SEROPREVALENCE OF HEPATITIS E VIRUS AMONG HEALTHY BLOOD DONORS IN NAIROBI CITY COUNTY, KENYA

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P150F/CTY/PT/37790

A RESEARCH THESIS SUBMITTED IN PARTIAL FULFILMENT OF REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTERS OF SCIENCE IN INFECTIOUS DISEASES IN THE SCHOOL OF MEDICINE, KENYATTA UNIVERSITY

March 2022

DECLARATION

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

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DEDICATION

I dedicate this thesis to my family and my late grandmother Yei Nelway. I also dedicate it to my wife, Sherlay Yarsiah Fardolo, and our sons; Oscar Yukelea Fardolo Jr., Anthony Nyan Fardolo, Sherkiel Miaway Fardolo. Thank for all the support you gave me throughout my academic journey.

ACKNOWLEDGEMENT

I acknowledge Kenyatta University for providing a favorable learning environment and Stella Maris Poltechnic University for financial and moral support through this study. My sincere thanks and appreciation goes to my supervisors; Dr. George Gachara Maina, Ph.D. and Prof. Wallace D. Bulimo Ph.D. Thank you for inspiring me to work hard and give my best every day. Your support and encouragement has helped me to be committed to this work. Besides, I acknowledge my my wife Sherlay Yarsiah Fardolo. May the Almighty God bless you for being there for me. To my sons, am grateful for the support and company you gave your mother while I was away. Thank you for the joy you have brought in our lives.

To the Almighty God; you are the Alpha and the Omega, your name is glorified.

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LIST OF ABBREVIATIONS AND ACRONYMS

HEV	Hepatitis E Virus
SOT	Solid Organ Transplant
PCR	Polmyerase Chain Reaction
ELISA	Enzyme-linked immunosorbent assay
HIV	Human immunodeficiency virus
HBV	Hepatitis B virus
ORFs	Opening reading frames
HCV	Hepatitis C virus
RNA	Ribonucleic acid
NT	Nucleotides
UTR	Untranslated region
RT-PCR	Reverse Transferase Polymerase Chain Reaction
ERC-KU	Ethical review committee Kenyatta University
IgM	Immunoglobulin M
IgG	Immunoglobulin G
WHO	World health organization
ANOVA	Analysis of Variance

HSPGs	Heparin sulfate proteoglycans
PCP	Papain-like Cysteine Protease
HVR	Hypervariable Region
NCR	Non-Code Region
Met	methyltransferase
RdRp	RNA-dependent RNA polymerase
ER	Endoplasmic Reticulum

ABSTRACT

Hepatitis E disease is caused by Hepatitis E virus (HEV). The virus is an enteric virus that is transmitted via fecal contaminated food, water, blood transfusion, and organ transplants. HEV causes approximately twenty million infections globally, of which over three million are asymptomatic. In 2015, The World Heal Organization (WHO) reported that this virus accounted for approximately 55,000 deaths representing a 3.3% mortality rate. HEV is classified into at least four human infecting genotypes. Out of these known genotypes, genotypes one and two are endemic in Asia and Africa, while genotypes three and four are sporadically described in developed countries. Two previous studies in Kenya have reported a seroprevalence of 25.7% and 77 % among acute febrile illness and jaundice patients. Despite these reports, most African countries, including Kenya, do not screen for the virus during blood donation. Besides, the seroprevalence, and the molecular characteristics of this virus in blood donors in Nairobi, Kenya, remain unknown. Therefore, this study aimed to determine the seroprevalence of Hepatitis E virus circulating genotypes and the relationship between socio-demographic risk factors and the HEV on voluntary blood donors in Nairobi using a retrospective study design. Archived plasma samples from 358 voluntary blood donors were used. The obtained blood plasmas were screened for Hepatitis E virus IgM and IgG antibodies using a qualitative membrane-based immunoassay (Biopanda reagents, Belfast, UK). The collected blood donors' socio-demographic characteristics were analyzed using Chi-square in SPSS software version 20 to determine the relationship between the blood donor's socio-demographics and HEV infection. The total virus RNA from the seropositive samples was extracted using the ThermoFisher PureLink™ Viral RNA/DNA Mini kit and used as a template to synthesize cDNA. A nested PCR was performed to amplify the HEV ORF2 gene. The blood donors recruited in this study were between 16-60 years and had a mean age of 29±14 years. Out of the 358 subjects, 36% (n = 130) were females, while 64% were male. In addition, 10.6% of the tested samples were HEV IgG seropositive, while 7.8% were HEV IgM seropositive. The Anti-HEV was distributed in all the study age groups. The blood donors aged 20-29 years reported of 4.5% IgM and 5.5% IgG seroprevalence rate compared to other age groups. HEV seroprevalence was statistically significant higher in males and donors with tertiary level of education. Based on RT-PCR, all the seropositive samples were HEV RNA negative. Therefore, the study was unable to determine the Hepatitis E virus genotypes circulating in Kenya. This study shows that in Nairobi, there is high HEV seroprevalence among healthy blood donors. Further studies among other population groups are desirable to evaluate the potential benefits of incorporating screening of HEV among blood donors.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Hepatitis E disease is caused by the primarily enteric Hepatitis E virus (HEV). The virus has a single-stranded RNA genome estimated to be approximately 7.2kb, and has three open reading frames. According to Zhang *et al*. (2017), Hepatitis E virus is characterized in the genera *Orthohepevirus* and the family *Hepeviridae*. There are different genotypes of the HEV. However, four of these genotypes are known to infect humans. Genotypes one (HEV-1) and two (HEV-2) are highly endemic in Africa and Asia. In these continents, the virus causes human waterborne diseases (Baki *et al*., 2018).

Additionally, genotypes three and four are common in developing countries and are known to have a zoonotic transmission (Woo *et al*., 2016; Zhang *et al*., 2010). Moreover, Hepatitis E virus is mostly spread from feces to water and food (Furukawa *et al*., 2016). In addition, also the virus is spread through blood transmission and organ transplant (Purpari *et al*., 2019). According to Kim *et al*. (2014), Hepatitis E virus which is associated with acute liver disease, is a threat to global blood safety. Furthermore, it is estimated that this virus causes approximately twenty million global infections annually. Out of the twenty million reported annually, three million are symptomatic infections (Horvatits *et al*., 2019).

According to a WHO 2015 report, Hepatitis virus causes approximately 55,000 global deaths annually. In most cases, many reported deaths occur in Latin America, Africa, and Asia. This is because the majority of the populations in these countries have low income; hence may not adhere to hygiene and cannot access good sanitation (Kmush *et al*., 2013). In Africa, several HEV outbreaks have been reported with varying seroprevalence across the different virus genotypes. In Zambia, a 42% seroprevalence among urban dwellers was reported by Jacobs *et al*. (2014), while in Ethiopia, 31.6 % prevalence has also been documented among pregnant women (Abebe *et al*., 2017). In northern Uganda, a 14% seroprevalence was reported in HIV-infected adults (Boon *et al*., 2018). In addition, a prevalence of 77.1% was reported in a refugee camp located in Eastern Kenya and 25.7% and 37.8% among febrile patients in Kibera (Ahmed *et al*., 2013; Furukara et al.,2016). Generally, the seroprevalence among blood donors ranges from 0-49% (Bagulo *et al*., 2020).

In Sub Saharan Africa, the asymptomatic Hepatitis E disease is common among healthy persons while the prevalence rate is high in immune compromised persons and pregnant women. Approximately 60% of immunocompromised individuals infected with HEV develop chronic hepatitis E (Neukam *et al*., 2013). In Kenya, the health ministry stipulates that all blood must be screened for possible blood transfusion pathogens before any transfusion is done. The infectious pathogens screened before transfusion include; syphilis, HCV, HBV, and HIV. However, Hepatitis E virus is not one of the pathogens screened before transfusion (Ware *et al*., 2018).

1.2 Problem statement

Despite being the prevalent cause of viral Hepatitis, HEV is the least diagnosed etiology of acute viral hepatitis. Some recent reports suggest that HEV is accountable for chronic Hepatitis in immunocompromised patients (Neukam *et al*., 2013). The virus could be transmitted through direct blood transfusion from infected blood donors (Purpari *et al*., 2019). In developing countries like Kenya, the HEV virus is not tested during blood donations. However, the virus is tested among blood donors some European and Asian countries like United Kingdom, Ireland, and Netherlands, Switzerland and Japan and many European countries are on above to approve of HEV among potential donors (Denner, 2019). Therefore, this study sought to determine the seroprevalence and molecular characteristics of HEV among potential blood donors in Nairobi, Kenya.

1.3 Justification

Globally, Hepatitis E virus is one of the public health challenges, especially in developing nations. Data has shown that the incidences and seroprevalence of the virus are high in Africa. Some studies have shown that the virus spreads through blood transfusion (Neukam *et al*., 2013). However, in Kenya, HEV is not considered when testing potential blood donors. Therefore, there is a need for data on HEV prevalence and genotypic characteristics in low-risk voluntary blood givers. This information will be useful in guiding policymakers on making policies regarding blood donation and screening of the donated blood to guarantee safety to the blood recipients.

1.4 Research questions

i. What is the overt seroprevalence of the Hepatitis E virus among voluntary blood donors in Nairobi, Kenya?
ii. What are the socio-demographics related to the Hepatitis E infection among the voluntary blood donors in Nairobi, Kenya?
iii. What are the Hepatitis E circulating genotypes among the voluntary blood donors in Nairobi, Kenya?

1.5 Objectives of the study

1.5.1 General objective

To determine the overt seroprevalence and the association between socio-demographic factors and HEV seropositivity among blood donors in Nairobi

1.5.2 Specific objectives

i. To determine the overt seroprevalence rate of HEV among voluntary blood donors in Nairobi, Kenya
ii. To determine the association between socio-demographic characteristics and HEV seropositivity among voluntary blood donors in Nairobi, Kenya.
iii. To identify the circulating genotypes of HEV among the voluntary blood donors in Nairobi, Kenya

1.6 Significance of the study

The study purposed to establish the seroprevalence and molecular characteristics of Hepatitis E on blood donors in Nairobi, Kenya. The study findings reported herein show the extent of exposure to HEV infection in healthy voluntary blood donors, a study

group not considered in previous studies. Consequently, the findings reported in this study provide a critical baseline data on HEV infection among low risk blood donors in the country.

1.7 Limitations of the study

In this study, the data on HEV viral load and variation in titers of different disease markers in the course of disease progression among the affected persons were not determined. This, therefore, assumes that there is no correlation between the occurrence of the infection and prognosis in the asymptomatic population. Despite being a retrospective study, this study did not consider doing a follow-up on the infected persons to assess the risks that could have resulted from exposure to infection.

CHAPTER TWO

LITERATURE REVIEW

2.1 Historical background

Hepatitis E virus was first reported in a waterborne epidemic in Kashmir, India. During this epidemic in 1978, the cause was suspected to be a non-A, non-B hepatitis virus (Balayan *et al*., 1983). The virus was also identified in 1983 during an outbreak in Afghanistan among Soviet militaries (Khuroo, 2011). Since the recognition of the virus as the main cause of infection, the virus was named Hepatitis E (HEV). The virus contains eight genotypes; one and two genotypes infect humans only (Smith *et al*., 2016). Genotypes three and four are known to be zoonotic and mostly infect several animals, especially swine, but occasionally cause infection in humans (Smith *et al*., 2014). These genotypes are abbreviated as HEV, followed by the genotype number. Additionally, each of the genotypes is further classified into several subgenotypes. The subgenotypes of HEV-1 are 1a, 1b, 1c, 1d and 1e. The genotype two (HEV-2) has two recognized sub-genotypes; 2a and 2b. Genotype three (HEV-3) has several sub-genotypes; 3a-3j and genotype four (HEV-4) has subgenotypes 4a-4j (Liu *et al*,. 2012).

2.1.1 Classification

Based on the Hepatitis E virion structure, its particle organization and overall genome structure, it was placed into the *Caliciviridae* family. Later, a thorough investigation of the viral genome showed that it had numerous features that were not in agreement with classification in any existing virus family (Reuter *et al*., 2016). Therefore, the virus was

classified in the *Hepeviridae* family, and it is the only representative of the genus *Hepevirus* (Sun *et al*., 2019).

2.1.2 HEV genotypes

There are eight recognized HEV genotypes, including four human pathogens. The eight major genotypes include; genotype one to eight. Humans are infected by genotypes one and two, whereas genotypes three and four are known to be zoonotic (Aslan and Balaban, 2020). Genotypes one and two are primarily human infecting genotypes, while genotypes three, four, and seven are diverse because they infect different hosts. Recently, there have been advanced techniques of identifying the HEV genotypes from animals such as; trout, deer, rabbits, rats, bats, and camels. The identification of these isolates from different hosts necessitated a review of the taxonomy of the *Hepeviridae* family (Himmelsbach *et al*., 2018). The primary host of genotypes three and four is swine, especially in developed countries (Olavinka *et al*., 2020).

Each of the different HEV genotypes has been shown to have distinct geographical distributions. In South and North America and Asia, many genomes of HEV strains have been fully or partly sequenced. There are four main groups of human Hepatitis E strains. In Asia and North Africa, human genotype one has been the main cause of waterborne epidemics and sporadic diseases. In contrast, human genotype two was isolated following outbreaks that occurred in Mexico and central Africa. In developed countries, Hepatitis E is caused by genotypes three and four. In most cases, Hepatitis E affects older people (Wehmeyer *et al*., 2018).

2.2 Biology of HEV

Hepatitis E virus is classified in the family *Hepeviridae,* and it is the only representative of the genus *Hepevirus* (Kamar *et al*., 2012; Sun et al., 2019). The virus is small (27-34nm long), naked, with an icosahedral capsid, a positive-sense single-stranded RNA genome (7.2kb long) and has three overlapping reading frames in feces. Moreover, the HEV virus occurs in a non-enveloped virion and membrane-associated and quasi-enveloped virion (eHEV) in blood (Nagashima *et al*., 2017). The virus enveloped uncoats in the biliary tract and leads to a non-enveloped virus that withstands harsh environmental conditions and possesses high infectivity than eHEV (Ji *et al*., 2021).

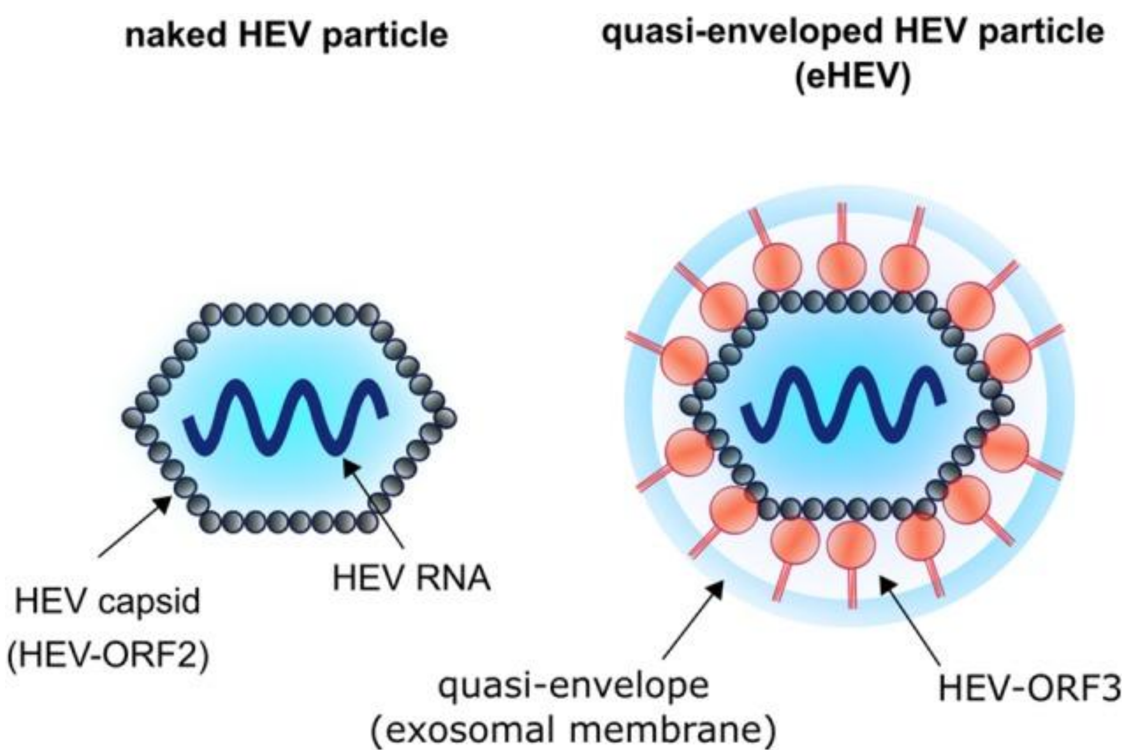

Figure 2.1: Virion structure of HEV (Himmelsbach *et al*,. 2018)

2.3 Hepatitis E Virus genome

Hepatitis E virus genome comprises a small 5′ end which is the untranslated region (UTR), three overlapping reading frames (ORFs), and a tiny 3′ untranslated region. The 5′ end is capped and the 3′ end is polyadenylated. The ORF 1 starts at the 5' end immediately after a non-coding region that is normally short and contains 26 nucleotides. This frame is one of the largest coding regions which is approximately 5,000nt in length and is involved in replication because it encodes the non-structural proteins responsible for viral replication. The second ORF (ORF 2) comprises 1820 nucleotides and has a polyadenylated chain that is 65 nucleotides long. The segment encodes the viral structural protein (capsid). The capsid helps in the attachment of the virus to the host cell membranes (Figuer 2.3). Also, it aids in the induction of neutralizing antibodies. The third ORF (ORF 3) is the smallest and consists of 263nt that encodes a minute immunogenic phosphorylated protein that is involved in viral morphogenesis and release (Arends *et al*., 2014).

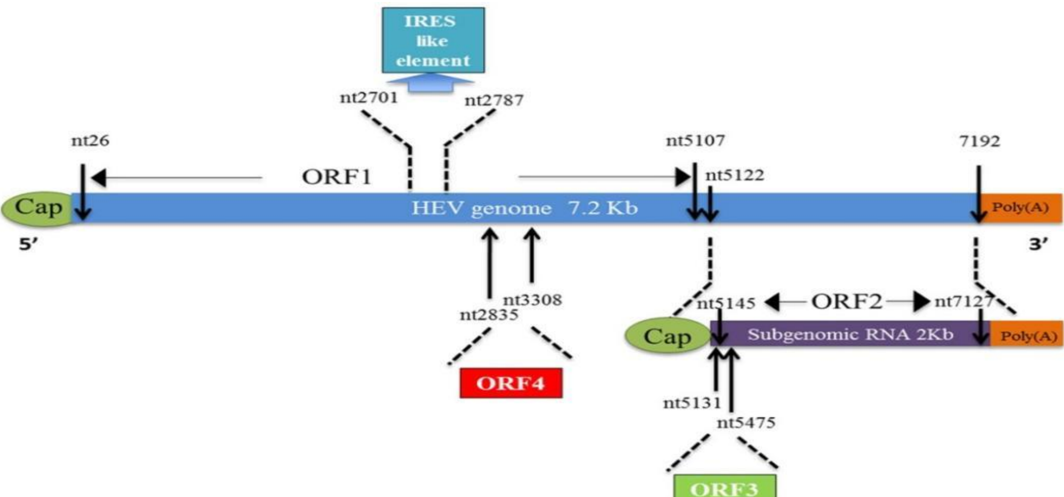

Figure 2.2: Hepatitis genome (Arends *et al*., 2014).

2.4 HEV proteins and their functions

The HEV genome is expressed into proteins that are classified as either structural or non-structural depending on their function.

2.4.1 Non-structural proteins

The ORF1 is one of the important genes in the HEV virus because it encodes for the non-structural proteins involved in viral replication. This coding region starts directly following the 5' non-coding region (NCR) and expands 5082bp. The open reading frame one encodes the 1693-amino acid (aa) polypeptide concerned with replicating and processing viral proteins (Ahmad *et al*., 2011). It is also involved in the mechanism of HEV pathogesis (Nan and Zhang, 2016). This region is comprised of many domains, including the papain-like cysteine protease (PCP) and methyltransferase (Met) (Wang *et al*., 2021); which caps the 5' of the virus genome (Muñoz *et al*., 2016). In addition, the open reading frame has the Y domain, which is rich in proline and has the hypervariable region. Also, the X domain is located next to the PCP region and helicase domain. Lastly, it contains the RNA-dependent RNA polymerase (RdRp), which helps the virus during replication (Agrawal *et al*., 2001).

The Methyltransferase domain is one of the useful domains of the ORF1 (Cao *et al*., 2017). Guanine-7-Met and guanylyltransferase activities were both discovered in a baculovirus-expressed 110-kDa ORF1 polyprotein. Some studies suggest that the HEV Hel protein domain may form capped HEV genomic RNA (Khateri *et al*., 2018). This Hel region of HEV has both nucleoside triphosphatase and contains 5´–3' RNA duplex unwinding activaties (LeDesma *et al*., 2019)

HEV ORF1 has been predicted to contain a Papain-like Crystal Protease domain (Proudfoot *et al*., 2019); though, there is no tentative proof for HEV ORFI polyprotein translation and post-translation process. A 185-KDa polyprotein of hepatitis E ORF1 was previously expressed in *Escherichia coli*, the cells of an insect, and the mammalian cells (Gupta *et al*., 2020). When using vaccinia virus-based techniques, ORF1 was expressed in mammalian cells, and two products were obtained, 107 and 78 kDa respectively, from the post-translation process (Kenney and Meng, 2019). Nevertheless, the processing of the protein products was not affected by the interruption of the predicted ORF1 PCP domain. Therefore, it remains uncertain whether the polyprotein of ORF1 performs as one protein with several functional domains or if the smaller cleaved domains work separately after cleaving. (Kanade *et al*., 2018).

There are eight motifs in the viral RdRp of HEV common to the RdRp of other RNA-positive sense viruses (Su *et al*., 2020). When expressed in *E. coli*, the Hepatitis E RdRp showed binding in the 3' NCR of the virus genome while the recombinant Hepatitis RdRp can make RNA *in vitro* using the Hepatitis E virus 3' polyadenylated RNA template in a primer dependent method. (Kumar *et al*.,2020). According to Paliwal *et al*., (2017), the RdPp in the endoplasmic reticulum are localized while cells that express the RdRp recombinant proteins showed that the endoplasmic reticulum could play a role in the Hepatitis E virus replication (Paliwal *et al*,.2017).

2.4.2 HEV structural proteins

The open readings frame two (ORF2) helps in encoding the Hepatitis E capsid. The ORF2 is 66 amino acids long and has a molecular weight of 72kDa. The capsid is arginine-rich and signals the peptide sequence and the possible N-linked glycosylation (Meng, 2010). The capsid proteins have been observed to vary in size when examined *in vitro* (Ankavay *et al*., 2019). Some studies suggest that HEV capsid protein has four products (72, 63, 56, and 53 kDa).

However, the analysis of the sequence of HEV ORF2 showed that 63, 56, and 53 kDa protein are the results of the cleavage of the full length 72 kDa protein (Lin and Zhang, 2021).

The viral particle assembly and its contact with host cells are carried on by the ORF2 capsid protein (Wei *et al*., 2018). In mammalian cells, most of the proteins are glycosylated. The glycosylation process is essential in the production of infectious viral particles (Kang and Meng, 2017). In addition, the capsid helps the virus during the viral assembly stage by binding onto the genomic RNA. Also, it aids the virus by attaching to the host and allowing the virus to interact with the heat shock protein 90 (HSP90) (Nimgaonkar *et al*., 2021), glucose-regulated protein, and heparin sulfate proteoglycans (HSPGs) (Slot *et al*., 2017). Previously, it has been demonstrated that the HSPG helps the virus attach to the host receptors and helps the virus enter the host cells. On the other hand, ORF2 can bind to the chaperone Grp78 helping in intracellular transport (Sari *et al*., 2019).

The HEV ORF2 capsid proteins are antigenic, and it has been observed that neutralizing antibodies aim at it. The aa residues in the C-terminal part of ORF2 have been identified for neutralizing linear and conformational antibodies (Xu *et al*., 2021). Previous studies have shown that antibodies that can identify conformational epitopes can be deactivated. The capsid proteins are vital in producing a deactivation-sensitive epitope, especially the aa residues (Harms *et al*., 2020). There is broad immunogenic cross-reactivity among the various genotypes of HEV. Regardless of the large antigenic cross-reactivity between the Hepatitis virus genomes that are known, the variations in immunogenicity have been identified using the monoclonal antibodies that are genotype and strain-specific. It has been reported that the aa residue 606 in the capsid has been documented to be important in preserving the antigenicity of the Hepatitis E opening reading frame 2 (ORF2) (Simanavicius *et al*., 2018).

Hepatitis E virus ORF3 gene encodes a protein of about 114 amino acids (aa) which has diverse functions in the Hepatitis E virus life cycle (Chandra *et al*., 2011). HEV ORF3 protein plays a vital role in virion secretion (Emerson *et al*., 2010) but is not involved in replication or virion assembly (Ju and Ding, 2019). Notably, the open reading frame three (ORF3) protein helps the Hepatitis virus express or release its content on the host cell through the exosomal pathway (Ding *et al*., 2017). The ORF3 is connected to the quasi-enveloped viral particle (Nagashima *et al*., 2017). Previously, it has been demonstrated that the ORF3 proteins have essential channel activity in releasing the virus infectious (Primadharsini *et al*., 2020).

According to Kenney *et al*. (2019), in genotype one, the ORF3 is phosphorylated at the serine (ser) 71, while in genotype three, it is phosphorylated at serine (ser) 70 by the mitogen-activated protein kinase of the cell Furthermore, phosphorylated ORF3 protein has been demonstrated to interact with the open reading frame two capsids (Tyagi *et al.,* 2001). Some early research had observed that the ORF3 protein associates with the cytoskeleton, particularly microtubules (Gouttenoire *et al*., 2018). In addition, the protein has also been shown to play a role in early and recycling endosomes and multivesicular bodies (MVBs) (Nagashima *et al*., 2014; Factor, 2008).

2.5 Replication of HEV

A receptor-dependent procedure allows the hepatitis E virus to enter a receptive cell, and Heparin Sulfate Proteoglycans (HSPGs) and other unknown factors assist the virus in entering the host cell (LeDesma *et al*., 2019). After that, the ORF1 is translated and processed to several functional domains once the viral genome is released, which together mediate the virus's asymmetric genome replication mechanism (Anang *et al.,* 2018). As a result, many copies of the capped genomic RNA and the subgenomic RNAs are generated. On the other hand, open reading frames two and three are synthesized by the sub-genomic RNA. The ORF2 genomic RNA and other viral or host factors cause the formation of additional virions, which are then expelled from the cell via the viral ORF3 protein-dependent endosomal sorting complex required for transport (ESCRT) (Figure 2.5).

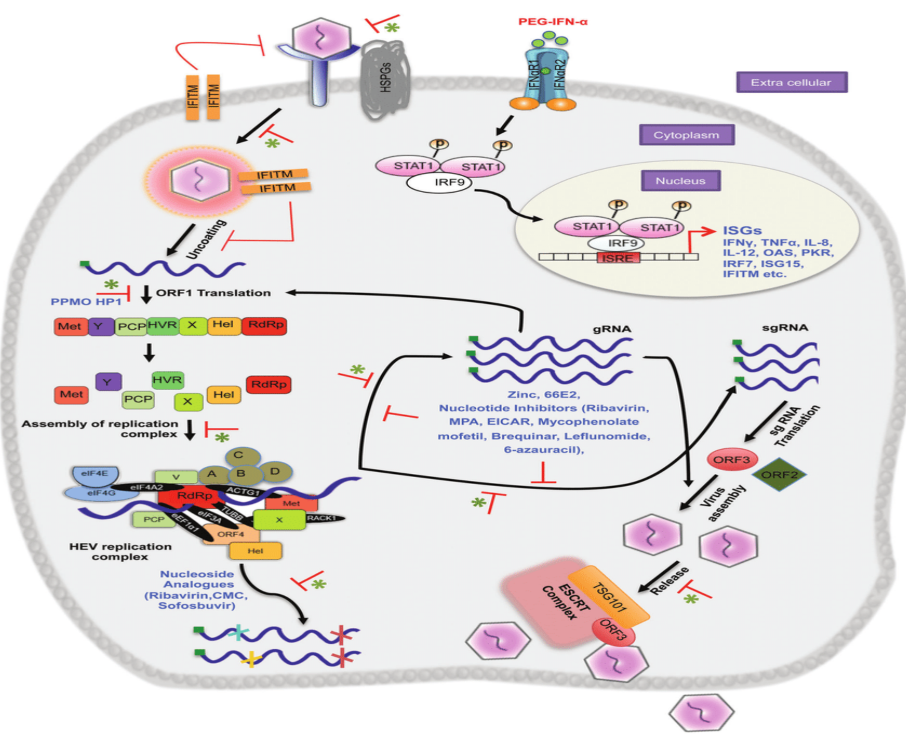

Figure 2.5: Replication of HEV, (Anang *et al*., 2018)

2.6 Transmission of HEV

Transmission occurs mainly through fecal contamination of water supplies in hyperendemic areas (waterborne transmission). Zoonotic and food transmission through the intake of poorly cooked pork and hunted meat also accounts for significant transmission (Ferri and Vergara, 2021). However, three additional modes of transmission of HEV have been described; transfusion-transmission related, vertical transmission, and human to human HEV transmission (Mirazo *et al*., 2014). Placental HEV transmission is one of the main transmission routes for HEV (Yu *et al.*, 2020). Vertical transmission of HEV often results in an undesirable result of pregnancy, especially stillbirth, preterm labor, and abnormal function of the neonate's liver (Zaki *et*

al., 2014). Previous studies have suggested that about 45 % to 50 % of the infected mothers transmit the virus to their children during pregnancy (Singh *et al*., 2003). While most worldwide HEV infections are mainly associated with food and water contamination, there has also been a growing concern about transfusion transmission HEV (TT-HEV) in blood and blood products (Bi *et al*., 2020). A blood transfusion may be a risk in the transmission of the HEV virus. This may pose a risk to the recipients because some recipients may be immunocompromised and have other unknown diseases requiring blood transfusion (Denner, 2019). A blood transfusion may include blood products such as; red cells, platelets, and plasma. These may be linked to TT-HEV, leading to chronic HEV in immune-suppressed patients (Gallian *et al*., 2019). Moreover, immune-suppressed patients may include patients who have undergone organ transplants and are at a higher risk to the evolving HEV chronic form, which can develop to cirrhosis and hepatomegaly, which has been reported to increase the overall HEV mortalities (Bi *et al*., 2020).

2.7 Risk groups

According to the previous study following groups of people are at higher risk of acquiring HEV; people living in local communities where neighborhood outbreaks exist and individuals traveling to areas of the world with high HEV endemicity (Krumbholz *et al*., 2014). Refugees residing in congested provisional camps due to the war, such as North Eastern Kenya, Somalia, and Ethiopia, are also at risk (Ahmed *et al*., 2013). People living with severe liver problems and individuals that are in close contact with

animals such as; pigs, cows, rabbits, sheep, goats, and persons with a compromised immune system are at more of HEV infection (Sayed *et al*., 2018).

tudies have suggested that pregnant women are at risk of HEV infection. HEV has been shown to contribute to stillbirths and maternal mortality (Rein *et al*., 2012). The mortality rate among pregnant women ranges from 20 to 25% and frequently occurs in the third trimester of the gestation period. (Labrique *et al*., 2010), indicating that HEV infection is often fatal among pregnant women (Dagnew *et al*., 2019). Mortality is high among pregnant women due to the physiological changes that occur during pregnancy. The mother's innate immune responses are suppressed to protect the foetus from immunological recognition and rejection. Shifts in immune states may aggravate HEV infection and result in adverse pregnancy outcomes (Yu *et al.,* 2020).

2.8 Epidemiology of HEV

The world epidemiology of HEV is about 939 million that is 1 in 8 persons have been exposed to HEV infection. Individual recently or have ongoing HEV infection range from 15 to 110 million worldwide (pengfei *et al*., 2020). In developing nations, HEV is a waterborne disease that has been proved to be caused by Hepatitis one and two. The virus is mainly transmitted to humans in poor sanitary conditions (Kamar *et al*., 2012). The occurrences of Hepatitis virus are seasonal; occurring mostly during the rainy seasons (Labrique *et al*., 2010). Besides, human-to-human transmission is not considered as one of the forms through which viruses can be spread.

However, it remains a concern since it is speculated to have resulted in an epidemic in the Republic of South Sudan in 2013 (Thompson *et al.*,2013). Previously, Howard *et*

al. (2010) demonstrated some of the factors contributing to the disease's spread, which including animal farming and consumption of uncooked animal products.

Several studies have demonstrated that hepatitis infection is high, especially in blood donors infected with the HEV virus. The virus can spread via human-human transmission through blood during a blood transfusion. For instance, in the United States and South-East England, 16% of blood samples were seropositive (Tedder *et al*., 2016), 21% in Denmark, 29.4% in Switzerland (Niederhauser *et al*., 2018), 7%-30% in Germany, and 52% in South-Eastern France (Mansuy *et al*., 2011, Domanović *et al*., 2017).

During the past two decades, it has been proven that HEV infection is not restricted to developing nations or tourists coming from these nations. In these countries, the commonest mode of Hepatitis E transmission is zoonotic primarily through the consumption of pork or game meat that is not adequately cooked. In developed, the transmission of HEV is mostly of foodchain (Webb et al.. 2019). An acute infection of HEV can be aggravated in pregnant women, organ transplant patients, patients with pre-existing liver disease and immunocompromised patients (Kamar *et al*., 2012).

2.9 Clinical presentation of HEV

In the last decade, there had been efforts to improve the understanding of the HEV natural history, and through these efforts, a lot has been achieved. Also, several reservoirs and methods of HEV virus transmission have been identified (Kamar *et al*., 2014). In the bulk of infected individuals, HEV causes a self-limiting illness with HEV

genotypes one and two, which lasts a few weeks. After incubation, which is usually two to six weeks, the symptoms of the disease, such as fever and nausea, may present in the patient. Other symptoms such as abdominal pain, vomiting, malaise, and anorexia may start showing with time. Also, inflammation of the liver may be evident at this stage. About 40 % of patients develop Jaundice (Nelson *et al*., 2018). Increased mortality may be common in pregnant women and patients who have pre-existing liver conditions such as chronic liver disease (Niguse *et al*., 2018). In developing countries, it is challenging to distinguish the HEV virus genotypes 3 and 4 by clinical presentations of acute infections compared to the clinical conditions of hepatitis E virus genotypes 1 and 2. In developed countries, Hepatitis E patients may be middle-aged or older men (Wehmeyer *et al*., 2018). Large outbreaks do not occur, and the cases are sporadic, even though there have been a few reported cases of outbreaks from food contamination (Syed *et al*., 2018).

HEV infection has many symptoms. These symptoms include nausea, abdominal pains, malaise, vomiting, and arthralgia, among others. However, about 75 % of the patients infected with HEV3 and four develop jaundice, and the alanine aminotransferase (ALT) level is typically higher than those with HEV1 and 2 (Dalton *et al*., 2007). The disease is self-limiting, especially in patients with HEV symptoms. Such a patient's ALT may return to normalcy after or within four to six weeks. However, there are significant differences in the natural history and prognosis of middle-aged and older patients. A poor prognosis among patients with underlying chronic liver disease and immunosuppression leads to chronic infection development (Hoffmann *et al*., 2020).

In developed countries, few studies have focused on the differences in disease manifestation between HEV3 and HEV4. Patients with HEV 3 and 4 have an increase level of ALT (Kanayama *et al*., 2015). In a study done in France by Jeblaoui *et al*. (2013), a small percentage of the hepatitis E genotype four patients had severe clinical presentation, some of clinical presentation includes fever, nausea, tiredness, stomach discomfort, vomiting and joint pain than patients with Hepatitis E genotype three.

2.9.1 Chronic infection of HEV

Hepatitis E infections are often known to be self-limiting illnesses that resolve by themselves in many patients. However, chronic hepatitis cases have been reported in patients who have HIV and those who have undergone organ transplants (Koenecke *et al*., 2012). Therefore, the development of HEV to chronic form is an issue of concern among solid organ recipient patients who are managed with immunosuppressive drugs. In recent times, chronic HEV has been linked with immunodeficiency, including HIV infection and cancer (Schlosser *et al*., 2012). The chronic form of HEV in immunocompromised patients is an emerging and essential health issue. One of the recent studies suggested that healthy individuals can equally develop chronic HEV infection (Senosiain *et al*., 2016). The chronicity of HEV can lead to inflammation of the liver, which results in liver cirrhosis. In severe cases, it results in liver damage and eventually liver failure, leading to death (Narayanan *et al*., 2019).

2.10 Immune response to HEV

Characteristically, both IgG and IgM antibodies are detectable at the beginning of a disease, which permits serologic diagnosis of the disease once the patient presents the clinical symptoms. IgM drops to undetectable levels over two to six months, and about 10-fold increase in IgG titer is seen over this period. Titer then becomes stable, but the time for protective immunity is unidentified (Krain *et al*., 2014). Patient's response by Immunoglobulin A (IgA) production to HEV has been observed in about 50% of patients (Ramdasi *et al.,* 2020). These agglutinins quickly decline to undetectable levels, even though IgA may continue somewhat longer than IgM (Cao *et al*., 2017). The IgA in hepatitis E virus disease immunity serves as a marker of active infection alongside IgM anti-HEV. This is because passive IgG immunization has been attributed to offering adequate protection while IgA may not be crucial (Nan *et al*., 2016). In HEV infection, the cellular immune response has not been fully understood (Li *et al*., 2019).

2.11 Hepatitis E diagnosis

Hepatitis E virus is usually diagnosed through the recognition of the hepatitis E virus-specific antibodies and the performance of the test kits in different environments due to the difference in manufacture companies and the level of detection is dependent on the kits involved (Moal *et al*., 2015). Viral RNA detection in clinical samples is also used for identification. However, there is a need for researchers' agreement on the technologies that can be utilized in serosurveys and the Acute Hepatitis virus's analysis (Lu *et al*., 2021).

2.11.1 Isolation of the virus

A cell culture system may be necessary to allow hepatitis E propagation *in vitro* especially for the classification of the virus, its diagnosis, and prevention. A number of *in vitro* culture systems, such as the lungs, liver, or kidney of human and primates liver cells for HEV replication, have been reported (Zhang *et al*., 2010). On the other hand, the majority of these cannot provide genuine particles of HEV or a high titer of viral particles and have poor reproduction (Fu *et al*., 2019).

2.11.2 Immune electron microscopy (IEM)

The immune electron microscope is one of the methods used to diagnose the Hepatitis E virus by detecting the viral particles in clinical specimens (Blasco-Perrin *et al*., 2016). The native antibody is precipitated with HEV particles to the Hepatitis E derived from the acute patient serum or the serum obtained from Hepatitis E patient during the recuperative stage. Anti-HEV antibody concentrations can be determined semi-quantitatively by rating the antibody coating. Even though immune electron microscopy (IEM) is a better-quality technique for specificity, the assay's sensitivity is insufficient for a routine test (Thodou *et al*., 2020). IEM is complicated to perform, and most clinical specimens do not have adequate virus-like particles (VLPs) to be detected (Bigoraj and Rzezutka, 2018).

2.11.3 Immune fluorescence microscopy (IFM)

A small number of specialized laboratories use this procedure for antibodies detection. IFM identifies agglutinins that react against the HEV agglutinogen semi-quantitatively.

Anti-HEV agglutinins inhibit the binding of fluorescein-conjugated anti-HEV IgG to HEV agglutinogen in frozen liver tissue. Semi-quantitatively, the concentration of the anti-HEV agglutinins is estimated. This procedure is difficult and costly and is not useful in a routine laboratory (Protzer *et al*., 2015).

2.11.4 Hepatitis E diagnosis using serological methods

The investigation of serum samples obtained from the patient at different stages of Hepatitis E infection (incubation, acute, and convalescent) show distinct patterns of anti HEV IgM and anti IgG (Tholen *et al*., 2016). The IgM anti-Hepatitis E antibodies are manifested at the onset of the acute stage. This can be diagnosed four days after the presentation of jaundice or jaundice-related symptoms and may persist for up to five months (Mazzola *et al.,* 2019). The IgG anti-HEV peaks shortly after the peaking of IgM. As a result, both seem to peak simultaneously in the acute stage of the illness. The IgG anti-hepatitis E may stay longer in the body and can be detected after several years and the IgM to HEV is the marker for recent infection while IgG serve as previous exposure to the HEV infection. (Khuroo *et al*., 2016).

Apart from the regular enzyme immunoassays, other rapid diagnostic assay formats have been developed for HEV serology. Serological tests such as immunochromatographic procedures detect Hepatitis E virus within a short time while utilizing simple procedures. These procedures have also been used in the production of rapid diagnostic assays, which can be utilized in regular testing of patients and blood donors. In addition, the assays have been developed to help in the detection of the IgM

anti-Hepatitis E virus in serum samples obtained from patients with acute Hepatitis E infection. These tests are specifically designed to bind the IgM in the lateral-flow immunochromatographic essays. In this design, the anti-HEV IgM binds to the anti-human IgM monoclonal agglutinins, which are immobilized into the membrane. The anti-HEV IgM is detected using the colloidal gold-labeled Hepatitis E virus agglutin attached to the Hepatitis E virus agglutinogen (Chen *et al*., 2005). The speed and simplicity of carrying out these tests are significant by advantage immunochromatographic HEV IgM test. Currently, these tests have been reported to be specific (100%) and sensitive (82%) in detecting acute Hepatitis E virus infection (Ferreira *et al*., 2018).

2.11.5 HEV molecular detection

Nucleic acid-based procedures, mainly nested reverse transcriptase-polymerase chain reaction and real-time polymerase chain reaction (RT-PCR), have become the most efficient and first approach options for identification of the RNA viruses because they are sensitive and specific (Germer *et al*., 2017). However, nested RT-PCR is subject to contamination, and virus quantification cannot be carried out. To conquer these intricacies, fast and sensitive real-time RT-PCR assays are preferred to identify the hepatitis E virus RNA in samples obtained from humans and swine (Cuevas-Ferrando *et al*., 2020). Notably, choosing an appropriate technique for RNA extraction to guarantee a sufficient recovery of intact viral RNA and the eradication of inhibitory substances is crucial for the successful discovery of HEV genomes (Cattoir *et al*., 2017).

2.12 Hepatitis E Virus disease management

Previously, ant-viral drugs such as Ribavirin monotherapy has been used to treat solid organ transplant (SOT) patients. According to earlier studies done by Kamar *et al.,* (2010), the findings on Ribavirin monotherapy in children and adults were positive. They encouraged the use of this therapy in the treatment of the Hepatitis E virus disease. Reducing the immunosuppressive agent remains the best way of preventing HEV illness in SOTs, especially the agents that target the T-cells. This works best through the introduction of antiviral agents (Suneetha *et al.*, 2012). Clinical guidelines agree with the recent data that demonstrated that Hepatis E virus-specific T-cell proliferations are decreased by using the Ribavirin in monotherapy in treatment of the SOT, especially in the case of chronic Hepatitis E disease (Suneetha *et al*., 2012). The process through which Ribavirin clear HEV is not so far understood but is thought perhaps to occur due to the agent inhibiting viral replication (Gorris *et al*., 2021).

2.13 Prevention and control of HEV infection

According to Van *et al* (2018), two subunit vaccines have been developed against the Hepatitis E virus, and they have proved to be effective against the virus during clinical trials. Presently, only one of these vaccines has been licensed in China. Additional information is needed before these vaccines can be used for hepatitis E virus prevention in the global population. In the meantime, the awareness about the epidemiology of hepatitis E allows for formulation and use of non-vaccine measures for avoiding this disease. In highly endemic areas, measures such as the use of safe drinking water, proper disposal of human feces, and hygiene education have been put into place to

prevent HEV. In addition, boiling and chlorination of water are useful during outbreaks. Additionally, hygienic handling and appropriate cooking of swine and deer meat may be crucial (Nan *et al*., 2018).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

This study was carried out in Nairobi regional blood transfusion center. The center is responsible for collecting blood from voluntary blood donors. In addition, the blood transfusion center screens and process the different blood products such as plasma, platelets among others. The processed products and tested blood are subsequently distributed to the various hospitals for transfusion to patients. The center is situated in Nairobi City County, which is the smallest county by landmass in the country after Mombasa. The population of the county is more than four million residents. It shares a border with Kajiado and Machakos counties to the South and Kiambu County to the North (appendix 4)

3.2 Study design

In this study, a retrospective study design was used. It involved 358 archived blood samples obtained from an earlier study done in the same center titled "Genotypic characterization of hepatitis B virus (HBV) among voluntary blood donors in Nairobi regional transfusion center, Kenya". These samples were collected from September 2018 to January 2019

3.3 Determination of the study sample size

The study sample size was determined using the formula by Israel *et al*., 1998. Based on this formula, the minimum sample size was calculated to be 303 samples.

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

Since there was no existing seroprevalence for HEV among blood donors in the country, the study used a 27 % HEV seroprevalence from a study among blood donors in neighboring Sudan (Ahmed *and* Hamedelnil, 2015).

Where N = the minimum sample size

Z = the Z value (1.96) at confidence interval of 95%

P = the prevalence at 27% (0.27)

C = estimated error at 5% (0.05)

By calculation; $N = \frac{1.96^2 \text{ x } 0.27(1\text{-o}.27)}{0.05^2}$

$$N = \frac{1.037232\text{x}0.73}{0.0025}$$

N = 303 (minimum sample size)

However, all the 358 archived serum samples obtained from the previous study were utilized in this study.

The samples included in this study were those from healthy and voluntary donors, as indicated by Kenya National Transfusion Services (KNBTS) donor recruitment guidelines (appendix VI).

3.3.1 Exclusion criteria

The blood donors who did not meet the KNBTS blood transfusion requirements (appendix VI) were excluded from participating in this study.

3.5 Laboratory procedures

All laboratory procedures were done at the laboratories of the department of medical laboratory science of Kenyatta University.

3.5.1 Detection of the antibodies against the hepatitis E virus

Serum samples were tested using a Hepatitis E virus rapid test kit obtained from Biopanda reagents Belfast UK. The kit is a quantitative assay based on membrane-based enzyme immunoassay and helps detect Hepatitis E virus antibodies in human serum. This test can differentiate the Hepatitis E virus IgG and IgM antibodies in human serum

The HEV rapid test kit is comprised of two different components; IgG and IgM. This kit has HEV IgG relative sensitivity of 90% and 98.7%. In addition, it has HEV IgM sensitivity of 93% and specificity of 98.6%. In the IgG component, the human anti IgG coats the test line region. During analysis, the specimen reacts with the Hepatitis E virus

antigen coated particles in the test cassette. The mixture moves up the kit by capillary action and along the membrane until it gets into contact and reacts with the human anti-IgG in the IgG test area. If the results are HEV positive, a line that is colored will be observed in the IgG-marked test line in the kit.

In the IgM component, the human anti-IgM is coated in the IgM test line region. When the samples are put in the test region, it reacts with the human anti-IgM. In addition, the Hepatitis virus IgM antibody in the sample reacts with the anti-human IgM and the Hepatitis E virus coated particles in the kit. The formed reaction complex binds into the human anti-IgM and results in forming a colored line in the test line. If the sample contains IgM antibodies, a colored line will appear in the IgM test line area. If it is Hepatitis E virus-negative, there will be no colored line in the test line. Besides, a colored line will always appear in the control area, which helps test the kit's effectiveness. It also helps indicate that the sample used is within the recommended volume.

3.5.1.1 Assay procedure

Hepatitis E virus rapid test kit from the Biopanda reagent Belfast UK Company was used to test the serum samples following the manufacturer's instructions. The test kits and the samples were brought to room temperature before running the test. Briefly, the test cassette was unwrapped, and 25µl of serum sample was pipetted into the sample well of the cassette. A 40µl of chase buffer was added to the sample. The result of the test was observed and recorded after 20 minutes.

3.6 Determination of HEV genotypes

Nested reverse transcriptase PCR methods were used to identify the HEV genotypes in the seropositive samples. In this process, four steps were utilized; genomic RNA extractions, reverse transcription of the genomic RNA, polymerase chain reaction (PCR), and Gel electrophoresis.

3.6.1 HEV RNA extraction

The RNA of the Hepatitis E virus was extracted from 400 µl of serum samples using the PureLink™ Viral RNA/DNA Mini Kit from the Thermo Fisher Scientific USA, according to the manufacturer's protocol. In this procedure, 50 µl of proteinase K was added into a 1.5 microcentrifuge tube, and 400 µl of the sample was added. To the sample mixture, 400 µl of the lysis Buffer (containing carrier RNA) was added and vortexed for fifteen seconds to give a homogeneous mixture. The mixture was incubated at 56° C for fifteen minutes. To this mixture, 500 µl of ethanol was added and mixed using a vortex for fifteen seconds, followed by incubation at room temperatures for five minutes.

The lysate with the ethanol was transferred onto a viral spin column containing collection tube and centrifuged at 6800 x g for one minute. The remaining lysate was transferred into the same column, centrifuged at 6800x g for one minute, and transferred to a clean collection tube. In the same column, 500µl of wash buffer (W1) was added into the 2ml spin column and centrifuged at 6800x g for one minute. In addition, the spin column was placed in another 2ml collection tube, and 500 microliters of the wash buffer (W2) were added and centrifuged at 6800x g for three minutes. Besides, a viral

spin column was placed in a new 2ml collection tube and centrifuged at 6800x g for one minute. Lastly, the spin columns were placed in a 1.5ml recovery tube and 50 micro liters of sterile RNase-free water (elution buffer) was added to the viral spin column for elution of the RNA. The mixture was incubated at room temperature for one minute and later centrifuged at 6800x g for one minute. The obtained RNA was stored at 80°c.

3.6.2 Complementary DNA (cDNA) synthesis

The cDNA synthesis was done using the RevertAid Strand cDNA synthesis kit from the Thermo Fisher Scientific, Waltham, U.S.A., following the manufacturer's protocols. A 20µl of the reaction volume contained; 8µl of the extracted RNA 1µl of random hexamer primer, 3 µl of nuclease-free water which was gently mixed. The following reagents were then added to the content of the first solution, 4µl of 5x reaction buffer, 1µl of RiboLock RNase inhibitor, 2 µl 10 mM dNTP mix, and 1µl Revert M-MuLV Reverse Transcriptase then the contents were gently mixed. Reverse transcription was achieved by incubating for 5 minutes at 25°C followed by 60 minutes at 42°C, and the reaction was terminated by heating at 70°C for 5 minutes. The synthesized cDNA was stored at -20°C. The positive control RNA template (from the kit) was also synthesized similarly to the HEV RNA and the no Negative template control. As for the negative control, the mixture remains the same except there was no RNA template.

3.6.3 RT-PCR for HEV RNA amplification

The resulting cDNA was used as a template to amplify the 5' region of the HEV ORF2 gene using a nested RT-PCR using two different sets of primers. The two sets of primer pairs were (table 3.1):

Table 3.1 HEV primers sequence Used

PRIMERS	SEQUENCE	REACTION	BAND SIZE(Bp)
Primer (+)	5´ -CCCTTATCCTGCTGAGCATTCTC-3	First Reaction	731
Primer (-)	5´ -AAYTATGCMCAGTACCGGGTTG-3´		
Primer (+)	5´ -GTYATGYTYTGCATACATGGCT-3´	Nested Reaction	438
Primer (-)	5´ -AGCCGACGAAATYAATTCTGTC-3´		

The amplification reactions were prepared in a total of 25µl volume per tube. This comprised of: 12.5µl of Dream Taq PCR master mix (Thermo Scientific™), 0.5 µl of the forward primer, and 0.5µl of the reverse primer (primers concentration of 20 µM). A 7.5 µl of RNAse-free water and 4 µl of the cDNA template were added and mixed well. In each amplification round, a positive and no-template negative controls were included. The cycling was carried out using a BIO-RAD T100 Thermal Cycler (BIO-RAD, Laboratory, Singapore). The thermocycler conditions were set as follows; initial

denaturation at 95°C for two minutes, denaturation of 35 cycles at 95°C for 30 seconds, and primer annealing for another 30 seconds at 55.6°C. Lastly, the extension at 72°C for one minute and the final at 72°C for seven minutes and these conditions were applied to both first and second rounds. The product of first round was used as template for the second using the second primer. The composition of master mix remains the same.

3.6.4 Agarose gel electrophoresis and amplicons virtualization

A 1.5 % (W/V) agarose concentration was prepared in 1x Tris Borate Ethylene diamine tetra acetic acid (TBE) buffer. A 1.5g of agarose powder was weighed and dissolved in 100 ml of 1x TBE buffer. The mixture was mixed and heated in a microwave until the agar was completely dissolved. The gel was allowed to cool in the conical flask for a few minutes at room temperature. Then a 10 µl SYBR™ Safe DNA gel stain was added to the gel and mixed well. The gel was then dispensed in a gel casting jar containing combs at room temperature. The gel was left to solidify for thirty minutes, and the combs were removed gently. A 2µl of the gel loading dye (Invitrogen, NY, USA) was mixed with 4µl of the PCR products and loaded onto the gel wells. A 100bp DNA ladder from Invitrogen, NY, USA, was loaded onto the first lane of the well while the samples were loaded on the rest of the wells. The tank was connected to Power Park and allowed to run for 45 minutes at 110volts. The gel images were visualized using an ultraviolet transilluminator.

3.7 Data analysis

The socio-demographic data collected in this study included; gender, age, and level of education. The collected data was entered into the Microsoft Excel® spreadsheet and imported into the SPSS version 20 for analysis. The seroprevalence among blood donors was determined based on the proportion of those who were HEV positive using the Hepatitis E virus rapid test Kit from the Biopanda Reagent Belfast UK. The Chi-square test in the SPSS software was used to establish the relationship between socio-demographic factors and HEV seropositivity. The differences were considered significant if $p < 0.05$

3.8 Ethical consideration

The ethical approval to conduct this study was obtained from Kenyatta University Ethics Review Committee (KU-ERC) under application number PKU/2103/I1251 and approved (Appendix I). In addition, a research permit was also obtained from NACOSTI (Kenya National Commission for Science and Innovation) (Appendix II)

CHAPTER FOUR

RESULTS

4.1 Demographic characteristics of blood donors

In this study, 358 archived samples from blood donors recruited in a previous study were used. The majority of the donors were male, n= 228 (63.7 %) and 130 (36.3 %) were female. The age range of the donors was 16-60 years (mean 29.42). The recruited donors were categorized into six different age ranges. The majority of the donors were within the 20-29 (47.8%) age group bracket, followed by those in the 30-39 (25.1%) age bracket.

Forty-one (11.5%) of the blood donors did not disclose their educational status. Seven (2%) of the donors had a primary-level education. Eighty-seven (24.3%) of the blood donors had a secondary level, while the majority of donors n = 223 (62.3%) had a tertiary level of education (Table 4.1).

Table 4.1: Demographic characteristics of the Blood donors

Demographic factors	Frequency	Percent
Gender		
Female	130	36.3
Male	228	63.7
Age group in years		
<20	48	13.4
20-29	171	47.8
30-39	90	25.1
40-49	33	9.2
50-59	13	3.6
>60	3	0.8
Level of Education		
Primary	7	2.0
Secondary	87	24.3
Tertiary	223	62.3
Undeclared	41	11.5

4.2 HEV seroprevalence among blood donors

Out of 358 samples analyzed 28 (7.8%) were IgM seropositive while thirty-eight (10.6 %) were HEV IgG seropositive. In relation to gender, more males than females were IgM seropositive (5.3% vs. 2.5%). Similarly, more males were IgG seropositive than females (7.0% vs. 3.6%). Among the 38 (10.6 %) IgG positive cases majority were aged between 20-29 years (5.5 %), while among the 28 (7.8 %) IgM positive cases majority (4.5 %) were also in the 20-29 years age bracket. Donors aged <20 and those aged above 50 years reported the lowest seroprevalence.

Based on the level of education, out of 28 (7.8 %) HEV IgM positive samples, 10 (2.8%) were from blood donors who had a secondary level education. Fifteen (4.2%) of the HEV IgM positive samples were from blood donors with a tertiary level ofeducation, while 3 (0.8%) were from blood donors who did not disclose their educational status. All the blood donors with primary education were HEV IgM negative. A total of eleven (3.3%) donors with a secondary level of education were HEV IgG positive. In comparison, 24 (6.5%) of HEV IgG positive blood donors had a tertiary level of education, whereas 0.8 % of blood donors who were HEV IgG positive did not declare their educational status. All the donors with primary education were HEV IgG negative. (Table 4.2)

Table 4.2 Distribution of HEV Seropositivity among Blood donors

Demographic factors	Frequency	Percent	IgM ve	Percent	IgG +ve	Percent
			N=28	7.8 %	N=38	10.6 %
Gender						
Female	130	36.3	9	2.5 %	13	3.6 %
Male	228	63.7	19	5.3 %	25	7.0 %
Age group						
<20	48	13.4	1	0.3 %	2	0.6 %
20-29	171	47.8	16	4.5 %	20	5.5 %
30-39	90	25.1	7	2.0 %	12	3.3 %
40-49	33	9.2	2	0.6 %	2	0.6 %
50-59	13	3.6	1	0.3 %	1	0.3 %
>60	3	0.8	1	0.3 %	1	0.3 %
Level of Education						
Primary	7	2.0	0	0.0 %	0	0.0 %
Secondary	87	24.3	10	2.8 %	11	3.3 %
Tertiary	223	62.3	15	4.2 %	24	6.5 %
Undeclared	41	11.5	3	.0.8 %	3	0.8 %
Serostatus						
Seropositive			28	7.8 %	38	10.6 %
Seronegative			330	92.2 %	320	89.4 %

4.3 Relationship between sociodemographic characteristics and HEV seropositivity

There was a statistically significant relationship between gender, Hepatitis E virus IgM and IgG seropositivity (P = 0.0142) with a high likelihood of males testing positive compared to females. This study also established a relationship between IgM and IgG seropositivity and blood donors' level of education and age (p = 0.0338, p = 0.025 respectively) There was a likelihood to test IgG positive for those aged between 20-29

years, while those with tertiary levels of education were likely to test IgM positive (Table 4.3).

Table 0 Sociodemographic factors and their association with HEV seropositivity

Demographic Factors	**IgM seropositive**	**IgM Seronegative**	**P-value**	**IgG Seropositive**	**IgG Seronagative**	**P-Value**
	n=28	n=330		n=38	n=320	
Gender			**0.0142**			**0.0163**
Female (%)	9(32.1)	121(36.7)		13(34.2)	117(36.6)	
Male (%)	19(67.8)	209(63.3)		25(65.8)	203(63.4)	
Age group			0.757			**0.025**
Below 20	1(3.6)	47(14.2)		2(5.3)	46(14.4)	
20-29	16(57.1)	155(46.9)		20(52.6)	151(47.2)	
30-39	7(25)	83(25.2)		12(31.6)	78(24.4)	
40-49	2(7.1)	31(9.4)		2(5.3)	31(9.7)	
50-59	1(3.6)	12(3.6)		1(2.6)	12(3.8)	
Above 60	1(3.6)	2(0.6)		1(2.6)	2(0.6)	
Level of Education			**0.0338**			0.639
Primary	0(0)	7(2.1)		0(0)	7(2.2)	
Secondary	10(35.7)	77(23.3)		11(28.9)	76(23.8)	
Tertiary	15(53.6)	208(63)		24(63.2)	199(62.2)	
Undeclared	3(10.7)	38(11.5)		3(7.9)	38(11.9)	

4.4 Determination of HEV genotypes

An RT-PCR to amplify the ORF 2 gene was used to determine HEV genotypes in all the seropositive samples. However, Hepatitis E virus RNA was not detected in any of the samples (Figure 4.4).

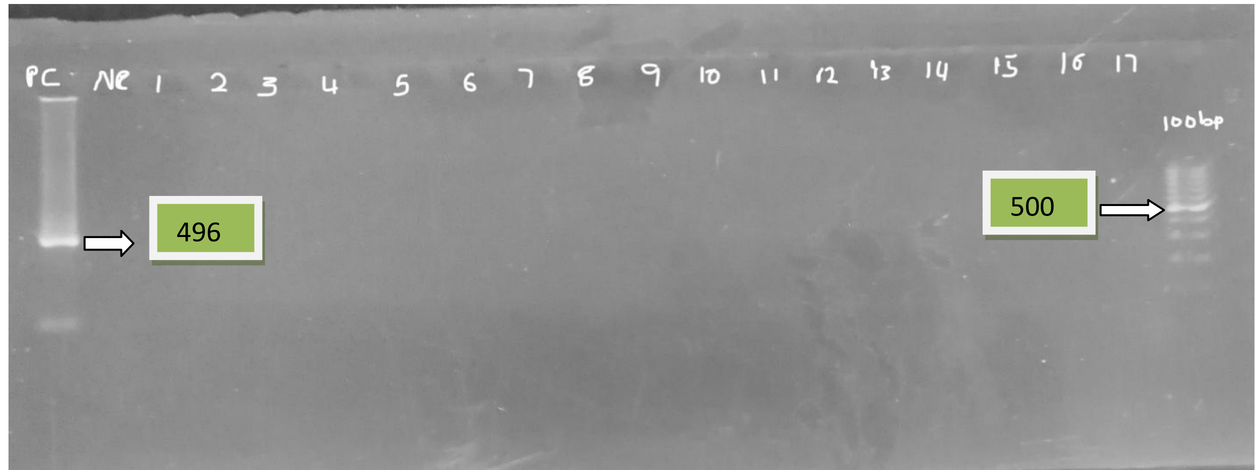

Figure 4.1 Gel electrophoresis of HEV RNA amplification. PC refers to the positive control of 496bp and and NC is the negative control. Lanes labelled 1 to lane 17 represent the HEV seropositive samples. Finally, lane 18 is the 100bp ladder.

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSIONS, AND RECOMMENDATIONS

5.1 Discussion

The transfusion of blood is one of the important portions of healthcare delivery. The screening of blood donors for transmissible transfusion infections is an essential component of blood transfusion. It is therefore essential to detect HEV antibodies in donors' blood because cases of transfusion mediated HEV infection are increasingly being reported. Additionally, it helps in understanding trends of Hepatitis E infection among blood donors. The findings obtained in this study showed the extent of exposure to HEV infection in healthy blood donors.

In this study, 358 archived plasma samples from blood donors were used. The screening of these samples showed that in the Nairobi regional blood transfusion center, the Hepatitis E virus seroprevalence in blood donors was 7.8 % for anti-HEV IgM and 10.6 % for anti-HEV IgG. In Africa, there have been a few studies on Hepatitis E virus seropositivity in blood donors. However, two studies have reported a 4.6-39 % seropositivity rate using the enzyme-linked immunosorbent assay (ELISA) testing method (Maponga *et al*., 2020). Thus, the seroprevalence in this study is in line with the continental trends. This anti-HEV antibody IgM seroprevalence is similar to a (7.8%) seroprevalence reported among the same population in Sudan using the ELISA testing method (Ahmed *and* Hamedelnil, 2015).

The anti IgM seroprevalence was however lower than the one reported in Accra blood transfusion center, Ghana, of 26.5 % (Tettey *et al*., 2011). The IgG seroprevalence in this study is higher than the 3.3 % seroprevalence reported among leukemic patients in Khartoum State, Sudan, using the ELISA testing method (Zoelnoon *et al*., 2017) but was lower than that reported among blood donors in Omdurman state in the same country (Ahmed and Hamedelnil, 2015).

Previously, HEV seroprevalence was carried out in a Nairobi informal settlement among febrile subjects. A higher seroprevalence of 25.7 % IgM and 37.8 % IgG was obtained (Furukawa *et al.,* 2016). This higher seroprevalence is not unexpected since healthy blood donors are expected to have low seroprevalence compared to the sick and also acute HEV infection may lead to a febrile syndrome. Additionally, the poor sanitation especially in informal settlements where they have inadequate provision of basic needs may be one of the factors contributing to the high seroprevalence since HEV is mainly transmitted enterically (Stramer *et al*., 2016).

In the current study, the collected sociodemographic data was limited to blood donor's gender, age, and level of education. The HEV IgM seropositivity was 5.3% in males. In females, the seropositivity was 2.5% which is lower when compared to their male counterparts. The seroprevalence among males in this study is similar to 4.78 % among male donors recorded in a study in Western India (Gajjar *et al*., 2014). The findings on the higher seroprevalence among males compared to females are in agreement with a previous study done in Burkina Faso by Traore *et al*., (2016), where IgM seropositivity

in blood donors was 2.6% in males and 1.9% in females. Considering that IgM is considered a marker of recent/acute infection, the low anti HEV IgM is an indication of reduced incidences in this blood donor population.

In this study, more males were recruited (63.7%) compared to females (36.7%). A comparison of Hepatitis E virus IgG and IgM seropositivity showed that HEV seropositivity was significantly higher in males compared to the seropositivity in females. This finding is in agreement with previous studies done in the Middle East and North Africa region by Al-Sadeq *et al*. (2017), where the HEV seropositivity rate in healthy males was significantly higher compared to the HEV seropositivity in healthy females. However, several studies on HEV prevalence in diverse study groups have shown discrepancies on gender being an HEV infection predisposing factor (Kim *et al*., 2014). However, there is consensus that HEV affects women, especially pregnant women, with severe outcomes than men (Kamar *et al*., 2014).

In this study, the blood donors aged between 20-39 years had the highest seroprevalence compared to those under 20 years and more than 50 years who reported a low seroprevalence. The finding is contrary to the previous finding by Al-Sadeq *et al*. (2017), where HEV seroprevalence was reported to increase with age while the exposure was high among the young more than the adults. The high seroprevalence among the middle-aged blood donor groups can be attributed to the blood donation criteria used in the country. In most cases, the major blood donors are high school and college students aged between 20-39 years who are assumed to have a low HEV

infection rate (Fearon *et al*., 2017). Therefore, 73% of the blood donors who participated in this study were aged between 20-39 years, which could probably be the reason of the high seroprevalence reported within this age group. However, it is expected that recruiting older blood donors would increase the seroprevalence because the seroprevalence rate is expected to be high in older persons.

Moreover, in this study, there was a significant relationship between Hepatitis E virus seropositive and blood donor's level of education. Out of the 38 IgG seropositive individuals, 24 individuals had tertiary education. This is contrary to a previous study done in Ethiopia by Abebe *et al*. (2017) where 22% of those who were IgG seropositive had a tertiary education while 36% of the IgG individuals had a primary education. It is important to emphasize that out of the 358 blood donors recruited in this study 213 (63%) had tertiary education.

The study sought to determine HEV genotypes in the study population. Consequently, all the HEV seropositive samples were subjected to RT-PCR for HEV RNA detection. However, none of them yielded any amplification and thus HEV genotypes could not be determined. While detection of HEV RNA in blood samples have been reported, it’s absence in HEV seropositive samples is not uncommon. Similar results have been reported among blood donors in New Zealand (Hewitt *et al*., 2018), Southern Brazil (Costa *et al*., 2021) and in Nigeria among healthy individuals (Osundare *et al*., 2020). This can be attributed to the small sample size and the use of archived samples that could have led to degradation of HEV RNA, affecting its amplification and detection. It

has also been suggested that viremia occurs within a short period during acute infection thus limiting the period of detection. Since blood borne HEV transmission has been demonstrated in other studies, future studies on the risks of HEV transmission through blood should consider larger sample sizes and use of fresh samples.

5.2 Conclusions

a) The current study has established a relatively high HEV IgM and IgG seroprevalence in the Nairobi regional blood transfusion center at 7.8 % and 10.6 %, respectively.

b) HEV seroprevalence appears disproportionate among various blood donor groups with the age group 20-29 years having the highest levels.

c) Males and donors with tertiary education had a statistically significant higher anti-HEV IgM seroprevalence.

d) Male donors and those aged 20-29 years had a significantly higher anti-HEV IgG seroprevalence.

5.3 Recommendations

I. The study recommends the screening of HEV IgM antibody in potential blood donors to minimize risks associated with HEV transmitted through blood transfusion. The WHO should provide guidelines on the testing platforms to be used.

II. Future studies should utilize fresh blood samples owing to the rapid degradation of RNA during storage..

III. This study recommends a broader study involving a greater diversity of demographics to get a clearer picture of HEV epidemiology in the country.

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Appendix I: Ethical Approval Letter

KENYATTA UNIVERSITY
ETHICS REVIEW COMMITTEE

Fax: 8711242/8711575
Email: chairman.kuerc@ku.ac.ke
Website: www.ku.ac.ke

P. O. Box 43844,
Nairobi, 00100
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Our Ref: **KU/ERC/COND.APPR.1/VOL.1** Date: 12/08/2020

Ezekiel Fardolo
P.O Box 43844-00100

NAIROBI.

Dear Ezekiel Fardolo

RE: APPLICATION NUMBER: PKU/2103/I1251 –'SEROPREVALENCE AND MOLECULAR CHARACTERIZATION OF HEPATITIS E VIRUS AMONG BLOOD DONORS IN NAIROBI CITY COUNTY KENYA'

This is to inform you that **KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE** has reviewed and approved your above research proposal .Your application approval number is **PKU/2103/I1251**.The approval period is **12th August 2020 - 12th August 2021.**

This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments) Will be used.
ii. All changes including (amendments, deviations and violations) are submitted for review and approval by **KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE.**
iii. Death and life-threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to **KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE** within 72hours of notification.
iv. Any changes ,anticipated or otherwise that may increase the risk or affected safety bor welfare of study participants and others or affect the integrity of the research must be reported to **KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE** within 72 hours.

v. Clearance for export of biological specimens must be obtained from relevant institutions.

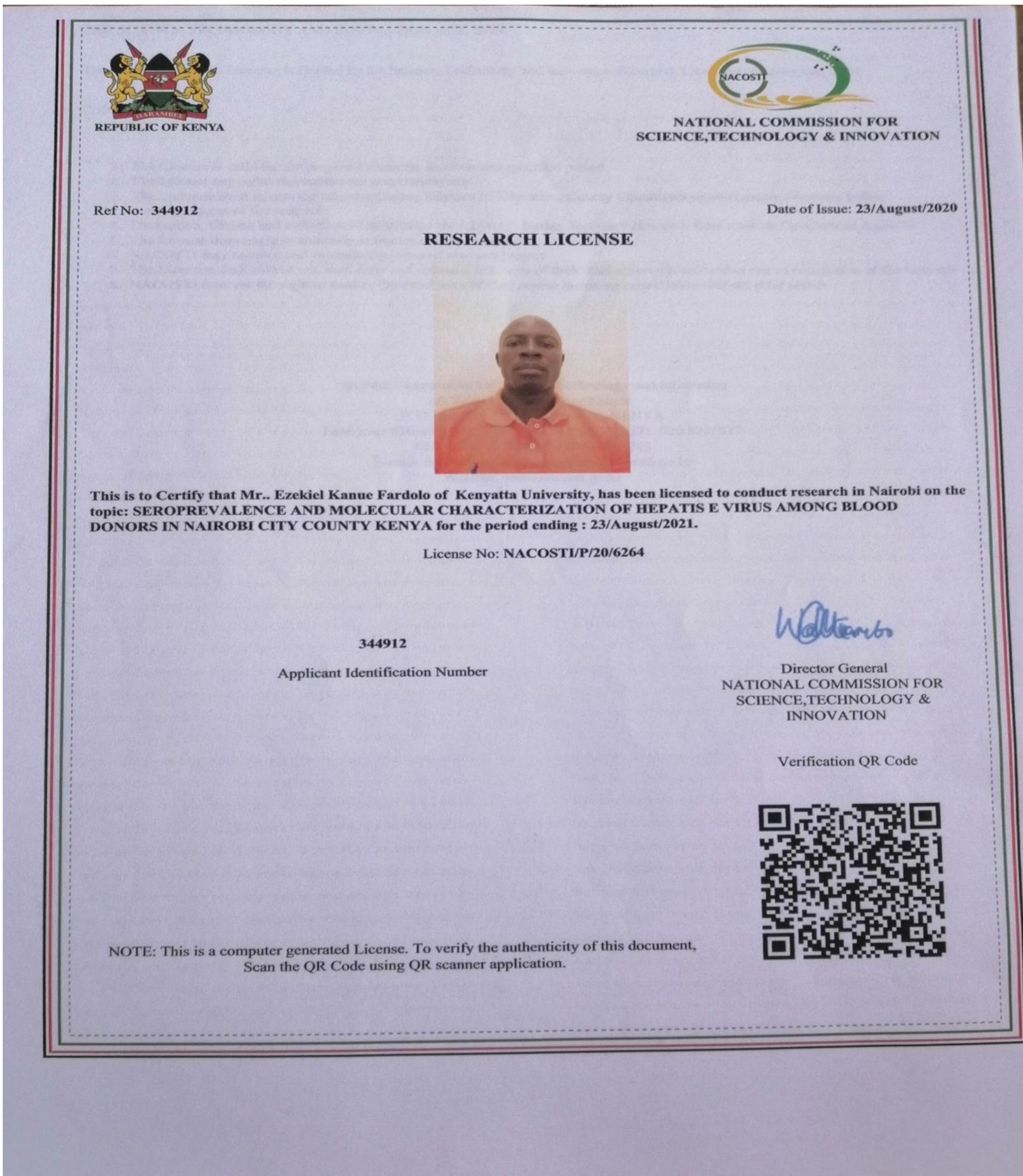
vi. Submission of requests for renewal of approval of atleast 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.

vii. Submission of executive summary report within 90 days upon completion of the study to **KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE** prior to commencing your study, you will be expected to obtain a research license from National Commission of Science, Technology Innovation (NACOSTI) https://oris.nacosti.go.ke and also obtain other clearance needed.

Yours. Sincerely

Prof.Judith Kimiywe
CHAIRPERSON-KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE

Appendix II: Research Permit



REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: 344912

Date of Issue: 23/August/2020

RESEARCH LICENSE

This is to Certify that Mr.. Ezekiel Kanue Fardolo of Kenyatta University, has been licensed to conduct research in Nairobi on the topic: SEROPREVALENCE AND MOLECULAR CHARACTERIZATION OF HEPATIS E VIRUS AMONG BLOOD DONORS IN NAIROBI CITY COUNTY KENYA for the period ending : 23/August/2021.

License No: NACOSTI/P/20/6264

344912

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

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Appendix III: Research Approval Letter from Graduate School

KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: kubps@yahoo.com
dean-graduate@ku.ac.ke
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P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 810901 Ext. 57530

Internal Memo

FROM: Dean, Graduate School

DATE: 3rd March, 2020

TO: Mr. Ezekiel K. Fardolo
C/o Department of Medical Laboratory Science
KENYATTA UNIVERSITY

REF: P150F/CTY/PT/37790/17

SUBJECT: APPROVAL OF RESEARCH PROPOSAL

This is to inform you that the Graduate School Board at its meeting 26th February,2020 approved your M.Sc Research Proposal entitled "Seroprevalence and Molecular Characterization of Hepatitis E. Virus among Blood Donors in Nairobi County, Kenya".

You may now proceed with your Data collection, subject to clearance with the Director General, National Commission for Science, Technology & Innovation.

As you embark on your data collection, please note that you will be required to submit to Graduate School completed supervision Tracking and Progress Report Forms. The Forms are available at the University's Website under Graduate School webpage downloads.

Thank you.

JULIA GITU
FOR: DEAN, GRADUATE SCHOOL

c.c. Chairman, Department of Medical Laboratory Science

Supervisors:

1. Dr. George Gachara
   C/o Department of Medical Laboratory Science
   KENYATTA UNIVERSITY

2. Prof. Wallace Bulimo
   Chief Virologist
   US-Army Medical Research Directorate, Nairobi
   C/o Department of Medical Laboratory Science

JG/cao

Appendix IV: Map of Nairobi City County.

NAIROBI NATIONAL BLOOD TRANSFUSION CENTRE

Legend

National_Blood_Donation_Centre

Major Roads

Nairobi County Boundary

Coordinate System: GCS WGS 1984
Datum: WGS 1984
Units: Degree

Sources: Esri, HERE, Garmin, Intermap, increment P Corp., GEBCO, USGS,

Appendix V: Research Pblication

Journal of Biosciences and Medicines, 2020, 8, 78-85
https://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

Seroprevalence of Hepatitis E Virus among Voluntary Blood Donors in Nairobi County, Kenya: A Pilot Study

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How to cite this paper: Fardolo, E.K., Bulimo, W., Aluora, P.O. and Gachara, G. (2020) Seroprevalence of Hepatitis E Virus among Voluntary Blood Donors in Nairobi County, Kenya: A Pilot Study. *Journal of Biosciences and Medicines*, **8**, 78-85.
https://doi.org/10.4236/jbm.2020.812008

Received: October 27, 2020
Accepted: December 12, 2020
Published: December 15, 2020

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Abstract

Hepatitis E is a common infection caused by the Hepatitis E virus (HEV), a primarily enteric virus. HEV disease is mainly spread via stool contamination of water and food supplies. The virus has recently been identified as a global threat to blood safety. The seroprevalence of HEV among blood donors in Kenya remains unknown. This study was carried out as a pilot study to determine the seroprevalence of the virus among blood donors at Nairobi Blood Transfusion Center during the period from September 2018 to January 2019. A total of three hundred and fifty eight (n = 358) plasma samples were used in this study. The plasma was tested for HEV IgM and IgG antibody using a qualitative membrane-based immunoassay (Biopanda reagents Belfast UK). Demographic characteristics of the blood donors were also collected. The age of the blood donors ranged from 16 to 61 years with a mean age of 29 years (±14). Of the study subjects, 36% (n = 130) were females while 64% (n = 228) were males. Overall, 10.6% (38) and 7.8% (28) of the samples were HEV IgG and IgM seropositive respectively. Anti-HEV was distributed among all age groups; however donors aged 20 - 40 years had the highest prevalence. This study shows a relatively high prevalence of anti-HEV among healthy blood donors in Nairobi, Kenya. Further studies would be needed in other population groups to assess the potential benefit of incorporating HEV screening of blood products to the current blood donor selection criteria. Moreover, further studies to determine the circulating genotypes of HEV among this group are required for epidemiological reasons.

Keywords

Hepatitis E Virus, IgG, IgM, Blood Donors, Transmission

Appendix VI: Kenya Blood Transfusion Service

KENYA NATIONAL BLOOD TRANSFUSION SERVICE

Donation Number

DONOR QUESTIONARE

Clinic Venue -------------------------------- **County** -----------------**Clinic Code:** -------- **Donor Number** ------------

SECTION 1: DAILY BLOOD DONOR REGISTRATION& SCREENING FORM (Donors please complete this section below)

Surname: ________________________**Other Names**: ____________ __________ **GENDER:** F / M

Student Number/ National ID Number: _________________ **Date of Birth:** ------/------/----- **(dd/mm/yy)**

Marital Status: ***(Mark in appropriate box)***

Single	Married	Divorced/Separated	Widowed

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:** ……………………..…

SECTION 2: HEALTH QUESTIONNAIRE **Circle the appropriate answer**

1. Are you feeling well and in good health today?	Yes/No
2. Have you eaten in the last 6 hours?	Yes/No
3. Have you ever fainted?	Yes/No
In the past 6 months have you:	
4. Been ill, received any treatment or any medication?	Yes/No
5. Had any injections or vaccinations (immunizations)?	Yes/No
6. Female Donors: Have you been pregnant or breast feeding?	Yes/No
In the past 12 months have you:	
7. Received a blood transfusion or any blood products?	Yes/No
Do you have or have you ever had:	
8. Any problems with your heart or lungs e.g. asthma?	Yes/No
9. A bleeding condition or a blood disease?	Yes/No
10. Any type of cancer?	Yes/No
11. Diabetes, epilepsy or TB?	Yes/No
12. Any other long term illness Please Specify	Yes/No

KENYA NATIONAL BLOOD TRANSFUSION SERVICE

SECTION 3: RISK ASSESSMENT QUESTIONNAIRE

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.

In the past 12 months have you:	
1. Received or given money, goods or favours in exchange for sexual activities?	Yes/No
2. Had sexual activity with a person whose background you do not know?	Yes/No
3. Been raped or sodomized?	Yes/No
4. Had a stab wound or had an accidental needle stick injury e.g. injection needle?	Yes/No
5. Had any tattooing or body piercing e.g. ear piercing?	Yes/No
6. Had a sexually transmitted disease (STD)?	Yes/No
7. Live with or had sexual contact with someone with yellow eyes or yellow skin?	Yes/No
8. Had sexual activity with anyone besides your regular sex partner?	Yes/No
Have you ever:	
9. Had yellow eyes or yellow skin?	Yes/No
10. Injected yourself or been injected, besides in a health facility?	Yes/No
11. Used non-medical drugs such as Marijuana, Cocaine etc?	Yes/No
12. Have you or your partner been tested for HIV?	Yes/No
13. **Do you consider your blood safe to transfuse to a patient?**	Yes/No

SECTION 4: DECLARATION (Please read this before you complete the form with your name and signature below)

I declare that I have answered all the questions truthfully and accurately.

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 National Blood Transfusion Service.

I understand that should any of the screening tests give a reactive result, I will be contacted by 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, and offered counselling to make an informed decision about further confirmatory testing and management.

I hereby give consent to KNBTS to use the contact details provided in this form to communicate to me as the need may be.

I understand the blood may be used for scientific research, main objective being to improve the safety of the blood supply to patients.

I consent to give blood; I understand that it may be used for transfusion for the benefit of others.

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:

Name of Phlebotomist: ------------------------------ **Date:** ------------------------------