Swahili for ease of communication with the pig farmers and better clarity of the questions and most farmers within Kiambu understand Swahili. The questionnaire captured information on household demographics, sources of income, length of experience in pig rearing, pig herd sizes, and litter size, types of housing, sources of feed, breeding methods and type of breeds kept, reasons for keeping pigs, sources of replacement stock, other livestock reared, frequency of observing diarrhea in the farms, source of water and disease control and prevention measures.

3.14 Data management and analysis

Data obtained from the laboratory analysis and the farm-level survey questionnaire were entered in Microsoft Excel, cleaned, and analyzed using R statistical software (version R version 4.1.2, x86_64-apple-darwin17.0 (64-bit)). The coded entries in the data were re-coded using tidyverse packages, whereas janitor package was used to clean data. Other packages like lubridate, Epidisplay, dplyr, ggplot were used to execute specific functions. For dichotomous and normally distributed parameters of all objectives, 95% confidence intervals were calculated for descriptive statistics (e.g. proportions, means), otherwise interquartile ranges and medians were determined. For the risk factors analysis for rotavirus infection and diarrhea in piglets, the significant level was

determined at *P*-value <0.05. The outcome variable was the presence or absence of rotavirus infection and presence or absence of diarrhea in piglets while the other factors of interest from the questionnaire were considered as the predictor variables. The predictor variables included gender of the farmers, level of education, source of income, length of pig keeping, herd size, litter size, type of pig house, source of feed, source of water, breeding method, source of replacement stock, history of diarrhea in the farm and reasons for keeping pigs. Univariate logistic regression analysis was employed in the analysis of the data. Univariate multi-level mixed models for all the predictor variables was fitted into separate logistic regression models, employing the functional logit. Those variables with P value < 0.05 were considered the significant variables.

CHAPTER FOUR: RESULTS

4.1 Detected rotaviruses

Reverse transcriptase quantitative PCR was used to detect rotaviruses A, B and C. As shown in figure 4.1 the quantitative RT PCR screen detected the rotavirus RNA with the positive samples revealing sigmoid curves. The curves representing the positive samples for rotavirus RNA were above the threshold value of 1217.6 and 1448.3 for RVA and RVC respectively (Figure 4.2). Out of the total 255 samples collected from piglets, rotavirus RNA was detected in 41 (16.08%) samples. Of these, 15 (5.9%) were detected for RVA, 25 (9.8%) for RVC. Co-infection of both RVA and RVC was detected in one sample representing 0.4%. Rotavirus B was not detected in any of the farms. The 41 positive piglets were from 20 farms (38.46%) out of the total 52 farms sampled. Rotavirus A (RVA) was detected in piglets from 7 (13.5%) farms, RVC detected in piglets from 12 (23.1%) farms. Co-infection with RVA and RVC was detected in one piglet from one farm (2%). Rotavirus C was detected in more farms as compared to RVA indicating that RVC is more prevalent in the farms than RVA (Table 4.1).

Table4. 1: Results showing farm and herd prevalence of RVA and RVC.

Detected rotavirus group	Farms (n=52) n (%)	Piglets (n=255) n (%)
A	7 (13.5)	15 (5.9)
B	0(0)	0 (0)
C	12 (23.1)	25 (9.8)
A and C	1 (2)	1 (0.4)
Total	20 (38.5)	41 (16.1)

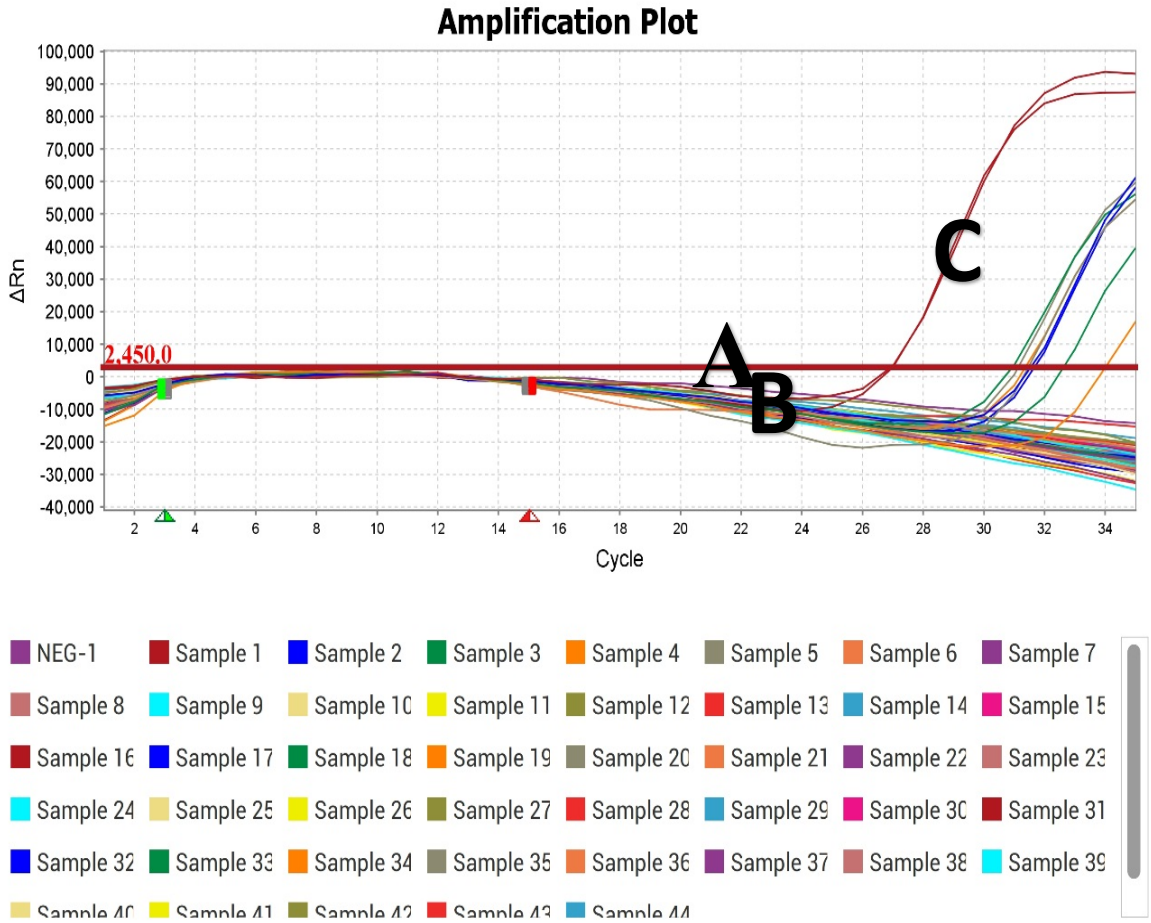

Figure4. 1: Amplification plot showing positive and negative curves for RVC. *(A) Shows the threshold (the straight line with dark red color) which is the value that distinguishes amplification signals that are relevant from the background. (B) Shows the baseline (C) is the exponential curve that shows positive detection of RNA. The numbers on the x axis which are the threshold cycle values (Ct). These are the values in which the fluorescent signals of the reactions crosses the threshold. Each sigmoid curve has its own threshold cycles and are inversely proportional to the quantity of detected RNA. Lesser Ct values means the curve crosses the threshold early in the cycle indicating more rotavirus RNA and vice versa. Sample 1 to sample 44 are the sample Identification numbers. NEG 1 is the negative control.*

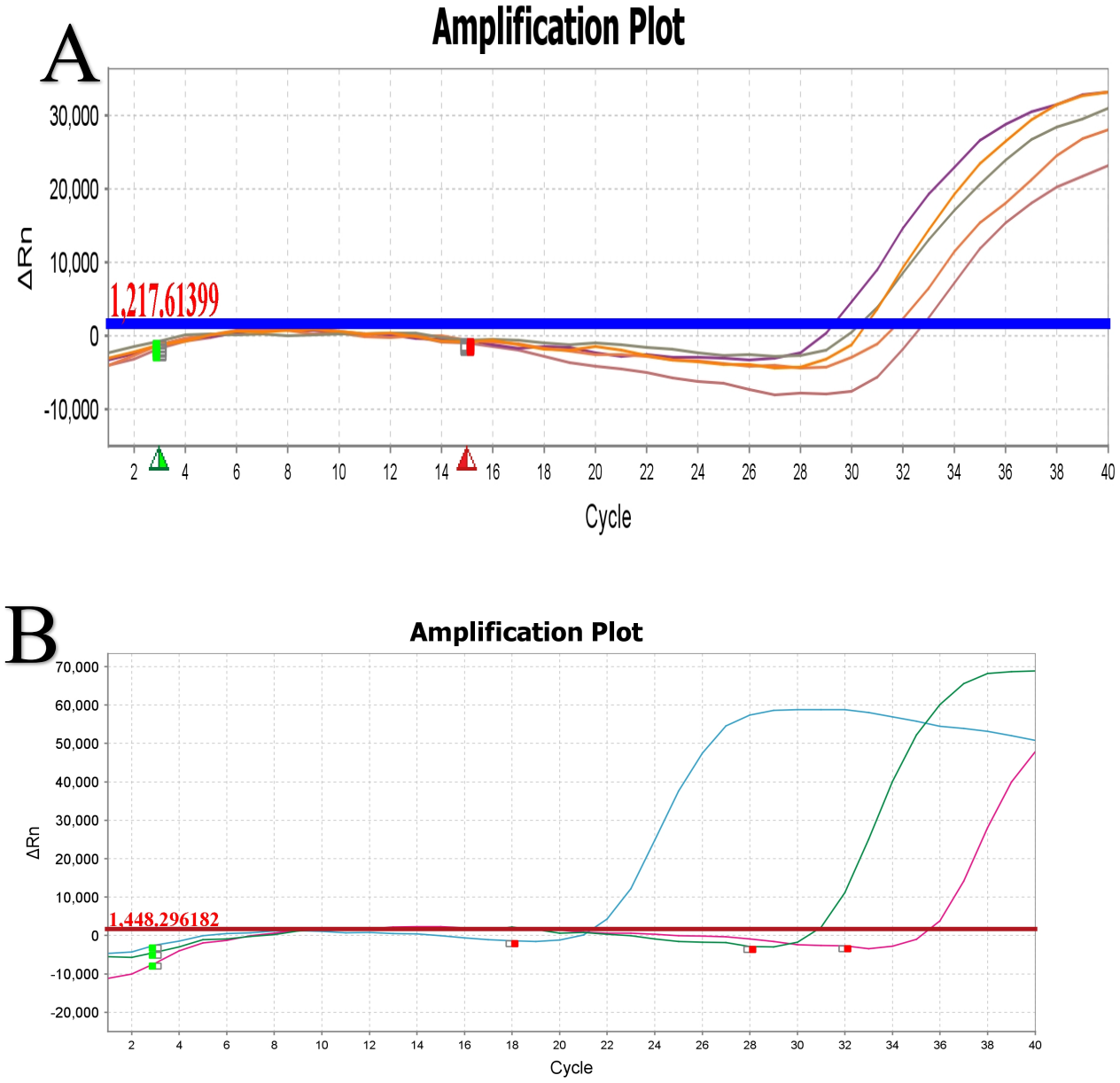

Figure4. 2: Amplification plots showing positive samples for RVA and RVC: *The thick red and blue straight lines are the threshold lines. (A) Shows the RVA RNA positive samples with the Ct values for the curves starting from the first as 29.2, 30.2, 30.5, 31.7 and 32.7. (B) Represents amplification curves for RVC RNA positive samples with Ct values of 21.4, 30.8 and 35.5.*

4.2 Rotavirus load detected in feaces

In order to detect RVA and RVC load in feaces the quantity of RNA were determined by generating standard curves for both rotavirus groups. As shown in figures 4.3 and 4.4 a standard curves were generated successfully for RVA and RVC as revealed by linear curves with R^2 values of 0.9994 and 0.9701 respectively. In this case, the Ct values were lower if the concentration of the RNA was higher (at lower dilutions). For lower RNA concentration, the Ct values were higher.

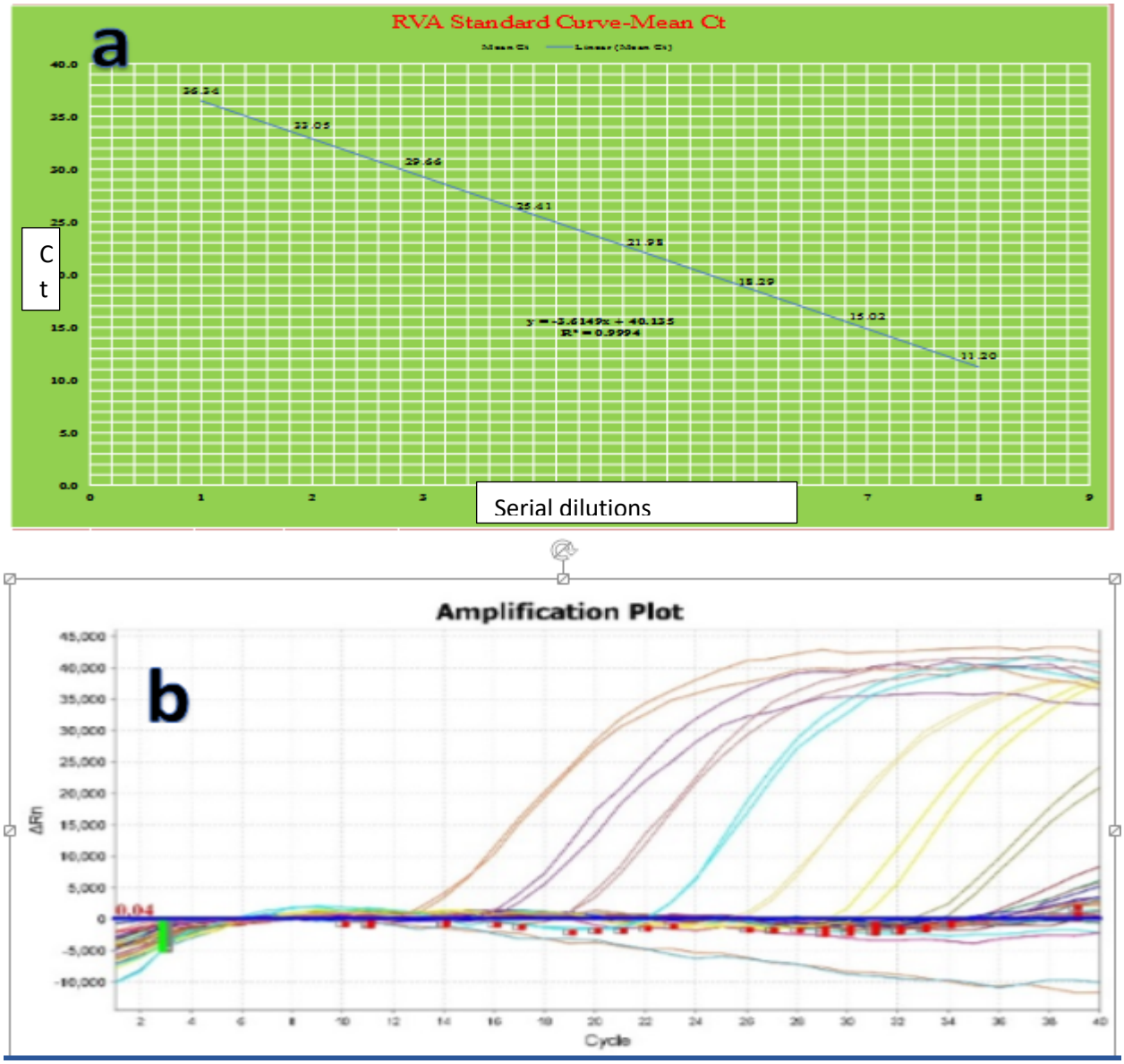

Figure4. 3: RVA standard curve and amplification plot: *(a) Is the log linear phase of the amplification reaction derived from the RVA standard curve for the different dilutions. On the y axis are the threshold cycles (ct) and on the x- axis are the starting RNA quantities for each dilution series. The slope is -3.614 and the y- intercept is 40.135 with fitness test of 99.97%. (b) amplification plot of a 10- fold dilution series of RVA plasmid of known concentration obtained from Nano drop spectrophotometer. Amplification plot is obtained from the RT qPCR.*

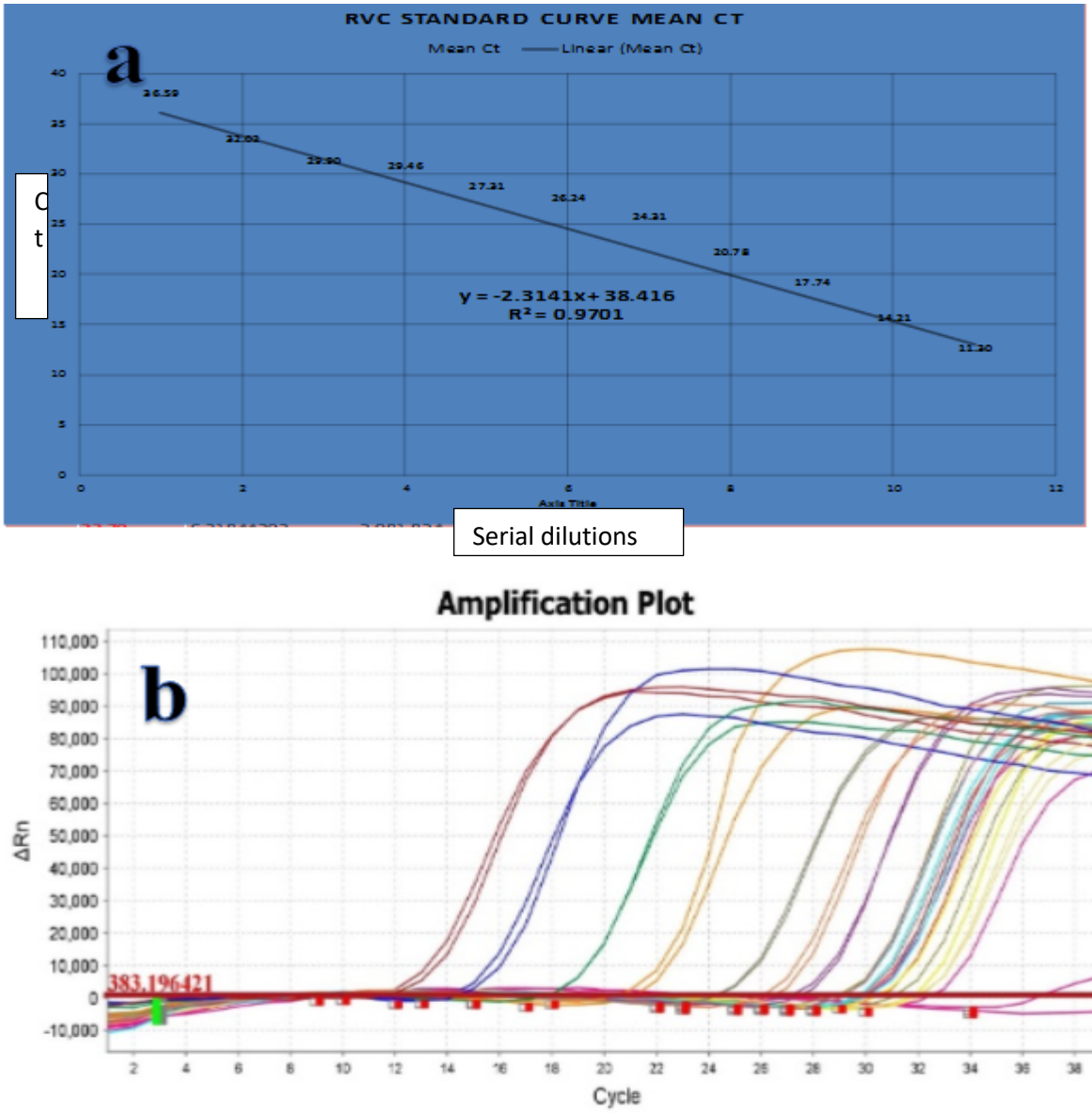

Figure4. 4: RVC standard curve and amplification plot: *(a) Is the log linear phase of the amplification reaction derived from the RVC standard curve for the different dilutions. On the y axis are the threshold cycles (ct) and on the x- axis are the starting RNA quantities for each dilution series. The slope is -2.314 and the y- intercept is 38.416 and R2 of 97.01% (b) amplification plot of a 10- fold dilution series of RVC plasmid of known concentration obtained from Nano drop spectrophotometer. Amplification plot is obtained from the RT qPCR.*

After the generation of the standard curves, the RVA and RVC load shed in feaces were determined. As shown in table 4.2, RVA shedding in feaces amongst the individual piglets varied with quantity of RNA (ng/µl) detected ranging from 40 to 1061ng/ µl. However, the viral load for RVC had a higher RNA quantity range (18-22777392 ng/ µl) (Table 4.3) indicating that RVC load shed in feaces is higher as compared to RVA. This is shown by the high number of samples having above 1000ng/µl of RVC RNA as compared with only one sample having above 1000ng/µl RVA RNA. From these findings, it shows that RVA was widely distributed across different locations, unlike RVC where most piglets that shed above 500ng/µl were from Uthiru Gichagi (UG). This indicates that RVC is circulating more in Uthiru as compared to other locations.

Table4. 2: Quantity of detected RNA from RVA positive samples

Sample Id	Ct value	RNA quantity (ng/ul)	Location
G137	29.20	1,061	Gikambura
KR100	29.33	978	Karura
KIS17	29.36	957	Kiserian
KR104	29.59	827	Karura
TH179	29.62	810	Thogotho
TK75	29.96	655	Thika
G135	30.21	556	Gikambura
G134	30.45	477	Gikambura
RT44	31.20	296	Ruthimithu
TH177	31.22	293	Thogotho
TK77	31.47	250	Thika
KR101	31.67	220	Karura
G136	31.71	215	Gikambura
RT41	31.97	181	Ruthimithu
RT25	33.56	66	Ruthimithu
N98	34.35	40	Ndumbuini

Table4. 3: Quantity of detected RNA from RVC positive samples

Sample Id	Ct values	Rna quantity(ng/µl)	Location

GA 238	21.39	22,777,392	Gatundu
UG1	26.89	95,669	Uthiru
KA300	28.85	13,581	Kanyariri
UM91	30.48	2,688	Muthua
TH114	30.61	2,362	Thogotho
RU66	30.82	1,914	Ruai
TH185	30.83	1,894	Thogotho
UG5	31.21	1,296	Uthiru
UG4	31.28	1,210	Uthiru
UG2	31.56	920	Uthiru
UG3	31.71	787	Uthiru
TH111	32.14	515	Thogotho
KA300	32.19	491	Kanyariri
KA308	32.42	390	Kanyariri
D173	32.64	313	Dagorreti
TK75	32.79	270	Thika
K109	32.95	229	Karura
G142	33.25	172	Gikambura
KA305	33.54	128	Kanyariri
G140	33.61	119	Gikambura
D171	33.73	106	Dagorreti
G240	34.40	54	Gikambura
G141	34.55	47	Gikambura
G143	34.80	37	Gikambura
D52	35.29	23	Ndumbuini
GT39	35.54	18	Githunguri

4.3 Rotaviruses detected by conventional reverse transcription PCR

Out of the 41 positive samples from the RTqPCR, 10 from group A and 10 from group C were randomly selected (ensuring they are from different regions in the study area) for conventional RT PCR. All these samples were positive on conventional RT PCR with varied clarity of bands from gel electrophoresis. The primers used had 1062bp and 1352bp for RVA and RVC respectively (Figure 4.5 and Figure 4.6).

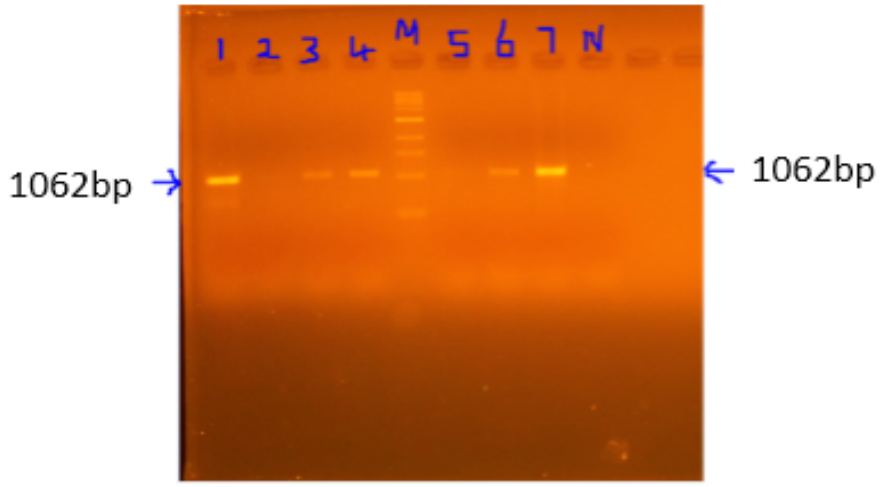

Figure4. 5: Conventional reverse transcriptase PCR amplification of viral protein 7(VP7) gene from RVA positive samples*: Gel electrophoresis was used to analyse the PCR products of RVA positive samples from q RT PCR. These were run on 1.5% agarose stained with ethidium bromide. Numbers1-7 represent gel wells loaded with the PCR products and loading dye. Lane (M) shows the molecular weight marker using 1Kb commercial ladder. The position of the amplicons are shown by the arrows. Lane (N) is the negative control.*

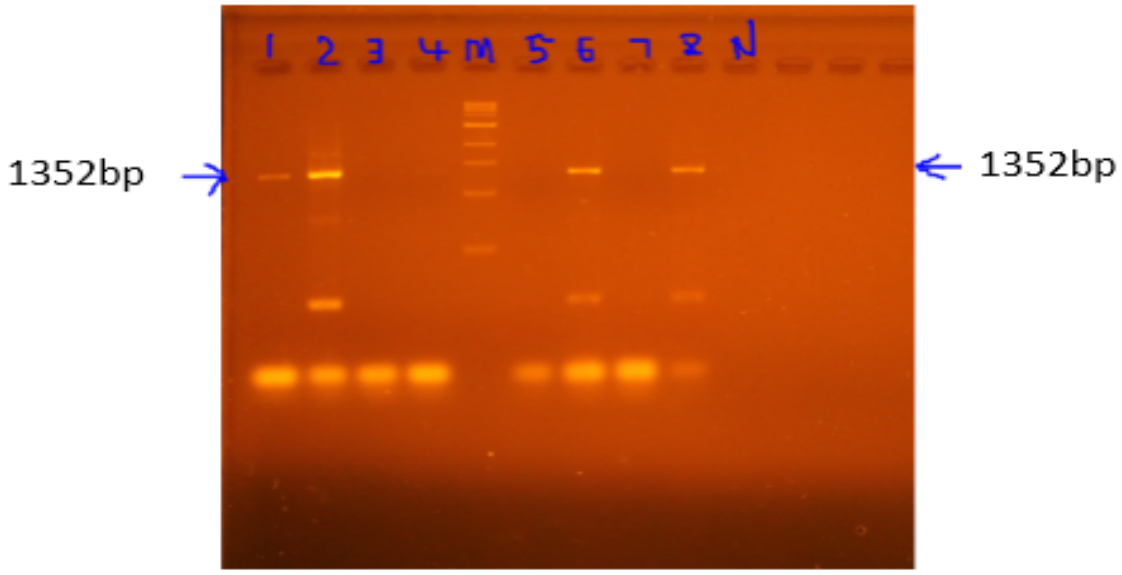

Figure4. 6: Conventional reverse transcriptase PCR amplification of viral protein 6 (VP6) gene of RVC positive samples*: This is a representative gel picture from gel electrophoresis of RVC positive samples. These were run on 1.5% agarose stained with ethidium bromide. Numbers 1-8 represent wells loaded with 5µl of the PCR products and 2 µl loading dye. Lane (M) is the molecular weight marker loaded with seven microlitre of commercial 1Kb ladder. The blue arrows shows the level of the amplicons with 1352bp. Lane (N) is the negative control.*

4.4 Socio-demographic characteristics of pig farmers

A total of 52 questionnaires were administered to pig farmers in Kiambu County. Higher proportion (61.5%) of the respondents were male and the remainder were females. Almost half (48.1%) of the respondents had obtained post-secondary level of education, 23.1% with secondary level education, 19.2% primary level and 9.6% did not have any formal education. Approximately 61.5% of the respondents interviewed owned pigs and other sources of income (self-employed) with 26.9% having both pigs and were also in formal employment while 11.6% relied only on pigs as their source of income. The time the respondents had kept pigs was categorized into <6, 6-10 and >10 years. Most of the respondents (57.8%) had kept pigs for a period less than 6 years with the other two categories reporting 21.1% each. About 34.6% of the respondents had a pig herd size of between 21-50 pigs in their farms compared to other categories. Those farms with herd sizes of between 1-10 and more than 100 were 15.4% each while those with herd sizes of 11-20 and 51-100 were 17.3% and 17.7% respectively. Most of the respondents (84.6%) kept pigs for business purposes, with 11.5% keeping them as a form of savings and business with about 3.8% reporting that the pigs were kept for home consumption. Slightly more than two thirds (67.3%) of the farmers practiced mixed livestock farming with different animal species mainly cattle and chicken, while the remaining 30.8% kept only pigs.

All the farmers interviewed confined their pigs in the pens until they attained selling/ slaughter weight. However, three quarters of them practiced other biosecurity measures such as restrictions on movement of visitors to the production areas (44.2%), farm hygiene (42.3%), disinfection (26.9%) and vaccinations (7.7%). None of the farmers reported having vaccinated their pigs against rotavirus and those who did practice vaccination reported having vaccinated against other diseases such as foot-and-mouth disease and parvovirus infections. More than half (55.8%) of the

respondents reported occasional diarrhea in pigs, 9.6% frequent diarrhea and 34.6% had not witnessed any case of diarrhea in their farms. More than half (55.8%) of the respondents had an average litter size of more than 10 piglets per sow while 40.4% reported having between five and ten litter size and only 3.8% reported an average litter size of below five.

Pig housing structures with both floor and walls made of concrete (57.7%) were the most common floor type followed by 28.8% those with floor made of concrete and walls made of wood and 13.5% having earthen floor with wooden or iron sheets walled pig houses.

Almost half (48.0%) of the farmers fed their pigs more than one type of feed (mixed) with only 5.8% feeding farm/kitchen waste to pigs. Those farms who feed commercial feeds were 30.8% while 15.4% feed home-made feed rations. Wells were the most common source (59.6%) of drinking water for the pigs with 40.4% using piped water from the county government supply.

Half of the farmers kept cross breed pigs with 40.4% of them keeping exotic breeds and 9.6% keep local breeds of pigs. Natural mating was reported to be practiced by 82.7% of the respondents as the common breeding method, 7.7% practiced artificial insemination and 3.8% practiced both artificial insemination and natural mating. However, 5.8% of the pig farmers did not breed their pigs but sourced from other sources. More than half (55.8%) of the farmers breed their pigs for replacement stock, 28.8% sourced pigs from pig breeders while 9.6% purchased their replacement stocks from their neighboring farms and only 5.8% sourced from the market (Table 4.4).

Table4. 4: Summary table for the questionnaire

Type of variables	Categories of variables	Respondents (n=52)	Percentage (%)
Gender	Male	32	61.5
	Female	20	38.5
Highest level of education	Primary	10	19.2
	Secondary	12	23.1
	Post-secondary	25	48.1
	Informal	5	9.6
Source of income	Formally Employed + pigs	14	26.9
	Own other businesses + pigs	32	61.5
	Pigs main source of income	6	11.6
Length keeping pigs (years)	<6	30	57.8
	6-10	11	21.1
	>10	11	21.1
Herd size	1-10	8	15.4
	11-20	9	17.3
	21-50	18	34.6
	51-100	9	17.3
	>100	8	15.4
Average litter size	<5	2	3.8
	5-10	21	40.4
	>10	29	55.8

Type of variables	Categories of variables	Respondents (n=52)	Percentage (%)
Pig houses	Concrete floor and walls	30	57.7
	Concrete floor and wooden walls	15	28.8
	Earthen floor and wooden/iron sheet walls	7	13.5
Source of feed	Commercial feeds	16	30.8
	Homemade feed	8	15.4
	Farm waste	3	5.8
	Mixed (mixture of either of the other types)	25	48.0
Source of water for pigs	Well	31	59.6
	Piped (county government)	21	40.4
Breed of pigs	Exotic	21	40.4
	Cross- breed	26	50.0
	Local	5	9.6
Breeding method	Artificial insemination	4	7.7
	Natural mating	43	82.7
	AI & natural mating	2	3.8
	Not breeding	3	5.8
Source of replacement stock	Market	3	5.8
	Breeders	15	28.8

Type of variables	Categories of variables	Respondents (n=52)	Percentage (%)
	Self- breed	29	55.8
	Neighbors	5	9.6
Reasons for	Business	44	84.6
keeping pigs	Home consumption	2	3.9
	Savings and business	6	11.5
Presence of other	Yes	36	69.2
animals	No	16	30.8
Diarrhea status	Frequent	5	9.6
	Occasionally	29	55.8
	Never	18	34.6
Biosecurity	Confinement	52	100.0
measures	Disinfection	14	26.9
	Hygiene	22	42.3
	Restriction	23	44.2
	Vaccination against rotavirus	0	0
	Other vaccination	4	7.7

4.5 Descriptive statistics for continuous variables

The average number of years the respondents had kept pigs was eight years with median of five years and a range of 1 to 30 years. Furthermore, the mean herd sizes were estimated at 104.3 pigs per farm with a median of 43, a standard deviation of 10.2 and range of 5-1,100 pigs. On the other hand, the average number of piglets per farm was 27 with a median of 17.5 and standard deviation of 30.2 and a range of 1-147 piglets. (Table 4.5)

Table4. 5: Continuous variables

Variable	Mean	Median	Standard deviation	Range
Number of years keeping pigs	8.0	5 .0	3.7	1-30
Number of piglets in farm	27.0	17.5	30.2	1-147
Pig herd sizes	104.3	42.5	10.2	5-1100

4.6 Factors associated with diarrhea in pig farms

In farms where pig houses had concrete floor and wooden walls, the likelihood of diarrhea cases were 5 times less likely as compared to farms with houses having earthen floor and wooden walls ($p = 0.01922$). Farms that relied on pigs as their main source of income were 12.5 times less likely to have diarrhea cases as compared with farms where owners had formal employment and owned other businesses as their source of income. Even though, most farms (61.5%) reported owning businesses in addition to raising pigs, there was no statistical difference on occurrence of diarrheal cases in farms. Farms which fed pigs with mixed types of feed were 5 times less likely to have cases of diarrhea reported as compared to other types of feeds ($p = 0.0240$). However, the use of farm wastes and preparing own feeds increased the risk of diarrhea in the pig farms. Finally, rearing other animal species together with pigs appeared to reduce the incidences of diarrhea since farms which had other animals present were 5 times less likely to report diarrhea in pigs (Table 4.6).

Table4. 6: Factors associated with diarrhea in pig farms

Types of variables	Estimate	s.e	z-value	P value	OR
Concrete floor and wooden walls	-1.5950	0.6813	-2.341	0.01922	0.2
Earthen floor and wooden/iron sheet walls	-0.2733	0.9415	-0.290	0.77160	0.8
Concrete floor and walls	Ref				
Pigs as main source of income	-2.5257	1.24499	-2.029	0.0425	0.08
Obtain income from own businesses and raising pigs	0.02198	0.71034	0.031	0.9753	1.0
Obtain income from formal employment and keeping pigs	Ref				
Uses mixed feeds (commercial, farm wastes, market wastes)	-1.7075	0.7567	-2.257	0.0240	0.2
Uses farm wastes as feeds	16.0997	2284.1019	0.007	0.9944	
Prepare their own feeds	0.4796	1.2462	0.385	0.7004	1.62
Use commercial feeds	Ref				
Responding “yes” to keeping other animals in farm	-1.7228	0.8270	-2.083	0.0372	0.2
Responding “NO” to keeping other animals in farm	Ref				

4.7 Factors associated with rotavirus infections in pig farms

On further analysis, it was shown that farms where the gender of the pig farmers was male had increased risk of testing positive for rotavirus infection. Indeed, the male headed farms were 4 times more likely to have rotavirus infection as compared to those farms where women were responsible ($p= 0.0361$) (Table 4.7). Rotavirus was detected in a higher proportion (41.2%; 14/34) of the farms that had history of diarrhea compared to that (33.3%; 6/18) of the farms with no history of diarrhea (Figure 4.7). However, there was no statistical difference ($p>0.05$) in rotavirus status and history of diarrhea in the farms.

Table4. 7: Factors associated with rotavirus infections

Types of variables	Estimate	s.e	z-value	P value	OR
Male farmers	1.3863	0.6614	2.0960	0.0361	4.0
Female farmers	Ref				

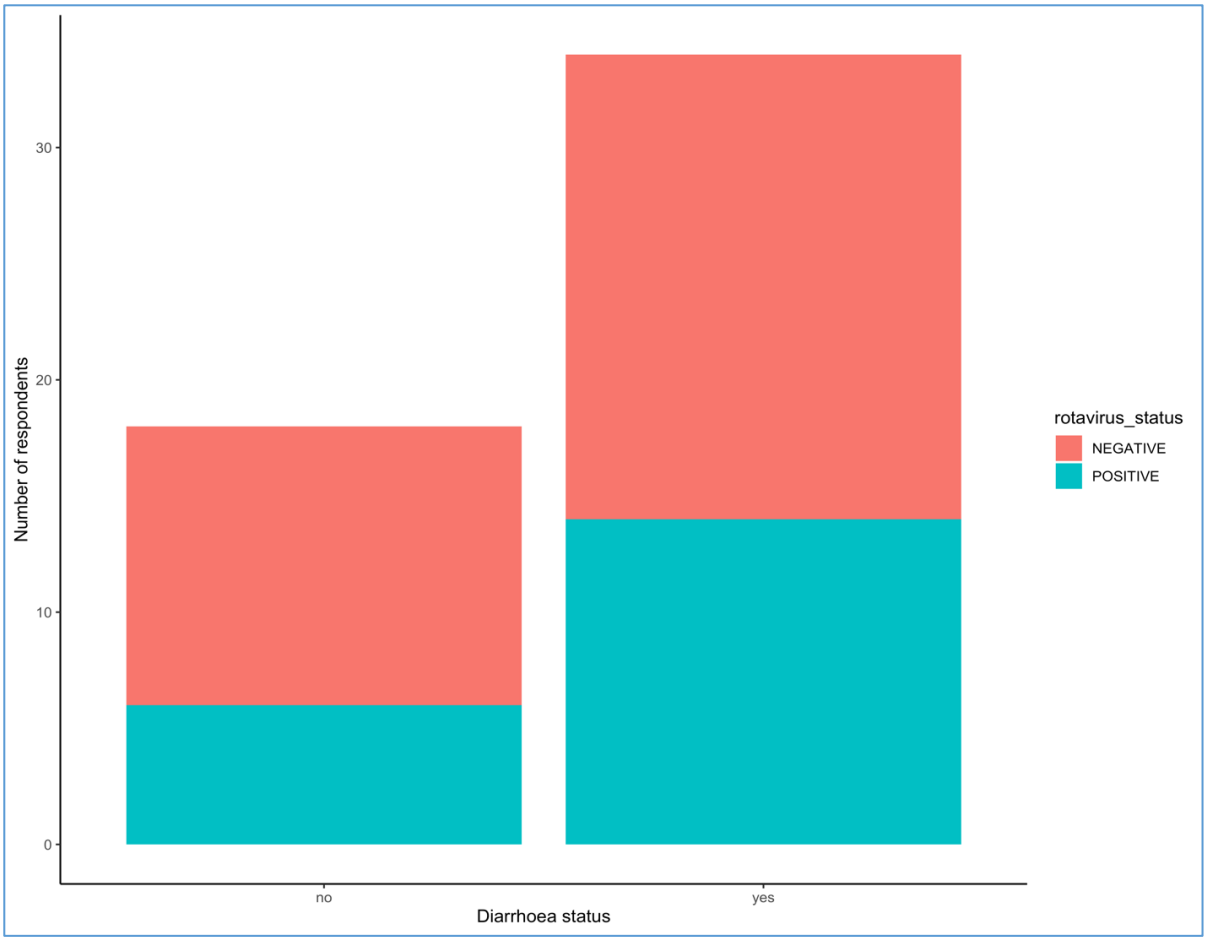

Figure4. 7. Relationship between reported history of diarrhea in farms and rotavirus status

CHAPTER FIVE: DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Discussion

Rotavirus has been documented in most parts of the world as a significant cause of losses in pig farms especially in smallholder pig farms (Vlasova *et al*., 2017; Amimo *et al*., 2015). Many studies have shown that rotavirus can be detected in both diarrhoeic and clinically healthy pigs (Flores *et al*, 2021; Theuns *et al*., 2016, Amimo *et al*., 2015; Collins *et al*., 2010). This current study reinforces this because the samples were collected from clinically healthy piglets and rotavirus geno-groups were detected. The presence of rotavirus in asymptomatic pigs could be due to continuous presence of maternal antibodies and animals sampled could have developed immunity earlier hence not showing signs yet they have the virus (Amimo *et al*., 2015; Flores *et al*., 2021). These positive asymptomatic pigs would act as reservoirs and continuously shed the virus to the environment promoting the spread to both humans and other animals. This therefore amplifies the need for frequent surveillance of rotaviruses in pig farms to successfully prevent and control it.

This study shows that rotavirus is circulating in pig farms in Kiambu County. Rotavirus geno-groups A and C were detected. For this current study, the farm level prevalence was 38.5% which is lower than what was reported in piglets from Belgium (Theuns *et al*., 2016) where 61% of the farms were rotavirus positive. The difference could be due to the target sampled piglets whereby, in this study, the piglets sampled were clinically healthy unlike the Belgian which were diarrhoeic. Notably, 23.1% of the farms had RVC which is slightly higher than 16.7% reported by Theuns *et al*. (2016) in diarrhoeic piglets in Belgium farms. On the other hand, 13.5% of the farms had RVA which was way below 86.7% reported in Phillippines from backyard farms (Murao *et al*., 2019). This study detected rotaviruses in 16.5% of the samples which is similar to 16.7% reported in Spain (Halaihel *et al*., 2010) but lower than 30.9% reported in a study in Brazil (Flores *et al*., 2021)

and 78% reported in Italy (Ferrari *et al*., 2022). For the Brazil the sampled pigs were diarrhoeic while for the Italy study the sampled pigs were for a wider range of age.

Similarly, animal level prevalence demonstrated RVC (10%) being more prevalent serogroup followed by RVA (6.3%) which agrees with reports of studies done in Brazil (Molinari *et al*., 2016) and the USA (Chepngeno *et al*., 2019; Marthaler *et al*., 2014a). However, this was contrary to what was reported in Italy (Ferrari *et al*., 2022) and Spain (Monteagudo *et al*., 2022) and in USA (Amimo *et al*., 2013a) where rotavirus A was more prevalent. The difference could be brought about by the age of the pigs sampled, different environmental conditions and the number of samples. Interestingly, one sample (0.4%) from one farm (2%) had co-infection with RVA and RVC. This was lower than 4.2% of samples reported in Brazil (Flores *et al*., 2021), Switzerland where more than two thirds of the samples had co-infections (Baumann *et al*., 2022) and 86.4% in USA (Marthaler *et al*., 2012). The findings from this study confirm that different serogroups of rotavirus are circulating in pig herds either as single or mixed infection.

In this study, RVB was not detected from any of the piglets sampled from the farms. This is similar with studies in USA (Chepngeno *et al*., 2019; Amimo *et al*.,2013a) where none of the piglets sampled tested positive for RVB and in Switzerland where no single infection with RVB was reported (Baumann *et al*., 2022). Contrary to this, studies in United States and Brazil reported 46.8% and 64.4% (Miyabe *et al*., 2020) respectively of the samples having RVB.

The quantitative real time PCR enabled the determination of the viral load through quantification of RNA in each positive sample. It detected as low as 18ng/µl and 40ng/µl for RVC and RVA respectively. Viral loads determine the severity of the rotavirus in the piglet and the manifestation of the clinical signs. Rotaviruses are highly infectious and only requires few particles to infect and also environmental contamination and spread to other hosts (Zimmerman *et al*., 2019). With the

validation of conventional RT PCR with longer base pair primers results using RT qPCR showed the same results. This indicate that these two methods can be used interchangeably. Contrary to this a study comparing the two methods found out that RT qPCR was more sensitive than conventional RT PCR (Dagher *et al*., 2004). In this study different primers were used for the two methods and this could explain the difference. In as much as the two methods can be used interchangeably quantitative RT PCR is more superior in terms of advantages. These include ability to monitor the amplification progress, requires less time as compared to conventional PCR since there are no post amplification activities such as gel electrophoresis. Another advantage is that viral load can be determined through quantification of RNA and also less risk of spillovers and contaminations as compared to conventional PCR. These are in agreement with other studies that have been done to compare the two methods (Staggemeier *et al*., 2015: Martherler *et al*., 2014b)

This study found out that most of the pig farms (61.5%) were owned by males, post-secondary level of education 48.1% was the most common and most farmers had less than 6 years in pig rearing. Also 84.6% of the farmers kept pigs for business reason. This shows that pig rearing is an upcoming business opportunity that people are starting to venture into. This could be attributed to increased urbanization and close proximity to the fast-growing Nairobi city and therefore increase in demand for pork as a relatively inexpensive source of animal protein. It has been shown that there is rising high increase in demand for pork (Murungi *et al*., 2021; FAO, 2021). Similar to this study, other studies have reported male dominance in pig ownership in Kenya (Embu County) 92.6% (Kithinji *et al*., 2017) and in Uganda 62% and 66% (Muhanguzi *et al*., 2012; Ikwap *et al*., 2014) of the pig farmers were males. Contrary to this finding, studies in western part of Kenya reports females being predominant in pig rearing sector and that most farmers had no formal

education and have kept pigs for more than six years (Mwabonimana *et al*., 2020; Eshitera *et al*., 2012). The difference could be due to differences in sociocultural practices in the two regions and also that western part of Kenya is mainly rural unlike Kiambu where most of it is urban. Other studies from other African countries also differ with this finding, in South Africa (Sibongiseni *et al*., 2016), Zambia (Abigaba *et al*., 2022) and rural part of Uganda (Ampaire and Rothschild, 2011) where most pig farmers are females. The difference could be the regions of the studies because the farmers for the three countries were from the rural parts and those of Zambia practiced traditional pig farming. In rural areas women are unemployed and they are home most of the time therefore available to own and take care of the pigs (Ikwap *et al*., 2014). Traditionally pigs are kept by women but when commercially reared the men are involved (Dick *et al*., 2004). In urban areas land resource is limited and therefore pig rearing is under intensive system and for commercial purposes (Muhanguzi *et al*., 2012). In line with this statement 100% of the farmers in this study confine their pigs all year round and 84.6% kept pigs for business reasons.

Majority of the farmers (71%) in this study were literate with secondary and post-secondary level of education. This group of farmers are flexible to changes in technology and innovations such as disease control, housing and reproductive technologies and other factors of production and therefore improve on their productivity and profit margins (Ume *et al.,* 2020; Muhanguzi *et al.,* 2012).

Another finding of this study is that 50% of the farmers kept crossbred pigs. Similar finding has been reported in Cameroon where 72.75% of the farmers kept crossbred pigs (Kouam *et al*., 2020). A study by Noguera and colleagues (2019) found out that crossbred pigs had more advantage than purebreds (heterosis effect) in terms of litter size and pork quality. More than three quarters of the farmers (82.7%) used natural mating with 11.5% using artificial insemination as the method of

breeding. The fact that artificial insemination is present though practiced by few farmers, shows that farmers are adopting to new technologies which is supported by the high literacy level of the farmers in this current study. More than half of the farmers (55.8%) obtained replacement stock from their own farms. This could be attributed to the finding that most farmers reported high litter size of more than 10 at birth and high number of piglets (mean of 27 and median of 18) on-farm which are source of replacement stock for future flocks. A study in Tanzania found out that most farmers had fewer piglets per farm and that most of them get their replacement stock from other farms or sellers (Karimuribo *et al*., 2011). Apart from keeping pigs, 69.2% of the farmers kept other animals in their farms and 61.5% own other businesses. This is in agreement with other studies that pig farmers engage in other activities such as other livestock keeping (Obala *et al*., 2021; Karimuribo *et al*., 2011; Kagira *et al*., 2010). This implies that even though pig production sector is a potential employment opportunity in the area it does not provide enough to the farmers and therefore have to do other activities in order to increase their earnings. Most of the farmers keep pigs for businesses and therefore if the government would provide market opportunities and proper policy regulations of the sector more jobs would be created.

Slightly above half of the farmers (55.8%) reported occasional diarrhea in their pig herds. This indicates that diarrhea is common in pig farms in Kiambu. Diarrhea could be caused by several factors such as change in feed, stress due to weaning or even confinement, poor hygiene levels or diseases such as rotavirus infections. There is low practice of biosecurity measures in farms among farmers in this study. For instance, only 26.9% of the farmers used disinfection and only 7.7% vaccinated their pigs against pig diseases such as foot and mouth and none of the farmers had vaccinated against rotavirus which was detected in this study area. In agreement with this study, other studies have also found low biosecurity levels in small holder pig farmers. In Cameroon

(Kouam *et al*., 2020) and in border of Kenya and Uganda (Nantima *et al*., 2016) where they found low level of biosecurity among pig farmers. This study has detected rotavirus in the study area and with the low biosecurity practiced by farmers will make this disease endemic in the area. Mutua and Dione (2021) in their review stated that improving and implementing farm level biosecurity not only reduce risk of diseases but also greatly minimize financial losses due to disease outbreaks. From this low level of biosecurity practices, it shows that the farmers may not be aware of the importance or the need of biosecurity measures in their farms. This therefore calls for speedy action by the veterinary officials and the government in offering trainings to farmers on different biosecurity measures they can implement and the benefits of implementation.

Pigs owned by men were 4 times more likely to have rotavirus infections compared to those owned by women. The reason could be that men may not be taking into consideration the hygiene of the pig pens so much and focusing more on the growth performance of the pigs. Studies have shown that females have better attitude, high level of practice for hygiene and higher hygiene compliance than males (Laskar *et al*., 2018; Bimerew and Muhawenimana, 2022)

From this study, factors such as type of pig house, type of feed, source of income and presence of other animals were significantly associated with diarrhea. Pig houses with concrete floor and wooden walls ($p=0.01922$) were 5 times less likely to report diarrhea as compared to those with earthen floor and wooden walls. This could be attributed to the fact that concrete floors are easy to clean and drain (Dione *et al*., 2022). This therefore reduces wetness of the floor and accumulation of pathogens. A study in Australia reported higher percentage of floor contamination in wet pens as compared to dry pens (Banhazi, 2013). Another study by Rantzer and Svendsen, (2001) showed that there was better pen hygiene and reduced gastrointestinal problems morbidity in slatted concrete floors. Another reason could be that fecal material removal is better in concrete

floor unlike earthen floor where not all fecal material can be removed and this can host several pathogens (Venglovsky *et al*., 2018).

Presence of other animals in the farm (p=0.0375) reduces the likelihood of diarrhea 5 times as compared to keeping only pigs. Explanation to this could be that the transmission of causes of diarrhea in pig farms are majorly within species and less between species. A review by Keesing (2006) explained different epidemiological models where increase in species diversity decreased disease risks. These include contact reduction between the pathogen and susceptible host and secondly decreasing the pathogen load by increasing other species (pathogen/pressure dilution). Contrary to this study, other studies have shown presence of other animals in pig farms increases the risk of diseases causing diarrhea. For example, a study in Vietnam found presence of chicken in pig farms having strong association with porcine epidemic diarrhea status citing movement of animals as a way of pathogen transmission (Mai *et al*., 2020). Studies in Philippines (Murao *et al*., 2018), India (Vinodhkumar *et al*., 2019) and Nigeria (Delia *et al*., 2019) also found keeping of other animals alongside pigs increases the risk of rotavirus infections.

Those farmers depending only on pigs as a source of income were less likely to report diarrhea compared to those having formal employment and pigs which were 2.5 times likely to report diarrhea. This could be attributed to, those depending only on pigs put all their focus on pigs and biosecurity measures as is the only source of income while those on formal employment do not focus much on pigs as they have another source of income.

Implications of the study

The groups of rotaviruses circulating in pig farms in the study area are now known. This will help in the prevention of rotaviruses especially with vaccine development. It should be a vaccine that is able to target all the groups present.

This study will also help the authorities and veterinary community in the area understand the characteristics of pig farmers in the area. This study found out that most of pig owners are male and most are literate. This will guide in identifying the target groups in pig production trainings and even identification of methods passing massage to pig farmers in the area.

This study highlights the need for farmer education on importance of biosecurity measures in disease prevention and control in order to reduce losses due to diseases.

Regarding research, this study could act as a baseline study for future advanced studies such as longitudinal study on rotaviruses in Kenya. This will also guide development of policies that help in prevention of rotaviruses in farms and protocols of regular surveillance of rotaviruses in pig farms.

Limitations of the study.

1. Limited resources limited the study from further characterization of the rotavirus groups through sequencing.
2. This study was carried out as a cross sectional study and could only capture information in one season. The results of different seasons are unknown and therefore could be underrated or overrated by this study.
3. Only piglets of three months and below were sampled and tested and therefore the status of the other age groups from farms in this study area are unknown.
4. Bias from the veterinarians and animal health practitioners whose referrals of pig farmers were from. This is because they only refer the researchers to those farmers they had an association with.

5. Only 52 farms in Kiambu were sampled and this might not represent all pig farmers in the area. These limitations could be managed with an advanced research study in the area that captures all the above mentioned areas.

5.2 Conclusion

1. Rotavirus is indeed present and circulating in pig farms in Kiambu County and that RVC is the predominant sero-group followed by RVA while RVB was not detected.
2. Reverse transcriptase quantitative PCR and conventional reverse transcriptase PCR can be used for detection of rotaviruses interchangeably though quantitative has more advantages.
3. Gender of the farmers influenced the occurrence of rotavirus in pig farms. Various management practices influenced the occurrence of diarrhea in the farms.
4. There is low practice of biosecurity measures in pig farms and this could lead to persistence of some diseases such as rotavirus in the area.

5.3 Recommendations

1. Active surveillance (serological and molecular) on rotavirus infections in pigs. Molecular characterization should be done to understand the strains and the relationship with other rotaviruses detected elsewhere in the world.
2. Introduction of new technologies such as RTqPCR for quick detection of rotaviruses.
3. Creation of awareness to pig farmers on the presence and risk of rotaviruses in pigs.
4. Farmers should improve on biosecurity measures to reduce the incidences of rotavirus infection and improve the production and productivity of their herds.

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Appendix Survey questionnaire

Questionnaire Survey on the Assessment of risk factors associated with Rotavirus infection in pigs in Kiambu County

Household No. _____ Date ..…/…/2022

I. Socio-demographic characteristics of respondents in the sample population

Name ----------

Location----------

1. Gender of respondent **A)** Male **B)** Female
2. What is your highest level of education?

A) Primary **B)** Secondary **C)** Postsecondary **D)** informal education

3. What is your household main source of income?

A) Employed

B) Housewife

C) Self-employed

D) Other specify____

4. How long have you kept pigs (years)?.....
5. How many pigs do you keep by category:

A) Sows ……

B) Boars……

C) Piglets…….

6. What type of pig houses do you have?.....

7. What is your main source of feed to the pigs?.....

   A. Commercial feeds,

   B. Hotel wastes,

   C. Home- made feed

   D. Pastures,

   E. Farm wastes

   F. Scavenging from the farm environment

8. Which breeds of pigs do you keep?.....

   A) Exotic B) local breeds C) crossbred

9. Why do you keep pigs? A) form of savings **B)** Household assets **C)** Farm business **D)** home consumption E) Others

10. Where do you obtain your replacement stock of pigs from? A) market B) breeders C) neighbors D) other

11. How do you raise your pigs?: A) Free range B) complete confinement C) mixed system

12. Which other livestock do you raise in the farm?

A) Cattle B) Goats C) Sheep D) chicken E) Any other specify……

13. Which type of breeding do you practice in the farm for the pigs?

   A) Artificial insemination B) Natural mating C) Other

14. What is the average litter size from the sows?

   A) <5 B) 5-10 C) >10

15. How frequent do you observe diarrohea in piglets in the farm?

A. Occasionally

B. Very frequent

C. Never

16. Where do you obtain water for the pigs? A) Borehole B) Dam C) River D) other

17. Have your pigs been vaccinated against diseases? (A) Yes (B) No

18. If yes, against which diseases.....

19. When was the last vaccination done? ……..…….

20. How do you prevent disease outbreaks in the farm?.....