HEPATITIS E VIRUS PREVALENCE AMONGST BLOOD DONORS IN SELECTED REGIONS OF KENYA

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DECLARATION AND APPROVAL

Declaration by the student

This thesis is my original work and has not been presented for a degree in any other university or for any other award.

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I confirm that the work reported in this thesis was carried out by the candidate under my supervision.

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DEDICATION

I dedicate this work to family, friends and those who in one way or another have been the pillar of my strength.

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ABSTRACT

Currently, Kenya is carrying out mandatory tests for four transfusion-transmissible infections for the provision of safe adequate blood as required by the World Health Organization. These mandatory tests include HIV 1 and 2, hepatitis B and C viruses and syphilis. Hepatitis E Virus is a transfusion-transmissible virus that can cause lasting liver illness and therefore, an important public health concern. Presently, there is no scientific documentation on Hepatitis E Virus prevalence of voluntary blood donors in Kenya. The objective of the present study was to evaluate Hepatitis E Virus prevalence of voluntary blood donors in Kenya. The findings from this study will aid in the development of safe blood transfusion policy, hence mitigate transfusion-transmissible Hepatitis E Virus via blood and blood products. Blood samples were obtained from voluntary blood donors upon consenting in six regional blood collection centers in Kenya. The blood samples were transported under cold chain to the national central testing laboratory in Nairobi, Kenya to test for IgG and IgM seroprevalence using ELISA. Confirmatory test was done using RT-PCR for 19 blood samples which tested seropositive for Hepatitis E Virus IgM and/or IgG antibodies. The present study recorded a Hepatitis E Virus seroprevalence of 4.9% which is similar to non-endemic countries. There was no association between Hepatitis E Virus seroprevalence and gender, age or region of blood collection. This study also recorded Hepatitis E Virus IgM and IgG seroprevalences of 3.4% and 2.6% respectively. There was an association between age and Hepatitis E Virus IgG seroprevalence. No Hepatitis E viremia was detected among IgM and/or IgG seropositive blood samples. This study confirmed for the presence of Hepatitis E Virus IgM and/or IgG seropositive blood samples among blood donors in Kenya and therefore, recommends screening of blood products to mitigate transfusion-transmissible Hepatitis E Virus infection.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Hepatitis E is a liver disease caused by Hepatitis E Virus. Hepatitis E is a self-limited, acute illness but may result in fulminant hepatitis (Khuroo and Khuroo, 2010; WHO, 2010b; CDC, 2012). It is the fifth known human hepatitis virus globally which cause most of the acute viral hepatitis (Chandra *et al*., 2010; Hoofnagle *et al*., 2012; Krawczynski, 2013). It is estimated that twenty million Hepatitis E Virus (HEV) infections, more than three million acute incidences of Hepatitis E Virus infection, and seventy thousand Hepatitis E related deaths are reported yearly (WHO, 2014a; Murali *et al*., 2015).

Most Hepatitis E deaths and infections are reported in endemic countries, but the number of incidences in low-endemic nations has increased (Murali *et al*., 2015). Developing countries, such as Southeast Asia and India have high Hepatitis E Virus seroprevalence ranging from 27% to 80% (BAPC, 2012; Hoofnagle *et al.*, 2012; Jong-Hoon *et al.*, 2014; Murali *et al*., 2015). Surprisingly, some developed countries including the United States of America (USA) and the United Kingdom (UK), have reported unexpectedly high Hepatitis E Virus seroprevalence of 21 to 25% (Meng *et al*., 2002; Murali *et al*., 2015). Acute Hepatitis E has a death rate of 1% to 4%, with risk being higher in immunodeficient patients and pregnant women (Murali *et al*., 2015).

Hepatitis E Virus is a small non-enveloped, positive-sense, single-stranded ribonucleic acid (RNA) virus, with a diameter of 27 to 34 nm (Chandra *et al.*,

2008b; Christensen *et al.*, 2008). Hepatitis E Virus replicates in hepatocytes cytoplasm and sometimes in the colon, lymph nodes and small intestine cells (Ahmad *et al*., 2011; Hoofnagle *et al*., 2012; Kumar *et al*., 2014). It presents itself in 3 discontinuous open reading frames (ORF): ORF1, ORF2 and ORF3 (Ahmed *et al*., 2011; Unzueta and Rakela, 2014)

Hepatitis E Virus exists in the family hepeviridae, which comprises 2 genera: *Piscihepevirus* and *Orthohepevirus* (Endale *et al.*, 2013). Human beings are the natural hosts for Hepatitis E Virus (Labrique *et al.*, 2010; CHP, 2011; WHO, 2014a). The mean incubation period of Hepatitis E Virus is 40 days and affects more men than women (Shrestha *et al*., 2007, Kamar *et al*., 2014). Individuals between the ages of 15 to 40 years commonly exhibit symptomatic infection of Hepatitis E Virus (Shrestha *et al*., 2007).

The Hepatitis E infection causes a moderate illness devoid of jaundice that goes unexamined. The major symptoms of hepatitis include jaundice, dark urine, pale stools, anorexia, hepatomegaly, fever, stomach pain and tenderness, vomiting and queasiness (Baylis *et al*., 2012a; Kamar *et al*., 2014; Ahmed *et al*., 2015). These symptoms are indistinct from those witnessed during any extreme period of hepatic ailment and exist even after two weeks (WHO, 2010b).

Intense Hepatitis E Virus infection can sometimes bring about acute liver failure (fulminant hepatitis) and death. Acute liver failure happens often during pregnancy (Arankalle *et al*., 1999). Chronic Hepatitis E Virus infection has been reported

among immunosuppressed and immune-compromised individuals (Kamar *et al.*, 2008; WHO, 2010b).

The very first epidemic of Hepatitis E ailment took place from 1955 to 1956 in New Delhi, India (WHO, 2010b; BAPC, 2012). Other Hepatitis E Virus episodes have been reported in Asia, Mexico and Africa. Between twenty to sixty percentage of overall acute hepatitis cases are estimated to be caused by Hepatitis E Virus (Mun-Hon *et al.*, 2010; Teshale *et al.*, 2010; Eyasu and Dale, 2011).

Hepatitis E Virus is egested in the fecal of infected people and is transmitted via the following routes: fecal-oral, contaminated water; ingested food, infected animals (undercooked pig, deer and wild boar meats); transfusion of contaminated blood products; transplant of Hepatitis E Virus infected grafts; maternal-fetal transmission (vertical transmission) and zoonosis through human exposure to body fluids of infected animals (Khuroo *et al.*, 2004; Christensen *et al.*, 2008; Matsubayashi *et al*., 2008; Qing-Shun *et al.*, 2010; Endale *et al.*, 2013; Jens and David, 2014; WHO, 2014b).

Hepatitis E disease is largely preventable by safe disposal and treatment of human waste, avoid contact with infected animals, avoid undercooked meat of wild deer and pig, blood screening before transfusion and provision of clean water, (Christensen *et al*., 2008; Aggarwal and Naik, 2009). Acute Hepatitis E infection usually does not require treatment, however, chronic infection is treated using antiviral therapy or by reducing immunosuppression in organ transplanted patients.

This is because chronic Hepatitis E may lead to rapid progression to cirrhosis and demise (Kamar *et al*., 2014; Murali *et al*., 2015; Guerra *et al*., 2017).

Acute Hepatitis E Virus infections have been reported among blood donors and confirmed through Hepatitis E Virus RNA detection (Khuroo and Khuroo, 2010). Recent reports on volunteer blood donors have revealed the predominance of Hepatitis E Virus antibodies in the blood samples using enzyme-linked immunosorbent assays (ELISA) (Mun-Hon *et al.,* 2010; Baylis *et al.,* 2012b; Hassan *et al.,* 2013). Transfusion-transmissible Hepatitis E Virus infection by contaminated blood components and products has been documented in the United Kingdom, Japan, and Europe (Colson *et al*., 2007; Matsubayashi, *et al*., 2008; Adlhoch *et al*., 2009). In Kenya, Hepatitis E Virus prevalence among blood donors and the general population has not yet been documented and therefore, it is against this background that the present study was designed to determine the Hepatitis E Virus prevalence of blood donors in Kenya.

1.2 Statement of the problem

It is estimated that twenty million Hepatitis E Virus infection, more than three million Hepatitis E infection cases, and seventy thousand Hepatitis E related fatalities are reported yearly (Murali *et al*., 2015). In Kenya, the cases of Hepatitis E Virus among blood donors remain unclear and underestimated due to asymptomatic infections, lack of testing and the fact that Hepatitis E is not a notifiable disease. Reports on seroprevalence are valuable and they largely depend on the different efficacy of the assays used (Jens and David, 2014).

Hepatitis E Virus infection and its connections to public health and safety of blood are uncertain in Kenya (Slot *et al.,* 2013). Studies have document that Hepatitis E Virus has been transmitted through blood donations in endemic nations. Besides, Hepatitis E Virus IgG seroprevalence is on the rise among blood donors in low endemic countries (Hassan *et al.,* 2013). High prevalence of IgM against Hepatitis E Virus has also been reported after blood transfusions in European and Asian countries (Mun-Hon *et al.,* 2010; Baylis *et al.,* 2012b).

Transfusion of blood and blood products which have not been tested for Hepatitis E Virus is associated with chronic Hepatitis E infection in immune-compromised and anemic individuals (Kamar *et al.*, 2008; WHO 2010a; CDC, 2015). Hepatitis E Virus infection is underreported and thus, it should be considered when other acute hepatitis causes are exhausted (Jens and David, 2014). Though there are Hepatitis E Virus vaccines in clinical trials, none has been approved for Hepatitis E Virus infection (Drobeniuc *et al.*, 2010; Eyasu and Dale, 2011).

In Kenya, Hepatitis E has been reported and mostly affects ages of 18 and 65 years due to the blood donor age provisions. This disease is therefore of critical health importance. Since the prevalence of Hepatitis E has not been established in Kenyan population and among blood donors, Kenya is unable to enact policies that will mitigate cases of Hepatitis E outbreaks.

1.3 Justification and significance of study

Although Hepatitis E Virus is not amongst the mandatory tests before blood transfusion, it remains a public health concern (WHO, 2014a). Prevention of

Hepatitis E Virus infection is the greatest effective approach. Screening for Hepatitis E Virus in potential blood donors and the general population usually add more knowledge on prevention measures for Hepatitis E Virus infection. This helps in the implementation of policies which will lower the rate of infection (Cyrille *et al.*, 2014). Screening for blood in Kenya will add more knowledge on Hepatitis E Virus prevalence among the blood donors and will aid in the enactment of policies which will ensure that the health of the blood recipient is prevented from Hepatitis E infection. Blood donors with Hepatitis E Virus will also be supported by health facilities in the treatment of the Hepatitis E infection before it becomes chronic and fatal.

1.4 Hypothesis

Blood donors have no Hepatitis E Virus in Kenya

1.5 Objectives

1.5.1 General objective

To determine Hepatitis E Virus prevalence amongst blood donors in in selected regions of Kenya

1.5.2 Specific objectives

1. To determine the IgM and IgG seroprevalence of Hepatitis E Virus among blood donors in selected regions of Kenya
2. To determine the demographic trends of Hepatitis E Virus seroprevalence of blood donors in selected regions of Kenya
3. To determine Hepatitis E Virus viremia among seropositive blood samples in selected regions of Kenya

1.6 Limitations

Several limitations were encountered during the study, these included, time for the study, finances, reaching the far flung areas and also confirmatory testing for the samples that turned positive during Elisa testing. The duration time was two months and this would have made it impossible to reach every corner of the country to come up with a national prevalence rate for HEV taking note that some of these areas are so remote and travelling over these distances would be near impossible within that time frame.

To acquire testing kits for the research was another of the challenges .Test kits were only available out of the country and the cost of each was dear. To have finalised this study within the stipulated period, availability of funds and the timely acquisition of the same was key. Blood donation activities are provided in the whole country and the ability to reach all these areas and also get samples from voluntary non remunerated donors was a tall order. Most parts of the hard to reach areas also source their blood from replacement donors of whom this research was not targeting.

To be able to publish the findings, confirmatory testing for HEV after the screening using Elisa was critical as per WHO requirements. Access to Nucleic Acid Testing (NAT)would only be carried out of country or another cost would be made for the procurement of the same from out of the country.

1.7 Delimitations.

To enable the timely execution of the study, collection of samples from selected areas of the country was found to be a game changer and for this to happen, within the two months, visitation to regional centres where donors visit for donation was

included in the design of the research. Constrains of finances to visiting all the selected areas , procure the testing kits both for ELISA and NAT was managed by mobilisation of finances and also support from the manufacturers of the kits who were able to donate some kits and also the ability to have linkages out of the country for NAT to be carried out.

On minimising the risk of having all blood donors being replacement donors and also having all donors being between 14 years and 16 years,timing for carrying out the study was key. During the two months, all donated blood was from across the population unlike the usual school going period when most blood is sourced from schools.

1.8 Operational definitions

Seroprevalence- The level of a pathogen (HEV) in a population, as measured in blood serum.

Screening-Testing for presence or absence of blood borne diseases.

Fulminant- a clinical syndrome of severe liver function impairment, which causes hepatic coma and the decrease in synthesizing capacity of liver,

Cut Off Value. Calculated Level/value at which a test is interpreted as being either being positive or negative

Blood products-Derivatives of plasma also called Plasma derived medicinal product

Viremia-Presence of virus in the bloodstream

Envelope- outermost layer of many types of viruses that protects the genetic material in their life cycle.

Replacement donors-Blood donors who are either family related or are brought in by family members for donation purposes who donate not voluntarily but only due to the need and relationship to the recipients.

CHAPTER TWO

LITERATURE REVIEW

2.1 Hepatitis E Virus

Hepatitis E Virus is a small, spherical, non-enveloped particle of about 27 to 34 nm known to cause acute viral hepatitis (Mohamand and Mehnaaz, 2008). Hepatitis E is the 5th human viral hepatitis known to cause most acute viral hepatitis globally (Chandra *et al*., 2010; Hoofnagle *et al*., 2012; Krawczynski, 2013). Hepatitis E Virus possesses a genome of about 7.2 kb in length which comprises of the single-stranded ribonucleic acid molecule, capped and polyadenylated at the 5' and 3' termini, respectively (Chandra *et al.*, 2008b; Christensen *et al.*, 2008).

The genome of Hepatitis E Virus possess short non-coding regions at 5′ and the 3′ ends, and 3 discontinuous and to moderately overlapping open reading frames (ORF). Open reading frame one (ORF1) is largest region and codes for non-structural viral proteins and possess a few conserved functional domains such as RNA-dependent RNA polymerase, cysteine protease, RNA helicase, and methyltransferase domains (Chandra *et al*., 2008a; Kamar *et al*., 2014).

Open reading frame two (ORF2) codes for viral capsid protein which interaction with target cells, virion assembly, and immunogenicity (He *et al*., 2008; Kalia *et al*., 2009; Xing *et al*., 2011). The capsid protein is highly immunogenic and antibodies against it are protective. Therefore, the capsid antigen is the preferred protein for the development of vaccines (Purcell and Emerson, 2008). Open reading frame three (ORF3) overlaps ORF2 and encodes for small phosphoprotein that are

involved in virion morphogenesis and release (Graff *et al*., 2006; Chandra *et al*., 2008a; Yamada *et al*., 2009; Emerson *et al*., 2010; Xing *et al*., 2011; Kumar *et al*., 2014).

Hepatitis E Virus replicates in the cytoplasm, with a subgenomic ribonucleic acid producing open reading frame 2 and 3 proteins and the full genomic ribonucleic acid encoding nonstructural proteins which serves as a template for replication (Kamar *et al*., 2014). Hepatitis E Virus replicates in the cytoplasm of hepatocytes and sometimes in the colon and small intestine cells and lymph nodes (Ahmad *et al*., 2011; Hoofnagle *et al*., 2012; Kumar *et al*., 2014).

2.1.1 Classification of Hepatitis E Virus

Hepatitis E Virus exists in the family hepeviridae, which comprises 2 genera: *Piscihepevirus* and *Orthohepevirus* (Endale *et al.*, 2013). The genus *Orthohepevirus* consists of avian and mammalian Hepatitis E Virus variants and comprises of four species: A, B, C, and D. The Hepatitis E Virus is classified into four HEV : 1 to 4 (Huang *et al.*, 2002; Lu *et al.*, 2006; Kamar *et al*., 2014; Murali *et al*., 2015; Sridhar *et al*., 2017).

Genotype 1 is the leading cause of waterborne epidemics and sporadic disease in North Africa and Asia (Purcell and Emerson, 2008; Tashale *et al*., 2010; Murali *et al*., 2015). Genotype 2 has caused an epidemic in Central Africa and Mexico (Purcell and Emerson, 2008). Genotype 3 is considered a reemerging zoonosis and is mainly found in South and North America, Europe, several Pacific countries and Japan (Teshale *et al*., 2010; Murali *et al*., 2015). Genotype 4 is the major cause for

sporadic cases in China, Taiwan, Japan, several European countries and Vietnam (Teshale *et al*., 2010; Murali *et al*., 2015). Epidemiology and clinical disease seem to be associated with the molecular structure of HEPATITIS E VIRUS and this justifies territorial occurrence of different virus HEV (Krawczynski and Foreword, 2013).

HEV 1 and 2 causes Hepatitis E epidemics and are considered human virus. They are transmitted via fecal-oral route due to consumption of contaminated water. HEV three and four are considered swine (pigs) viruses and have the ability to infect humans as accidental host and therefore regarded zoonotic. They do not seem to cause disease in pigs but infect them. Chronic Hepatitis E cases are largely caused by genotype 3 virus as well as genotype 4 in children (Aggarwal and Naik, 2009; Geng *et al*., 2014; Murali *et al*., 2015).

2.1.2 Origin of Hepatitis E disease

An epidemic acute Hepatitis E was first reported between 1955 and 1956 in Delhi, India. The epidemic caused more than twenty-nine thousands symptomatic embittered cases (Boutriouille *et al.*, 2007; WHO, 2010). The second Hepatitis E epidemic occurred in Kashmir valley in 1978 in India. Other HEPATITIS E VIRUS epidemics have occurred in Africa, Asia, and Mexico (Mohamand and Mehnaaz, 2008).

2.1.3 Epidemiology of Hepatitis E disease

It is estimated that twenty million Hepatitis E Virus infection, more than three million acute Hepatitis E Virus incidences, and seventy thousand Hepatitis E Virus related demise are reported yearly (Murali *et al*., 2015). The mean incubation time

of Hepatitis E Virus is forty days, and usually affects individuals between 15 and 40 years old (Shrestha *et al*., 2007). Hepatitis E disease affects more men than women, with a ratio of >3:1 in developed countries and 2:1 in developing countries (Kumar *et al*., 2014). Anti- Hepatitis E Virus rates are lower in the United States of America and Europe compared to Africa and Asia (Hoofnagle *et al*., 2012; Murali *et al*., 2015).

Two epidemiological patterns of Hepatitis E exist in different geographic regions: hyperendemic and low endemic regions. Hyperendemic regions have large outbreaks which affect large populations in developing countries and are usually caused by consumption of contaminated water (WHO, 2011). Hepatitis E occurs as both epidemic and sporadic diseases in hyperendemic regions and is commonly caused by genotype 1. Hepatitis E Virus seroprevalence of 30 to 80% have been reported in hyperendemic regions (Hoofnagle *et al*., 2012). Hepatitis E affects adolescents and adults, and mortality is higher among pregnant women (Hoofnagle *et al*., 2012).

Zoonotic transmission plays an important role in low endemic regions. These regions include developed countries (Europe and United States), where Hepatitis E occurs as isolated cases and small outbreaks, which have been attributed to consumption of undercooked pork and exposure to pigs (Hoofnagle *et al*., 2012). These cases are usually caused by genotype 3 and genotype 4, which are less virulent (Purcell and Emerson, 2008; Hoofnagle *et al*., 2012). In low endemic

regions, the disease usually affects older adults, in whom mortality is higher (Hoofnagle *et al*., 2012).

2.2 Transmission of Hepatitis E Virus

Scientific studies have established that Hepatitis E Virus infections can be transmitted via 4 routes: waterborne transmission, parenteral transmission (bloodborne), consumption of infected wild (deer and boars) and domestic (pig and camels) animals, exposure to body fluids of infected animals (zoonosis), from mother to child (vertical transmission) and transplant with HEPATITIS E VIRUS infected grafts (Chandra *et al*., 2008b; Mohamand and Mehnaaz, 2008; Jens and David, 2014). Consumption of contaminated water is the most prevalent route for Hepatitis E Virus transmission (Chandra *et al*., 2008b**;** Aggarwal and Naik, 2009). However, it is impossible to ascertain the route of transmission, especially in regions of low endemia and in sporadic cases in hyperendemic regions, but zoonotic reservoirs among pig, camels, wild boars and wild deer may play a big role (Woo *et al*., 2014; Lee *et al*., 2016).

Other risk factors associated with Hepatitis E Virus outbreaks include: inadequate hygiene practices (hand-washing), lack of waste disposal and management, inadequate or inappropriate sanitation facilities, lack of basic hygiene items (soap), personal and communal practices, risk behavior, overcrowding and sub-optimal living/shelter conditions (Carolina *et al.,* 2008; Audrey *et al.,* 2012; UNHCR, 2014).

2.3 Symptoms of Hepatitis E Virus infection

Individuals between 15 and 40 years old consistently show symptomatic infection. The incubation duration of Hepatitis E following exposure of Hepatitis E Virus varies with an average of 5 to 6 weeks. Individuals affected by Hepatitis E excrete Hepatitis E Virus viremia between third and fourth week after the commencement of the disease (Aggarwal and Naik, 2009; WHO, 2014a).

Hepatitis E symptoms include abdominal pain, anorexia, fever, nausea, jaundice (sclera of the eyes and yellow discoloration of the skin), enlarged tender liver, vomiting and dark urine (Ohnishi *et al*., 2006; WHO, 2014a). These symptoms are associated with high levels of liver enzymes alanine (aminotransferase (ALT), and aspartate aminotransferase (AST)), mild increases in alkaline phosphatase (ALP) activity and elevated serum bilirubin levels (Teshale *et al*., 2010).

The severity of Hepatitis E Virus infection may range from subclinical to fulminant (Ohnishi *et al*., 2006). Fulminant hepatitis occurs when women are pregnant (Arankalle *et al*., 1999). Hepatitis E infection causes obstetrical complications and deaths in pregnant women. Hepatitis E illness can result in a death rate of between twenty to forty percentage in pregnant mothers in their third trimester (Hughes *et al.*, 2010; WHO, 2014b).

Victims of genotype 4 have extreme clinical symptoms compared to genotype 3 infections (Ohnishi *et al*., 2006). Chronic Hepatitis E infection has been documented in immunosuppressed patients, particularly, organ transplant

recipients being administered with immunosuppressive drugs, with genotype 3 or genotype 4 Hepatitis E infection (WHO, 2014a).

2.4 Diagnosis of Hepatitis E Virus

Hepatitis E is an underdiagnosed illness as a result of the use of serological tests with low sensitivity (Kamar *et al*., 2014). Hepatitis E Virus infection can be determined either directly by detecting Hepatitis E Virus genome in feces or blood samples or indirectly by detecting anti-Hepatitis E Virus antibodies in the serum (Kamar *et al*., 2014). Diagnosis of Hepatitis E Virus infection by detecting serum anti- Hepatitis E Virus is carried out on specific IgG and IgM antibodies to the virus from the patient blood using enzyme-linked immunosorbent assays (ELISA). These enzyme immunoassays have vast activity and provide reproducible results on specific IgM and IgG antibodies to Hepatitis E Virus (Drobeniuc *et al*., 2010).

The anti- Hepatitis E Virus IgM antibody is usually detectable days prior to the commencement of Hepatitis E Virus symptoms and disappears from 4^{th} to 6^{th} month amid acute Hepatitis E infection (Teshale *et al*., 2010). The detection of anti-Hepatitis E Virus IgM is an indicator of acute Hepatitis E Virus infection. Individuals infected with the four Hepatitis E Virus have been compared using six anti-Hepatitis E Virus IgM enzyme-linked immunosorbent assays (Drobeniuc *et al*., 201).

Anti-Hepatitis E Virus IgG is detected soon after the IgM response and may remain up to twelve years after infection (Teshale *et al*., 2010). Presently, the available anti-Hepatitis E Virus IgG enzyme immunoassays have been confirmed against

sera collected from individuals with recent Hepatitis E infection. However, their capability to detect distant infection remains uncertain. The extremity of detection of these assays ranges between 0.25 WHO unit/ml and 2.5 WHO units/ml (Bendall *et al*., 2008). The detection of anti-Hepatitis E Virus IgG alone is a marker of prior exposure (Ferguson *et al*., 2005). Test for the anti-Hepatitis E Virus IgG concentration may be important in determination of the levels of anti-Hepatitis E Virus IgG which mitigates natural infection in clinical trials or infection after vaccine administration (Brendall *et al*., 2008)

Besides, reverse transcriptase polymerase chain reaction (RT-PCR) to detect Hepatitis E Virus ribonucleic acid in stool and blood has been developed. Two specific and sensitive real-time polymerase chain reaction (PCR) assays (Primer-Probe Energy Transfer (PriProET) and TaqMan® assays) have been developed to detect Hepatitis E Virus and variant. These molecular diagnostic methods are practical tools that can be employed to diagnose Hepatitis E infection in endemic regions (Gyarmati *et al*., 2007).

2.5 Management of Hepatitis E infections

2.5.1 Antiretroviral Therapy

Currently, there is no specific antiviral therapy that is capable of managing acute Hepatitis E infection (Purcell *et al.*, 2003; CHP, 2011). However, immunosuppressed patients with chronic Hepatitis E infection are prescribed for the specific antiviral drug (ribavirin). Besides, interferons are also prescribed in some specific situation (WHO, 2014a).

2.5.2 Prevention of Transmission

Prevention of Hepatitis E Virus is the most effective approach. Fecal-oral is the most common route of Hepatitis E Virus transmission and therefore, proper disposal of human waste, adequate supply of clean water and good personal hygiene forms the key prevention measures. Besides, more emphasis should be implemented in sanitary food-handling practices, Hepatitis E Virus screening before blood transfusion and avoid consumption of undercooked or uncooked meat (Emerson *et al*., 2005; Christensen *et al*., 2008; Aggarwal and Naik, 2009).

Since Hepatitis E Virus possesses no envelope, it is resistance to cooking temperatures of up to sixty degrees Celsius. The virus is also not affected by detergents (Jens and David, 2014). Nano-filtration offers an appropriate measure to remove Hepatitis E Virus from water. However, filters with pore size of about 20 nm should be used due to the size of Hepatitis E Virus (Jens and David, 2014). In endemic regions, efforts to boost the quality of water by boiling and chlorination may be useful in eradicating the virus. In non-endemic areas, preventive measures may be directed on avoidance eating porcine and deer meat as the zoonotic route is the common mode of Hepatitis E Virus transmission (Emerson *et al*., 2005).

Two recombinant Hepatitis E Virus vaccines have successfully gone through phase 3 trials. However, their efficacy is uncertain and they are not yet available on the market. The first vaccine to prevent Hepatitis E Virus infection has been produced and licensed in China, though it is not yet available in the market globally (WHO 2014a).

2.6 Prevalence of Hepatitis E Virus

Hepatitis E Virus infection is endemic in Asia, Middle East, Western and Northern Africa and South America. However, Hepatitis E infection is rare in developed nations such as the USA and the UK. Individuals from developed countries are usually infected with Hepatitis E Virus when they visit endemic countries. Globally, it is approximated that twenty million Hepatitis E Virus infections, 3.3 million Hepatitis E Virus symptomatic incidences and 56, 600 Hepatitis E Virus related mortalities are reported yearly (Chugh *et al*., 2013, WHO, 2014a).

In Africa, different Hepatitis E Virus episodes have been reported among individuals residing in internally displaced person's camps or refugee camps (Teshale and Hu, 2011; Audrey *et al.*, 2012; Endale *et al.*, 2013; UNHCR, 2014). Individuals residing in these camps may lack adequate access to sterile conditions and clean water. In addition, such populations might be at risk to infectious diseases due to overcrowding which prompts dangers of infections after exposure (UNHCR, 2014). Availability of healthful care services may inadequate and therefore, deaths from severe complications of Hepatitis E Virus infection might be higher. This may be attributed to high death rate during Hepatitis E Virus epidemic in Africa countries (Boccia *et al.*, 2006; UNHCR, 2014). Hepatitis E outbreaks are also reported among migrant workers who live in overcrowded cities and slums (Eyasu and Dale, 2011).

Hepatitis E Virus infection is responsible for over fifty percent of acute sporadic hepatitis (CDC, 2012). High Hepatitis E infections occur in areas with inadequate

clean water and poor sanitation. This has been confirmed by major episodes of waterborne Hepatitis E in 1991 in Kanpur city, Uttar Pradesh, India, where more than seventy-nine thousands clinical incidences were reported. Contamination of drinking water with human fecal was reported as the cause of the outbreak (Takeda, 2010; CHP, 2011).

In a normal population, the mortality rate of Hepatitis E infection is majorly greater than 1%. However, the mortality rate can rise up to forty percent among pregnant mothers (BAPC, 2012; Khuroo, 2011). Currently, two surveys have indicated that the death rate of Hepatitis E infections is on the rise among children. For example, in Kazakhstan, the mortality rate of children lower than two years of age was 5%, while in Uganda it was eight percent (Teshale *et al.*, 2010). Current surveys on the occurrence of Hepatitis E infections has ascertained that Hepatitis E is a deadly disease not only in pregnant mothers but also in young children (WHO, 2012; WHO, 2014a). Hepatitis E Virus super-infection may also increase mortality rates in stable individuals with the chronic liver ailment (Eyasu and Dale, 2011).

2.7 Blood Donation and Transmission

Blood is a natural body fluid which comprises of leucocytes, erythrocytes, plasma, and platelets. Blood transport oxygen and nutrients to the cells and deliver metabolic waste away from the cells (Rogers, 2010). Blood transfusion is a safe common procedure in which blood or blood products are given via permanent intravenous or vein. Blood transfusion is crucial in patients with blood-related diseases, pregnancy complications, surgical conditions, accident injuries among other medical complications (WHO, 2014b, Pule *et al.*, 2014; CDC, 2015).

Blood requirements in any given society is about 1 to 2% of its population (WHO, 2014b). Kenya needs approximately four hundred thousand pints of blood to guarantee adequate blood supply to all individuals at the time of need. Kenya National Blood Transfusion Service (KNBTS) is the major body in Kenya which mobilize, collect, screen and distribute most of the donated blood. Kenya National Blood Transfusion Service is a department of the government serving in the Ministry of Health. It supplies the transfusing hospitals with eighty percent of blood and blood products needs which are obtained from voluntary blood donors.

2.7.1 Blood-borne pathogens

Blood-borne pathogens are pathogens which can be transmitted through blood the transfusion to blood recipient from the blood donor. Diseases caused by pathogens among blood recipient through blood transfusion are known as transfusion-transmitted infections (TTIs) (WHO, 2010a). Some of the transfusion-transmissible pathogens include parasites, viruses, bacteria, and prions. Majority of the infections caused by these pathogens may result in chronic disease with damaging repercussions to blood recipients while others can be fatal (WHO, 2010a). The standard donor screening questionnaire and laboratory test reports are crucial in the reduction of dangers associated with blood-borne pathogens transmitted through blood transfusion (CDC, 2015).

In some instances, blood banks may lack funds to carry out all the possible transfusion transmissible pathogens tests. However, it is recommended as mandatory to screen for hepatitis B and C viruses, human immunodeficiency virus

(HIV), and *Treponema palladium* (Syphilis) before blood transfusion. Infections from these pathogens may result in chronic ailments to blood recipients after blood transfusion (WHO, 2010b; WHO, 2015).

2.7.2 Hepatitis E Virus transmission through blood transfusion

Although Hepatitis E Virus is not among the mandatory tests before blood transfusion, it remains an important public health challenge as it affects approximately 20 million individuals in the world yearly (WHO, 2014a). Hepatitis E Virus has been reported as a transfusion-transmissible virus from the year 2002. Recently, epidemiological reports have documented that Hepatitis E Virus may affect the safety of blood distribution (BPAC, 2012; CDC, 2015).

Incidences of transmission of Hepatitis E Virus via blood transfusion have not been documented in number of countries Kenya included (Stoszek *et al.,* 2006a; Dalton *et al.,* 2008; Teshale et *al.,* 2010; Endale *et al.,* 2013; Pule *et al.,* 2014; Tamas *et al.,* 2014). Transmission of hepatitis through blood transfusion has also been reported in Botswana, Egypt, the USA, Japan, France, the UK among others nations (Meng *et al.,* 2002; Boutrouille *et al.,* 2007; BAPC, 2012; Baylis *et al.,* 2012b; Mansuy *et al.,* 2011; Cleland *et al.,* 2013; David *et al.,* 2013; Hewitt *et al.,* 2014; Slot *et al.,* 2013).

2.7.3 Hepatitis E Virus in blood donors and recipients

Hepatitis E Virus viremia has been detected among blood donors during acute Hepatitis E Virus infections. The dynamics of recent reports on cases of transfusion-transmissible Hepatitis E Virus infection and detection of Hepatitis E Virus RNA among asymptomatic blood donors have demonstrated the importance

of this virus (Khuroo and Khurro, 2010). Recent studies among blood donors in developed nations have revealed the predominance of anti-Hepatitis E Virus antibodies with a prevalence of 2.0% in the Netherlands, 2.3% in Brazil, 2.8% in Spain, 3.2% in France, 2.6% to 3.9% in Japan, 4% in New Zealand and 4.9% in Southwest Switzerland (Bortoliero *et al*., 2006; Beale *et al.,* 2011; Kaufmann *et al.,* 2011; Sakata *et al.,* 2008; Baylis *et al.,* 2012b).

Blood donors in endemic nations have exhibited higher Hepatitis E Virus IgG seroprevalence. Hepatitis E Virus IgG seroprevalence of 12.1%, 16.9% and 45.2% have been reported in Albania, Saudi Arabia and Egypt respectively (Boutrouille *et al.,* 2007; Hassan *et al.,* 2013). A high seroprevalence of Hepatitis E Virus IgM antibody have been reported after blood transfusions in European and Asia countries (Kamar *et al.,* 2008; Khuroo and Khuroo, 2010; Mun-Hon *et al.,* 2010; Baylis *et al.,* 2012b). In Germany and Sweden, 1 of 4525 and 1 of 7986 plasma donations tested positive for Hepatitis E Virus viremia, respectively. In Germany, ten percent of plasma pools tested positive for Hepatitis E Virus viremia (Labrique *et al.,* 2010; David *et al.,* 2013, Cyrille *et al.,* 2014).

Though the cases Hepatitis E Virus RNA in the blood donated by blood donors is not frequently detected, only several incidences of transfusion-transmitted Hepatitis E infections have been reported in different countries (Sakata *et al.,* 2008; Beale *et al.,* 2011; Baylis *et al.,* 2012b). Fresh frozen plasma has been linked to Hepatitis E infection during archive test and succession analysis using nucleic acid amplification technique. The Hepatitis E Virus RNA identified from the blood

donor exhibited complete identity for two distinct regions of Hepatitis E Virus genome which differed with those detected in the blood recipient (Bradley, 1995; Tanaka *et al.,* 2009; Ijaz *et al.*, 2012).

A transfusion-transmitted Hepatitis E Virus infection through a red blood cell unit has been documented in the United Kingdom (David *et al.,* 2013; Hewitt *et al.,* 2014). Whereas the recipient Hepatitis E infection was asymptomatic separated from higher levels of liver enzymes and mellow jaundice, the blood donor revealed intense Hepatitis E Virus infection. The diagnosis of Hepatitis E Virus infection and sickness of the donor prompted to the evaluation of the recipient (David *et al.,* 2013). In another incident, a child experienced transfusion-transmitted Hepatitis E Virus infection after receiving platelet unit in France (Mansuy *et al.,* 2011). Sequence homology in donor and recipient in the two incidents in the United Kingdom and France suggested an association amid transfusion (Jens and David, 2014).

Another incident of transmission of Hepatitis E Virus during blood components transfusion was reported in Japan (Takeda *et al*., 2010). It was discovered that the platelet donor consumed grilled pork twenty-three days prior to platelet donation. The Hepatitis E infection was thus transmitted to the recipient during platelets transfusion (Jens and David 2014).

CHAPTER THREE

RESEARCH METHODOLOGY

3.1 Introduction

The present study was carried in the months of November and December in the year 2016 on blood samples collected from six regional blood collection centers in Kenya. Blood samples were collected after securing an informed consent, and then stored at two to eight degrees Celsius until anti- Hepatitis E Virus assays were carried out. A total of 384 serum samples which comprised 22057 individual blood donations which were collected from different regional centers were screened for anti-Hepatitis E Virus IgG and IgM using ELISA. The seropositive IgM and IgG blood sample were confirmed using RT-PCR.

3.2 Study Area

This study was carried out at the National central testing laboratory located at KNBTS building within Kenyatta National Hospital in Nairobi, Kenya. Blood samples were obtained from Eldoret, Embu, Kisumu, Mombasa, Nairobi and Nakuru regional blood collecting centers located within the respective county level 5 hospitals formally provision hospitals.

3.2.1 Study design

A cross sectional study design was used where presence or absence of Hepatitis E Virus was carried out on blood samples collected from six blood collecting centers in Keya upon donor consent.

3.2.2 Population under study

This study was executed on blood specimens collected from donors during the months of November and December in the year 2016.

3.2.4 Sample size and Sampling

The sample size was computed using Daniel formula (Fischer, 1999):

$N = Z^2P\ (1\text{-}P) \div d^2$

Where:

N= desired sample size

Z= Standard, corresponding to 95% confidence; (1.96)

d = significant level = 5% (0.05%)

P= National HEPATITIS E VIRUS prevalence of 0.5%

$N = 1.96^2*0.5(1\text{-}0.5) \div 0.05^2 = 384$

The sample size was comprised 384 blood samples

3.2.5 Sampling Procedure

A total of 22057 blood specimens were collected from the six collection centers in Kenya as shown in Table 3.1.

Table 3. 1 Blood units collected in November and December 2016 per region

Regions	November	December
Eldoret	2200	1311
Embu	2209	1480
Kisumu	2796	1559
Mombasa	1103	1093
Nairobi	2750	2789
Nakuru	1184	1583
Total	12242	9815
Sum total	22057	

A systematic random sampling of all collected blood specimens using random numbers from the table of random numbers in the two months was performed where each 23rd blood sample was picked. The allocation of sample size per region was computed as shown below:

$$\text{Blood sample per region} = \frac{\text{Units of blood collected in a region}}{\text{Total blood units collected}} \times \textit{sample size}$$

Table 3. 2 Sampling frame

	November 2016		December 2016		Total	
Stratum	**Donors**	**Sample**	**Donors**	**specimens**	**Donors**	**specimens**
Eldoret	2200	38	1311	23	3511	61
Embu	2209	39	1480	26	3689	65
Kisumu	2796	49	1559	27	4355	76
Mombasa	1103	19	1093	19	2196	38
Nairobi	2750	48	2789	48	5539	96
Nakuru	1184	21	1583	27	2767	48
Total	12242	214	9815	170	22057	384

3.3 Inclusion criteria

All fresh blood samples that were received from the six regional blood collection centers in Kenya and stored at a temperature of 2^0C to 8^0C during the months of November and December 2016 were used in this study. The blood samples were obtained from blood donors aged between 16 and 65 years upon consenting.

3.4 Exclusion criteria

Samples which were received before and after the study period and samples which were not obtained from Kenya National Blood Transfusion Service facilities were excluded from this study.

3.5 Limitations of the study

The study period lasted for two months and never test for blood recipient after blood transfusion.

3.6 Ethical approval

Ethical approval was obtained from Mount Kenya University Ethical Research Committee before the commencement of study (Appendix III). Informed consent from the donor as part of the pre-counseling process on a donor questionnaire was administered before blood donation was carried out. A consent form was administered to the donors who upon consenting, signed to confirm part of the study (Appendix I).

Relevant permissions were obtained from the Kenya National Blood Transfusion Service (Appendix II) and National Commission for Science Technology and Innovation (Appendix IV) to carry out this study. Besides, the donated blood was identified using unique codes which could not disclose the identity of donors.

3.7 Sample collection and handling

Two milliliter of blood sample was collected from each blood donor. Of the 2ml blood sample, 1ml was put in plain blood collecting tubes, while the other 1ml was put in blood collecting tubes containing ethylenediaminetetraacetic acid (EDTA). The blood samples containing EDTA were used for blood typing, while those in plain blood collecting tubes were centrifuged at 2500 revolutions per minute to obtain blood serum for serological testing. The serum samples were stored under cold chain (2^0C -8^0C) before they were tested for Hepatitis E Virus IgM and/or IgG seroprevalence using ELISA kit.

3.8 Testing protocol

3.8.1 Enzyme-linked immunosorbent assay

Screening for human IgM and IgG Hepatitis E Virus antibodies in 384 human serum samples which were obtained from blood donors in Kenya was carried out using human MP Diagnostics (0721150096T-96 wells) Hepatitis E Virus ELISA kit. To test for IgM and IgM anti- Hepatitis E Virus antibodies, microplates with 96-well plate were used. Every microplate well contained adsorbed recombinant Hepatitis E Virus proteins and was stored at two to eight degree Celsius. The diluent was used to fill the reagent reservoir (MP Diagnostics).

Two hundred microliters of the diluent were added to the wells using a multichannel pipette. Wells A1 and B1 were left blank and human serum was added. However, diluent (10µl) was added to these blank wells. Ten microliters of the human serum were added to all wells giving a dilution of 1:21. Ten microliters of non-reactive control were added to wells C1, D1 and E1. One microliter of reactive control was added per well to wells F1 and G1 and mixed well by tapping gently on all sides of the microplate.

The plates were carefully kept flat on the bench and then covered with a plate cover during incubation to avoid evaporation. The incubation period was carried out at thirty-seven degrees Celsius for thirty minutes to allow binding of Hepatitis E Virus specific antibodies with solid phase of Hepatitis E Virus antigens. One volume of plate wash concentrate and 19 volumes of distilled water were used to prepare wash buffer. After incubation, the plate covers were removed, discarded and the

microplates washed with diluted wash buffer (300ml/plate). The wells were thoroughly washed to remove unbound materials. Automated microplate washer (Figure 3.1) was used to wash the microplates six times with at least 300µl/well wash buffer. Aspirator tip was gently lowered to the bottom of each well to completely aspirate all wells content without scratching the well surface.

Figure 3. 1 ELISA microplate washer (Diatect, China)

Blot drying of plates was carried out by inverting the microplates and tapping onto the absorbent paper. This was because the color formation is inhibited amid substrate incubation by the residual buffer in the plate. The fresh conjugate of rabbit anti-human IgG labelled with horseradish peroxidase was prepared by making a 1:500 dilution of the conjugate with diluent in the commercial test kit. This labelled antibody usually binds to any antigen-antibody complexes previously formed and excess unbound labelled antibodies are removed by washing. Using a multichannel pipette, 100µl of working conjugate was put in each well, cover plate applied and then incubated at room temperature (20^0C to 25^0C) for 30 minutes. After incubation, the plates cover were removed, discarded and wash procedure repeated 6 times to remove excess unbound labelled antibodies.

The substrate solution containing 3, 3', 5, 5'-tetramethylbenzidine (TMB) was filled in reagent reservoir and one hundred microliters of substrate solution added to each well, cover plate applied and then incubated at room temperature (25 ± 3^0C) for 15 minutes in darkness to avoid edge effects. After the incubation period, the plates were removed and the content and one hundred microliters of stop solution (hydrochloric acid) were put in each well and mixed well by tapping the plate to stop the reaction. This was followed by detection of absorbance (optical density) using an enzyme immunoassay microplate reader (Figure 3.2) with two filters of 420nm per 620nm in the period of ten minutes for each well.

The intensity of the absorbance was directly proportional to the concentration of the IgM or IgG present in the human serum sample.

Figure 3. 2 Microplate ELISA reader (MR 9600, Azure Biosystems, USA)

Each blood sample was tested separately for IgG and IgM using the same method. Assay procedure was strictly followed to ensured optimal assay performance as the

immunoassay are time-dependent and temperature sensitive. The absence or presence of IgM or IgG anti- Hepatitis E Virus antibodies were determined by relating the absorbance of the specimens to the cut-off value of the plate.

The reactive and blank controls were replicated twice, while the non-reactive control was replicated thrice on each plate with each run of specimens. Each microplate was considered separately when calculating and interpreting results after reading spectrophotometrically.

3.8.2. Reverse transcriptase polymerase chain reaction (RT-PCR) test

The RT-PCR test was used as the confirmatory test for 19 blood samples which were seropositive for IgG and/or IgM Hepatitis E Virus antibodies which turned negative for IgG and/or IgM Hepatitis E Virus.

3.8.2.1 RNA extraction

Blood samples (4.8ml) which tested seropositive for Hepatitis E Virus IgG or IgM antibodies were extracted with the Chemagic viral RNA reagent kit (viral 5k; PerkinElmer Chemagen Technologie GmbH, Baesweiler, Germany). Blood sample (4.8ml) was mixed with 30µl protease, 4.8 ml lysis buffer, 5µl Hepatitis E Virus internal control (IC) and 7 µl poly (A) (RealStar Hepatitis E Virus reverse transcriptase polymerase chain reaction kit).

The samples were incubated for ten minutes at fifty-five degrees Celsius. Lysates were afterward mixed with fifteen milliliters binding buffer consisting of one hundred microliters magnetic beads. Automated magnetic separation module I (MSMI; PerkinElmer Chemagen Technologic GmbH) shown in Figure 3.3 was

used to carry out nucleic acid extraction as well as binding, washing (twice) and elution using elution buffer (100µl).

Figure 3. 3 Magnetic separation module I (MSMI; PerkinElmer Chemagen Technologic, Germany)

3.8.2.2 Real-time reverse transcriptase PCR

Reverse transcriptase polymerase chain reaction assays were carried out using Rotor-Gene 3000 system (Figure 3.4) according to the manufacturer's instructions (Corbett Life Sciences, Sydney, Australia). The reaction mixture of polymerase chain reaction protocol consisted of a forty microliters reaction mixture possessing twenty five microliters of 2× Superscript III one-step RT-PCR buffer, 6mM $MgSO_4$, 200nM each IC (integrated circuit) primer, 100nM IC fluorescent probe, 400nM each Hepatitis E Virus primer, 100 nM Hepatitis E Virus probe, 10µl RNA extract and 1µl Superscript III Platinum Taq polymerase mix. In the reaction mixture, a 278-bp polymerase chain reaction product of the lambda gene was added as an exogenous IC sequence.

The polymerase chain reaction conditions included: reverse transcription for ten minutes at 50°C and preliminary denaturation for two minutes at 95°C, forty five cycles of denaturation for fifteen seconds at 95°C, annealing for twenty seconds at 55°C, and extension for thirty seconds at 72°C, with a single fluorescence acquisition step at the end of the annealing step (Jothikumar *et al*., 2006).

Figure 3. 4 Rotor-Gene 3000 system (Corbett Life Sciences, Sydney, Australia)

2.8.2.4 Hepatitis E Virus genotyping

Hepatitis E Virus RNA was amplified by a nested reverse transcription-PCR in the open reading frame 1 (ORF1) region using the following primers that were modified according to the method of Preiss et al. (2016): outer primers, ORF1-F (5′-CTGGCATCACTACTGCTATTGAG-3′) and ORF1-R (5′-CCGTCGAGGCAGTAAGGTGCGGTC-3′); inner primers, ORF1-Fn (5′-CTGCCCTGGCGAATGCT-3′) and ORF1-Rn (5′-AGCAGTATACCAGCGCTGAACATC-3′). Sequencing analysis of the 242-bp polymerase chain reaction products was performed with inner Hepatitis E Virus

primers as described by Dreier *et al*. (2005) and sequences were taken to the GenBank database. The BLASTn search facility and the GenBank nr/nt database were used to carry out sequence similarity searches.

3.9 Data analysis and management

The obtained data was recorded in a data handbook, tabulate in Microsoft Excel spreadsheet, cleaned and then exported to Statistical Package for Social Sciences (SPSS) Version 21.0 for statistical analysis. Data were expressed as frequency and percentages. Categorical data were evaluated in 2-way contingency table analyses using Pearson Chi-Square test. Odds ratios were also computed. Statistical differences were analyzed at 95% level of significance. Data was presented in graphs or tables.

CHAPTER FOUR

RESULTS

4.1 Seroprevalence of Hepatitis E Virus among blood donors in Kenya

Three hundred and eighty-four mini pools, which comprised 22057 individual blood donations were screened and 19 blood donations containing anti-Hepatitis E Virus IgM and/or IgG antibodies were identified, resulting to a seroprevalence of 4.9% (one out of twenty samples) (Table 4.1).

Table 4. 1 Hepatitis E Virus seroprevalence among blood donors by gender, age and region in Kenya

Variable	No. of blood donors	HEPATITIS E VIRUS seroprevalence	p value
Gender			
Male	208 (54.2%)	5.3% (11/208)	0.74
Female	176 (45.8%)	4.5% (8/176)	
Age in years			
≤24	24 (6.5%)	4.0% (1/25)	0.16
25-34	189 (49.2%)	2.6% (5/189)	
35-44	150 (39.1%)	8.0% (12/150)	
≥45	20 (5.2%)	5.0% (1/20)	
Region			
Eldoret	61 (15.9%)	4.9% (3/61)	0.91
Embu	64 (16.7%)	4.7% (3/64)	
Kisumu	76 (19.7%)	3.9% (3/76)	
Nairobi	38 (9.9%)	4.1% (4/97)	
Nakuru	97 (25.3%)	8.3% (4/48)	
Mombasa	48 (12.5%)	5.3% (2/38)	
Total	384	4.9% (19/384)	

Key: HEPATITIS E VIRUS = Hepatitis E Virus ; No. = Number

4.1.1 Seroprevalence of Hepatitis E Virus by gender

Among the 384 blood donors who were tested in this study, 54.2% (208 blood donors) were male, while 45.8% (176 blood donors) were female (Table 4.1). The association between gender and Hepatitis E Virus seroprevalence failed to achieve

significance (Table 4.1; χ^2 = 0.112; df = 1; p = 0.74). The male blood donors exhibited a seroprevalence of 5.3% (11/208), while the female respondents recorded a seroprevalence of 4.5% (8/176) (Figure 4.1; Table 4.1). Male blood donors were 1.17 (odd ratio) times more likely to be Hepatitis E Virus seropositive compared to female blood donors.

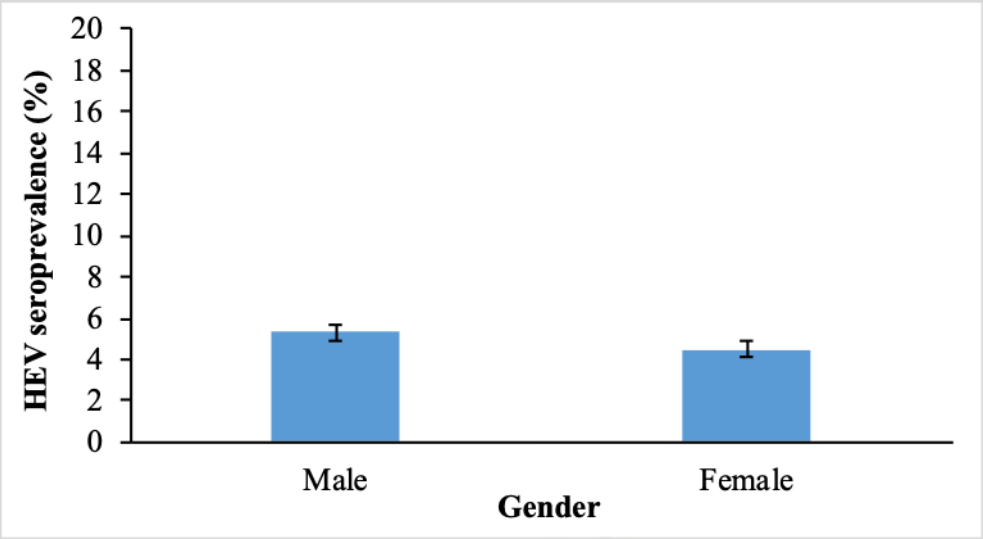

Figure 4. 1 Seroprevalence of Hepatitis E Virus by gender

4.1.2 Seroprevalence of Hepatitis E Virus by Age

The age of blood donors was categorized into four groups of less or equal to 24 years, between 25 to 34 years, between 35 to 44 years, and above or equal to 45 years. Of the 384 blood donors, 6.5% (24 blood donors) were in the age group of less or equal to 24 years old, 49.2% (189 blood donors) were between 25 to 35 years old, 39.1% (150 blood donors) were between 35 to 44 years old, while 5.2% (20 blood donors) were above or equal 50 years old (Table 4.1).

The age groups of the blood donors showed no significant association with HEPATITIS E VIRUS seroprevalence among blood donors in Kenya (Table 4.1;

χ^2 = 5.149; df = 3; p = 0.16). The age group of blood donors less or equal to 24 years old recorded a seroprevalence of 4.0% (1/25), the age group between 25 and 34 years old recorded a seroprevalence of 2.6% (5/189), the age group between 35 and 44 years old recorded a seroprevalence of 8.0% (12/150), while the ages above or equal to 45 years old recorded a seroprevalence of 5.0% (1/20) (Figure 4.2; Table 4.1). The seroprevalence of Hepatitis E Virus between the ages of 35 and 44 years old was 1.65 (odd ratio) times higher compared to the age group of blood donors above or equal to 45 years old. However, the Hepatitis E Virus seroprevalence of blood donors above or equal to 45 years old was 1.26 and 1.80 (odd ratios) times higher compared to ages below or equal to 24 years old and between 25 and 34 years old respectively.

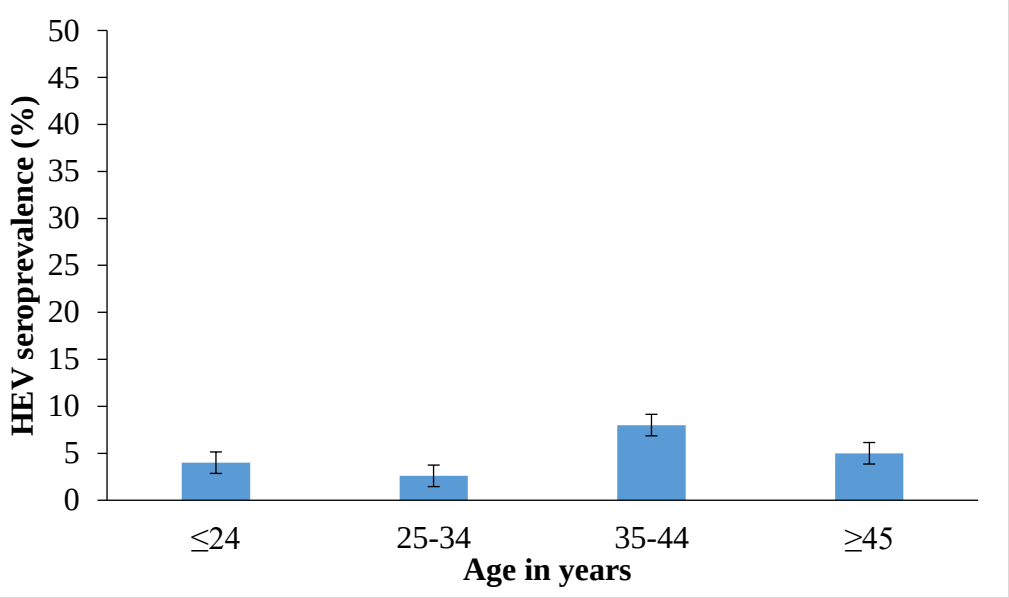

Figure 4. 2 Seroprevalence of Hepatitis E Virus by Age

4.1.3 Seroprevalence of Hepatitis E Virus by region

Blood samples were collected from six regional blood centers in Kenya. Among blood samples that were screened, 61 samples were obtained from Eldoret, 64 from Embu, 76 from Kisumu, 38 from Mombasa, 97 from Nairobi and 48 from Nakuru blood collection center (Table 4.1).

The regions of blood collection exhibited no significant difference in the seroprevalence of Hepatitis E Virus (Table 4.1; $\chi^2 = 1.489$; df = 5; p = 0.91). The regions of Eldoret, Embu, Kisumu, Nairobi, Nakuru and Mombasa exhibited a seroprevalence of 4.9% (3/61), 4.7% (3/64), 3.9% (3/76), 4.1% (4/97), 8.3% (4/48) and 5.3% (5.3%) respectively (Figure 4.3; Table 4.1). The seroprevalence of blood donors in Nakuru blood collection centers was 1.64 (odd ratio) times higher compared to Mombasa blood collection center.

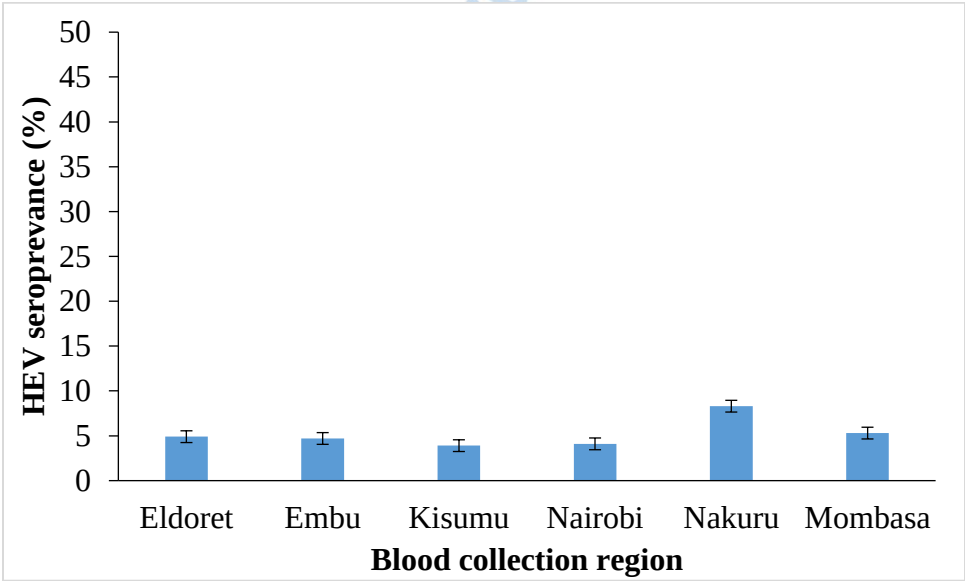

Figure 4. 3 Seroprevalence of Hepatitis E Virus by region

4.2 Anti-Hepatitis E Virus IgM and IgG prevalence of blood donors in Kenya

The Hepatitis E Virus IgM seroprevalence was determined using ELISA (MP Diagnostic's Hepatitis E Virus ELISA protocol). Human serum samples with optical density (OD) value greater than the cut off value of 0.483 were tested twice before they were regarded anti- Hepatitis E Virus IgM positive. Among the 384 blood samples that were screened, only 13 blood samples were seropositive for Hepatitis E Virus IgM, corresponding to a seroprevalence of 3.4% (Table 4.2). Only four samples among the Hepatitis E Virus IgM seropositive samples were Hepatitis E Virus IgG seropositive.

Anti-Hepatitis E Virus IgG prevalence was also determined using ELISA (MP Diagnostics Hepatitis E Virus A protocol). Human serum sample with an optical density (OD) value greater than the cutoff value (<0.559) was tested twice before it was confirmed seropositive. Of the 384 blood samples analyzed, a total of 10 blood samples were found to be Hepatitis E Virus IgG seropositive representing a seroprevalence of 2.6% (Table 4.2). Only six blood samples among the anti-Hepatitis E Virus IgG positive samples were Hepatitis E Virus IgM seropositive.

4.2.1 Anti-Hepatitis E Virus IgM and IgG prevalence among blood donors by gender

There was no significant association between gender and Hepatitis E Virus IgM seroprevalence among blood donors in Kenya (Table 4.2; $\chi^2 = 0.001$; df = 1; p = 0.98). The male and female blood donors had similar Hepatitis E Virus IgM seroprevalence of 3.4% (Figure 4.4; Table 4.2). The female blood donors were 1.01

(odd ratio) times likely to be Hepatitis E Virus IgM seropositive compared to male blood donors.

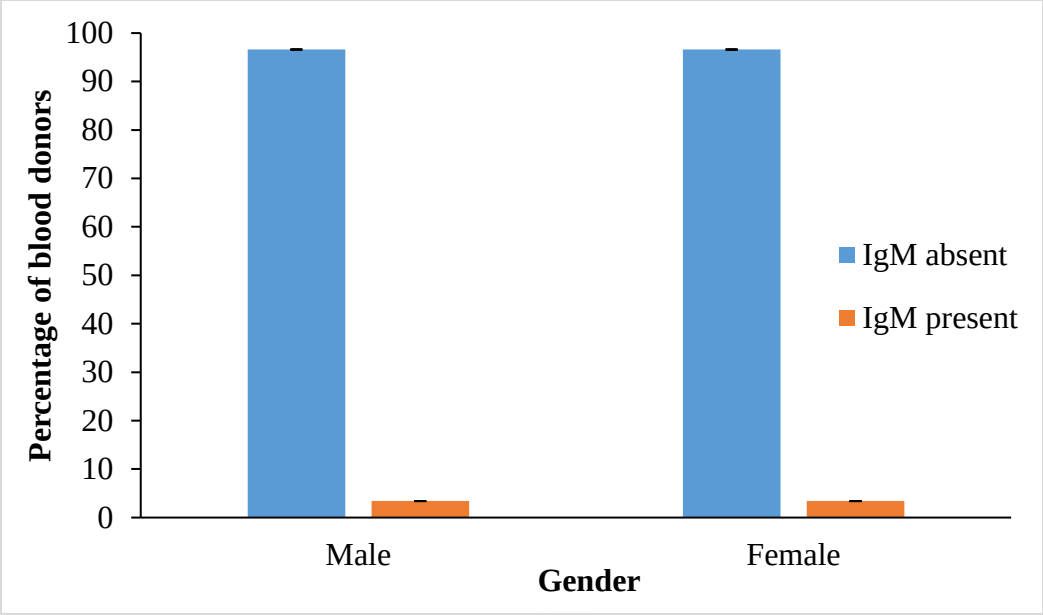

Figure 4. 4 Anti-Hepatitis E Virus IgM prevalence by gender

Similarly, there was no significant association between gender and seroprevalence of Hepatitis E Virus IgG antibodies among blood donors in Kenya (Table 4.2; χ^2 = 0.072; df = 1; p = 0.79). The male blood donors had a Hepatitis E Virus IgG seroprevalence of 2.4% (5 blood samples), while the female blood donors exhibited a Hepatitis E Virus seroprevalence of 2.8% (5 blood samples) (Figure 4.5; Table 4.3). The female blood donors were 1.19 (odd ratio) times likely to be anti-Hepatitis E Virus IgG seropositive compared to male blood donors.

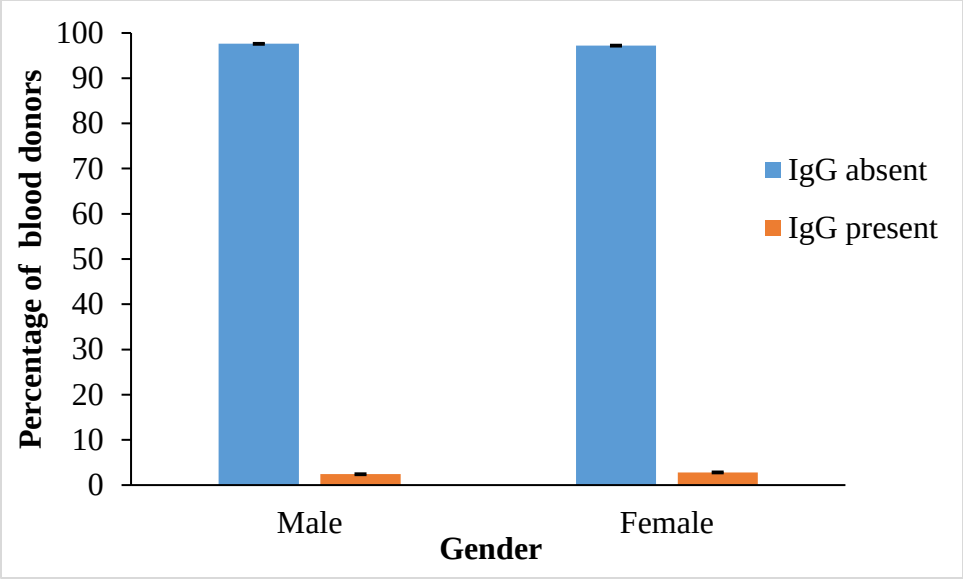

Figure 4. 5 Anti-Hepatitis E Virus IgG prevalence by gender

Table 4. 2 Anti-Hepatitis E Virus IgM and IgG prevalence among blood by gender

4.2.2 Anti-Hepatitis E Virus IgM and IgG prevalence among blood donors

		Anti-HEPATITIS E VIRUS IgM			Anti-HEPATITIS E VIRUS IgG		
Gender	**No. of samples**	**Negative samples**	**Positive samples**	**p value**	**Negative samples**	**Positive samples**	**P value**
Male	208	201(96.6%)	7 (3.4%)	0.98	203 (97.6%)	5 (2.4%)	0.79
Female	176	170(96.6%)	6 (3.4%)		171 (97.2%)	5 (2.8%)	
Total	384	371(96.6%)	13(3.4%)		374(97.4%)	10(2.6%)	

by age

There was no significant association between seroprevalence of Hepatitis E Virus IgM and age groups of the blood donors in Kenya (Table 4.3; $\chi^2 = 2.105$; df = 3; p = 0.55). The age group below or equal to 24 years never revealed active anti-

Hepatitis E Virus IgM. However, the age group between 25 and 34 years old had a seroprevalence of 2.6% (5 blood samples), the age group between 35 and 44 years old had a seroprevalence of 4.7% (7 blood samples), while the age above or equal to 45 years old recorded a seroprevalence of 5.0% (1/188 blood sample) (Figure 4.6; Table 4.3). The anti- Hepatitis E Virus IgM seroprevalence increased with increase in age.

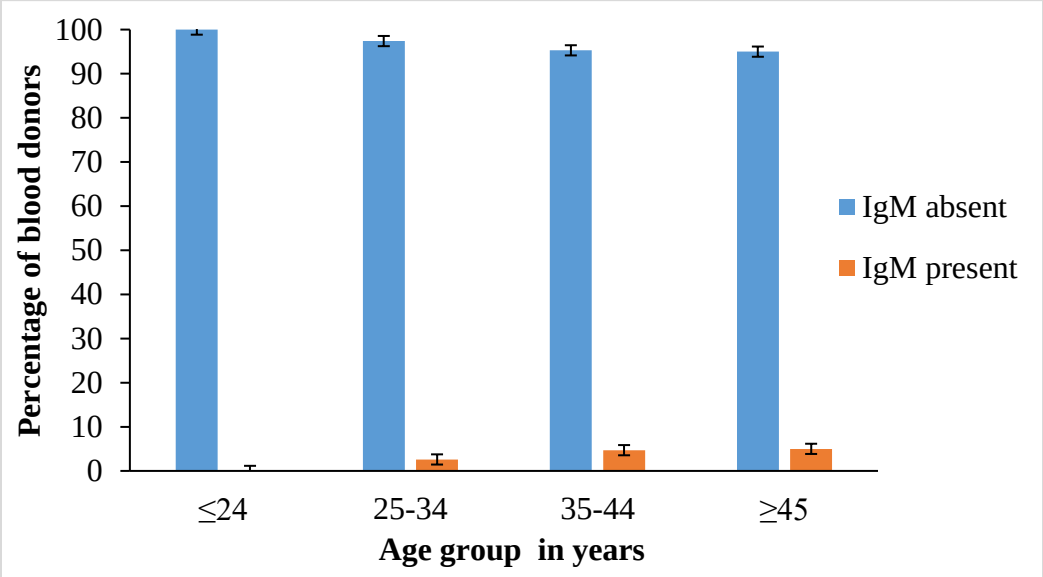

Figure 4. 6 Anti-Hepatitis E Virus IgM prevalence by age

However, there was a significant association between age and Hepatitis E Virus IgG seroprevalence among blood donors in Kenya (Table 4.3; $\chi^2 = 8.340$; df = 3; p = 0.04). The age group of blood donors between 35 and 44 years old recorded the highest anti- Hepatitis E Virus IgG prevalence of 5.3% (8/142 blood samples), followed by age group below or equal to 24 years old with a prevalence of 4.0% (1 blood sample), then the age group between 25 and 34 years old with a prevalence

of 0.5% (1 blood samples), while the age group above or equal to 45 years old never recorded the presence of anti- Hepatitis E Virus IgG (Figure 4.7; Table 4.3).

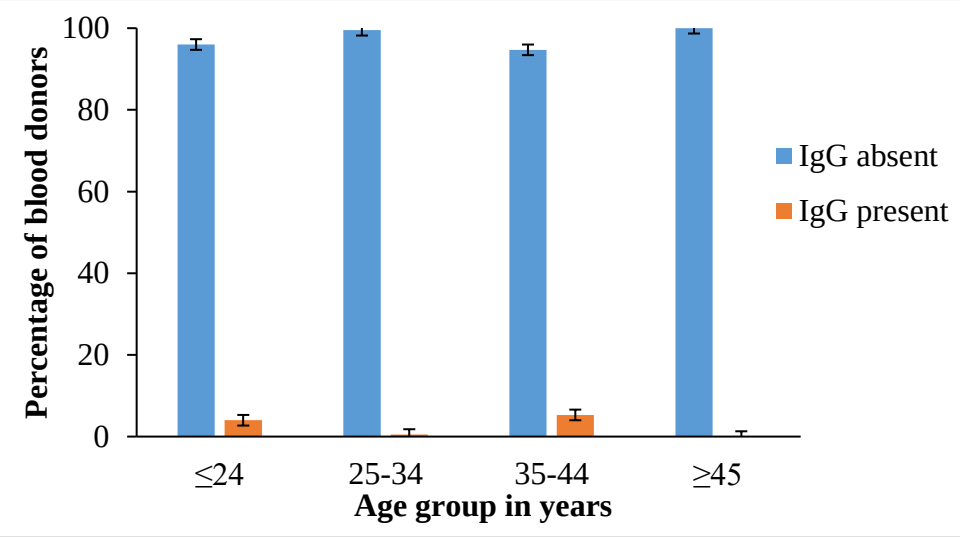

Figure 4. 7 Anti-Hepatitis E Virus IgG prevalence by age

Table 4. 3 Anti-Hepatitis E Virus IgG prevalence by age Anti-Hepatitis E Virus IgG prevalence by age

		Anti-HEPATITIS E VIRUS IgM				Anti-HEPATITIS E VIRUS IgG			
Age (years)	**No. of samples**	**Negative samples**	**Positive samples**	**Odds ratio**	**p value**	**Negative samples**	**Positive samples**	**Odds ratio**	**P value**
≤24	25	25 (100%)	0 (0.0%)	0.034	0.55	24 (96.0%)	1 (4.0%)	0.026	0.04
25-34	189	184(97.4%)	5 (2.6%)			188 (99.5%)	1 (0.5%)		
35-44	150	143(95.3%)	7 (4.7%)			142 (94.7%)	8 (5.3%)		
≥45	20	19 (95.0%)	1 (5.0%)			20 (100.0%)	0 (0.0%)		
Total	384	371(96.6%)	13(3.4%)			374(97.4%)	10(2.6%)		

4.2.3 Anti-Hepatitis E Virus IgM and IgG prevalence among blood donors by region

The Hepatitis E Virus IgM seroprevalence exhibited no significant difference among regional blood collection centers in Kenyan (Table 4.4; $\chi^2 = 0.65$; df = 5; p = 0.99). Higher Hepatitis E Virus seroprevalence of 4.2% and 4.1% were recorded in Nakuru and Nairobi respectively, while lower Hepatitis E Virus IgM seroprevalence of 2.6% was recorded in Kisumu and Mombasa (Figure 4.8; Table 4.4). The blood donors from Nakuru blood collection center were 1.61 (odd ratio) times likely to test positive for anti- Hepatitis E Virus IgM compared to Mombasa blood collection center.

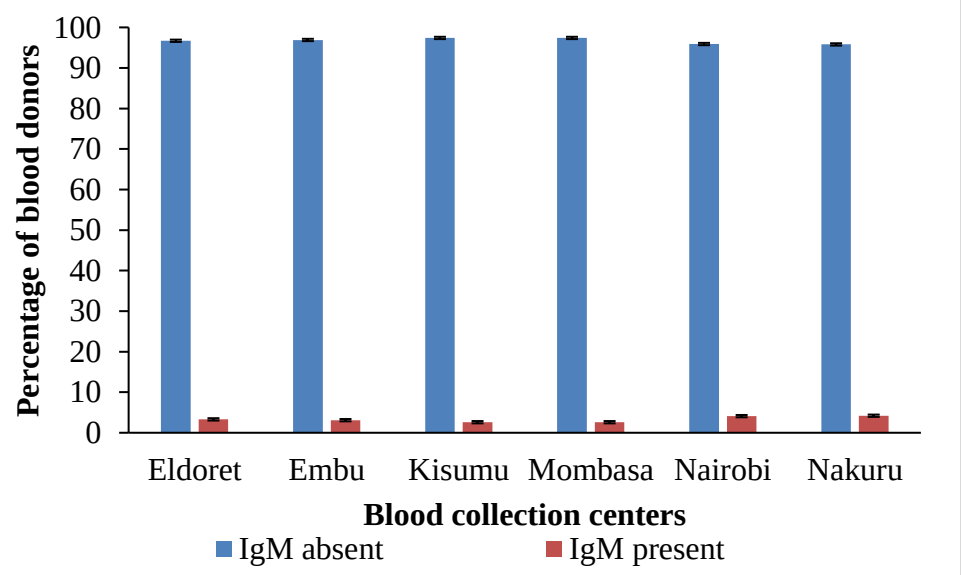

Figure 4. 8 Anti-Hepatitis E Virus IgM prevalence by region

There was no significant difference in the prevalence of anti- Hepatitis E Virus IgG of blood collected in various regions in Kenyan (Table 4.4; $\chi^2 = 3.126$; df = 5; p =

0.68). Higher seroprevalence of 6.2% was recorded in Nakuru blood collection center (Figure 4.9; Table 4.4). The blood samples collected from Nakuru were 2.47 (odd ratio) times likely to be anti- Hepatitis E Virus IgG seropositive compared to

		Anti- Hepatitis E Virus IgM			Anti- Hepatitis E Virus IgG		
Region	**No. of samples**	**Negative samples**	**Positive samples**	**p value**	**Negative samples**	**Positive Samples**	**P value**
Eldoret	61	59(96.7%)	2 3.3%)	0.99	60 (98.4%)	1 (1.6%)	0.68
Embu	64	62(96.9%)	2 (3.1%)		63 (98.4%)	1 (1.6%)	
Kisumu	76	74(97.4%)	2 (2.6%)		74 (97.4%)	2 (2.6%)	
Mombasa	38	37(97.4%)	1 (2.6%)		37 (97.4%)	1 (2.6%)	
Nairobi	97	93(95.9%)	4 (4.1%)		95 (97.9%)	2 (2.1%)	
Nakuru	48	46(95.8%)	2 (4.2%)		45 (93.8%)	3 (6.2%)	
Total	384	371(96.6%)	13(3.4%)		374(97.4%)	10(2.6%)	

blood samples collected from Mombasa.

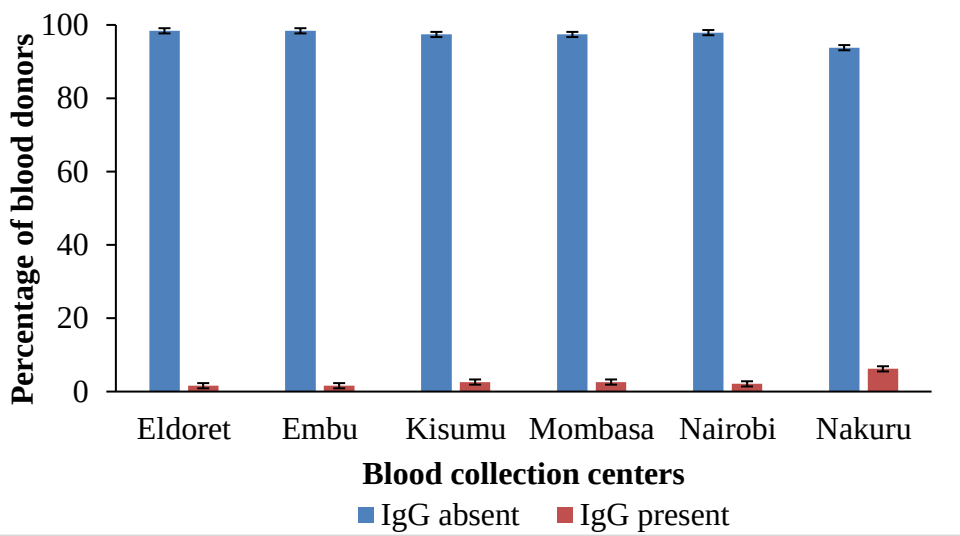

Figure 4. 9 Anti-Hepatitis E Virus IgG prevalence by region

Table 4. 3 Anti-Hepatitis E Virus IgM and IgG prevalence among blood by Region

4.3 Hepatitis E Virus RNA test

All the 19 Hepatitis E Virus IgM and/or IgG seropositive blood samples tested negative for Hepatitis E Virus RNA since no bands were detected using RT-PCR technique (Appendix V).

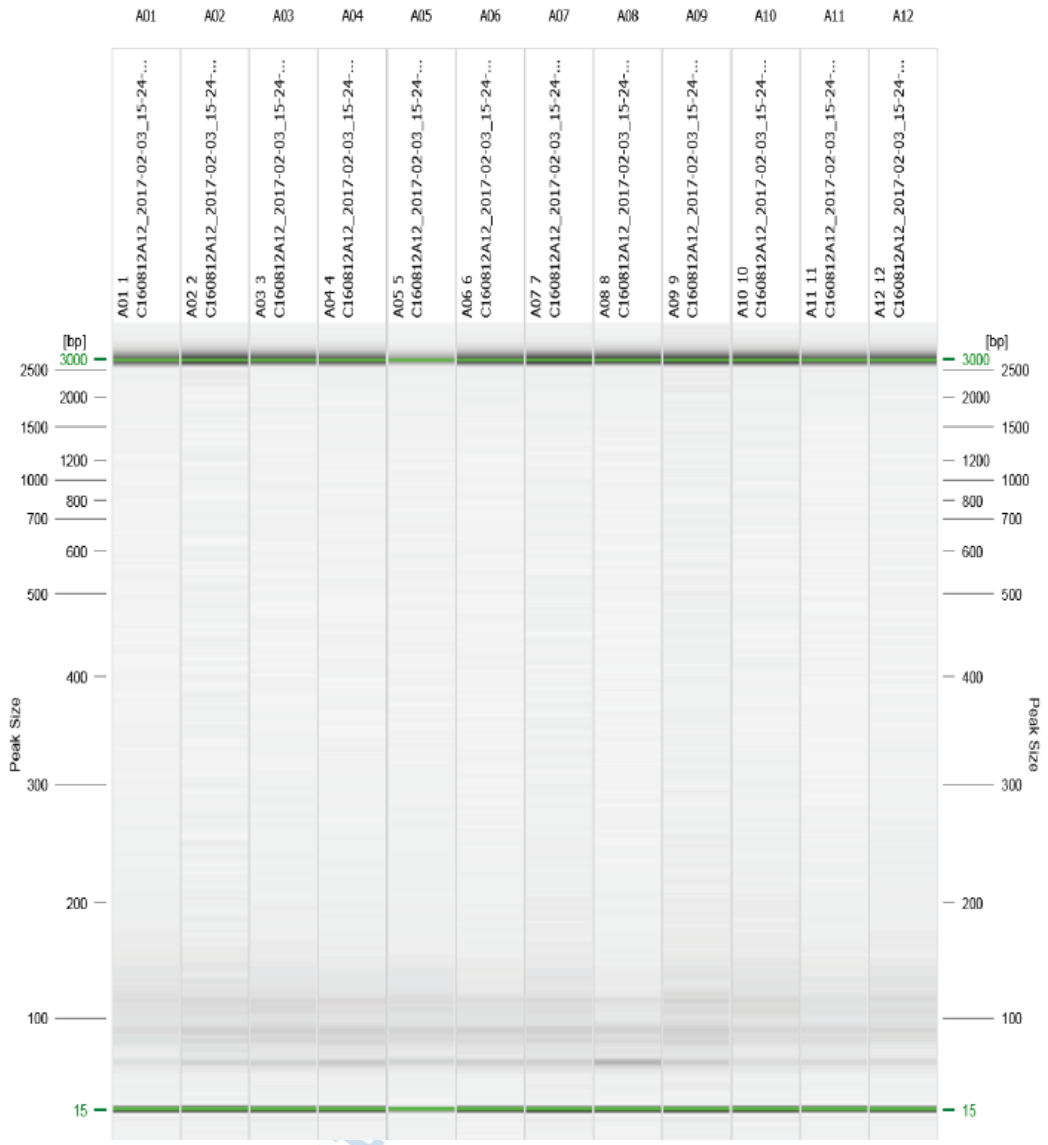

Figure 4. 10 Gel image of open reading frame 1 PCR products separation of the 4 HEV

4.4 Results Discussion

4.4.1 Seroprevalence of Hepatitis E Virus among blood donors

It is estimated that twenty million Hepatitis E Virus infections, more than three million acute incidences of Hepatitis E Virus, and seventy thousand Hepatitis E Virus related deaths are reported annually and therefore, an important public-health concern (WHO, 2014a). Hepatitis E Virus is transmitted through transfusion of

contaminated blood products, consumption of contaminated water, consumption of infected wild (deer) and domestic (pig) animals, exposure to infected animal fluids, vertical transmission from mother to child and transplant of infected grafts (Mohamand and Mehnaaz, 2008; Jens and David, 2014).

Transfusion-associated transmissions of Hepatitis E Virus through contaminated blood products have been documented in the United Kingdom, Japan, and France among other countries (Boutrouille *et al*., 2007; Colson *et al*., 2007; Matsubayashi, *et al*., 2008; Adlhoch *et al*., 2009). Besides, the detection of Hepatitis E viremia in asymptomatic blood donors has revealed the importance of this Hepatitis E Virus (Khuroo and Khurro, 2010; Ijaz *et al*., 2012; Baylis *et al*., 2012b). This has raised the question of blood safety during blood transfusion (Adlhoch *et al*., 2009).

This study revealed exposure of Hepatitis E Virus in blood donors among Kenyan populations. A Hepatitis E Virus seroprevalence of 4.9% (19/384) was computed on blood donated from different regional blood collection centers in Kenya, which was similar to non-endemic countries (Qing-Shun *et al*., 2009). Similarly, studies carried on Hepatitis E Virus seroprevalence of blood donors in Ghana, Scotland, Tunisia and Southwest Switzerland recorded similar Hepatitis E Virus seroprevalence of 4.6% 4.7%, 4.8% and 4.9% respectively (Kaufmann *et al*., 2011; Cleland *et al*., 2013; Meldal *et al*., 2013). However, the Hepatitis E Virus seroprevalence among blood donors in Kenya was higher compared to a seroprevalence of 2.3% and 1.3% in blood donors from Londrina, state of Parana, Brazil, and Italy respectively (Bortoliero *et al*., 2006; Scotto *et al*., 2012).

Besides, the Hepatitis E Virus seroprevalence of blood donors in Kenya was lower compared to Southwest Iran, Albania, Southwest France, USA, Denmark, Southwest England, and Netherlands which recorded a seroprevalence of 7.8%, 12.1%, 16.6%, 18.0%, 20.6%, 25.0%, and 26.7% among blood donors respectively (Meng *et al*., 2002; Kondili *et al*., 2006; Assarehzadegan *et al*., 2008; Christensen *et al*., 2008; Dalton *et al*., 2008; Mansuy *et al*., 2008; Slot *et al*., 2013).

Zoonotic transmission of Hepatitis E Virus plays a vital role in low endemic regions and therefore, the low Hepatitis E Virus seroprevalence in Kenya may be attributed to contact with infected animals (wild deer, camels, and pigs) body fluids or consumption of infected undercooked meat (Hoofnagle *et al*., 2012). HEV 3 and 4 may be attributed to low Hepatitis E Virus seroprevalence in Kenya as they appear less virulent (Purcell and Emerson, 2008).

4.4.2 Demographic trends of Hepatitis E Virus seroprevalence of blood donors

There was no significant association between gender of blood donors and Hepatitis E Virus seroprevalence in Kenya ($p>0.05$). These findings were consistent with a study carried by Boutrouille *et al*. (2007), on the seroprevalence of Hepatitis E Virus among French blood donors which reported that gender of blood donors was insignificant in terms of Hepatitis E Virus seroprevalence. Similarly, the gender of blood donors in Southwest Switzerland exhibited no significant difference in terms of Hepatitis E Virus seroprevalence (Kaufmann *et al*., 2011).

Although the age of blood donors exhibited no significant difference in prevalence of Hepatitis E Virus among the blood donors in Kenya ($p>0.05$), the ages of 35 to 44 years old recorded higher Hepatitis E Virus prevalence of 8.0%, while the ages between 24 to 35 years old recorded the lower Hepatitis E Virus prevalence of 2.4%. These findings disagreed with a study carried out by Boutrouille *et al*. (2007), which reported that the ages of 45 years and above recorded the highest Hepatitis E Virus prevalence among blood donor in France.

The blood donors revealed no association between Hepatitis E Virus IgG seropositivity and gender ($p>0.05$). However, the Hepatitis E Virus IgG seroprevalence was significantly associated with increasing age ($p<0.05$). This may be due to cumulative lifetime exposure to the virus. The ages of 35 to 44 years old of the blood donors recorded the highest anti-IgG Hepatitis E Virus prevalence. These results were in agreement with studies conducted by Cleland *et al*. (2013), and Dalton *et al*. (2008), which revealed that Hepatitis E Virus IgG seroprevalence increased with age among blood donors in southwest England and Scottland respectively.

The Hepatitis E Virus seroprevalence was not dependent on the six blood collections centers in Kenya. However, Nakuru recorded higher Hepatitis E Virus seroprevalence of 8.3%, while Kisumu exhibited lower Hepatitis E Virus seroprevalence of 3.9%. Hepatitis E Virus is transmitted through consumption of infected wild deer meat (Tei *et al*., 2003). The higher Hepatitis E Virus seroprevalence in Nakuru regional blood collection center may be attributed to consumption of wild deer meat due to the high population of wild deer.

Immunoglobulin M is the first antibody to appear at week 4 during Hepatitis E Virus infection, followed by IgG at week 5 (Boxall *et al*., 2006). The presence of anti- Hepatitis E Virus IgM class antibodies with or without the presence of IgG class antibodies indicate a recent or an acute phase of Hepatitis E Virus infection (Drobeniuc *et al*., 2010). The usual length of IgM positivity is between 2 and 3 months (WHO, 2010b).

This study recorded a Hepatitis E Virus IgM seroprevalence of 3.4% (13/384) among blood donors in Kenya. This implied that blood donors had serological evidence of recent infection. A similar study by Shrestha *et al*. (2016), recorded almost similar anti- Hepatitis E Virus IgM prevalence of 3.0% among blood donors in Nepal. A study on Hepatitis E Virus seroprevalence among blood donors in China reported lower Hepatitis E Virus IgM seroprevalence of 1.02% compared to Kenyan blood donors (Ren *et al*., 2014). A study on Hepatitis E Virus infection in English and North Welsh blood donors exhibited a higher Hepatitis E Virus IgM seroprevalence of 7.0% compared to Kenyan blood donors (Beale *et al*., 2011). Only four samples (4/13) among the anti- Hepatitis E Virus IgM seropositive samples were anti- Hepatitis E Virus IgG seropositive. There was no association between Hepatitis E Virus IgM seropositivity and gender, regional blood collection centers as well as age ($p>0.05$).

The Hepatitis E Virus IgM antibodies are succeeded by the emergence of specific IgG antibodies. The detection of IgG anti-Hepatitis E Virus antibodies is an affirmation of prior exposure to Hepatitis E Virus. The persistence of IgG anti-

Hepatitis E Virus antibodies in circulation is unknown. The IgG anti-Hepatitis E Virus antibodies are found in healthy individuals living in all geographical regions, although the seroprevalence varies. Generally, prevalence rates of IgG anti-Hepatitis E Virus are higher in developing countries where Hepatitis E Virus clinical cases are common compared to countries with low clinical cases (WHO, 2010b).

4.4.3 Hepatitis E Virus viremia among seropositive blood samples

Only six blood samples among the Hepatitis E Virus IgG seropositive samples were Hepatitis E Virus IgM seropositive. The presence of detectable anti- Hepatitis E Virus IgG in absence anti- Hepatitis E Virus IgM among blood donors indicated prior exposure to an infection through infection and individuals are considered immune to Hepatitis E Virus infection (WHO, 2010).

Viremia appears during acute Hepatitis E Virus infection at the second week and usually lasts for two to three weeks but can last for up to fifty-two days (Jothikumar *et al*., 2006). The RNA in the blood becomes undetectable approximately three weeks after the commencement of symptoms. However, the Hepatitis E Virus RNA can be detected in feces for another two weeks (Kamar *et al*., 2014). Therefore, suppose individuals are sampled late in the symptomatic phase of Hepatitis E Virus infection, a negative Hepatitis E Virus RNA result does not exclude recent infection (Abravanel *et al*., 2013).

No Hepatitis E Virus RNA was detected in the 19 Hepatitis E Virus seropositive IgM and/or IgG blood samples. This could be attributed to the small number of

samples to detect a low occurrence event and thus, much larger surveys are required (Ren *et al*., 2014; Gallian *et al*., 2014). Hepatitis E Virus viremia is also short-lived in serum (Yazbek, *et al*., 2016). Similar studies on assessment of Hepatitis E RNA in the United States and English and North Welsh blood donors never detected HEV RNA on HEV seropositive samples (Beale *et al*., 2011; Xu *et al*., 1013). However, studies on Scotland, China, France, Germany, Netherlands and England blood donor have reported Hepatitis E Virus RNA positive blood sample among the seropositive blood samples (Baylis *et al*., 2012b; Ijaz *et al*., 2012; Vollmer *et al*., 2012; Cleland *et al*., 2013; Slot *et al*., 2013; Ren *et al*., 2014; Gallian *et al*., 2014).

CHAPTER FIVE

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1 Summary

This study recorded a Hepatitis E Virus IgG seroprevalence of 2.6% (10/384) among blood donors which was lower compared to Hepatitis E Virus IgM seroprevalence of 3.4% (13/384). This implied that most of the blood donors were in the acute phase of Hepatitis E Virus infection compared to prior exposure. The Hepatitis E Virus IgG seroprevalence in Kenya was similar compared to Hepatitis E Virus IgG seroprevalence of 2.3% among blood donors from Londrina, state of Parana, Brazil (Bortoliero *et al*., 2006). However, the Hepatitis E Virus IgG seroprevalence in Kenya was higher compared to Hepatitis E Virus IgG seroprevalence of 1.3% among Italian blood donors (Scotto *et al*., 2012). In addition, the anti-Hepatitis E Virus IgG prevalence among the blood donor in Kenya was lower compared to Hepatitis E Virus IgG seroprevalence reported in Scottish (4.7%), English and North Welsh (10%), Germany (16.8%), China (27.42%) and Southwestern France (52.5%) blood donors (Cleland *et al*., 2013; Beale *et al*., 2011; Faber *et al*., 2012; Mansuy *et al*., 2011; Ren *et al*., 2014).

There was no significant association between gender of blood donors and Hepatitis E Virus seroprevalence in Kenya ($p>0.05$).

Although the age of blood donors exhibited no significant difference in prevalence of Hepatitis E Virus among the blood donors in Kenya ($p>0.05$), the ages of 35 to 44 years old recorded higher Hepatitis E Virus prevalence of 8.0%, while the ages between 24 to 35 years old recorded the lower Hepatitis E Virus prevalence of 2.4%.

The blood donors revealed no association between Hepatitis E Virus IgG seropositivity and gender ($p>0.05$). However, the Hepatitis E Virus IgG seroprevalence was significantly associated with increasing age ($p<0.05$). This may be due to cumulative lifetime exposure to the virus. The ages of 35 to 44 years old of the blood donors recorded the highest anti-IgG Hepatitis E Virus prevalence.

No Hepatitis E Virus RNA was detected in the 19 Hepatitis E Virus seropositive IgM and/or IgG blood samples. This could be attributed to the small number of samples to detect a low occurrence event and thus, much larger surveys are required

5.2 Conclusion

The present study revealed a Hepatitis E Virus seroprevalence of 4.9% in the blood donated in various regional blood collections centers in Kenya which is similar to seroprevalence reported in non-endemic countries. The Hepatitis E Virus seroprevalence was not dependent on age, gender and regional blood collection centers. Most blood donors had recent Hepatitis E Virus infection compared to past infection. This was due to the presence of more seropositive anti- Hepatitis E Virus IgM blood samples compared to seropositive anti- Hepatitis E Virus IgG blood samples. No Hepatitis E Virus viremia was detected among seropositive IgM and/or IgG blood samples. Unscreened blood products during blood transfusion may be a risk factor associated with Hepatitis E Virus infection in Kenya. Therefore, there is a need for continued vigilance for Hepatitis E Virus in the blood donors and the blood recipients.

5.3 Recommendation

i. Hepatitis E Virus may be transmitted through blood transfusion in Kenya. Hence, preventive measures such as serological screening of anti-Hepatitis

E Virus IgM and IgG of blood donors should be introduced to prevent transfusion-transmitted Hepatitis E Virus infections in future. This may be of special importance to individuals who are unable to achieve viral clearance or who are immune-compromised.

ii. Large surveys on Hepatitis E Virus viremia should be carried out in future due to the low occurrence of viremia in few blood samples.

iii. A prospective study on blood recipient should be carried out to investigate transfusion-transmitted Hepatitis E Virus cases.

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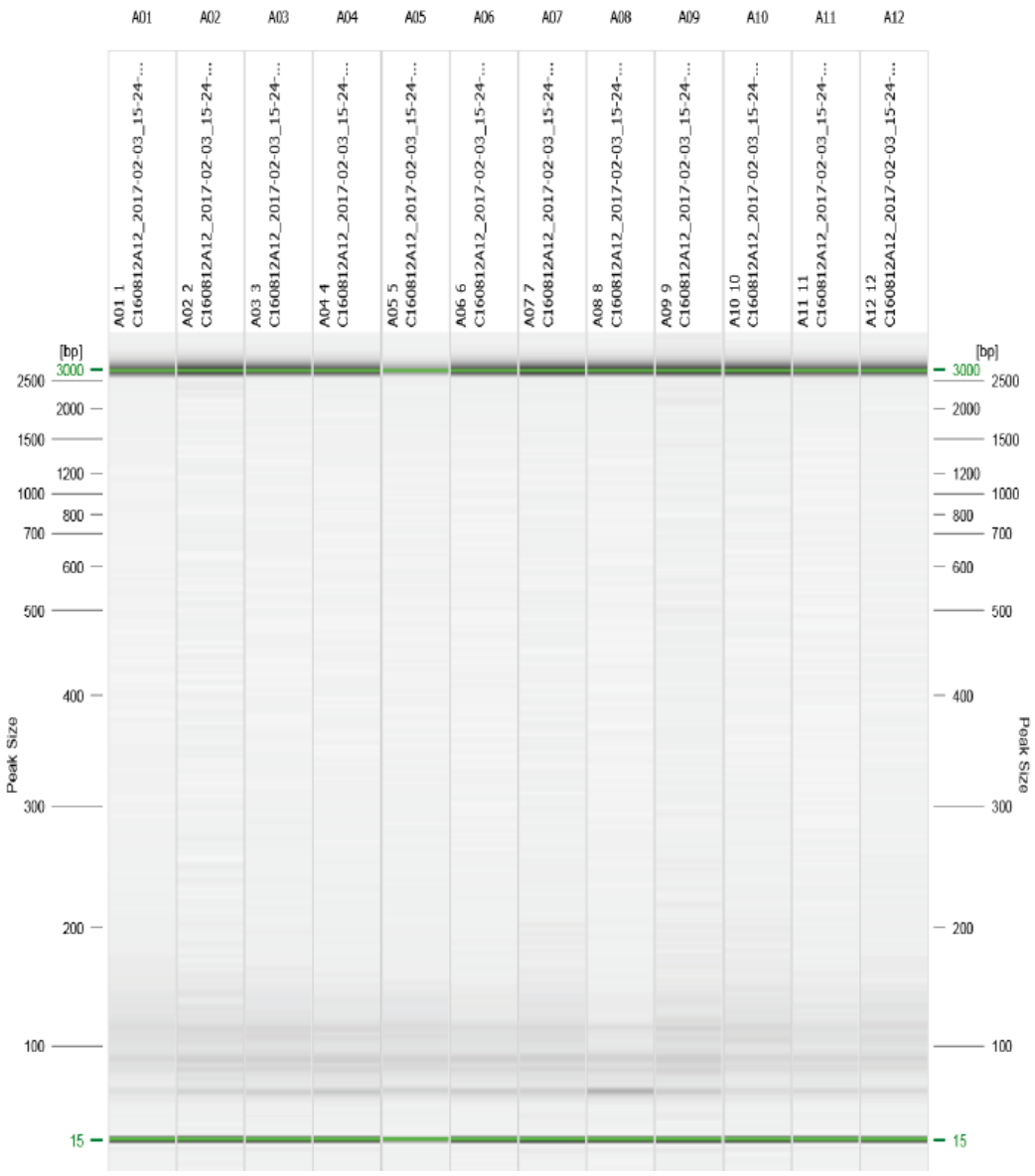
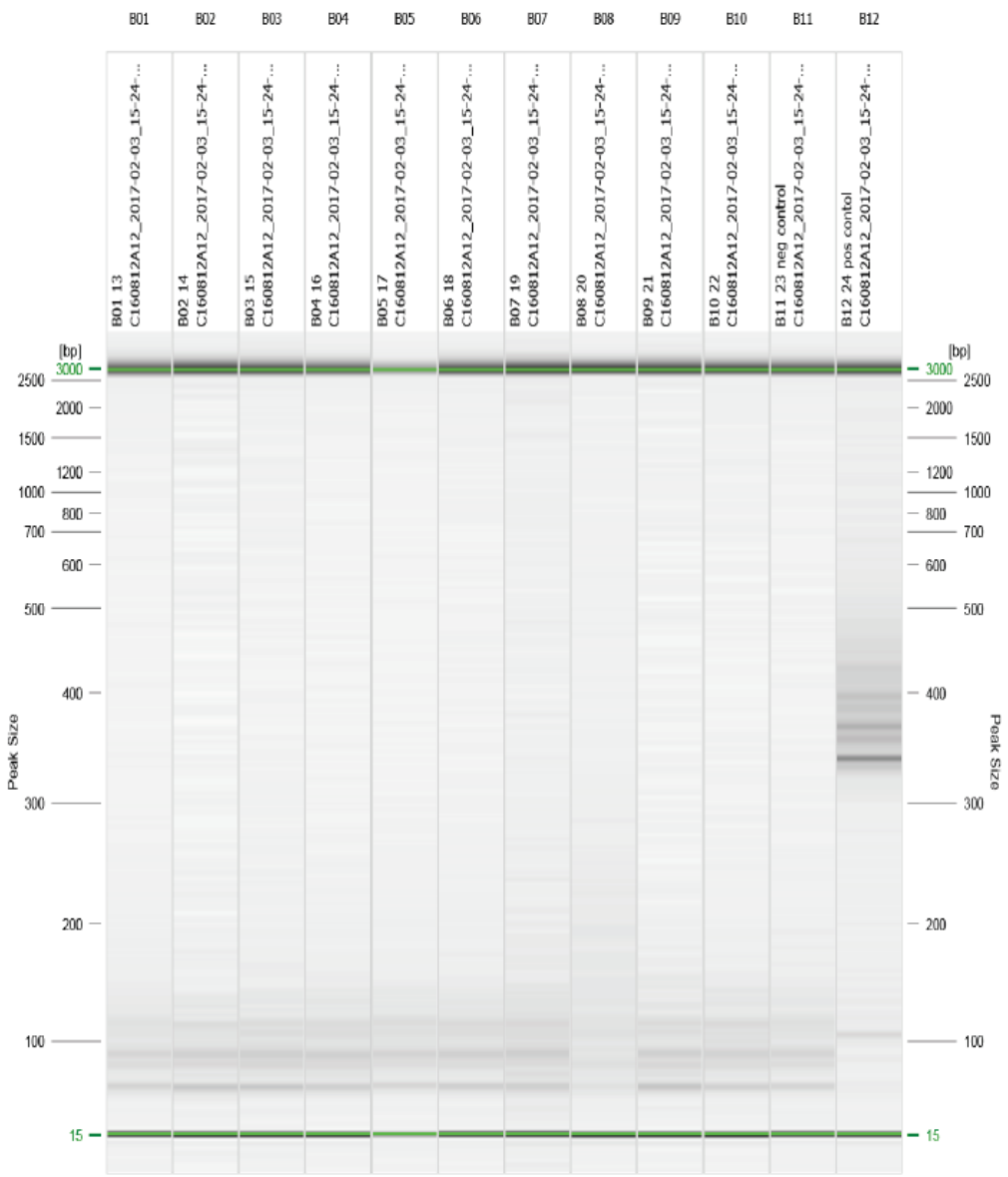
APPENDICES

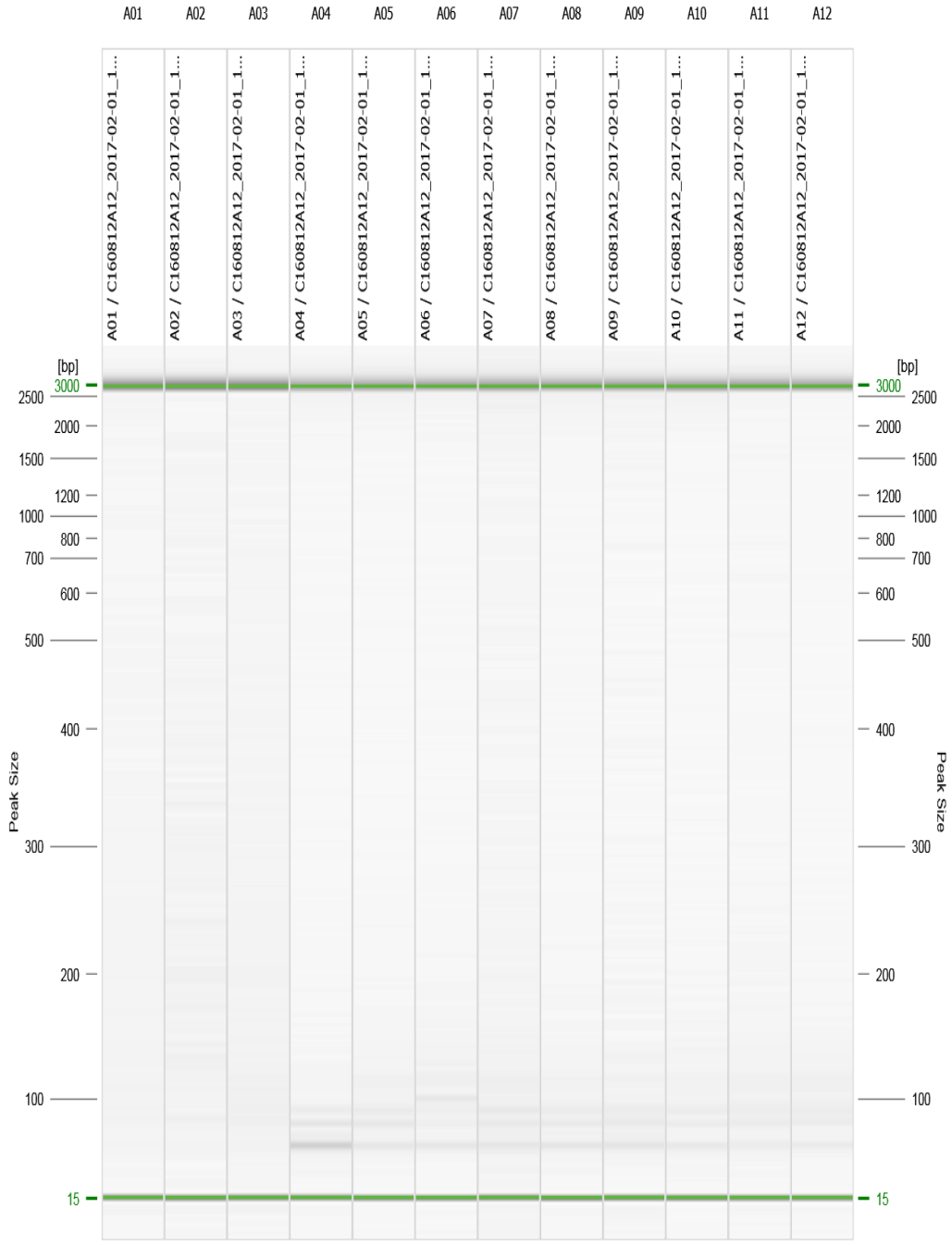
Appendix I: Gel image showing open reading frame 1 PCR products separation of the 4 HEV



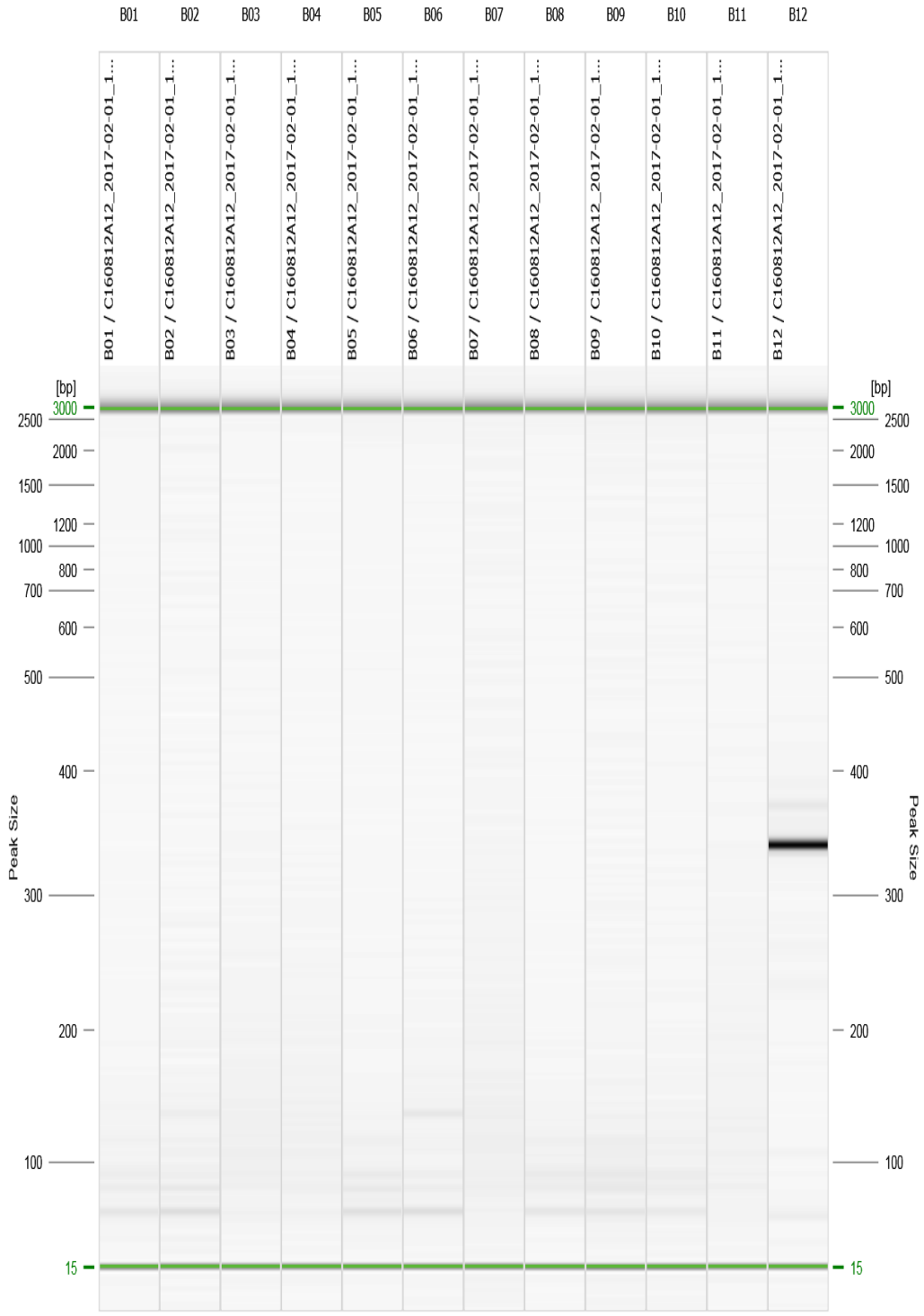
Open reading frame I HEPATITIS E VIRUS genotype 1



Open reading frame I HEPATITIS E VIRUS genotype 2



Open reading frame I HEPATITIS E VIRUS genotype 3



Open reading frame I HEPATITIS E VIRUS genotype 4

Appendix II: Kenya national blood transfusion service forms

Donation Number

KENYA NATIONAL BLOOD TRANSFUSION SERVICE

Blood Donor Questionnaire

Clinic Venue ---------- County ----------Clinic Code: -------

Donor Number ----------

(Donors please complete this section below)

Surname: _____Other Names: _____ _____

GENDER: F / M

Student Number/ National ID Number: _____ Date of Birth:----

--/------/-----(dd/mm/yy)

Single	Married	Divorced/Separated	Widowed

Marital Status:

Contact Details: Postal Address (where you would like to receive your correspondence)

Code

Home phone number: ---------- Cell phone number:----------

Email: ---------- Residence (County) ----------

Level of education: None/ Primary/ Secondary/ Tertiary

Occupation: ……..…

When did you last donate Blood? ……

Blood Group: ……...…

HEALTH QUESTIONNAIRE Circle the appropriate answer

1	Are you feeling well and in good health today?	Yes/No
2	2. Have you eaten in the last 6 hours?	Yes/No
3	3. Have you ever fainted in the last 4 months?	Yes/No
4	4. In the past 6 months have you been ill, received any treatment or any medication?	Yes/No
5	5. In the past 6 months have you had any injections or vaccinations (immunizations)?	Yes/No
6	6. In the past 6 months have you Female Donors: Have you been pregnant or breast feeding?	Yes/No
7	7. Do you have or have ever had any problems with your heart or lungs e.g. asthma?	Yes/No
8	8. Do you have or have ever had a bleeding condition or a blood disease?	Yes/No
9	9. Do you have or have ever had any type of cancer?	Yes/No
10	10. Do you have or have ever had diabetes, epilepsy or TB?	Yes/No

RISK ASSESSMENT

The lives of patients who receive your blood are totally dependent on your honesty & frankness in answering the questions below. Your answers will be treated in a confidential manner.

Circle the appropriate answer

12	Received blood transfusion in the past 12 months	Yes/No
13	Received or given money, goods or favours in exchange?	Yes/No
14	Had sexual activity with a person whose background you do not know in the past 12 months?	Yes/No
15	Been raped or sodomized in the past 12 months?	Yes/No
16	Had a stab wound or had an accidental needle stick injury e.g. injection needle in the past 12 months?	Yes/No
17	Had any tattooing or body piercing e.g. ear piercing in the past 12 months?	Yes/No
18	Had a sexually transmitted disease (STD) in the past 12 months?	Yes/No
19	Live with or had sexual contact with someone with yellow eyes or yellow skin in the past 12 months?	Yes/No
20	Had sexual activity with anyone besides your regular sex partner in the past 12 months?	Yes/No
21	Have you ever had yellow eyes or yellow skin?	Yes/No
22	Have you ever Injected yourself or been injected, besides in a health facility?	Yes/No

23	Have you ever used non-medical drugs such as Marijuana, Cocaine etc?	Yes/No
24	Have you ever have you or your partner been tested for HIV?	Yes/No
25	Do you consider your blood safe to transfuse to a patient?	Yes/No

DECLARATION

I declare that the information I have given above is correct.

I understand that my blood will be tested for HIV, Hepatitis B & C, and Syphilis and the results of my tests may be obtained from the Kenya National Blood Transfusion Service.

I understand that the Kenya National Blood Transfusion Service may use any communication medium(s) to send me important information. Such medium(s) shall include but not limited to e-mail, post office, mobile telephone and/or fixed telephone. I hereby give consent to KNBTS to use the contact details provided in this form to communicate to me as the need may be.

Signature: ---------- Date: ----------

For Official Use:

Weight (kg)	Hb>12.5g/dl	BP	Pulse

Donor is Accepted	
Yes	No

Report:

Name of Nurse / Counselor: Date:

Low Volume	> 1 Venepuncture	Hematoma	Faint		
			Mild	Moderate	Severe

Time Needle In		Time Needle Out	

Report:

Appendix III: TTI Worksheet

Name of KNBTS Facility……...…… Date of Testing…… Type of Test……..

Test Kit……..... Lot Number……..... Expiration date…….

Date of Reconstitution……

	1	2	3	4	5	6	7	8	9	10	11	12
A	NC											
B	NC											
C	NC											
D	PC											
E	PC											
F	PC											
G	KNC											
H	KPC											

Are all negative controls within specification? Yes No Are all positive controls within specification? Yes ☐ No ☐

Calculated cut-off…….....

Is the test run valid? Yes No

List all positive samples .....……...… ……..…..

…… ..... ……

.... ……..…..

....

.... ……..…

....

.... ……..

..... ……. ..……..

Name of Technologist…….....…..

Sign…… Checked by.....

Sign.....

Appendix IV: University research approval letter

ALEX K. MUTAI

MMLS/2013/41772

P.O BOX 26673- 00506

NAIROBI

DATE 24/09/2015

THE HEAD,

NATIONAL BLOOD TRANSFUSION SERVICE

P.O BOX 29804-00202

NAIROBI.

Approved
MOduer.
29/9/2015

APPROVAL TO UNDERTAKE A RESEARCH WITHIN THE KENYA NATIONAL BLOOD TRANSFUSION SERVICE.

Am a bonafide student at the Mount Kenya University currently undertaking a Master of Science course in Hematology and Blood Transfusion and employed the Ministry of Health working at the KNBTS .

As part of the course, I intend to carry out my research entitled"DETERNINATION OF HEPATITIS E VIRUS AMONGST BLOOD DONOR POPULATION IN KENYA" and do hereby request for your approval to carry out this research within the organistion.

To do this, I will randomly collect blood samples after routine testing for transfusion transmissible infections from the six regional blood transfusion centers and carry out the test for Hepatitis E virus antibodies on the selected samples. Confirmatory testing using PCR will be carried out on every sample that tests positive for the markers.

All the findings will be shared with your office and it's my commitment to abide by all the rules and regulations set out by the organization.

I will be grateful if my request will be considered.

Yours Sincerely,

Alex Kiprotich Mutai

P/N 1997018882

Appendix V: Letter of approval from National Commission for Science, Technology and Innovation

CONDITIONS

1. You must report to the County Commissioner and the County Education Officer of the area before embarking on your research. Failure to do that may lead to the cancellation of your permit.
2. Government Officer will not be interviewed without prior appointment.
3. No questionnaire will be used unless it has been approved.
4. Excavation, filming and collection of biological specimens are subject to further permission from the relevant Government Ministries.
5. You are required to submit at least two(2) hard copies and one (1) soft copy of your final report.
6. The Government of Kenya reserves the right to modify the conditions of this permit including its cancellation without notice

REPUBLIC OF KENYA

National Commission for Science, Technology and Innovation

RESEACH CLEARANCE PERMIT

Serial No.A 13566

CONDITIONS: see back page

THIS IS TO CERTIFY THAT:
MR. ALEX KIPROTICH MUTAI
of MOUNT KENYA UNIVERSITY,
27663-506 NAIROBI,has been permitted
to conduct research in *Embu , Kisumu , Machakos , Nairobi, Nakuru Counties*
on the topic: *SEROLOGICAL DETERMINATION OF HEPATITIS E AMONGST BLOOD DONOR POPULATION IN KENYA*
for the period ending:
30th March,2018

Permit No : NACOSTI/P/17/35066/16065
Date Of Issue : 31st March,2017
Fee Recieved :Ksh 1000

Applicant's Signature

Director General
National Commission for Science, Technology & Innovation