SEROLOGICAL PREVALENCE OF HEPATITIS E VIRUS (HEV) AMONG PREGNANT WOMEN ATTENDING HEALTH-CARE CENTERS IN EKITI STATE.

BY

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CERTIFICATION

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DEDICATION

This work is dedicated to almighty God for his unmerited favour over my life. May His name be praised both now and evermore, Amen.

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I thank the Almighty God for His guidance over my life, His indescribable love for me, wisdom and the successful compilation of this project.

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ABSTRACT

The current prevalence of HEV among pregnant women attending antenatal care at major health-care centers in Ekiti State Nigeria was investigated. Ninety (90) serum samples and demographic information were collected from consenting pregnant women aged between 15-45 years and screened for anti-HEV specific IgM antibody using rapid ELISA test kits (CTK Biotech, Inc. USA). Data were analysed using SPSS software version 20.0 and Chi-square test. Three out of ninety (3.3%) serum samples within the age group of 25-35 years tested positive for HEV IgM. Chi-square analysis shows a statistically significant association between HEV infection and blood transfusion as against socio-demographic characteristics and household characteristics. This study reveals a generally low prevalence of HEV among the subjects; however, public enlightenment about environmental and personal hygiene before and during pregnancy must be maintained in order to keep the rate of HEV infection very low in Ekiti State.

CHAPTER ONE

1.1 INTRODUCTION

Hepatitis E is a liver disease caused by infection with a virus known as Hepatitis E virus (HEV). HEV is a non-enveloped, single-stranded RNA virus, 7.2 kb in size that belongs to the genus *Orthohepevirus* in the family *Hepeviridae*. HEV Infection can lead to acute (subclinical) liver disease, like that associated to hepatitis A infection. Every year, there are an estimated 20 million HEV infection worldwide leading to an estimated 3.3 million symptomatic cases of Hepatitis E. Hepatitis E has historically been referred to as enterically transmitted non-A, non-B hepatitis. There are five genotypes of HEV. Genotypes 1 to 4 are human pathogens, while genotype 5 is fund only in birds (Pischke *et al.*, 2014).

Hepatitis E is generally transmitted by the fecal-oral route, usually due to the consumption of contaminated water or food. In developing countries, this is the route of infection that leads to outbreaks. In contrast to the hepatitis A virus and other enteric viruses, human-to-human transmission of HEV is rare. Alternative transmission routes, such as blood transfusions and vertical transmission, are becoming increasingly relevant as more cases are reported each year. Transmission via blood transfusion has become one of the main transmission routes in developed countries (Bouamra *et al.*, 2014).

Vertical transmission of HEV from a pregnant woman to unborn fetus is very well documented (Khuroo, 2009). The risk factors for HEV infections are related to resistance of HEV to environmental conditions and host factors such as surviving in sewage and surviving passage through acidic milieu of the host's stomach, poor sanitation in large areas of the world, and HEV shedding in faeces (James, 2001). The infection affects primarily young adults and is generally mild; however, the mortality rate is higher among women, especially during the second or third trimesters of pregnancy (Purcell *et al.*, 2008; Aggarwal 2002; Benait *et al.*, 2007).

The major symptoms of hepatitis E are fever, nausea, abdominal pain and loss of appetite, vomiting, hepatomegaly, jaundice, itching, pale stools, and darkened urine (Mirazo *et al.*, 2014). Epidemiological studies indicate that most cases of hepatitis E occur in young adults (15–45 years old). The overall mortality rate of hepatitis E is approximately 2%, but can be more than 20% among pregnant women in some regions because of fulminant hepatic failure (Kumar *et al.*, 2013). It also leads to severe complications which may result in fetal and/or maternal mortality, abortion, premature delivery, or death of a live-born baby soon after birth (Shukla *et al.*, 2011; Blut, 2009). Numerous studies from developing countries have linked high mortality in pregnant females to hepatitis E virus infection. Pregnant women die of obstetric problems, including hemorrhage or eclampsia, or develop fulminant hepatic failure. Stillbirths are common, as is vertical transmission to infants who survive, with an attendant increase in neonatal morbidity and mortality (Kamar *et al.*, 2014).

Hepatitis E virus (HEV) remains a major public health concern in resource limited regions of the world. Yet data reporting is suboptimal and surveillance system inadequate. Despite its high mortality rate in pregnant women, studies on HEV infection are still scanty in Nigeria including Ekiti State. In a previous study carried out about ten years ago, on the detection of anti HEV antibodies in Ekiti State, out of 186 serum samples obtained from healthy and sick individuals which also included pregnant women. There was a recorded 13.4% (25/186) seroprevalence (Adesina *et al.*, 2009). Similar study conducted on anti-HEV antibody detection among pregnant women showed 9.9 % (18/182) seroprevalence rate in Sokoto State (Alkali *et al.*, 2017). With no recent study on seroprevalence of HEV among pregnant women in Ekiti State, this study seeks to investigate the current prevalence of HEV among pregnant women attending antenatal care at major hospitals in Ekiti State.

CHAPTER TWO

LITERATURE REVIEW

2.1.1 Discovery of Hepatitis E Virus

The initial investigation of an infection that looks like hepatitis E infection occurred during the 1978-Kashmir epidemic. In that year, Dr. Mohammad S. Khuroo investigated an epidemic of jaundice in and around a town 50 km from Srinagar, Kashmir, India. The epidemic had hit two hundred villages with a population of 600,000 which resulted in about 52,000 patients with icteric disease and 1,700 fatalities. The disease was a self-limiting and did not cause chronic viremia, chronic hepatitis and cirrhosis (Khuroo and Khuroo, 2010). It was postulated that the disease was caused by yet unrecognized human hepatitis virus (Khuroo, 1981).

In the 1980s, an outbreak of what was known as non-A, non-B hepatitis (NANBH) was reported in Russian military personnel posted in Afghanistan (Balayan *et al.*, 1983). The epidemic had similar epidemiological features as seen in the 1978-Kashmir epidemic. Dr. Mikhail S Balayan, in a self-experimentation, ingested pooled stool extracts from 9 such patients. On the 36th day after ingestion of the stool extracts, he developed severe acute hepatitis with jaundice and elevated liver tests. Stool samples of 28th, 43th and 45th day showed virus like-particles (VLP) on immune electron microscopy leading to the discovery of the virus in 1983 (Balayan *et al.*, 1983).

2.1.2 Virology of Hepatitis E Virus

HEV is a non-enveloped virus with an icosahedral capsid and a size of 27 to 34 nm. The virus has a positive-sense, single-stranded, 7.2kb RNA genome which is capped and polyadenylated at the 5'and 3'termini, respectively (Reyes *et al.*, 1990; Tam *et al.*, 1991). A taxonomic scheme of the viral family was proposed to create a consensus classification based on the complete genome

sequences of HEV isolates (Tam *et al.*,1999). The family *Hepeviridae* comprises two genera: *Orthohepevirus* and *Piscihepevirus*. *Orthohepevirus* contains four species: *Orthohepevirus* A–D. *Orthohepevirus* A includes four major genotypes (HEV-1 to HEV-4) that infect humans, with HEV-1 and HEV-2 occurring only in humans. HEV-3 has been isolated from humans and several animal species including pigs. HEV-4 has been isolated from humans and pigs. It is proposed that additional genotypes including HEV-5 and HEV-6, which were recently identified in wild boars, and HEV-7, which was identified in camels, belong to *Orthohepevirus* A (Guerra *et al.*, 2017).

The HEV genome contains three discontinuous open reading frames (ORFs) designated ORF1, ORF2, and ORF3 that encode nonstructural proteins including RNA-dependent RNA polymerase, a capsid protein, and a small protein, respectively (Smith *et al.*, 2014). It replicates in the cytoplasm of cells and can replicate in hepatocytes, small intestine and colon cells, and lymph nodes. The capsid protein is highly immunogenic and antibodies against it neutralize and are protective. Thus, the capsid antigen is the preferred protein for the development of vaccines (Harrison, 1999; James, 2001).

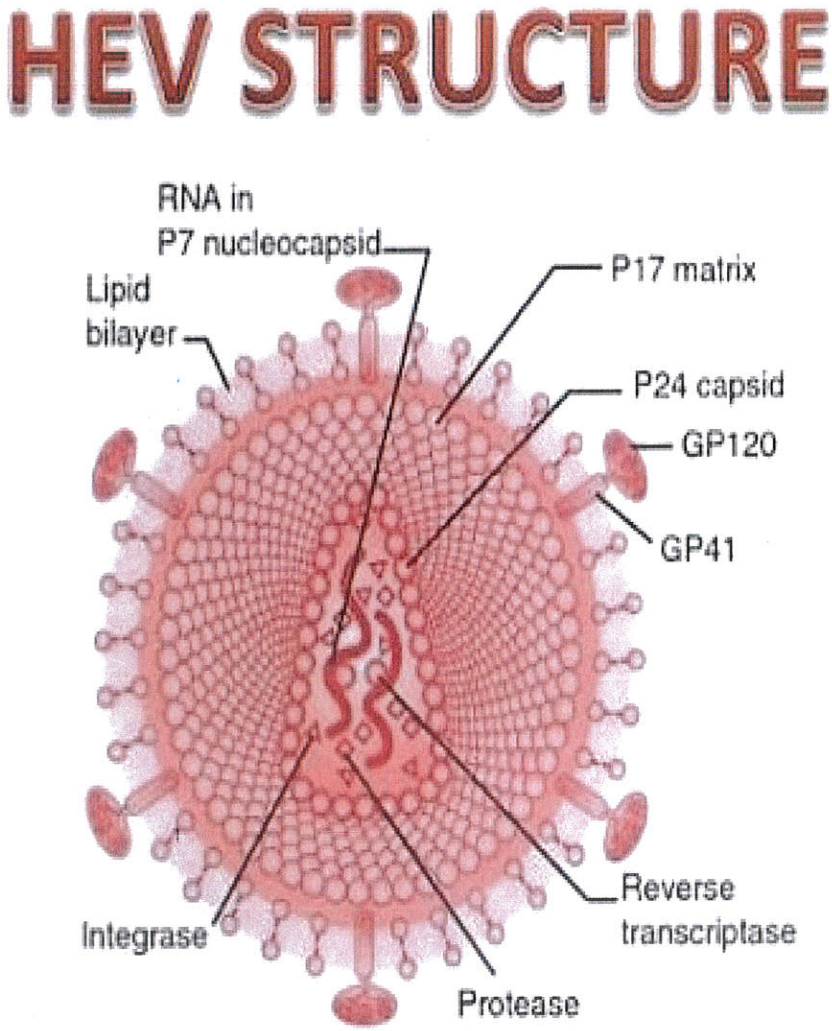

Figure 1: Structure of HEV (Source: Cao and Meng, 2012).

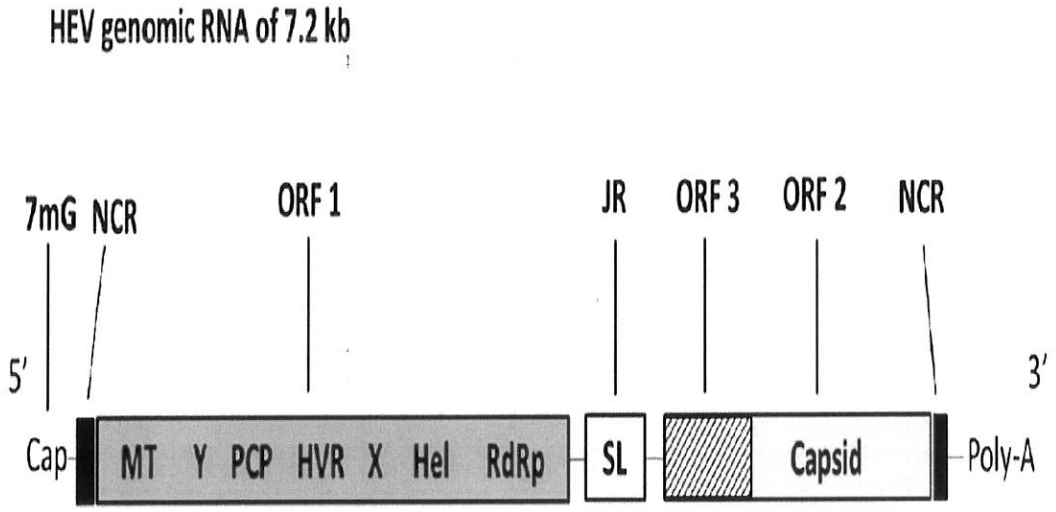

Figure 2 – A schematic representation of the genomic organization of the hepatitis E virus (HEV).

ORF: open reading frame; MT: methyltransferase; Y: Y domain; PCP: a papain-like cysteine protease; Hel: helicase; HVR: hypervariable region; X: macro-domain; RdRp: RNA-dependent RNA polymerase; JR: junction region; SL: stem-loop structure; NCR: non-coding region.

(Source: Cao *et al.,* 2010).

2.1.3 Replication of HEV

The precise mode of replication of HEV is still being studied owing to the lack of robust in-vitro propagation system. The assumed course of events has been mostly based on analogy with other viruses having a positive-sense RNA genome and on the knowledge of conservative segments of non-structural HEV domains. The positive-sense chain of viral RNA in their cytoplasm is translated into a non-structural protein necessary for viral replication. Negative-sense RNA serves as a template for genome replication (Worm *et al.*, 2002).

The primary target cells for hepatitis virus replication are the hepatocytes even though other sites of replication have been suggested in swine and rhesus monkeys. The presence of negative-strand HEV RNA replicative intermediates in the liver of infected rhesus monkeys constituted the first direct evidence of HEV replication in the liver (Khuroo *et al.*, 2016).

Attachment and entry of HEV into cells is thought to occur via a specific receptor on the host cell. Recent findings suggest that HEV attaches to the susceptible host cell by specific high affinity receptor and enters the cytoplasm by clathrin-mediated endocytosis (Kamar *et al.*, 2010). Upon entry, the HEV genome undergoes cap-mediated translation to produce the non-structural polyprotein (Panda *et al.*, 2007). Post translational processing of non-structural polyprotein could help to switch the synthesis of negative-sense RNA to positive sense RNA, which also helps in maintaining a high ratio of positive to negative-sense RNA.

Replication complex, assembly and membrane compartmentalization is an essential step in the replication of all positive-strand RNA viruses. The proposed sub-genomic positive-strand HEV RNA is translated into the structural proteins to package the viral genome to form progeny virions (Rehman *et al.*, 2008).

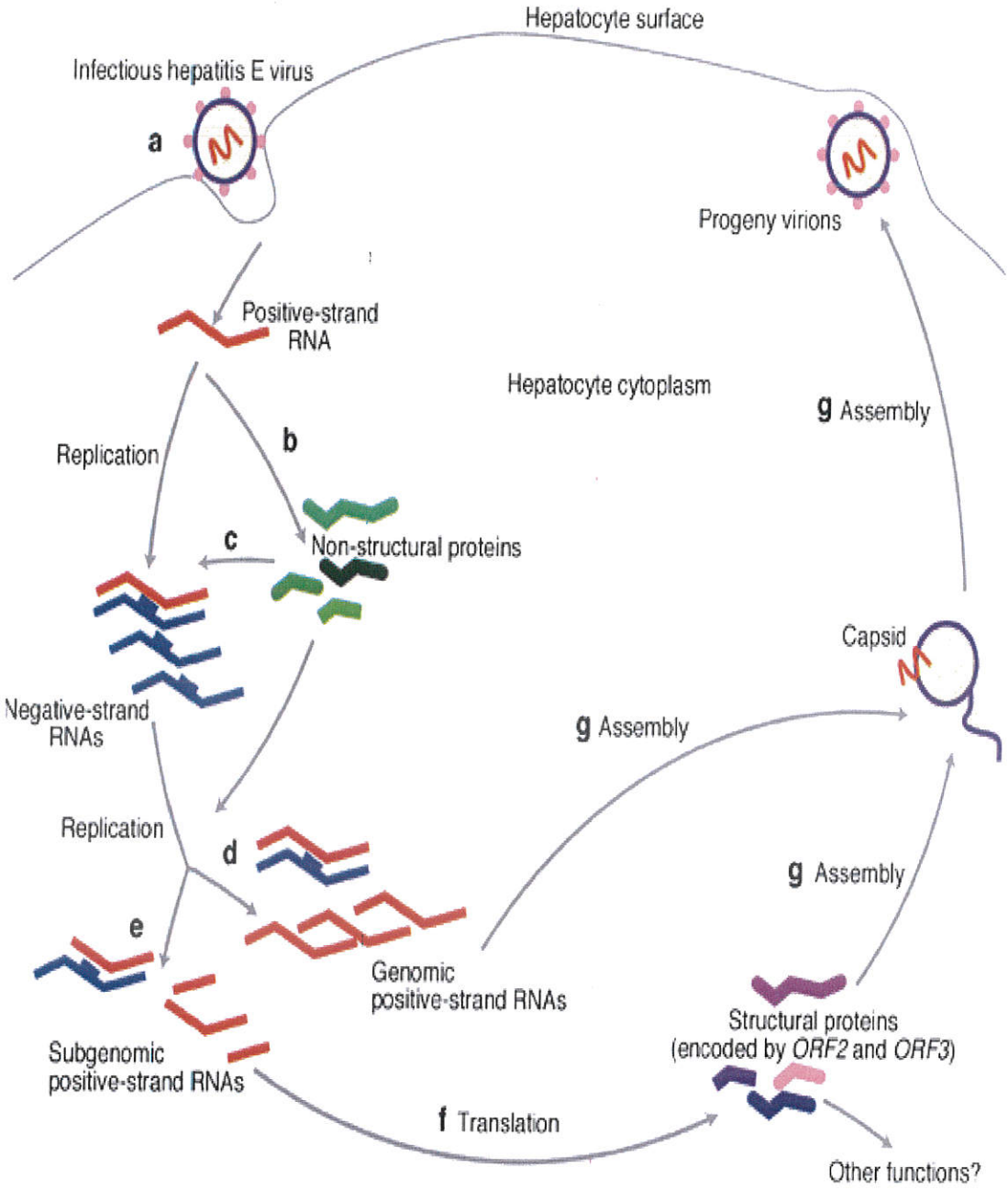

Figure 3: Proposed model of HEV replication. (Source: Panda and Varma, 2013)

2.1.4 Classification and Characteristics of HEV Genotypes

Currently, there are eight described genotypes (HEV 1–8) of HEV (Table 1), of which HEV1-4 usually affect humans and hence have been better studied than other genotypes (Guerra *et al.*, 2017).

Within species *Orthohepevirus* A, there are four genotypes described that infect humans. They include;

- Genotype 1 comprises the human Burma strain (prototype) and strains from Asia and Africa.
- Genotype 2 comprises a human Mexican strain (prototype) and several strains isolated in outbreaks from Nigeria and Chad.
- Genotype 3 comprises human and animal strains from the USA, Canada, Argentina, Spain, France, UK, Austria, the Netherlands, New Zealand, and others.
- Genotype 4 includes human and animal strains identified in China, Taiwan, Japan, India, Vietnam, France, and Italy.

Genotypes 1 and 2 have been responsible for outbreaks in Asian and African countries and in Mexico. On the other hand, genotype 3 and 4 are responsible for the acute autochthonous cases reported in the USA, Argentina, European countries, Japan, and China.

These four genotypes have been divided into different subtypes based on the phylogenetic analysis of many HEV isolates (Ijaz *et al.*, 2005). Genotype 1 has been divided into five subtypes, genotype 2 into two subtypes, whereas genotypes 3 and 4 show a greater diversity and have been divided into 10 and seven subtypes, respectively. Genotypes 1 and 2 have been isolated in all human epidemic outbreaks in developing countries, whereas genotypes 3 and 4 have been isolated not only in humans but also in animals in both developing and industrialized countries (Table 2). These data support the hypothesis that genotypes 3 and 4 have a zoonotic nature (Jung *et al.*, 2007).

Table 1: Current classification of HEV

Family	Genera	Species	Genotypes
Hepeviridae	*Orthohepevirus*	A, B, C, D	I, II, III, IV, V, VI, VII, VIII
	Piscihepevirus		

(SOURCE: Guerra *et al.*, 2017).

Table 2: Characteristics of more prevalent HEV Genotypes.

Characteristics	Genotype 1	Genotype 2	Genotype 3	Genotype 4
Viral discovery	1983	1986	1995	2003
Geographic distribution	Developing countries	Mexico, West Africa	Developed countries	China, Taiwan, Japan
Food-borne transmission	No	No	No	No
Fecal-oral transmission	Yes	Yes	?	No
Water-borne transmission	Yes	Yes	?	No
Person-person transmission	Yes	Unknown	Yes	Yes
Zoonotic transmission	No	No	Yes	Yes
Occurrence of epidemics	Common	Smaller scale epidemics	No epidemics	Uncommon
Highest attack rate	Young adults	Young adults	Persons ≥40yr of age	Young adults
Gender	Male preponderance	Not discriminatory	Mostly male	Not discriminatory
Mortality rate	0.5%-3%	0.5%-3%	Not determined	0.5%-3%
Mortality among pregnant women	High	High	Not determined	High
Chronic infection	None	None	Yes	None
Severe disease among immune-compromised	Not reported	Not reported	Yes	Not reported
Interspecies transmission	Only humans and non-human primates	Only humans and non-human primates	Humans and Pigs	Humans and Pigs
Subtypes	5	2	10	7

(Source: Teshale and Hu, 2011).

2.1.5 Routes of Infection of HEV

The various routes of infection of HEV that have been identified are;

Fecal-oral route

In endemic countries, the fecal-oral route of HEV transmission is predominant. Contamination of drinking water through sewage disposal by humans and animals into water bodies and consumption of contaminated food (seafood and undercooked animals) and is the main cause of the transmission of Gt1 and Gt2 HEV to human beings (Reyes *et al.,* 1990; Acharya *et al.,* 2011; Yugo *et al.,* 2013).

Person-to-person transmission

In contrast to hepatitis A virus (HAV) infection, secondary transmission among household members of patients with acute hepatitis E is an uncommon event, both in the context of outbreaks and sporadic infections. (Kamar *et al.,* 2010; Baylis *et al.,* 2011). Person-to-person transmission has been reported but is uncommon (0.7-2.2%) (Thomas *et al.,* 2013; Aggarwal *et al.,* 1992), although evidence of domestic HEV transmission during a large outbreak in Uganda was recently reported (Teshale *et al.,* 2010) and dissemination of the virus by close contact with infected patients was also documented by (Khuroo *et al.,* 1992).

Nosocomial and Parenteral transmission

Nosocomial and parenteral transmission in hemophiliacs (Toyoda *et al.,* 2008) and in hemodialysis patients (Hossseni-Mòghaddan *et al.,* 2010) has also been reported. HEV-infected individuals can transmit the infection by donating blood during the viremic period. Viremia can be detected even in asymptomatic infections and during the incubation period, even in the absence of amino-transferase elevation. Transmission of HEV via blood transfusion has been documented in several countries, including Saudi Arabia, Japan, and the UK, where matching

RNA sequences were found in blood donors and their recipients. Cases have also been described in recipients of a variety of transplants, including kidney, liver, heart, bone marrow, and lung. In solid organ recipients the prevalence of immunoglobulin G (IgG) anti-HEV was reported as 11.6% and that of genomic viral RNA as 2% (Hoerning *et al.,* 2012; Versluiz *et al.,* 2013; Waggoner *et al.,* 2013).

Mother-to-child transmission

Mother-to-child transmission of HEV has been scarcely documented; however, the available data suggest a significant rate of HEV vertical transmission among the HEV RNA positive mothers with worsening liver disease (Sood *et al.,* 2000;Dixit *et al.,* 2006; Shukla *et al.,* 2011). Transplacental vertical transmission of HEV was described in the third trimester of pregnancy and, mainly in endemic countries (Khuroo *et al.,* 2009).

Sexual transmission

Although sexual transmission is not frequent, homosexual males show a higher prevalence of HEV antibodies (20%) than the general population (Psichogiou *et al.,* 1996) and the sexual transmission of HEV is still under debate.

Direct contact with HEV-infected animals

This is another possible route of transmission of HEV. Seroprevalence studies show that veterinarians and swine handlers are more likely than the general population to be anti-HEV IgG positive (Martelli *et al.,* 2008).

2.1.6 Pathogenesis Of HEV Infection

It is thought that HEV infection initiates via cells lining the alimentary tract (primary site of virus replication). The virus then reaches the liver through the portal vein and replicates in the cytoplasm of hepatocytes without causing direct cytolytic damage. Several observations suggest that, in analogy with other hepatitis viruses, liver injury is largely immune-mediated: first, viremia precedes the onset of alanine transaminase elevation and liver histopathological changes; secondly, experimental infection of non-human primates has shown how the liver damage coincides with the detection of serum anti-HEV antibodies and with a decreasing level of HEV antigens in the hepatocytes; and finally, the lymphocytes infiltrating the liver have a cytotoxic/suppressor immunophenotype. HEV RNA is detectable in blood from as early as two weeks before and for two to four weeks after the onset of symptoms (Wedemeyer *et al.*, 2012).

Viremia and fecal shedding beyond the duration of biochemical hepatitis are uncommon, suggesting that prolonged fecal shedding is not important in maintaining the environmental reservoir of HEV.The antibody responses are directed primarily against epitopes in the ORF2 and ORF3 proteins and are typically detectable at the onset of the disease, with IgM antibodies persisting for two to six months. Anti-HEV IgG appears soon after IgM, and persists for a longer period of time (Kumar *et al.*, 2013).

2.1.7 Clinical Features

After a short prodromal phase, the most common symptom of hepatitis E is jaundice, which can be accompanied by asthenia, fever, malaise, arthralgia, vomiting, and abdominal pain (Wedemeyer *et al.*, 2013). In symptomatic patients, the rate of mortality ranges from 0 to 10% (some studies report 0.2 to 4%); it is higher in infants under 2 years of age for unknown reasons, and ranges from 10 to 25% in pregnant women. Maternal mortality occurs mainly in the third trimester caused by fulminant hepatic failure and obstetric complications (Kamar *et al.*, 2013; Hoofnagle *et al.*, 2012).

Acute hepatitis E

Acute HEV infection is usually a self-limiting illness lasting less than 6-7 weeks. The clinical pictures range from subclinical or asymptomatic forms to fulminant hepatic failure. In industrialized countries the most common clinical presentation is acute hepatitis but sporadic cases are frequently misdiagnosed as drug-induced liver injury or autoimmune hepatitis, and HEV infection is frequently detected only with retrospective serological testing (Davern *et al.*, 2011). Alcohol consumption is also a risk factor because it favours overt disease and is related to its severity.

Chronic hepatitis E

Chronic infection is defined by the persistence of (Gt3) HEV RNA and/or HEV IgM antibodies in serum or stools for more than 6 months in association with increased liver enzyme levels. HEV infection can progress to chronic liver disease mainly in immunocompromised patients but also in immunocompetent individuals (Fiore *et al.*, 2009).

Most of the patients are asymptomatic. The chronicity rate is very high in transplant recipients (more than 50%), with a rapid progression to liver fibrosis. Interestingly, a low risk of HEV

reactivation was reported after allogenic stem cell transplantation and no reactivation after kidney transplantation (Teshale *et al.*, 2010).

A recent study of Gt3 isolate from a patient with a chronic infection revealed a recombinant viral-host genome that was infectious to swine, deer and human hepatocytes *in vitro*. This cross-species adaptation of zoonotic HEV strains and their pathogenicity in humans could also possibly explain why chronic HEV infection is unique to Gt3 (González-Tallón *et al.*, 2011).

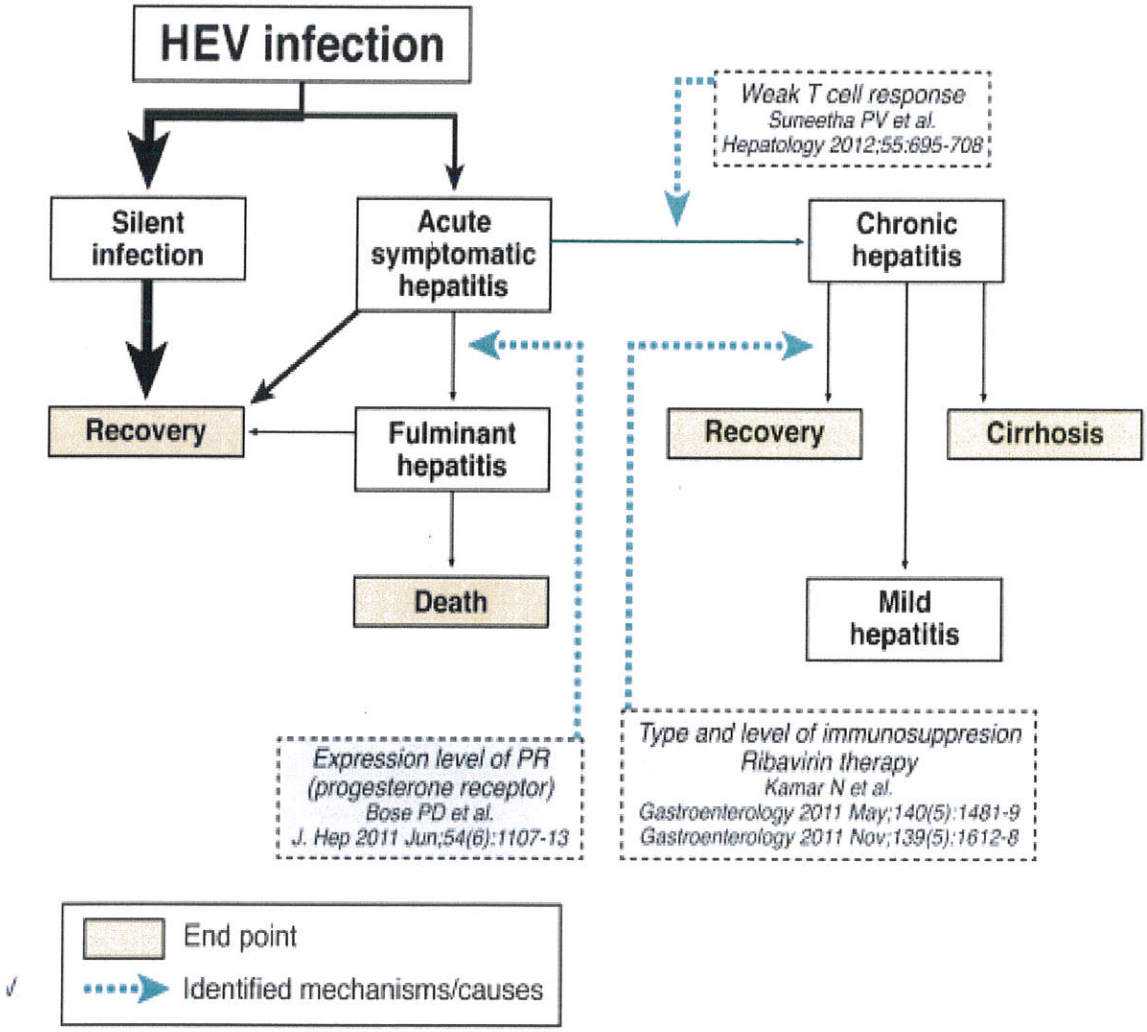

Figure 4: Clinical course of infection (Source: Wedemeyer *et al*., 2012).

2.1.8 Laboratory Diagnosis of Hepatitis E Infection

HEV can be diagnosed either directly by detecting its nucleic acids or more frequently indirectly, due to the relative short duration of viraemia, by detecting the immune response in the host through serological techniques (Zhu *et al.*, 2010). Hepatitis E virus can be detected in the laboratory by the following methods:

I. RT-PCR

RT-PCR is a conventional method that can be used to detect viral RNA, in sera and faeces during the acute stage of the disease in humans (Inoue *et. al.*, 2006). Rapid and sensitive real-time RT-PCR assays have also been developed for the detection of HEV RNA in clinical samples (Ahn *et al.*, 2006; Enouf *et al.*, 2006; Jothikumar *et al.*, 2006).

II. Serology

Enzyme immunoassay (EIA) is a practical, highly sensitive and inexpensive diagnostic method for detection of anti-HEV antibodies. The detection of IgG HEV antibody indicates a past infection whereas the detection of HEV IgM antibody is a marker of acute or recent infection.

III. Immune fluorescence microscopy (IFE)

A few specialised laboratories use this technique for the detection of antibodies. IFE detects antibodies that react against the HEV antigen semi-quantitatively. Anti-HEV antibodies block the binding of fluorescein-conjugated anti-HEV IgG to HEV antigen in frozen liver tissue. This method is labor-intensive and expensive and thus not useful for routine diagnosis.

IV. Cell culture

Establishment of a practical cell culture system to allow the propagation of HEV in vitro is vital for virological characterisation as well as for diagnosis and prevention of HEV infection. Several in vitro culture systems, such as human lung, kidney or liver and

macaques hepatocytes for HEV replication have been used. Most of these, however, cannot provide authentic HEV particles or a high titre of VLPs and have poor reproducibility (Worm *et al.*, 2002).

V. Immune electron microscopy (IEM)

IEM is used to detect VLPs in clinical specimens (Vascoski *et al.*, 2007). HEV particles are precipitated with the native antibody to HEV derived from acute- or convalescent-phase sera. Anti-HEV antibodies concentrations can be determined semi-quantitatively by rating the antibody coating. Although IEM is a superior technique for specificity but the sensitivity of the assay is insufficient for routine analysis. IEM is difficult to perform and most clinical specimens do not contain sufficient VLPs to be detected (Vascoski *et al.*, 2007).

2.1.9 Treatment, Prevention and Control

Treatment

The treatment of HEV infection is supportive and aimed at dealing with symptoms or complications, which are not frequent as the disease is usually self-limited and without consequences. Hospitalization is indicated only for patients unable to maintain oral intake and with hepatic complications. Ribavirin or pegylated α-interferon monotherapy- is an effective treatment for most patients with severe or chronic HEV infection (Kamar *et al.*, 2010; Gerolami *et al.*, 2011).

Reduction of immunosuppression and administration of antiviral medicines can be considered in immunocompromised patients. Antiviral treatment of HEV infections has only been reported in patients infected with Gt3 HEV. In patients infected with Gt1 and Gt2 HEV or in patients with underlying chronic liver disease the best treatment option has not yet been defined (Debing *et al.*, 2014).

Prevention and Control

There are two recombinant vaccines: one tested in Nepalese military and the other one tested in the Chinese adult population. In 2007, the Nepalese recombinant Gt1 HEV vaccine was tested in a phase II-controlled trial and showed 95.5% efficacy in preventing infection and clinical disease; its safety and efficacy in women was not established. Three years later, the HEV vaccine was registered in China and is now marketed in that country alone.

HEV infection can be prevented with an effective human immunization program. Since HEV has only one serotype and natural infection leads to protective antibodies, HEV is a good candidate for the development of an effective vaccine. A large vaccination campaign in developing countries would reduce large waterborne epidemics for thousands of people (Teshale *et al.*, 2011).

Reduction of exposure to the virus via improving sanitary facilities and providing clean drinking water play key roles in prevention and control in developing countries. In developed countries, prevention is more complex because the several possible routes of transmission are not yet fully understood (Guerra *et al.*, 2017). Strategies for prevention and control of HEV outbreaks include;

- Improving quality of drinking water.
- Prenatal tests in pregnant women.
- Development of vaccines such as the Chinese vaccine HecolinH.
- Timely referral to a health-care facility with prompt diagnosis and management of cases.
- Treating and disposing of human wastes correctly.
- Avoiding administration of unnecessary hepatotoxic drugs.
- Preparing safe and clean food and improving personal hygiene.
- Travelers to highly endemic regions should strictly consume only bottled or boiled water.
- Infected people should refrain from food handling and food preparation (Kamar *et al.*, 2012).

2.1.10 Epidemiology

Serological and molecular studies have shown that HEV is globally distributed. It is estimated that two billion people have been infected with HEV with 14 million symptomatic cases, and 300,000 deaths occurring annually around the world (Kamar *et al.*, 2012; Pischke *et al.*, 2014). The geographical distribution of HEV genotypes is depicted in (Figure 5).

Genotype 1 extensively circulates in Asia (including India, Pakistan, Nepal, Bangladesh, China, Kyrgyzstan, and Uzbekistan) and Africa (including Egypt, Algeria, Morocco, Namibia, Sudan, and Chad) (Perez-Gracia *et al.*, 2012), whereas genotype 2 has been isolated only in Mexico and in some African countries (Nigeria, Namibia, Chad, and Sudan) (Kumar *et al.*, 2013). Genotype 3 has been detected worldwide (America, Europe, Asia, Australia, and New Zealand) with the exception of Africa, whereas genotype 4 is restricted to India and East Asia (Archarya *et al.*, 2011). Although genotypes 1 and 2 are considered human viruses, genotypes 3 and 4 have been isolated from both humans and animal (Dalton *et al.*, 2013).

In Nigeria, HEV is very common although with limited information on the various modes of spread. The virus has been reported from pigs with prevalence rate of 76.7% as well as domestic animals with prevalence rate of 24.1% (Owolodun *et al.*, 2014; Junaid *et al.*, 2014). Acute HEV infection has been investigated from the serum and faecal samples of patients in Port-Harcourt, Nigeria by RT-PCR with a prevalence rate of 70% and pregnant women in Sokoto with a prevalence rate of 9.9% (Alkali *et. al.*, 2017).

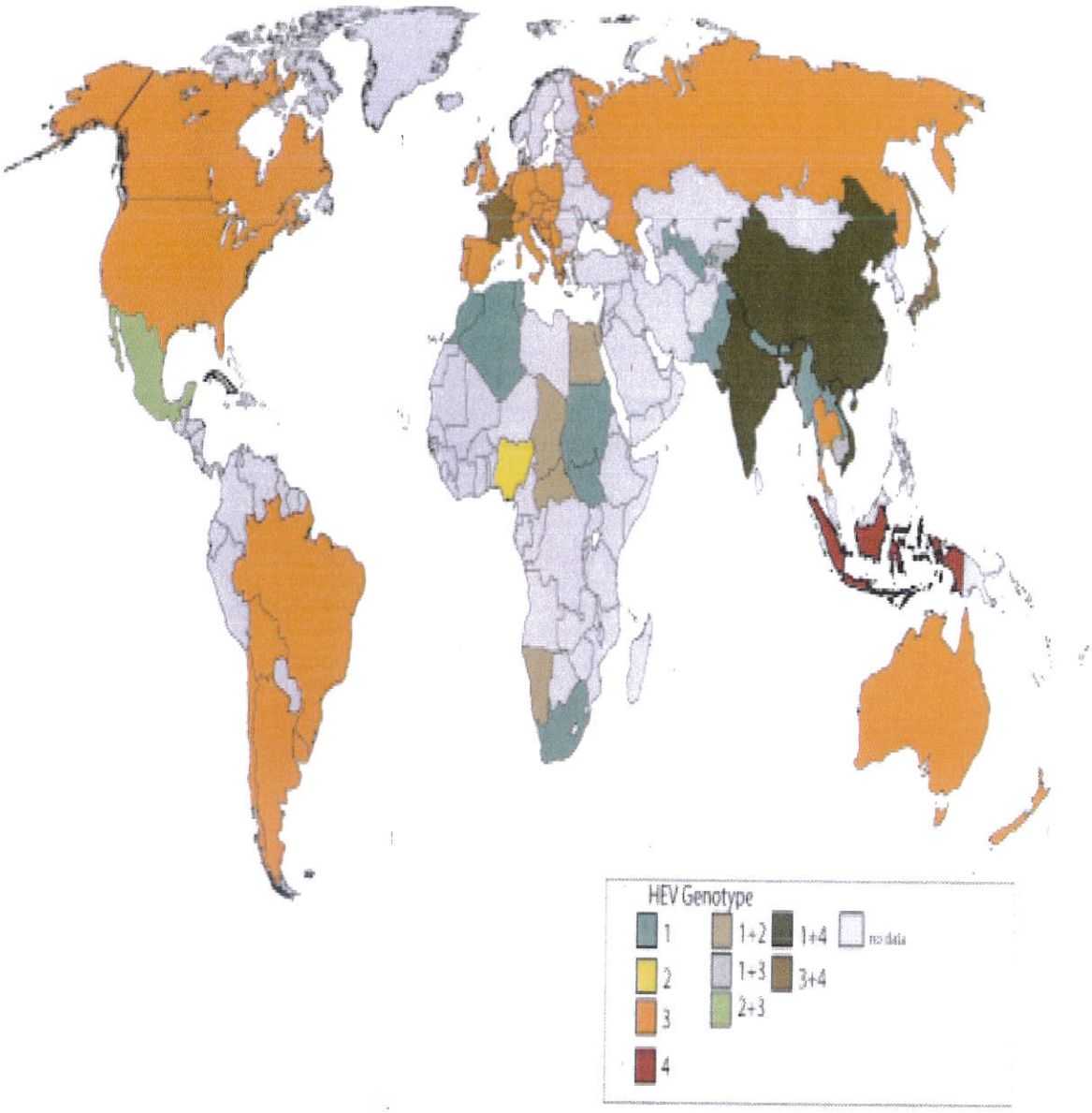

Figure 5: The global distribution of HEV genotypes. (Source: Pischke *et al.*, 2014).

2.2 AIM OF STUDY

The aim of this study is to determine the current seroprevalence of HEV among pregnant women in Ekiti State using ELISA techniques.

Specific Objectives

1. To screen for HEV IgM antibody from serum samples of pregnant women between (15-45 years old) using rapid HEV ELISA kits.

2. Determine the possible risk factors of HEV infection among the study subjects.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

This study was carried out in selected hospitals and health care facilities including *Comprehensive Health Centre Okeyinmi/Okesa, Ado-Ekiti, Basic Health Centre Odo-Ado, Ado-Ekiti, State Specialist Hospital Ikole-Ekiti, Heirs Specialist Hospital, Oye-Ekiti and State Specialist Hospital, Ikere-Ekiti* covering the three senatorial districts in Ekiti State.

3.2 Study population

The study population comprises of pregnant women (aged 15-45 years) who had enrolled for ante natal screening in the selected hospitals for the study.

3.3 Study design

This cross-sectional study was conducted between March 2018 and June 2018. A structured, closed-ended questionnaire was administered to the participants to obtain information on socio-demographic variables such as age, education, occupation, type of family, geographic location, gestation week or period, profession, source of water, sanitary condition and pet ownership. Only informed and consented participants were enrolled in the study.

Ethical approval was also obtained from Ekiti State Ministry of Health (MOU/EA/U/02).

3.4 Sample size determination

This was done according to Kanai *et al.* (2012). The sample size was determined to be minimum of 90 samples.

3.5 Collection of blood samples

- Structured questionnaire was offered to would-be participants (pregnant women) and inform consent was sort.
- 5ml of intravenous blood sample was then collected aseptically from inform consented participants, transferred into a vacutainer tube and marked with a unique identifier.

- Collected blood samples were collected in EDTA bottles and were transferred from the hospital to the laboratory in cold transport chain (cooler with ice packs) to microbiology laboratory.
- These samples were stored at -80^{O}C freezer until use for assay.

3.6 HEV ELISA Assay

The samples were tested for anti-HEV IgM using lateral flow chromatographic immunoassays. The Commercial test kits were from CTK Biotech, Inc. (USA) having sensitivity, specificity and accuracy of 98.1%, 99.2% and 98.9% respectively for anti-HEV IgM. Tests were performed in accordance to the manufacturer's instructions.

3.6.1 Assay Procedure

i. The specimen and the test component were brought to room temperature after refrigeration.

ii. The pouch of the kit's notch was opened to bring out the device which was placed on a clean, flat surface.

iii. The test device was appropriately labelled with the sample's identification number.

iv. The capillary tube was used to dispense 10µl of the blood into the sample well while ensuring that there was no air bubble; this was immediately followed by the addition of the sample diluent (85µl) into the sample well in a vertical position.

v. Then the timer was started for the period of 15mins.

vi. The results were read in 15 mins.

3.7 Data analysis

Chi square was used to determine the association between positive results and risk factors of HEV. Other analyses were performed using Statistical Package for Social Science (SPSS)

version (20.0) proportions were expressed at 95% Confidence Interval and p value-<0.05 to determine significance.

CHAPTER FOUR

4.1 RESULTS

A total of ninety (90) pregnant women aged between 15 to 45 years participated in the study. Of the 90 serum samples analyzed, three (3) were positive for HEV IgM antibodies giving a seroprevalence of 3.3% (95% CI) (Table 3).

The socio-demographic characteristics distribution of study population is shown in Table 4 The age group, 25-30 years had the highest prevalence of HEV positives with 2 out of 3 (66.7%) followed by age group 31-35 years with 1/3 (33.3%) HEV seropositivity. Conversely, there was no statistically significant association between HEV infection and socio-demographic characteristics (age group, type of family and type of residence) ($p>0.05$).

Table 5 shows the health status distribution of the study population. There was a higher HEV seroprevalence in women who had more than one pregnancy compared with those who had a single pregnancy with 2/3 (66.7%) and 1/3 (33.3%) respectively. Also, seroprevalence among women in their third trimester was 2/3 (66.7%) of the positive samples, while that of second trimester was only 1/3 (33.3%). Also, none of the positive HEV participants had history of blood transfusion. Hence, there was no statistically significant association between HEV infection and health status (first pregnancy, age of present pregnancy, history of abortion and blood transfusion) analyzed ($p>0.05$) (Table 5).

Table 6 shows the relationship of the HEV IgM antibody positivity with household characteristics of the study population. The highest prevalence (2/3) of pregnant women positive for HEV was recorded among those that used Borehole (66.7%) as water source for drinking, followed by Well water 1/3(33.3%). Nevertheless, the

prevalence of those using Well water for bathing and domestic use was 2/3(66.7%) while those using Borehole was 1/3(33.3%). Also, HEV positive participants with pets/animals at home had higher prevalence rates of 2/3(66.7%) and those who did not have pets/animals at home had a lower prevalence rate of 1/3(33.3%).Meanwhile, those HEV positive participants with contact with pets/animals was 1/3(33.3%) while those who did not have contact were 2/3(66.7%). Seroprevalence was highest in participants in contact with dogs with 11.1%. In addition, those that consume bush meat or pork were HEV IgM positive by 2.8% lower than those who consume no bush meat or pork had 3.7%. All 3(100%) of HEV IgM seropositive participants consume sea foods. However, there was no statistically significant association between HEV infection and household characteristics (water source, dealings with pet/animals and consumption) among participants ($p>0.05$).

Table 3: Distribution of HEV IgM antibody among pregnant women in Ekiti State.

Anti-HEV	IgM n (%)
Positive	3(3.3)
Negative	87(96.7)
Total	**90**

Table 4: Distribution of socio-demographic characteristics among pregnant women in Ekiti State.

Characteristics	Hepatitis E Virus		Total
	Positive (%)	Negative (%)	
Age Group			
15-20	0(0.0)	3(100.0)	**3**
21-25	0(0.0)	14(100.0)	**14**
25-30	2(7.1)	26(92.9)	**28**
31-35	1(3.9)	25(3.9)	**26**
36-40	0(0.0)	14(100.0)	**14**
41-45	0(0.0)	5(100.0)	**5**
Total	**3(3.33)**	**87(96.7)**	**90 (100.0)**
Chi- Square	**X^2 = 2.5237**	**P = 0.773**	
Type of Family			
Nuclear	2(2.6)	76(97.4)	**78**
Extended	1(8.3)	11(91.7)	**12**
Total	**3(3.3)**	**87(96.7)**	**90 (100.0)**
Chi- Square	**X^2 =1.0743**	**P =0.300**	
Type of Residence			
Room and Parlour	1(3.0)	32(97.0)	**33**
Flat	1(2.4)	41(97.6)	**42**
Self-Contain	1(6.7)	14(93.3)	**15**
Total	**3(3.3)**	**87(96.7)**	**90(100.0)**
Chi- Square	**X^2 =0.6449**	**P = 0.724**	

Table 5: Distribution of health status among pregnant women in Ekiti State.

Health Status	Hepatitis E Virus		Total
	Positive (%)	Negative (%)	
First Pregnancy			
Yes	1 (2.9)	33(97.1)	**34**
No	2(3.6)	54(96.4)	**56**
Total	**3 (3.3)**	**87(96.7)**	**90 (100.0)**
Chi- Square	**X^2 =0.0261**	**P =0.872**	
Age of Present Pregnancy			
First trimester	0 (0.0)	16 (100.0)	**16**
Second trimester	1(2.8)	35 (97.2)	**36**
Third trimester	2 (5.3)	36 (94.7)	**38**
Total	**3 (3.3)**	**87 (96.7)**	**90 (100.0)**
Chi- Square	**X^2 =1.0254**	**P =0.599**	
Ever Had Abortion?			
Yes	0 (0.0)	11(100.0)	**11**
No	3 (3.9)	74 (96.1)	77
Total	**3 (3.4)**	**85 (96.6)**	**88 (100.0)**
Chi- Square	**X^2 =0.4437**	**P =0.505**	
Ever Had Blood Transfusion?			
Yes	0 (0.0)	8 (100.0)	**8**
No	3 (3.7)	79 (96.3)	**82**
Total	**3 (3.3)**	**87 (96.7)**	**90 (100.0)**
Chi- Square	**X^2 =0.3028**	**P =0.582**	
Ever Heard of HEV?			
Yes	0 (0.0)	17 (100.0)	**17**
No	3 (4.5)	64 (95.5)	**67**
Not sure	0 (0.0)	5 (100.0)	**5**
Total	**3 (3.4)**	**86 (96.6)**	**89(100.0)**

Chi- Square	**X^2 =1.0194**	**P =0.601**	
Knowledge of Mode of Transmission?			
Yes	0 (0.0)	11 (100.0)	**11**
No	2 (2.9)	67 (97.1)	**69**
Not sure	0 (0.0)	2 (100.0)	**2**
Total	**2 (2.4)**	**80 (97.6)**	**82 (100.0)**
Chi- Square	**X^2 =0.3862**	**P =0.824**	
Prior Liver Disease?			
Yes	0 (0.0)	3 (100.0)	**3**
No	3 (3.5)	83 (96.5)	**86**
Total	**3 (3.4)**	**86 (96.6)**	**89(100.0)**
Chi- Square	**X^2 =0.1083**	**P =0.742**	

Table 6: Distribution of household characteristics among pregnant women in Ekiti State.

Characteristics	Hepatitis E Virus		Total
	Positive (%) (%)	Negative	
Source of Drinking Water			
Tap water	0 (0.0)	30 (100.0)	**30**
Sachet water	0 (0.0)	31 (100.0)	**31**
Well	1 (9.1)	10 (90.9)	**11**
Borehole	2 (13.3)	13 (86.7)	**15**
Others	0 (0.0)	1 (100.0)	**1**
Total	**3 (3.4)**	**85 (96.6)**	**88 (100.0)**
Chi- Square	**X^2 =7.7532**	**P =0.101**	
Source of Water For Bathing and Domestic Use			
Well	2 (3.6)	54 (96.4)	**56**
Stream	0 (0.0)	2 (100.0)	**2**
River	0 (0.0)	2 (100.0)	**2**
Borehole	1 (4.2)	23 (95.8)	**24**
Rain water	0 (0.0)	1 (100.0)	**1**
Others	0 (0.0)	4 (100.0)	**4**
Total	**3 (3.4)**	**86 (96.6)**	**89 (100.0)**
Chi- Square	**X^2 = 0.3675**	**P =0.996**	
Pets/Animals at homes?			
Yes	2 (7.4)	25 (92.6)	**27**
No	1 (1.6)	62 (98.4)	**63**
Total	**3 (3.3)**	**87 (96.7)**	**90 (100.0)**
Chi- Square	**X^2 =1.9869**	**P =0.159**	

Contacts With Animals?			
Yes	1(7.7)	12 (92.3)	**13**
No	2 (2.7)	72 (97.3)	**74**
Total	**3 (3.5)**	**84 (96.5)**	**87 (100.0)**
Chi- Square	**X^2 =0.8268**	**P =0.363**	
If Yes, Which Animals Were You In Contact With?			
Cat	0 (0.0)	3 (100.0)	**3**
Dog	1 (11.1)	8 (88.9)	**9**
Sheep	0 (0.0)	1 (100.0)	**1**
Goat	0 (0.0)	5 (100.0)	**5**
Cow	0 (0.0)	3 (100.0)	**3**
Pig	0 (0.0)	1 (100.0)	**1**
Others	0 (0.0)	12 (100.0)	**12**
Total	**1 (2.9)**	**33 (97.1)**	**34 (100.0)**
Chi- Square	**X^2 = 2.8620**	**P =0.826**	
Consumption of Bush Meat or Pork?			
Yes	1 (2.8)	35 (97.2)	**36**
No	2 (3.7)	52 (96.3)	**54**
Total	**3 (3.3)**	**87 (96.7)**	**90**
Chi- Square	**X^2 =0.0575**	**P =0.811**	
Consumption of Sea Foods?			
Yes	3 (4.5)	63 (95.5)	**66**
No	0 (0.00)	22 (100.0)	**22**
Total	**3 (3.4)**	**85 (96.6)**	**88**
Chi- Square	**X^2 = 1.0353**	**P =0.309**	

CHAPTER FIVE

5.1 DISCUSSION

HEV is responsible for about 3.4 million asymptomatic illnesses, 70,000 deaths and 3000 stillbirths across the world (WHO, 2017). Hepatitis E is one of the important liver diseases with high incidence in developing countries, mainly Asia and Africa (Dalton *et al.* 2008). Significant morbidity and mortality are seen in pregnant women (Mushahwar, 2008). In this study, the current seroprevalence of HEV was investigated in Ekiti State.

Findings from this study are comparable to that of (Khameneh *et al.,* 2013) with prevalence of 3.6% reported in their study among pregnant women in Urmia, Iran although higher than 2.6% prevalence recorded at Switzerland (Kovari *et al.,* 2010). However, from previous studies in Nigeria, the prevalence (3.3%) observed in this study is lower than a 5.5% prevalence recorded in Ogbomoso (Oladipo *et al.,* 2015) among individuals infected with HIV, 8.3% prevalence observed in Ekiti (Adesina *et al.,* 2009), 31.1% in Plateau (Junaid *et al.,* 2014), 7.7% in Cross river (Ekanem *et al.,* 2015) also, lower than that of (Bello *et al.,* 2016) with prevalence rate of 9.9% among pregnant women for HEV infection in Sokoto State, Nigeria. Furthermore, in contrast with other studies, the prevalence rate recorded in this study is lower than that of (Huang *et al.,* 2013) who reported a prevalence rate of 10.24% among pregnant women in Yunnan, China, and (Mamani *et al.,* 2015) that reported 7.4% seroprevalence among pregnant women in Hamadan, Iran. Higher prevalence rate than the finding of this study of 61.2% was also reported by (Musa *et al.*, 2016) among pregnant women in Khartoum, Sudan. The disparity in the prevalence rates may be ascribed to differences in the study participants, endemicity of the virus, sensitivity and

specificity of HEV antibody detection assays used, difference in study groups, socio-economic, cultural, hygienic and climatic conditions. The low prevalence (3.3%) recorded in this study may be attributed to an improved standard of living, improved environmental and hygienic conditions and health care in Ekiti State.

Findings in this study revealed a highest prevalence rate among age group 25-30 years with 7.1% followed by age group 31-35 years with 3.9% This suggest an higher prevalence rate of HEV among the youths (25-35 years) compared to the older age groups (Table 4). This finding is also consistent with Adesina *et al.* (2009), who observed the prevalence of anti-HEV antibodies to be highest in ages 20–40 years. In developing countries, the age-specific seroprevalence profiles reveal that HEV infection is largely limited to adult population between ages 15 -35 years (Kamar *et al.*, 2014). This study showed a decreasing seroprevalence, from 7.1% in the age range 25-30 years to 3.9% in the age range 31-35 years which follows a similar trend reported by Oladipo *et al.* (2017), with a decreasing seroprevalence, from 4.1% in the age range 15-25 years to 3.2% in the age range 26-35 years. This age-specific prevalence might be due to increased exposure to high-risk factors like contaminated food and water among the age group 15-35 years compared to older age group 35-45 years. This is in agreement with the reports of Ekanem *et al.* (2015). Although, there was no statistically significant association between HEV infection and age group ($p>0.05$).

Findings from this study showed higher prevalence in women who had more than one pregnancy compared with those who were having their first pregnancy with 3.6% and 2.9% respectively. Prevalence of women in their third trimester of present pregnancy was higher with 5.3% while that of second trimester was 2.8%. Significantly, two of the three (2/3) of the pregnant women in which Anti-HEV IgM was detected were in

their third trimester of pregnancy (Table 5). The fact that two pregnant women with HEV IgM in this study were in their third trimester (39th week of gestation) signifies a recent exposure to HEV and medical significance. The reason being that acute HEV in pregnancy can progress to fulminant hepatitis with a high mortality rate, especially, if it occurs in the 3rd trimester (Stoszek *et al.,* 2006; Perez-Gracia *et al.,* 2017). Furthermore, reports have shown that HEV infection during pregnancy can lead to maternal mortality rate of 15% to 25%, especially, with genotype 1, which together with genotype 2 are prevalent in the developing countries (Ranger-Rogez *et al.,* 2002). It is worth mentioning that there is no information on the outcome of these women's pregnancies. Hence, the impact of the HEV infection in the 3rd trimester of pregnancy on either the mother or child could not be ascertained.

Findings from this study showed a highest prevalence of 2/3 (66.7%) in pregnant women that used Borehole as water source for drinking, followed by Well water 1/3(33.3%). However, the prevalence of those using well water for bathing and domestic use was 2/3(66.7%) while those using borehole was 1/3. Also, HEV positive participants with pets/animals at home had higher prevalence rates of 2/3(66.7%) and those who did not have pets/animals at home had a lower prevalence rate of 1/3(33.3%). All 3(100%) of HEV IgM seropositive participants consume seafood (Table 6). This agrees with the report of La Rosa *et al.,* (2011) in Italy that the consumption of seafood may be proposed as major risk factors associated with HEV infection. Moreover, there was no statistically significant association between HEV infection and Household characteristics (water source, dealings with pet/animals and consumption of bush meat/pork or seafood) among participants ($p>0.05$).

5.2 CONCLUSION AND RECOMMENDATIONS

HEV IgM antibody which is a marker of recent HEV infection was detected among pregnant women among selected hospitals in Ekiti State. The overall prevalence rate was approximately 3.3% (3/90). Although, the prevalence rate is relatively low, however, this study showed the evidence of acute HEV infection among pregnant women from major hospitals in Ekiti State. There was no statistically significant association between HEV infection and socio-demographic characteristics, health status and household characteristics.

It is recommended that HEV infection among pregnant women needs to be studied in further details incorporating a larger population size, and carrying out RT-PCR to enable us understand the viral load (rate of infectivity) as well identifying the HEV genotype(s) possibly circulating among pregnant women. These were the limitations for the present study. Public enlightenment about environmental and personal hygiene before and during pregnancy may be useful in keeping the rate of HEV infection very low. In addition, HEV routine screening together with other viral hepatitis agents should be in included in the routine antenatal screening for pregnant women due to the global public health importance of HEV among this group (pregnant women).

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APPENDICES

Materials used:

EDTA bottles

Hand gloves

Face mask

EDTA bottles

Cotton wool

Syringes

Aria HEV rapid test kits

Ice packs

Hypochlorite solution

Vaccine carrier box

Table 7: Sample ID, Result of HEV ELISA Assay.

LAB NO	HEV(-/+)
CHC01	-
CHC02	-
CHC03	-
CHC04	-
CHC05	-
CHC06	-
CHC07	-
CHC08	-
CHC09	-
CHC10	-
CHC11	-
CHC12	-
CHC13	-
CHC14	-
CHC15	-
CHC16	-
CHC17	-
CHC18	-
CHC19	-
CHC20	-
CHC21	-
CHC22	-
CHC23	-
CHC24	-
CHC25	-
CHC26	-
CHC27	-
CHC28	-
CHC29	-
CHC30	-
CHC31	-

CHC32	-
CHC33	-
CHC34	-
CHC35	-
CHC36	-
CHC37	-
CHC38	-
CHC39	-
CHC40	-
CHC41	-
CHC42	-
CHC43	-
CHC44	-
CHC45	-
CHC46	-
CHC47	-
CHC48	-
CHC49	-
CHC50	-
CHC51	-
CHC52	-
CHC53	-
CHC54	-
CHC55	-
CHC56	-
CHC57	-
CHC58	-
CHC59	-
CHC60	-
SSH01	-
SSH02	-
SSH03	-
SSH04	-

SSH05	-
SSH06	-
SSH07	-
SSH08	-
SSH09	-
SSH10	-
SSH11	-
SSH12	-
SSH13	-
SSH14	-
SSH15	+
SSH16	-
SSH17	-
SSH18	-
SSH19	-
SSH20	-
SSH21	+
SSH22	-
HSH01	-
HSH02	+
HSH03	-
HSH04	-
HSH05	-
HSH06	-
HSH07	-
HSH08	-

MINISTRY OF HEALTH

Phase III, State Secretariat Complex, Ado - Ekiti, Ekiti State, Nigeria.

All Communications should be addressed
to the Permanent Secretary quoting
Our Ref. NO: MOH/PRS/040

Date: 12th March 2018

Kayejo Gbenga Victor
Undergraduate Student,
Federal University Oye Ekiti

Dear Sir,

RE: Application for Ethical Approval

Your application for ethical approval on the topic 'Sero-epidemiological and molecular surveillance for Hepatitis E virus (HEV) among pregnant mothers in Ekiti State' is hereby granted.

Your approval number is **MOH/EA/U/02** and can be quoted in future correspondence. It remains valid from March 2018 through August 2018.

You are strongly implored to abide by the basic health research principles of respect of persons, beneficence and justice, and also protecting all data obtained from the study participants.

Wishing you success in your investigation.

OFFICE OF THE
PERMANENT SECRETARY
MINISTRY OF HEALTH. ADO-EKITI
13 MAR 2018
Dr. O. O. Alabi
SIGN. ..... DPRS .....

Figure 6: Ethical Approval of study

Questionnaire on Sero-Epidemiological and Molecular Surveillance for Hepatitis E Virus (HEV) among Pregnant Mothers in Ekiti State.

Hepatitis E virus (HEV) is the causative agent of hepatitis E, a viral infection that attacks the liver and can cause both acute and chronic disease. The virus is transmitted mainly by the fecal-oral route, usually due to the consumption of contaminated water or food.

I, Kayejo Gbenga Victor from Department of Microbiology, Faculty of Science, Federal University Oye-Ekiti, is conducting a research on the Sero-epidemiological and Molecular Surveillance for Hepatitis E Virus (HEV) Among Pregnant Mothers in Ekiti State and hereby implores you to participate in the study. You are qualified to participate in the study because you meet the inclusion criteria *(being a pregnant mother)*. Your participation will entails your willingness to supply us about 3-5ml of your blood sample to be included in the study population as well as kindly filing-out this questionnaire accurately. You are hereby assured of the confidentiality of all information supplied in this form. Thank you.

Instruction: kindly tick the appropriate box [✓] ***for answer to each question or fill the gap where necessary.***

(A) Sex

Male [ ] Female [ ]

(B) Age group

15-20yrs [ ] 21-25yrs [ ] 26-30yrs [ ] 31-35yrs [ ] 36-40yrs [ ]

41-45yrs [ ] 46yrs and above [ ]

(C) Occupation/Profession: .....

(D) Geographic location: .....

(E) Type of family`

Nuclear [ ] Extended [ ]

(F) Type of Residence

Room and parlour [ ] Flat [ ] Self-Contain [ ]

(G) Pregnancy

- Is this your first pregnancy? Yes [ ] No [ ] Not sure [ ]. If No, how many children do you have? .....
- What is the age of your present pregnancy?
  First trimester [ ] Second trimester [ ] Third trimester [ ]
- Do you have any history of abortion? Yes [ ] No [ ] If yes, What year? .....

(H) History of blood transfusion

- Have you ever received blood transfusion before? Yes ☐ No ☐ Not sure ☐

(I) Knowledge of HEV

- Have you ever heard of Hepatitis E virus? Yes ☐ No ☐ Not sure ☐

If yes, do you know its modes of transmission? Yes ☐ o ☐ t sure ☐

(J) History of liver disease

- Have you had any liver disease before or at present? Yes ☐ o ☐

(K) Water Source

- What is the source of your drinking water? Tap water ☐ Sachet water ☐
  Well ☐ Boreholes ☐ Others? please kindly specify.....

- What is/are your source(s) of water for bathing and domestic uses? Well ☐
  Stream ☐ River ☐ Boreholes ☐ Rain water ☐ Others?
  Kindly specify.....

(L) Dealing with Pet/animals

- Do you have pets/animals in your house? Yes ☐ No ☐

- Did you have any contact with the animals? Yes ☐ No ☐

If yes, which animals were you in contact with? (Please tick all that apply) Cat ☐
Dog ☐ Sheep ☐ Goat ☐ Cow ☐ Pig ☐ Other
(specify).....

(M) Food consumption

- Do you consume bush meat or pork? Yes ☐ No ☐

- Do you consume sea foods? Yes ☐ No ☐. If Yes how often?
  Kindly specify.....

Signature **Date**

INFORMED CONSENT

Department of Microbiology (Virology Unit),
Faculty of Science,
Federal University Oye-Ekiti,
Ekiti State.

Title of Research Project: Sero-epidemiological and Molecular Surveillance for Hepatitis E
Virus (HEV) Among Pregnant Mothers in Ekiti State.
Name of Principal Investigator: Mr S. A. Osanyinlusi
Phone Number of Principal Investigator: +2348067434709

PURPOSE AND BACKGROUND

KAYEJO, Gbenga Victor with Matric number MCB/14/2324 is a 400L student of the Department of Microbiology, Federal University Oye-Ekiti is conducting a final year research on the prevalence of Hepatitis infection caused by Hepatitis E virus (HEV) among pregnant mothers in Ekiti State. The purpose of your participation in this research is to help in determining the prevalence, and possible risk factors of HEV among pregnant women. You were selected as a possible participant in this study because you meet the inclusion criteria-as the study is to be conducted among pregnant mothers. This research is supervised by Mr. S. A. Osanyinlusi, a Lecturer/Researcher, in the Department of Microbiology (Virology Unit), Federal University Oye-Ekiti.

PROCEDURES

If you agree to participate in this research study, the following will occur:

- You will be required to fill a structured questionnaire requesting for personal information like age, educational background, occupation, geographic location, type of family, age of pregnancy, e.t.c. Also, your source of drinking water, dealings with animals (pets), previous history of any liver disease, knowledge of Hepatitis E virus and possible routes of exposure to the virus will be accessed through the questionnaire.
- After complete filling of the questionnaire, 5ml of intravenous blood sample will be collected from you for just once by trained health personnel.

RISKS

There are no risks involved in your participation in this research.

CONFIDENTIALITY

The records from this study will be kept totally confidential. No individual identities will be used in any reports or publications resulting from the study. All questionnaires and informed consent forms will be given codes and stored separately from any names or other direct identification of participants. Research information will be kept in locked files at all times. After the study is completed by September 2018, all documents will still be treated confidentially.

BENEFITS OF PARTICIPATION

There will be no direct benefit to you from participating in this research study. The anticipated benefit of your participation in this study is to determine your HEV status

and be informed of its possible routes of infection as well as its preventive measures and control.

VOLUNTARY PARTICIPATION

Your decision whether or not to participate in this study is voluntary and will not affect your relationship with the Federal University Oye-Ekiti. If you choose to participate in this study, you can withdraw your consent and discontinue participation at any time without prejudice.

QUESTIONS

If you have any questions about the study, please contact Mr S. A. Osanyinlusi by calling +2348067434709.

CONSENT

I.....vo luntary consent to participate in the above named research study being conducted by the Virology Unit of the Department of Microbiology, Federal University Oye-Ekiti.

I have been given the following information:

That the research is undertaken to investigate the Sero-epidemiological and Molecular Surveillance for Hepatitis Virus (HEV) Among Pregnant Mothers in Ekiti State.

That all information provided in this questionnaire will be kept confidential.

That result of each participant's test will be kept confidential too, and made known to each participant only on request.

_____ _____

Signature **Date**