EPIDEMIOLOGY OF HEPATITIS E VIRUS INFECTION IN PREGNANT WOMEN IN SEKONDI-TAKORADI METROPOLIS, GHANA

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ABSTRACT

Infections caused by hepatitis E virus (HEV) constitute a global public health burden. Worldwide, over 20 million new cases of asymptomatic infections, 3.4 million illnesses and 70 000 deaths due to HEV occur each year. Infections with HEV are the leading cause of oro-fæcally acquired viral hepatitis and outbreaks have occurred in more than 61 countries. In developing countries particularly in Asia and Africa HEV outbreaks cause 10-30% case fatality rates in pregnant women and the infection is more common and fatal during the third trimester of pregnancy. Several reports in Ghana have pointed to the country being HEV endemic and sporadic maternal deaths and abortions due to HEV infections. However, there is not a surveillance system for HEV infections and the Ghana Ministry of Health (MOH) expressed concerns over knowledge gaps about the infection and particularly among the most HEV-vulnerable pregnant population.This is important because in the absence of vaccine to control HEV infection individual actions by the affected pregnant women to lower the effect of risk factors becomes necessary. There is also the policy implication of this study. In the absence of vaccines to control HEV infections the strategy of education becomes an important tool to lower the effects of the risk factors. The objectives of this thesis therefore were to determine seroprevalence of subclinical infections of HEV in the third trimester of pregnancy, risk factors and the delivery outcomes due to infections with HEV in the Sekondi-Takoradi Metropolis, Ghana. Asymptomatic and apparently healthy pregnant women in third trimester, of 18 years and above were purposively selected in a cross-sectional study. The third trimester was selected because this is the period reported to be associated with most HEV-related vulnerabilities of abortions, faetal and maternal mortalities. To reduce confounding effects on estimates pregnant women with hepatitis B, C and active liver disease profiles

examined earlier as part of antenatal care in routine checks and found not to be free of these infections were excluded from the study. Because reports have associated human immunodeficiency virus (HIV) with increased infections of HEV infections those with HIV have also been excluded. Blood samples were collected and analyzed for HEV infection. Socio-demographic and household data were collected. Household proximity to wetlands and domestic pig farms were estimated and farmed swine from two districts from where the sampled pregnant women originated were also tested for HEV infection. Data on diagnoses and mode of delivery of the pregnant women were collected from books on their discharge and also from the Regional Hospital Database. Bivariate and multivariate logistic regression (LR) analysis was done in SPSS version 20 and by Microsoft Excel, respectively. R code version 4.1 was used to check model assumptions and geographical dependence in the dataset. Anti-HEV IgM was 22.5% (81/360) 95% CI:18.2-26.8: and the anti-HEV IgG 11.0% (11/100) 95% CI: 5.6-18.8. In bivariate LR analysis statistically significant associations were found between recent HEV infections and age-groups, level of education, access to household flash water toilet systems. Three out of 12 (25%) domestic pig farms were infected with HEV. Proximity to 20% (5/20) farms were significantly associated with recent infections with HEV. Infection with HEV was significantly associated with complications at delivery (P = 0.029: OR 1.24 95% CI 1.021-1.496). Surprisingly, among 22 diagnoses recorded in ward discharge log books, normal pregnancy (NP) was the only significant outcome (P = 0.000: OR 0.349 95% CI 0.232-0.525), an indication of recent infections with HEV protective of normal deliverys. Seroprevalence of active infection with HEV in the third trimester of pregnancy is 22.5% in Sekondi Takoradi Metropolis of Ghana. The absence of water closet toilets and proximity to domestic pig farms are risk factors for HEV infection, whilst proximity to wetlands is not indicative of an infection risk to HEV. Active infection with HEV is not

indicative of having an adverse outcomes on deliveries at delivery. Metropolitan, municipal and local government authorities should increase support for water closet toilet facilities in households. Improved swine farm effluent management systems should also be encouraged. Health authorities should encourage public awareness creation about HEV infection in pregnancy. Even though the outcomes during delivery of recent HEV infections appear to be protective of normal pregnancy, clinical realities may be different. Therefore, research institutions should consider swine HEV as a One Health challenge to reduce the potential of swine-HEV risks for the development of liver cancers in pregnant women. The current study provided insights for dealing with geospatial analysis and provided additional analytic approach in dealing with geospatial data. The seroprevalence data on recent HEV infections adds to existing data on HEV during pregnancy and the first study of HEV in swine in Ghana. In the absence of vaccine against HEV infections education against HEV during pregnancy by the Ministry of Health and related institutions will improve disease prevention and control.

DECLARATION

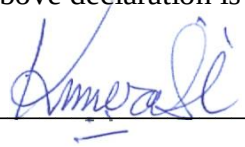
I, Reuben Mliwomor Komi Tettey, do hereby declare to the Senate of Sokoine University of Agriculture that this thesis is my original work done within the period of registration and that it has neither been submitted or being concurrently submitted to any other institution for degree award.

27/08/2019

Reuben Mliwomor Komi Tettey — Date

(PhD Candidate)

The above declaration is confirmed by;



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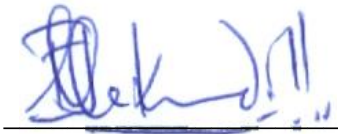

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DEDICATION

I dedicate this thesis to my family who bore the blunt of my absence since 1998 when I began my passionate academic pathway. My wife, Irene, carried 100% of the weight of the responsibility of caring for the daily needs of our children and to her I say ‘there’s no one like you’. To the pregnant women, I say the Ghanaian palance of “Ayekoo” “Congratulations for having supported this research.” There have been both challenges and opportunities created by persons that I met during this previous and current research journey. To every one who contributed to those realities of life, I do dedicate this piece of research work. To my mother I dedicate this book because she taught me early in life to follow my passion, to intensely love what I do. So, gradually I am passing this trait to my dear children Edem, Kujo, Paula and Malike. May God bless my Mother Luciana Geyewu wherever she is. Thank you God.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS, ACRONYMS AND SYMBOLS

ANC	Antenatal clinic
ArcGIS	Arc Geographic Information System
BLAST	Basic Local Alignment Search Tool
°C	Degree Centrigrade
DCD	Disease Surveillance Department
DNA	deoxyribose nucleic acid
EH	Essikardo Hospital
ENRH	Efia Nkwanta Regional Hospital
Fig	Figure
g	gram(s)
GAM	Geographical Analysis Machine
GHS	Ghana Health Service
HBsAg	Hepatitis B surface antigen
HE	Hepatitis E
HEV	Hepatitis E virus
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IRB	Institutional Review Board
IU	international unit
JSS	Junior Secondary School
kb	kilobytes
KBTH	Korle-Bu Teaching Hospital
KH	Kwesimintsim Hospital
min	minutes

ml	milliliter
NANBH	non-A, non-B hepatitis
ng	nanogram (s)
NIACUC	Noguchi Institutional Animal Care and Use Committee
nm	nanometer(s)
NMIMR	Noguchi Memorial Institute for Medical Research
OGD	Obstetrics and Gynecologic Department
ORF	open reading frame
PCR	polymerase chain reaction
PPH	post-partum haemorrhage
QGIS	Quantum Geographic Information System
RNA	Ribonucleic acid
RT	Reverse transcription
RT-PCR	reverse transcription-polymerase chain reaction
rt-RT-PCR	real-time reverse transcription polymerase chain reaction
Sec	Seconds
SHS	Senior High Secondary School
SUA	Sokoine University of Agriculture
TH	Takoradi Hospital
VH	Viral hepatitis
VSD	Veterinary Services Department
WHO	World Health Organization
μg	microgram(s)
μl	microliter(s)

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background

There are five main human viral hepatitides (VH) types: A, B, C, D and E. Among these hepatitis E virus (HEV) causes both human and animal infection with the unique characteristics of the relatively high mortality rates (10-28%) among pregnant women (Hussaini *et al.,* 1997; Guthman *et al.,* 2006) and the immunocompromised (Motte *et al.,* 2012). The incubation period ranges from 2 to 9 weeks and the clinical signs and symptoms include high fever, headache, loss of appetite, abdominal pains, jaundice and diarrhea, even though these signs and symptoms may not be typical for HEV alone (Tsega and Hansson, 1992).

Infections of HEV are zoonotic. Genotype 1 and 2 are found only in humans (Meng, 2009), whereas Gt 3 and 4 are associated with food-borne zoonotic transmission from domestic pigs, wild boar, and deer (Huang *et al.,* 2004; Kamar *et al.,* 2012). Household sheep, goats, swine and rabbits have also been reported by Zhao *et al.* (2009) as reservoirs of HEV Gt 4. Gt 3 and 4 share similar nucleotide identity (Wibawa *et al.,* 2007; Sung-Eun, 2008; Meng, 2009). HEV infections in animals is asymptomatic. However, there is scanty evidence of the zoonotic role of HEV infection in Africa. There has not been a study involving domestic animals in general and swine for that matter. Though in sub-Saharan Africa women work closely with animals (Karimuribo *et al.,* 2009), the potential risk that HEV-infected animals pose to them have not been reported.

Fæcal contaminated water sources have caused massive outbreaks of HEV infection in humans in Asia, on the Indian sub-continent. It has been reported that since 1979, HEV

has been implicated in 17 outbreaks involving 35 300 cases and 650 deaths in Africa (Kim *et al.,* 2014). In refugee camps and among displaced persons in the Democratic Republic of Congo, Sudan, Uganda and Tanzania hygiene-related risk factors have been attributed to the HEV outbreaks (Teshale *et al.,* 2007; Hogan *et al.,* 2010; WHO, 2013). During the HEV outbreak in Uganda, Guthmann *et al.* (2006) reported complications of pregnancy and maternal deaths. Futurity Group (2016) of Johns Hopkins University reported that pregnant women with low bodyweight, anemia and deficient in vitamin D and zinc were at greater risk of HEV infection. The course of HEV is no different in pregnancy than in non-pregnant women, the difference is that the incidence of acute liver failure complicated with hepatic encephalopathy is higher in pregnancy (Bhatia *et al.*, 2008).

In Nigeria, Junaid *et al.* (2014) found rural-dwelling to be a risk factor for infection with HEV. Reddick *et al.* (2011) demonstrated that pregnancy outcomes are positively associated with HEV infection. In Ghana, a study suggested that deaths of mothers and fetuses, abortion, premature delivery, or death of a live-born baby soon after delivery are common complications of hepatitis E infection during pregnancy (Bonney *et al*., 2012). HEV seroprevalence (<28.6%) in Accra were reported among pregnant women and among pig farmers (Tettey and Adjei, 2009; Ofosu-Appiah *et al.,* 2015) and in Cape Coast Metropolis (Obiri-Yeboah, 2018). However, the contribution of household factors, such as the level of sanitation and hygiene practices, contact with animals and consumption of undercooked products of animal origin to HEV infection have not been reported. Hepatitis E infection could not be reliably diagnosed by bio-chemical tests in health institutions but the more reliable serological and nucleic acid tests are not routinely done nor is screening of blood done for vulnerable population groups such as for pregnant women.

Globally there is no vaccine for HEV infection, though China in 2012 came up with a vaccine, *Hecolin.* This vaccine was safely used in healthy subjects in phases 1, 2 and 3 clinical trials. However, *Hecolin* was not safe to use in pediatric subjects (<16 years of

age), the elderly (>65 years of age), persons with underlying diseases or conditions such as those who are immunosuppressed or have liver disease. Further trials are recommended to assess the safety of *Hecolin* in these subpopulations and pregnant women; a post-marketing study is yet to be conducted (Soo, 2012). To date, *Hecolin* has been registered for use in China but yer to be accepted by WHO for use in other HEV-affected countries and for pregnant women in particular (Wu *et al.,* 2012).

Bonney *et al.* (2012) in a case-series reported pre-term deliveries, abortions, severe pre-eclampsia and fatalities involving 3 HEV-infected pregnant women at the Department of Obstetrics and Gynecology (OGD) of the Korle Bu Teaching Hospital (KBTH), which is the largest referral hospital in Ghana. However, data on HEV infections and the potential risk factors among vulnerable populations is limited. National surveillance data from the Disease Surveillance Department (DSD) of the Ghana Health Service shows an increasing annual trend of reported cases of clinical viral hepatitis (VH) from all the ten regions of Ghana. However, the data lacks information on the specifics of VH types, the population groups most affected and the vulnerabilities due to VH. Apart from the study of Adjei *et al*. (2009) that reported HEV seroprevalence (28.6% (45/157) among pregnant women at the OGD of the Korle-Bu Teaching Hospital in Accra, no other known report exists that quantifies the infection among pregnant women. The Effia Nkwanta Regional Hospital (ENRH) in the Western region of Ghana recorded 661 ante-natal hospital registrations during the first half-year of 2015 but none were tested for HEV to identify who are at risk of pregnancy complications at delivery. The aims of this research were to identify pregnant women with markers of acute phase infection with HEV, the associated risk factors and pregnancy outcomes.

1.2 Rationale

Ghana belongs to the West African region where the prevalence of infections of viral hepatitis is high:chronic hepatitis B virus (HBV) infection is ≥8% and that of hepatitis C

virus (HCV) is also 5-10%. Thus, the Ghanaian population has an unquantified burden of prevalence of chronic liver disease and liver cancer due to the HBV and HCV. Ghana Health Service expressed concerns over gaps in undiagnosed cases of suspected viral hepatitis (VH), which is only 14.8% (7 581 out of 51 052). However, in Ghana, anti-HEV IgM seroprevalence was mentioned in a research report by Adjei *et al.* (2009) in Accra in the Greater Accra Region, of 64.40% (29 /45). There are case reports of deaths due to HEV among pregnant women and their neonates (Bonney *et al.*, 2012; Ofosu-Appiah *et al.*, 2016) in Cape Coast in the Central Region. Both reports indicated prevalence of HEV infections. Literature search revealed no reports of infections of HEV in the Western Region of Ghana, and none of the previous studies also reported how infections with HEV complicates pregnancy outcomes in Ghana. Particularly in the Western Region, even though maternal mortality recorded decressed during 2014, 2015 and 2016 from 243, 272 to 200 per 100 000 live-deliverys respectively the rate is still high to warrant attention and investigation.

Pregnant women get infected everyday but unaware of that they risk complications during delivery. Wheras hepatitis E infection in the third trimester is characterized by a more severe infection, morbidity and mortality, there is no quantification of the infection to delivery outcomes, hence, the HEV etiology is often missed. Even though HEV is implicated in 50% of all VH outbreaks world-wide (Khuroo, 2016) and reports in the Ghana reports indicate data on HEV seroprevalence at sampling locations, a national prevalence indicator is yet to be determined (MOH, 2014). There are also gaps on what household and environmental factors drive the transmission of the infection in the community. There was therefore the need to bridge these knowledge-gaps and Goal 5 of the Sustainable Development Goals (Hogan *et al.*, 2010) stipulates the closing of these

gaps. This is to enhance effective surveillance, education, prevention and control of maternal and infant morbidity and mortality.

1.3 The key research questions were

i. How prevalent is HEV infection in pregnant women in their third trimester of pregnancy and in swine in the Sekondi-Takoradi Metropolis?
ii. What are the risk factors for HEV infection in the Metropolis?
iii. What pregnancy outcomes during delivery could be associated with HEV infection in the Sekondi-Takoradi Metropolis?

1.4 Objectives of the study

1.4.1 General Objective

The general objective of this study was to determine the distribution and determinants of recent HEV infection among apparently healthy pregnant women in Sekondi-Takoradi Metropolis in Ghana.

1.4.2 Specific Objectives

i. To determine recent infections with HEV in apparently healthy pregnant women in their third trimester of pregnancy and in domestic pigs in Sekondi-Takoradi Metropolis,
ii. To determine the associated socio-demographic, zoonotic and environmental risk factors for infection with HEV in Sekondi-Takoradi Metropolis, and
iii. To determine the outcomes at delivery of recent infection with HEV in Sekondi-Takoradi Metropolis.

CHAPTER TWO

LITERATURE REVIEW

2.1 Evolutionary and recent historical perspective of HEV

Purdy and Khudyakov (2010) investigated the most recent common ancestor for modern HEV and estimated that the progenitor of HEV existed between 536 and 1344 years ago. The common ancestor appears to have given rise to anthropotropic and enzootic forms of HEV, which evolved into genotypes (Gt) 1 and 2 and genotypes 3 and 4, respectively. Population dynamics suggest that Gt 1, 3 and 4 expanded in infected regions during the 20^{th} century. Genotype 1 increased in infected population size ~30–35 years ago, while Gt 3 and 4 increased in population size starting late in the 19th century until the years 1940-45. Subsequently, Gt 3 experienced additional rapid expansion until the 1960s.

The authors suggested that the effective population size for both Gt 3 and 4 rapidly declined to pre-expansion levels starting in the 1990s. Genotype 4 further experienced different population dynamics, suggesting that this genotype exhibited different evolutionary history. The author of the current study speculates that the Gt 3 spread and established in Europe in the last three decades due to the rapid growth of the swine industry to cause local HEV infections in humans. Ungulates became the carrier hosts of the Gt 4 that dominated in China and Japan to cause disease in humans, as much as the Gt 3 is currently spreading to human population in Europe.

2.2 Re-emergence of hepatitis E as a public health threat

In the three decades of 1950-1980, the Indian sub-continent particularly and to date many countries of the developing world continue to experience waves of hepatitis E outbreaks,

with genotype 1 or 2 virus, where faecal contamination of water is common. However, sporadic outbreaks with genotype 3 or 4 virus are reported in developed countries, where faecal contamination of water is less common (Khuroo *et al.,* 2016; WHO, 2014). However, the developed world experience only sporadic outbreaks of hepatitis E infection potentially from zoonotic sources (Figure 1). In the early 1980s, while the author of the current thesis was a veterinary student in the the Societ Union, hepatitis E further spread among Soviet soldiers returning from Afghanistan. There is a tell-tale narrative about how the Soviet scientist, Balayan smuggled the virus in faecal matter to his Moscow laboratory. He had mixed the infected faecal matter in yogourt, which he drank to enable viral multiplication in his own guts, in a bid to outwit his superiors. Using electron microscopy, Balayan (1983) discovered the new viral agent, HEV. Subsequently, partial cloning and full-length genome sequencing were done by Reyes *et al.* (1990) and Tam *et al.* (1991), respectively. Seven years on, in 1997 in Illinois, United States of America, the first animal strain of HEV, designated swine HEV was identified.

Irrespective of the absence of HEV reports from a number of sub-Saharan African countries (Figure 1), the author of the current study speculates that the presence of HEV infection in these countries should not be discounted. In Ghana, swine breeds have been imported from Europe to improve local breeds and potentially could be the source of zoonotic HEV variants into the sub-region. For above reasons, the detenmination of risk factors for HEV in this study potentially investigates domestic pig farms for likely source of HEV infection to humans. Hence, the investigation of animal sources of human infections of HEV is an on-going effort with reported finds in Nigeria, DRC and Cameroon, that the current study complements (Kaba *et al.,* 2010; Salete de Paula *et al.*, 2013; Modiyinji *et al.,* 2018).

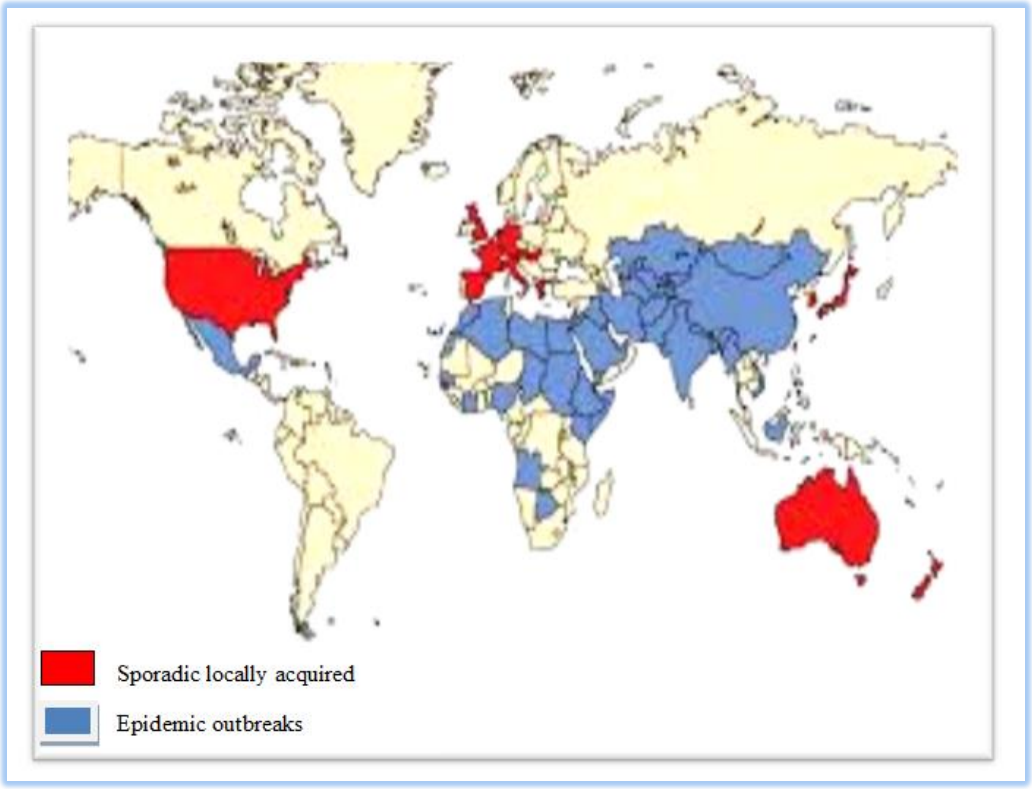

Figure 1: HEV endemic and sporadic regions of the world

The emergence of HEV infections in Africa has been catastrophic (Figure 1). Since 1979, HEV have been implicated in 17 outbreaks involving 35 300 cases and 650 deaths (Kim *et al.*, 2014). HEV infection is characterized by the relatively high mortality rate in infected pregnant women, which can reach up to 28% (Hussaini *et al.*, 1997). In Sudan, Democratic Republic of Congo (DRC) and Somalia mortalities among pregnant women have been reported (Hogan *et al.*, 2010). The course of HEV is no different in pregnancy than in non-pregnant women, the difference is that the incidence acute of liver failure complicated with hepatic encephalopathy is higher in pregnancy (Bhatia *et al.*, 2008). Military personnel and refugees were equally not spared the scorches of HEV. From 1988 to 1989, 423 military personnel in Ethiopia were hospitalized due to an outbreak of acute sporadic HEV infection (Tsega and Hansson, 1992). Outbreaks of HEV infection among Pakistani and Italian soldiers have also been reported (Alecci and Bonciani, 1997; Renou

et al., 2007). Guthmann *et al.* (2006) reported hepatitis E outbreak in southern Sudan that resulted in 126 deaths out of 8 060 illnesses. One refugee camp in southern Sudan accounted for 1 908 cases, including 39 deaths, or 71 percent of all reported new cases.

Genotypes 1 and 2, which are primarily human infections, were identified in these outbreaks with human excreta noted as the source of contamination of drinking water systems. Hepatitis E virus in humans results in 0.5-4% mortality in young adults and 15-25% in diseased pregnant women, including massive foetal loss (Bhatia and Singhal, 2008; Navaneethan *et al.,* 2008). Hepatitis E virus, which is zoonotic, has recently been reported in swine in sub-Saharan Africa (Kaba *et al.,* 2010; Salete de Paula *et al*., 2013). In sub-Saharan Africa, these reports are of concern as there is dearth of data on swine as a source of HEV transmission to humans. In Ghana, Adjei *et al.* (2009) described presence of HEV infection among pregnant women, regular blood donors and swine handlers in Accra. In Ghana, Bonney *et al.* (2012) suggested that deaths of mothers and fetus, abortion, premature delivery, or death of a live-born baby soon after delivery are common complications of hepatitis E infection during pregnancy. HEV seroprevalence in Accra was reported among pregnant women (28.6%) by Tettey *et al.* (2009) and in Cape Coast Metropolis by Obiri-Yeboah *et al.* (2018).

2.3 Genomic structure, classification and genotypic affinity of hepatitis E virus

Hepatitis E virus is a single stranded RNA, positive sense, non-enveloped, of virion size 32-34 nm and length 7.2 kb, respectively. According to Doceul (2016) the genome of HEV is made up of three open reading frames (ORF): ORF 1, 2 and 3. While the ORF 1 and 2 sligtly overlap each other, ORF 3 overlaps none of the two ORFs. The virion

belongs to the family *Hepeviridae* and of the genus *Hepevirus*. The species name of the virus is *Hepatitis E virus* (Figure 2).

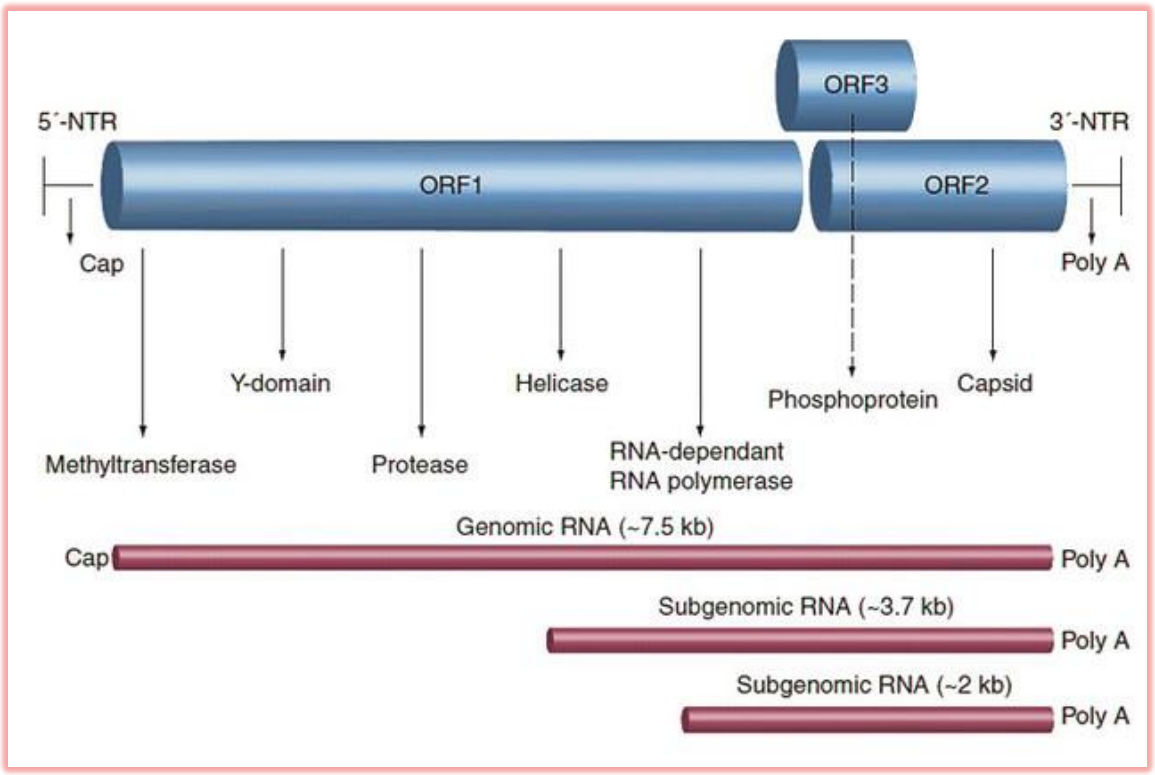

Figure 2: Structure and genomic organization of HEV. Source: Doceul (2016)

There are four mammalian genotypes: 1, 2, 3 and 4, and a fifth genotype that infected camels has recently been reported (Lee *et al.,* 2016). The four earlier known genotypes are distributed to specific geographic regions (Aggarwal and Krawczynski, 2000). HEV genotypes 1 and 2 cause acute hepatitis only in humans and are more prevalent in poorer countries where there is limited access to portable water (Meng, 2009). Huang *et al.* (2004) reported that the HEV genotypes 3 infect both humans and swine, and so is genotype 4 as was reported by Sung-Eun (2008). Household sheep, goats and rabbits have also been reported by Zhao *et al.* (2009) as reservoirs of HEV genotype 4, which also Wibawa *et al.* (2007) demonstrated in Indonesia. However it is swine and humans that

apparently share similar HEV genotypes 3 and 4 nucleotide identity Consistently, reports by Wibawa *et al.* (2007), Sung-Eun (2008) and Meng (2009) have shown that HEV from swine can infect humans. Genotype 1 and 4 are the most common subtype causing HEV infection in endemic countries such as Pakistan, Genotype 1 and 2 are endemic to Africa and Mexico, while genotype 3 predominates in the US and Central Europe.

2.4 Pathophysiology of hepatitis E

There is a complex interaction among viral, host, immunological and hormonal factors, producing a paradigm of severe liver damage in pregnancy. The maternal immune system is clearly altered to tolerate a genetically different fetus (Navaneethan *et al.*, 2008). These immunological changes promote the maintenance of the antigenic fetus in the maternal environment by suppression of T-cell-mediated immunity. There is a clear shift in the T-helper type 1 (Th1): Th2 cell paradigm during pregnancy, with a definite skew toward Th2 cells. The levels of most cytokines are depressed, particularly during the initial 20 weeks of pregnancy. CD4 counts are generally lower in HEV positive pregnant patients, while CD8 counts are higher. The CD4/CD8 ratio in these patients with fulminant hepatic failure was significantly lower when compared to HEV negative patients or controls.

Drebber *et al.* (2013) evaluated liver 221 biopsises from human patients with acute hepatitis of clinically unexplained origin. Amplified HEV RNA was detected in 7 patients of which four were sequenced and the Gt 3 determined. Histopathology revealed a classic acute hepatitis with cholestatic features and in some cases confluent necrosis in zone 3. Subtyping of liver infiltrating lymphocytes showed circumstantial evidence of adaptive immune reaction with CD 8 positive cytotoxic T lymphocytes (CTLs) being the dominant lympthocyte population. This find provided the evidence that HEV is not cytopathic but liver damage is due to immune reaction. Viral load and genotypes have also been

implicated in the severity of liver disease, and HEV viral load was found to be significantly higher in pregnant when compared to the non-pregnant.

Risalde *et al.* (2017) applied real-time PCR methods to evaluate the livers of naturally HEV infected wild boars but found no viremia. However, when the same authors used immunohistochemistry methods HEV antigen were detected. Further evaluation found the evidence of infection, mainly in Kupffer cells and liver sinusoidal endothelial cells, without apparent associated hepatitis lesions. in evaluated the histopathology of that were non-viremic by RT-PCR.

2.5 Possible risk factors for HEV infection

Farshadpour *et al.* (2018) reported that pregnancy is a potential risk factor for viral replication, especially of HEV genotype 1, which is also the result of extreme low immune status of women during pregnancy and particularly among Indian/Asian and African pregnant women. Mortality rates among pregnant women, especially those infected in the third trimester, have ranged between 30% and 100% (Gautam *et al.,* 1918). It has been reported that a significant proportion of pregnant women with acute hepatitis E (up to 70%) progress to acute liver failure with a short pre-encephalopathy period, rapid development of cerebral edema and high occurrence of disseminated intravascular coagulation (Khuroo *et al.,* 2003).

Several epidemiological studies have identified age of persons (Divizia *et al.,* 1999; Daniel *et al.,* 2004) and of swine (Di Bartolo *et al.,* 2011); sex (He *et al.,* 2009; Said *et al.,* 2009); and location either in the urban and local settings (Divizia *et al.*, 1999; Lin *et al.,* 2004; Dong *et al.,* 2007) as risk factors for HEV infection. Pregnancy (Tsega and

Hansson, 1992; Tsega and Krawczynski, 1993), and in a case-control study the eating of undercooked deer meat (Yamatani *et al.,* 2004) were also identified with increased risk for HEV in infection. However, there was no significant difference in age and overseas travel for HEV infected Brazilian blood donors (Bortoliero *et al.,* 2006). Eight (17.7%) of the subjects but only one (2.2%) of the controls had measurable serum anti-HEV IgG levels (P=0.014). Transmission of infection may occur postpartum, especially in the presence of acute maternal disease (Wang and Chibber, 2004). Blood borne transmission in blood donation and transfusion is a potential risk factor (Santiago *et al.,* 2017; Elduma *et al.,* 2017).

A systematic review of the evidence on risk factors and transmission routes of autochthonous HEV infection and hepatitis E was conducted in Europe in order to develop recommendations for future research, prevention and control (Lewis *et al.,* 2010). The study reviewed primary reports and studies published during 1998–2008 on hepatitis E infection in humans and animals in Europe. Each of the 106 included studies was categorized into one of three evidence levels (EL) based on study design and diagnostic methodology. Persons with autochthonous hepatitis E infection were on average older than the general population and predominantly male. Another study reported no significant associations between anti-HEV incidence and demographic or socioeconomic factors in an 18-month follow-up of apparently healthy persons (Labrique *et al.,* 2010). Few of the seroconverting subjects reported hepatitis-like illness. Overall incidence was calculated to be 64 per 1 000 person-years, with 1,172 person-years followed.

Another study concluded that drinking faecal contaminated water and exposure to infected swine were risk factors for HEV infection (Galiana *et al.,* 2010). A British study

determined whether coming in contact with people with jaundice, eating shellfish (Formiga-Cruz *et al.*, 2002), eating uncooked animal products, keeping pets, and contact with live swine were risk factors for hepatitis E (Withers *et al.*, 2002). However, zoonotic transmission seemed likely and person-to-person transmission appeared inefficient to cause clinical disease. In North Carolina, USA, it was reported that swine workers had 4.5-fold higher anti-HEV prevalence (10.9%) than the controls (Withers *et al.*, 2002). Indeed, the zoonotic risk of HEV should not be limited to developed countries (Wibawa *et al.*, 2004).

However, the evidence is not always available and the contrary evidence has been described. In a Northern Ugandan case control study (Howard *et al.*, 2010), storage of drinking water in large-mouthed vessels (adjusted odds ratios [AOR] = 2.83; 95% confidence interval [CI] = 1.16–6.94) and washing hands in a group basin (AOR = 1.90; 95% CI = 1.07–3.38) were associated with HEV infection. HEV RNA was detected from communal hand-rinse and surface water samples. The epidemiologic and environmental water-testing results suggest that household-level factors played an important role in the transmission of HEV—modalities that have been previously under-appreciated (Howard *et al.*, 2010). Furthermore, there are evidences indicating that higher steroid hormone levels, as presented during pregnancy, may influence viral replication and acute liver damage.

2.6 Epidemiology of zoonotic HEV

Infections with HEV in animals are globally distributed. The IgG anti-HEV has been reported in dogs in Vietnam and India, in cows from India, Somali, Tajikistan, Turkmenistan, and Ukraine, and in sheep and goats from the USA (Tien and Clayson,

1997; Arankalle and Chobe, 2003; Ruggeri *et al.,* 2013). However, the source of infection for these animal species could not be definitively identified. Nevertheless, the existence of a population of animal species positive for IgG anti-HEV suggests that swine may not be the only animal reservoir for HEV. While HEV genotypes 3 and 4 may spread from domestic swine and boars to humans, swine and human HEV isolates from the same geographic region are genetically and anti-genetically more similar to each other (Wibawa *et al.*, 2004) than they are to swine or human HEV isolates from other regions (Takahashi *et al.*, 2003; Clemente-Casares and Pina, 2003).

The potential for zoonotic transmission and cross-species infections with HEV genotypes 3 and 4 have been demonstrated experimentally (Emerson and Purcell, 2004; Meng, 2009). Several lines of evidence indicate that, in some cases involving HEV genotypes 3 and 4, animal to human transmissions occur (Mizuo *et al.,* 2002; Meng and Wiseman, 2009). Furthermore, individuals with direct contact with animals are at higher risk of HEV infection (Chang *et al.,* 2009). When the genotypes of swine HEVs identified in a Korean study were determined, these were all grouped into genotype 3. They were further subdivided into subtype 3a together with human and swine HEVs isolated in the U.S.A. (Sung-Eun, 2008). In New Zealand, anti-HEV IgG seroprevalence was 4% in human blood donors (Dalton *et al.,* 2007). However, in Bali, 20% anti-HEV antibodies were found in apparently healthy individuals (Wibawa *et al.,* 2004), in remarkable contrast with 4% and 0.5% in two other sub-districts. In 2007, the same authors identified genotype 4 hepatitis E virus strains from a patient with acute hepatitis E and farm swine in Bali, Indonesia. As indicated earlier, genotype 1 and genotype 2 mammalian strains are restricted in humans and mainly associated with large waterborne epidemics in endemic regions (Tyagi and Surgit, 2004; Lu and Hagedom, 2006). Genotype 3 and 4 mammalian

HEV strains are found both in human and other mammalian reservoirs (swine, wild boar, deer, mongooses) and are mainly responsible for sporadic cases of hepatitis E. However, swine HEV is considered to be a new zoonotic agent due to its close genomic resemblance to the human HEV and its ability to infect nonhuman primates (Zhang *et al*., 2004; Liu *et al.*, 2015).

A nested reverse transcription (RT)-PCR was developed by Choi and his cohorts to detect a part of the swine HEV open reading frame 2 (Choi *et al.*, 2004). Three Korean isolates of swine HEV were identified in 128 swine sera (2.3% prevalence) by this nested RT-PCR method. They were isolated from 2- to 3-month old swine showing an age-specific prevalence of HEV viraemia. A phylogenetic tree analysis with a number of swine and human HEV isolates indicated that these Korean swine isolates belong to genotype III. They were closely related to the swine and human HEV isolates that were identified in the United States and Japan. In addition, they formed a distinct branch in genotype III, showing a 92.7 to 99.8% identity at their nucleotide sequences.

In China, high prevalence of 31.6% and two times more risk of infection in US swine-veterinarians has been reported among swine handlers (Meng, 2009). Eighteen per cent (18%) of blood donors in Korea were found to be positive for HEV antibodies (Choi *et al.*, 2003). A Japanese study has determined that the faeco-oral route is likely to cause HEV transmission among swine (Bouwknegt and Teunis, 2010). There are few studies on zoonotic transmission of HEV in sub-Saharan Africa. Sources for human hepatitis E virus (HEV) infections of genotype 3 and 4 are largely unknown in Africa; even though recently the genotype 3c was reported in swine in the Democratic Republic of Congo and then later in the Cameroon (Kaba *et al*., 2010; Salete de Paula *et al.*, 2013). In Ghana,

Adjei *et al.* (2009) reported a high seroprevalence of HEV infection among swine handlers (38.1%) and blood donors: Anti-HEV IgG and anti-HEV IgM were 25.6 and 45.9%, respectively.

2.7 Diagnosis of HEV infection

Serological and nucleic acid tests for detecting hepatitis E virus (HEV) have been developed for both epidemiologic and diagnostic purposes. The laboratory diagnosis of HEV infection depends on the detection of HEV antigen, HEV RNA, and serum antibodies against HEV (immunoglobulin [Ig]A, IgM, and IgG). The commonly used tests for HEV infection include detection of IgM and IgG anti-HEV antibodies and detection of HEV RNA (Chobe and Chadha, 1997). IgM anti-HEV antibodies can be detected during the first few months after HEV infection, whereas IgG anti-HEV antibodies represent either recent or remote exposure (Agarwal, 2013).

Because HEV infection is rare, doctors will typically test for other conditions first. A physical examination and blood tests cannot confirm presence of HEV.According to the Expert Review in Molecular Medicine (*www-ermm.cbcu.cam.ac.uk*; accessed on 14th September, 2011), a first test will measure whether liver enzymes of aspartate aminotransferases (AST) and alanine aminotransferases (ALT), particularly ALT are elevated. This could be followed by serological tests to measure the presence of HEV antibodies and molecular assays for HEV nucleic acid detection could also be performed (Chobe and Chadha, 1997).

2.8 Clinical signs and symptoms

No specific clinical signs exist for hepatitis E infection. According to the WHO Technical Report (2014), cases of hepatitis E are not clinically distinguishable from other types of

acute viral hepatitis. Hepatitis A virus (HAV) and HEV are almost indistinguishable clinico-epidemiologically (Chandra and Sharma, 2010). Liver cancer and congestive heart failure also produce similar symptoms. A Chinese study (Zhang *et al.*, 2011) reported that of 210 HEV patients, 85.2% were male and the most common clinical symptoms were jaundice (85.7%), fatigue (70.5%) and anorexia (64.8%). Patients presenting with jaundice, vomiting, hepatalgia, asthenia, hepatomegaly, or distended abdomen with no signs of uncomplicated malaria in tropical developing countries are suspects (Naik and Aggarwal, 1992). During the Central African Republic outbreak (Hirsch, 2011), the most frequent clinical signs found were jaundice (93.4 percent), vomiting (50.7 %), hepatalgia (47.4%), hepatomegaly (30.9 %), and asthenia (26.8 %), which are the general clinical signs of hepatic disease.

2.9 Laboratory detection of HEV infection

Enzyme-linked immunosorbent Assays (ELISA) for the detection of total HEV immunoglobulins M and G (IgM &/or rising IgG) together and/or separately are important markers for recent and previous exposuresfor HEV diagnosis, respectively. The test for anti-HEV IgM and IgG are based on indirect ELISA method using purified activated recombinant HEV antigen (ORF2, ORF3), coated on the multi-wells plate. The horseradish peroxidase (HRP) conjugated mouse anti-human IgG (r chain) and IgM (μ chain) monoclonal antibodies serve as tracer. A chromogenic substrate, 3,3',5,5'-Tetramethylbenzidine (TMB) is catalyzed by HRP and produces a color change, and the intensity of the absorbance at 450 nm indicates the presence or absence of anti-HEV IgM or IgG antibodies in the sample.

Sensitive molecular assays are used for the detection and characterization of HEV nucleic acids. The sensitivity and specificity of the tests for IgM and IgG are 100.0% and ≥95.0%,

respectively. Considering the heterogeneity of the HEV strains circulating in humans and animals, these assays enable detection of the various HEV strains. Katano *et al.* (2011) reported of a novel real-time PCR system for the simultaneous detection of human viruses in clinical samples from pregnant women with uncertain diagnoses. Others include real-time RT-PCR assay such as the Jothikumar assay (Jothikumar *et al.,* 2006) for the detection of HEV nucleic acids in human and swine samples with high detection capabilities, even for suspected cases when the clinicals are not evident; 98% sensitivity and 87% specificity, respectively.

2.10 Immune prophylaxis and vaccination

Immunoglobulin prepared from donors in non-HEV-endemic countries did not prevent infection (Purcell and Ticehurst, 1988). Studies have been conducted on the development of vaccines against hepatitis (Xing *et al.*, 1999; Yarbough *et al.,* 1999). Experimental immune prophylaxis against HEV based on recombinant antigens appears to confer short-term protection (Agrawal and Gupta, 2001; Wang and Zhuang, 2004). Immune prophylaxis be useful for pregnant women in endemic areas and travellers coming into these regions (Worm and Wirnsberger, 2004; Shrestha *et al.,* 2007). Currently, a new vaccine, *Hecolin,* has been approved by China‘s State Food and Drug Board, and awaiting prequalification authorization from WHO (Soo, 2012). Vaccine safety and effectiveness is yet to be established for pregnant women. However, Cooper *et al.* (2017) estimated that reactive and pre-emptive vaccinations in refugee camps that included groups for whom the vaccine is not currently licensed led to the mean reduction in mortality from 35 to 65% to 66 to 82%.

2.11 Management of HEV infection

According to the WHO Global Alert/Response guidelines (WHO, 2011), surveillance and control procedures should include: provision of safe drinking water and proper disposal of

sanitary waste, monitoring disease incidence, determination of source of infection and mode of transmission by epidemiologic investigation, detection of outbreaks and spread containment. Recommendations include a patient should stay hydrated, avoid alcohol, and stay away from medicines that can damage the liver (WHO, 2011). Don't overexert, and try to rest as much as possible. Don't become sedentary: stay active and engage in forms of mild exercise. As there is no cure for HEV, treatments involve lifestyle changes‖. It is unknown the contribution of household hygiene practices to HEV infection during pregnancy and how the infection impacts pregnancy in Ghana. Hepatitis E infection could be diagnosed by liver function tests in health institutions. No treatment or commercial vaccination is available in Ghana. Also, clinicians/physicians have no experience with clinical management of hepatitis E and apparently are not able to deal with suspected cases (Adjei *et al.*, 2009). Early detection of infection could be achieved by surveying asymptomatic pregnant women for viremia. For the time being, although there is no consensus on how to treat patients with HEV infection in pregnancy, early delivery of the fetus, if possible, should be attempted, to prevent maternal mortality.

2.12 Tests for geographical dependence

Tests of geographical dependence in a set of environmental data is important to account estimates based on such data. According to Webster (2001) the variogram is used for the geoanalysis of data with geographical dependence, which is ideal for visual inspection (Pfeiffer, 2008). Mathematitically, a variogram is defined, as:

$$\gamma(\Delta x,\Delta y)=1/2\varepsilon\left[\{Z(x+\Delta x, y+\Delta y)-Z(x,y)\}^2\right]$$

where Z(x,y) is the value of the variable of interest at location (x, y), and Ɛ [ ] is the statistical expectation operator. Note that the variogram, ɣ( ), is a function of the separation between points (Dx, Dy), and not a function of the specific location (x, y).

According to Barnes (Golden Software, Inc) the variogram is statistically-based, quantitative description of spatial continuity or roughness of a data set. Ordinary one dimensional statistics for two data sets may be nearly identical, but the spatial continuity may be quite different. Variogram analysis consists of the experimental variogram calculated from the data and the variogram model fitted to the data. The experimental variogram is calculated by averaging onehalf the difference squared of the z-values over all pairs of observations with the specified separation distance and direction. If there are n observed data, there are n(n - 1)/2 unique pairs of observations. For each of these pairs we can calculate the associated separation vector when plotted as a two-dimensional graph.

The variogram model is chosen from a set of mathematical functions that describe spatial relationships, which can be assessed by visual inspection (Pfeiffer, 2008). The appropriate model is chosen by matching the shape of the curve of the experimental variogram to the shape of the curve of the mathematical function. To account for geometric anisotropy (variable spatial continuity in different directions), separate experimental and model variograms can be calculated for different directions in the data set. In the presences of spatial autocorrelation, the subsequent step in the geospatial analysis procedure is to conduct krigging. Kriging is an advanced geostatistical procedure that generates an estimated surface from a scattered set of points with z-values. As mentioned, kriging is most appropriate when you know there is a spatially correlated distance or directional bias in the data. Krigging has been the domain of soil science and geology, and currently finding various applications in public health (Auchincloss *et al.*, 2012). The R geo, GS+ version 10 (Gamma Design, Michigan, USA), ArcGIS and Surfer (Golden Software, Inc) are three programmes available to this researcher used to calculate and plot experimental variograms. The GS+ demonstrative graphical interfase is shown in Appendix 6.

CHAPTER THREE

GENERAL MATERIALS AND METHODS

Chapter three presents the general materials and methods of this study and thereafter outlays the section by each objective.

3.1 Study area

The Sekondi-Takoradi Metropolis (STM) is the study area for this research, and shown are communities and road network (Figure 3). The metropolis is the fastest growing industrial and business hub of the Western Region (WR) and for Ghana.

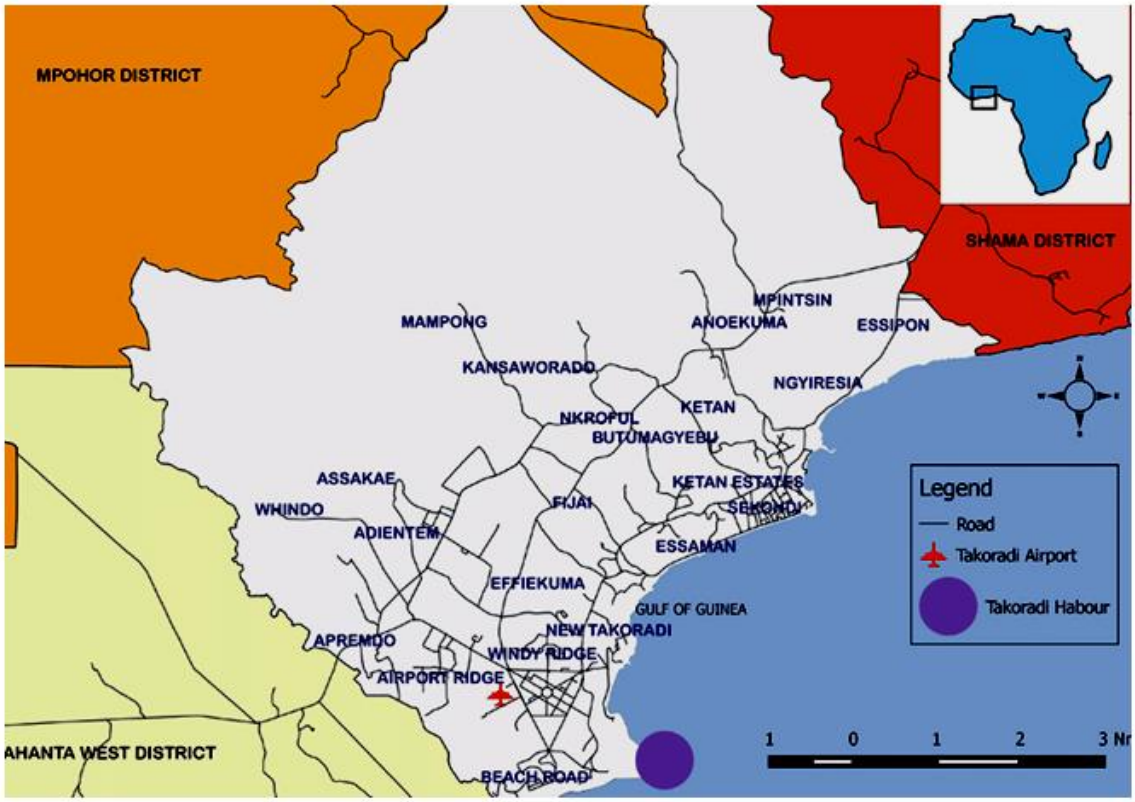

Figure 3: Sekondi-Takoradi Metropolis of Western Region of Ghana

Geographically, Ghana is bounded to the West by Cote D'Ivoire, to the East by Togo, to the North by Burkina Faso and to the South by the Atlantic Ocean. The country lies

between latitudes 4° and 12°N, and longitudes 4°W and 2°E of Greenwich Meridian with a total surface area of about 238 535 km^2. The climate is tropical and there are two main rainy seasons in the South from May to June and from August to September. In the North, the rainy seasons merge. Ghana is divided into 10 political regions with 216 districts. Ghana's population figure as at the last census in 2014 was approximately 27 million. The official language and *"de facto lingua franca"* of Ghana is the English language. There are over seventy different tribal groups, each with its own distinct languages; however eleven of the languages have the status of government-sponsored languages.

The WR is the most westerly situated region in Ghana and lies between 4.88° (4° 53' 0") North, 1.75° (1° 45' 0"). The Western region with a total human population of 2 376 021 (2010 census) and still increasing within the total land area of 23 921 km^2 Western Region is the fastest growing region in terms of population due iimigration as a result of the new oil and gas industry and increasing pig production.

The Sekondi-Takoradi Metropolis (STM) lies between the geographical coordinates of 4.88° (4° 53' 0") North and 1.75° (1° 45' 0"). The STM is Western Region's largest city and an idustrial and commercial centre, with a population of 445 205 (2012). There rapid increase in urban population due to the influx of the citizenry in search for better conditions of life has resulted in poor environmental conditions in most urban and peri-urban settlements in the country. Municipal solid waste management (MSW) for that matter has become problematic within Sekondi-Takoradi Metropolis.

The STM has been selected for the study because the Greater Accra and Central regions, which are two regions of east of the Western Region have HEV-prevalence studies in pregnant women reported. The three regions represent of the four coastal regions of Ghana and a holistic view of viral hepatititis infections and hepatitis E in particular is

important for control and preventive programming. Additionally, maternal performance indices during the years 2014, 2015 and 2016 are relatively high to warrant concern. The region is administratively divided into twenty-two districts of which the STM is the location of the regional and referral hospital, the Efia Nkwanta Regional Hospital (ENRH) and three other satellite public hospitals from where the pregnant women were selected for the study.

3.2 Operational definitions

Acute viral hepatitis E is defined in a pregnant woman with a positive anti-HEV IgM and or molecular reverse transcription-polymerase chain reaction (RT-PCR) test without a clinical jaundice.

Asymptomatic pregnant women are pregnant women with acute phase infection with HEV as measured by the presence of anti-HEV IgM/HEV ribonucleic acid (RNA) and without manifestations of acute hepatitis.

A pregnant woman classified with complicated delivery must have satisfied both condititions: a) returns from delivery with diagnoses other than Normal Delivery; b) not given delivery by spontaneous vaginal delivery.

The Euclidean distance (E) is the metric of the distance, in a straight line from the household location of a pregnant woman to potential risk locations of infection with HEV, as were identified in this study. Prevalence of HEV infection is defined in this study as the seroprevalence of anti-HEV IgM immunoglobulins, which are the markers of acute phase infectionThe outcomes of pregnancy as defined in this study are the diagnosis at delivery as recorded in ward discharge books as well as captured in the regional hospital database.

3.3 Ethical considerations

The human and animal research protocols were approved for the study, by the Noguchi Memorial Institute for Medical Research Institutional Review Board (NMIMR-IRB, CPN 088/15-16 IORG 0000908 of March 8, 2016) and the Noguchi Institutional Animal Care and Use Committee (NIACUC, 2014-01-1E), respectively (Appendix 2). Each pregnant woman gave a written informed consent to participate in the study. The Metropolitan Director of Health Services gave a written approval for this study to be undertaken at the four health care institutions. Each pregnant woman signed an informed consent to participate in this study. The owners of the domestic pig farms gave verbal consent for sampling on their farms for this study.

3.4 Study one

3.4.1 Human study population

The human study population consisted of pregnant women in their third trimester of pregnancy. Included in this study were pregnant women who registered at any one of the four antenatal clinics at the only four public healthcare facilities in the Sekondi-Takoradi Metropolis (STM). Their registration was 6 months prior to the start of this study in April, 2016.

3.4.1.1 Study design and sample size

This research was a cross-sectional study is based on the Kish Lisle formula:

$n = Z^2p\ (1\text{-}p)/d^2$

The formula was described by Thrusfield (1995) and applied for the calculatio of the maximum samples required for the determination of seroprevalence of HEV in the study area.

Hence, the sample size of 350 was calculated by using the following: {(Z = 1.96, 95% CI, d = 0.05 and p, the expected prevalence = 65 % (Adjei *et al.*, 2009)}. To make up for the anticipated losses to due to possible attrition, first, and to get enough samples for molecular studies, the size of 700 was decided upon and 699 blood samples were collected. This number was considered adequate also because not all of the women reported to give delivery at the sampling centres at the time of labour; to the Effia Nkwanta Regional Hospital (ENRH), Takoradi Hospital (TH), Effia-Kwesimintsim Hospital (KH) and Essikardo Hospital (EH) where they were sampled. Having timely access to their delivery records was important to minimize missing data on account of them delivering at different location and, or changing their recorded locations of residence. Pregnant women who reported at other facilities other than ENRH did not have their pregnancy outcomes recorded. This was to avoid classification bias of diagnoses at delivery. Because the hospitals have different electronic systems of record keeping that did not allow for the verification and validiation of diagnosis. Secondly, there was the need to pool blood.

3.4.1.2 Sampling technique and selection of pregnant women

The pregnant women were selected by a non-probability sampling using the purposive technique as described by Thrustfield (1995). Those who did not fit the inclusion criteria were removed and replaced by the next on the list.

Pregnant women included in the study were apparently healthy women, presumably asymptomatic for infection with HEV. Selected were pregnant women in their 3rd trimester, who must also have attained the legal age set in Ghana of 18 yrs and above, attending any of the 4 antenatal clinics from April to September, 2016. Also included were those pregnant women who agreed to the visit to their homes for the purpose of geo-

referencing using the GPS machine *etrex* version 10. Excluded were the study participants who were routinely screened and negative for Hepatitis B, C, PLW-HIV/AIDs, and those who reported sick and 6-months and more at current abode of residence. Excluded were also pregnant women who failed to report on their next antenatal scheduled dates after their initial selection.

Pregnant women (360) who met the inclusion criteria and consented were randomly sampled from the 699 sampled for the serological study. Subsequently they were georeferenced during homestead visits. The participants were consecutively selected as they waited at Antenatal clinics (ANC) to receive routine antenantal care services. They gave informed consent and were selected according to the set inclusion criteria and have signed consent forms.

3.4.1.3 Duration, data collection and storage

Data collection began in April 2016 and ended in April 2017. Blood samples, 5 ml per subject were collected in the **arm** by trained phlebotomists into sterile vacutainer tubes. Serum was extracted, labeled and ice-packed at -20 °C and stored at the initial collection facilities and thereafter shipped to the Department of Virology for testing and for longer storage at -80 °C. Socio-demographic and household data of the pregnant women were collected using a structured questionnaire instrument after pre-testing at a near-by antenatal clinic. Data was then entered into a coded database that was developed for the study. Data of cases that were found to be incomplete were eliminated and not included in the analysis. In the absence of available secondary global positioning system (GPS) data of household locations of the pregnant women, georeferencing of study participants was conducted by visiting individual homes and geographic coordinates were collected using a

GPS instrument (Version etrex 10)(Garmin, USA). Geographic coordinates were entered into Quantum Geographical Information System (QGIS, Finland) and Arc GIS and maps generated.

3.4.2 Detection of HEV infection in pregnant women

3.4.2.1 Serological analysis

For serological analysis a commercial ELISA kit (recomLine IgM/IgG, Mikrogen, Germany) was procured for the detection of anti-HEV in human. Samples were tested for infection with HEV by immunoblot for the detection of IgG and IgM antibodies, based on the recommended procedures of the manufacturer. In the first step, test strips of highly purified recombinant HEV antigens presented as nitrocellulose membranes were incubated at room temperature. The strips rocked gently for specified time intervals while the diluted serum and the specific antibodies (Ab) bind to the pathogen antigens on the test strips. Unbound antibodies were then flushed away under a running tap water.

In the second step, the strips are incubated with anti-human immunoglobulin Ab (IgG or IgM), which are coupled to horseradish peroxidase. Unbound conjugate Ab are then fushed away. Specifically bound Ab are detected with a staining reaction catalysed by the peroxidase. When an antigen-antibody reaction took place, a dark band appeared on the strip at the corresponding point (Figure 4).

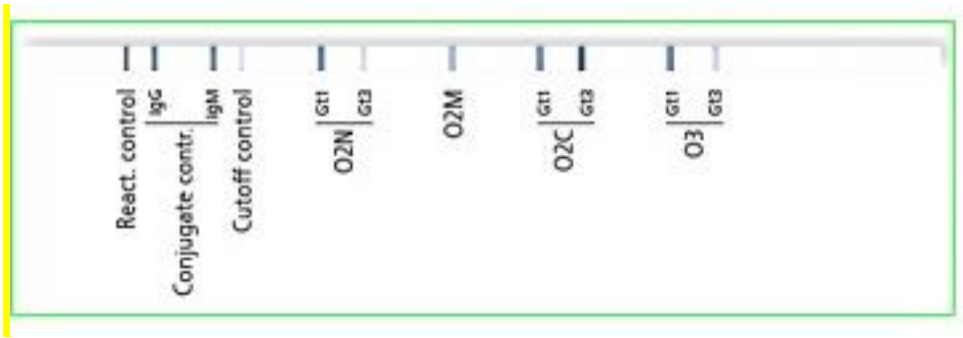

Figure 4: Test evaluation based on algorithm of antigen impregnated test strips (Mikrogen, Munchen, Germany)

Test performance was assessed by individual allocation of weigted points based on which specific location a dark band appeared on a test strip. The specific locations on a strip were designated by the manufacturer as: 02N, O2C, O2M and O3, each representing an Open Reading Frame (ORF) of the HEV genome. Each designation represented the presence of specific antigen, however, only an IgM or an IgG could be evaluated by a test at a time on a test strip depending on wherther an IgM or IgG conjugated was added to a test strip. For the purpose of detecting a marker of active HEV infection in human sera the evaluation was based on IgM detection only. Hence, 02N gained 1 point; O2C, 4 points; O2M, 1 point and O3, 2 points. A test strip for a particular sample was assessed as a negative result when the sum of points = $\leq$ 3; as a borderline result when the sum of points = 3 and as a positive result when the sum of points = $\geq$ 4. Generally, because HEV has one serotype the test can determine any of the genotypes present in a serum sample. Evaluation of a positive case was by the template of an recombinant-derived antigen-impregnated strips from ORF 2 and ORF 3 genes of Gt 1 and Gt 3 HEV:

The sensitivity and specificity of the kits are 96.6% and 97.1% for samples from asymptomatic infection, respectively. A number of the test runs were repeated for consistency in the results. After reading the results from the strips representing the positive and negative reactions, the strips (Appendix 3) were archived in cellophane for reference.

3.4.2.2 Molecular analysis

For molecular analysis all the 81 serum samples that were positive for anti-HEV immunoglobulin M (IgM) were extracted of their ribonucleic acid (RNA) using the QIAmp Viral RNA minikit protocol as described by the manufacturer (Qiagen, Hilden, Germany). The extraction process was carried out in the Biosafety Class 2A cabinet after

samples were equilibrated at room temperature of 20 °C. The process involved lysis of viral cells in 560 µl Buffer AVL-carrier RNA solution into which 140 µl of sample was added and pulse-vortexed. Subsequently, 650 µl absolute ethanol was added to complete the lysis process. Then 630 µl of the solution was added to the QIAamp Mini column and centrifuged at 8 000 rpm for a minute salvage the raw RNA. Thereafter, in a two-step washing process involving the addition of 500 µl wash buffer (Buffer AW1 and Buffer AW2). The process was completed by the addition of 60 µl Buffer AVE and centrifuging to collect the extracted viral RNA after an incubation at room temperature. The RNA samples were stored at -30 °C for one month or -80 °C for longer period.

To ascertain the yield of the extracted RNA samples 18 were randomly selected from the 81 extracted RNA samples. The RNA purity and concentration was quantified by A260/280 measurement using a ND-2000c NanoDrop spectrophotometer (Nanodrop Thermao Scientific, USA). The RNA parameters were found to be satisfactory at 50 µl/l and A 260 (Appendices 3 and 4). Further, 600 serum samples representing 86% of 699 serum samples that were collected from the pregnant women were ramdomly selected and extracted of their RNA to undergo amplification procedures.

The commercial assay (ampliCube HEV 2.0, Mikrogen, Germany) was used for HEV RNA detection by real time transcription PCR. A master-mix procedure and addition of the extracted RNA were performed according to protocol of the manufacturer Real-time RT-PCR analyses were conducted using the Applied Biosystem 7 500 Fast Real-Time PCR System, USA. The manufacter's procedures, using the recommended protocol and assays procured for this study were followed during the analysis. To ensure that the nucleic acids isolated from the sample did not contain any PCR-inhibiting substances, samples were subjected to an internal control prior to isolation. The internal control was

transcribed to compliment deoxyribonucleic acid (cDNA), amplified and detected in the RT-PCR preparation. In this way, false negative results die to inhibition of the RT-PCR reaction can be excluded. Probes for the specific detection of HEV RNA were marked with the reporter dye FAM. Probes for the detection of the internal control were marked with ATTO 647N, however, this dye was not available for use. The amplification process involves the use of specific primer and marked probes for the amplification and detection of HEV RNA of the Gt 1, 2, 3 and 4 (Table 1).

Table 1: The forward and reverse primers together with probe used in qRT-PCR assay

Oligonucleotide	Sequences (5'-3')	Assay
Hep-E-F	CGGTGGTTTCTGGGGTGAC – 3'	Real-time PCR (primer)
Hep-E-R	5'- AGGGGTTGGTTGGATGAA - 3'	Real-time PCR (primer)
Hep-E-P	6-FAM 5'--TGATTCTCAGCCCTTCGC- 3' TAMRA	Real-time PCR (probe)

GenBank M73218, nt 5261-5330, Amp = 70 bp, (Jothikumar *et al.,* 2006)

Hence, a slight modification to the RT-PCR protocol was done by the replacement of the reporter dye ATTO 647N of 660 nm with the Cy 5, which has similar wavelength detection range as the recommended dye. This was because the recommended dye was not readily available at laboratory. A test run was repeated to ensure that the same results were obtained.

3.4.3 Nested study of swine population for HEV infection

It is known that swine are infected by the genotype 3 (Gt 3) HEV that equally infect humans to cause chronic hepatitis, fibrosis and liver cancer in humans. Hence, a nested study was carried out to find out the HEV-infection status of swine. Swinefarms from two

of the four sub-metropolitan districts were purposively selected due to concerns of accessibilty from where the pregnant women were sampled. The were subsequently georeferenced.

The swine population selected are located on small-scale farms. They are of average holdings of 17 animals per farm and the farms are built near large gutters, streams and drains. Effluent from these farms flow freely southward to serve the four major wetlands located in each of the four sub-metropolitan areas into the Atlantic ocean. Included were swine of all production groups and breeds. The breeds are large-white, landrass, the large Ashanti black and their cross-breeds. Swine less than 1-month of age and pregnant swine were excluded because of previous reports that maternal antibodies neutralize viral particles and also to avoid spontaneous abortions during handling, respectively (Kunio and Hiroshi, 2007). Production data and *immunoLine* strips from serological reactions have been stored manually in a book file and electronically archived.

3.4.3.1 Sample size determination of swine

To the best experience and knowledge of the researcher no swine study for HEV in Ghana has been reported. However, Owolodun *et al.* (2014) reported anti-HEV IgG of 36% in swine in the Delta state of Nigeria, which share apparently similar riverine ecological features as the study area in the Sekondi-Takoradi Metropolis of Ghana. Hence, the prevalence p = 36% was used for the calculation of the sample-size determination in this cross-sectional study. The Kish Lisle formula described by Thrusfield (1995):

$N = Za^2 * p\ (1-p)/d^2$, was applied {Using , $Za^2 = 1.96$, p = 36%, (100-p) = 64% and d, the precision was decided upon as 7.2% (20/100*36)}. For this cross-sectional study involving swine, both the exposed and unexposed women were derived from the same

population at risk of HEV infection as such no other subgroups were required. The sample size of 170 at 95% CI (162.8 – 177.2) swine was calculated and arbitrarily rounded up to 200.

The selection of swine pens was by probability proportional to size method (www.who.int/tb/advisory_bodies/impact), assessed 15th July, 2013) were used for sampling the swine. The sample frame of the number of swine involving, young swine, boars, growers and sows were listed from the swinestirs, each with their corresponding animal sizes. A cumulative sum for the swine population which is equalled the sample frame was generated. A sampling interval (SI) was calculated by dividing the total population (sample frame) by the sample size. A random number between one (1) and the value of the sampling interval was generated to get the random start (RS) value. The cumulative total column was examined until the last number found that is equal to or greater than the randomly selected number. The swinesty containing that number was selected. The random start number and the sampling interval were used to compute the sampling series in the sequence: RS, RS+SI, RS+2SI, RS+3SI, RS+4SI. This series were used to select the corresponding swine pens until the sample size for the study is attained.

3.4.3.3 Sampling of swine

To select individual swine the selected pens were visited by the investigator together with the farm owner. The swine were examined to determine those that satisfied the inclusion criteria. The owner counted the swine loud to allow for the assignment of a temporary identification number to each swine. After counting, the selected swine were examined and specimens collected. The process was repeated at each of the selected pens until the required sample size is reached.

3.4.3.4 Detection of HEV infection in swine sera

To test the hypothesis that swine from the sample study area were previously exposed to HEV, serological and virological methods as previously described in the previous section were applied to the 200 swine sera randomly selected for the quantification of HEV infection This was possible because both human and animal sera cross react to the selected assay. It is known that HEV is a single serovar virus and higly conserved between different HEV strains (Gt 1 to 4). Sera was harvested from 5 ml of blood collected from the jugular veins of the pigs and the manufacturer's instructions were used during the conduct of the analysis. However, for the swine HEV detection IgG instead of IgM was evaluated, particularly, due to the limited availability of funds to procure more test kits. A random qRT-PCR analysis was done on a number of the swine samples to detect swine HEV. In the second round of the 2-step process, final PCR products were checked for appearance of possible bands by procedure of a 2% agarose gel electrophoresis.

3.5 Study 2: Association of HEV infection with socio-demographic and household factors

Logistic regression (LR) was used to predict the probability that an observation falls into one of two categories of a dichotomous dependent variable based on one or more independent variables that were either continuous or categorical (Peng, 2002). For a single exposure variable E, the LR model takes the form

Natural logarithm, In {p/1-p} = a + bx (1)

For n explanatory variables entered in the LR model $X_1, X_2, \ldots, Xn$,

Probability, $p = 1/1 + \exp\left(-a - \sum_{j=1}^{n} b_j x_j\right)$ (2)

Substituting x = 1 in Equation (1) gives a + b, or exp (a + b) for the odds.

Similarly, the odds of the outcome given no exposure is exp (a)

Thus, the Odds Ratio, OR = exp (a + b)/exp (a) = exp (b)

The odds ratio was the preferred measure of association used for the analysis because it is valid for cross-sectional study type and any level of risk investigated in this study.

By extention, for n explanatory variables entered in the LR model X_1, X_2, …, X_n, the coefficients of the explanatory variables = B_1, B_2, … Bn.

In((y/1-y) = $B_0 + B_1*X_1 + B_2* X_2$……..+ $B_n* X_n$

By generalization, the parameter B in any derived logistic model is interpreted directly as the (natural) logarithm of the odds ratio.

Analysis was done by choosing one of the exposure levels as the referent exposure, and computing the odds ratio for each other exposure with respect to it. A separate confidence interval was computed for each odds ratio. For the purpose of this study, the lowest risk group was chosen as the referent category, so that the OR are greater than 1.

3.5.1 Modelling of HEV infection

A logistic regression model of Paul (2005) was assessed in which the response variable is either negative infection with HEV or a positive infection, with the dummy variables of 0 and 1, respectively.

Log((y/1-y) = $B_0 + B_1*X_1 + B_2* X_2$……..+ $B_n* X_n$

B_1 to B_n = coefficients of the explanatory variables, X_1 to X_n = explanatory variables entered in the logistic regression model.

In this study, Log(y/(1-y) = the odds of infection with HEV or not. Where: X_1 = Antenatal clinic (ANC) attended: Essikardo =1, Effia-Nkwanta = 2, Takoradi = 3, Kwesimintsim =

4, X_2= Age group of pregnant woman: 18-22 = 1, 23-27 = 2, 28-32 = 3, 33-37 = 4, 38 &> 5, X_3 = level of education: none = 1, primary = 2, middle/JSS = 3, secondary/SHS = 4, tertiary = 5, X_4 = marital status: living together = 1, not married = 2, married = 3), X_5 = religion: christian = 1, moslem = 2, X_6 = occupation: unemployed = 1, housewife = 2, farming = 3, trading = 4, artisan = 5, civil servant = 6, cleaner = 7, police reserve = 8, self-employed = 9, X_7 = tap water in household: yes =1, no = 2, X_8 = water closet in household: yes = 1, no = 2, X_9 = district of residence: Ketan-Essikardo = 1, Effia-Kwesimintsim = 2, Takoradi = 3, Sekondi = 4), X_{10} = housing classification type: type 1 = 1, type 2 = 2, type 3 = 3, type 4 = 4.

3.5.2 Swine HEV as a risk factor for infection with HEV

It was hypothesized that pregnant women are at a risk for HEV infection due to the potential presence of infected swine in the study area. To ascertain the HEV status of the farms, swine on twenty farms out of 57 were purposively listed, bled and tested for anti-HEV IgG using recomLine IgM/IgG assays (Mikrogen, Germany) and by real time RT-PCR (AmpliCube HEV 2.0, Mikrogen, Germany) and also by conventional RT-PCR.

3.5.3 Proximity as a risk exposure factor for infection with HEV

To determine the role of proximity to domestic pig farms (zoonotic source) and wetlands (environmental source) as as exposure factors for infection with HEV the distance of each potential environmental risk zone was geovisualized. The geocoding of residences of the pregnant women in their respective districts was done by individually collecting their GPS coordinates using GIS machine, version *etrex 10* (Garmin, USA). The georeferenced households and domestic pig farms (Appendix 7) were plotted on the database map collected from the Metropolitan Physical Planning Offfice of the STM and converted to the Cartesian coordinates (x, y). Proximity to each of the 20 domestic pig farms holding

the swine as well as the four wetlands were calculated. It is presumed that swine are infected with HEV and was potentially a reservoir and a source of HEV transmission to humans. The geographic coordinates were collected using a GPS instrument, version etrex 10 (Garmin, USA), as well as to capture the geographic coordinates of the centroid of the wetlands. The centroids of the wetlands were used as the geographic references of the wetlands. It was assumed that the perceived risks for HEV infection were distributed homogeneously across the wetlands, as such a centroid position averages those risks. The Euclidean distances of the homesteads of each of the pregnant women to the swinefarms and to the centroids of the wetlands, each having different distances were estimated. The latitude and longitude coordinates were inputed into the Mikrosoft Excel version 10-programmed formula to batch-estimate the Euclidean distances, *E*:

E=ACOS(COS(RADIANS(90.Lat1))*COS(RADIANS(90.Lat2))-SIN(RADIANS(90-Lat1))*SIN(RADIANS(90.Lat2))*COS(RADIANS(Long1.Long2)))*6371,
Where Lat1 = latitude of a pregnant woman, Lat2 = latitude of a risk location, Long1 = longitude of a pregnant woman, Long2 = Longitude of a risk location

3.5.4 Logit modelling of proximity as an exposure factor for infection with HEV

In this study, Log(y/(1-y) = the odds of infection with HEV or not. The coefficients of the explanatory variables are B_1 to B_n. The explanatory variables entered in the logistic regression model (Peng, 2002).

3.6 Study 3: Determination of outcomes of infection with HEV at delivery

This section of the study presents the materials and methods applied to the third objective, which was to determine HEV infection on pregnancy outcomes. In this study, Log(y/(1-y)= the odds of infection with HEV or not. Where: X1 = Diagnosis at delivery: Breech =

1, Normal Pregnancy = 2, *PIH = 3, Fetal Distress = 4, *PPH = 5, Transverse Lie = 6, Preeclampsia/*HELLP = 7, Anaemia = 8, Previous CS = 9, *FIoL = 10, Large Fetus = 11, Prolonged Pregnancy = 12, *MUF = 13, Fresh Stilldelivery = 15, Malaria = 16, *FMP = 17, Placenta Previa = 18, LAP = 19, *DwD = 20, Abruptio Placentae = 21, X2 = Delivery mode:*SVD = 0 ,*AoMD = 1.

The logistic regression model was assessed (Paul, 2005) in which a negative HEV infection and a positive wHEV infection carried dummy variable of 0 and 1, respectively.

$\text{Log } (y/1-y) = B_0 + B_1*X_1 + B_2* X_2 \ldots\ldots\ldots\ldots\ldots\ldots + B_n* X_n$

B_1 to B_n = coefficients of the explanatory variables, X_1 to X_n = explanatory variables entered in the logistic regression model.

Thus, the log(y/(1-y) = the odds of infections or Not, Where: (Infection with HEV: Yes = 1, No = 0). Missing data was dealt with by restricting analysis to what data is available.

Pregnancy discharge data was collected from the Regional Hospital Database at the termination of each pregnancy. The data on termination of pregnancy collected was not limited to spontaneous vaginal delivery but includes caesarean section (CS) and any other method of delivery but also includes diagnoses and complications at delivery. Other data sources were patient folders, ward registers and laboratory registers and the also from the database of the regional hospital, which is the main referral center of the Western Region. Where in doubt additional information was sought from attending physicians. The author attended a number of weekly medical review and mortality audit meetings to collate and triangulate data. The collected data were used as explanatory variables in binomial and multiple logistic regression analysis.

3.7 Tests for spatial dependence

Once the first-order effects have been accounted for in LR models, possible second-order effects were investigated. Analysis of spatial dependence was done to provide firstly, informative description of the data. Secondly, to gain additional insight into potential risks: the risks that could be associated with geo-proximity to the domestic domestic pig farms and the wetlands. Hence, spatial dependence of HEV status and households of the pregnant women in the study area investigated. Because geostatistical methods are optimal when data are normally distributed and stationary: the parameters of the conditional means and variances were evaluated. Deviations in normality and stationarity can cause problems in the interpretation of our result.

To evaluate model stability against spatial dependency geographical data points were analyzed against the null hypothesis of no difference. This enables a follow-up to evaluate individual risks within the space. Variograms were contructed from the standardized residuals from logistic regression analysis that, attempt to convey explicit information in detail. Because a variogram checks for spatial correlation (statistical significance) in the dataset, a semivariogram analyses were conducted by plotting spatial lag on the x- and semivariance on the y-axis. Spatial dependence is displayed as a scatter plot. This is an isotropic variogram as a function of spatial lag, which are standardized residuals from the fixed-effects logistic regression models. Additionally, a directional variogram was constructed; since HEV infection may be affected by common risk sources, the spatial distribution of the infection may be potentially directional.

3.8 Data analysis

The prevalence of HEV with their corresponding 95% confidence intervals were estimated at 95% confidence level, using the exact binomial online calculators developed by John C.

Pezzullo (www.sample-size.net/confidence-interval-proportion/). When checking for assumptions data of cases identified to be significant outliers and or having high leverage as well as highly influential points were eliminated. Descriptive statistics, bivariate analysis, and multivariate logistic regression were used to derive logistic regression models on seroprevalence of anti-HEV IgM at 5% level of significance and explanatory variables were predicted and quantified in terms of risk factors and pregnancy complications. Variogram analysis was used to examine spatial bias in the data. The statistical significance level acceptable was $P < 0.05$.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Socio-demographics, clinical and swine production characteristics of participants of these studies

Over 62% of pregnant women were registered at the antenatal clinic at Effia Nkwanta Regional Hospital and nearly 40% had no formal education. Over 35% are engaged in trading and nearly 56% reside in relatively improved housing areas of the metropolis (class 2). Nearly 40% of the women were in the 28–32 years age group with the mean age of 30 years (± 5.43SD) and nearly 74% of the women are not married. However, 56% and 71% have water closet toilet and tap water facilities in their households, respectively. Nearly 69% of the women gave delivery by spontaneous vaginal delivery, whilst 25% gave delivery by caesarean section and 6% opted for caecerean section (elective caesarean section). Over half of the women had complicated pregnancies. The deliverys of over half of the women (51%) who were infected with HEV were complicated and 43% of the women not infected with HEV had complicated pregnancies. Over half of the pigs (53%) were young males pigs and female pigs and nearly one-fourth of them were either mature males male pigs (boars) or sows that were not pregnant (Table 2).

Table 2: Socio-demographical, clinical and swine production characteristics of pregnant women, STM.

Background characteristics	Total number of Women, N	Number anti-HEV IgM positive, n
1. Individual level characteristics		
Antenatal clinic (ANC) sampled	**N=360**	
Essikardo ANC	50 (13.9)	6(12.0)
Regional ANC	225 (62.5)	54 (24.0)
Takoradi ANC	49 (13.6)	14 (28.6)
Kwesimintsim ANC	36 (10)	7 (19.4)
Age (years) (Mean = 30.27 (SD ± 5.43)	**N=344**	
18-22	30 *(8.7)	11*(36.7)
23-27	104 (30.2)	20 (19.2)
28-32	131 (38.1)	32 (24.4)
33-37	70 (20.3)	12 (17.1)
>38	9 (2.6)	2 (22.2)
Educational level	**N=245**	
None	97 (39.6)	26 (26.8)
Middle/JHS	66 (26.9)	14 (21.2)
Secondary/SHS	37 (15.1)	10 (27.0)
Tertiary	45 (18.4)	6 (13.3)
Marital status	N=269	
Married	71 (26.4)	17 (23.9)
Never Married	198 (73.6)	43 (21.7)
Occupation	**N=267**	
Unemployed	31 (11.6)	6 (19.4)
Trading	94 (35.2)	24 (25.5)
Artisan	69 (25.4)	20 (28.9)
Civil Servant	45 (16.9)	6 (13.3)
Cleaner	28 (10.5)	4 (14.3)
Access to Tap Water	**N=133**	
No	39 (29.3)	11 (28.2)
Yes	94 (70.7)	31 (33.0)
Access to Water Closet	N=135	
No	60 (44.4)	24 (40.0)
Yes	75 (55.6)	18 (24.0)
2. Community level characteristics		
Submetro/District	**N=326**	
Ketan-Essikado	101 (31.0)	22 (21.8)
Sekondi	49 (15.0)	21 (42.9)
Takoradi	87 (26.7)	18 (20.7)
Effia-Kwesimintim	89 (27.3)	13 (14.6)
Residential classification	**N=334**	
First class	12 (3.6)	4 (33.3)
Second class	186 (55.7)	37 (19.9)
Third class	102 (30.5)	22 (21.6)

Fourth class	34 (10.2)	11 (32.4)
3. Clinical characteristics		
Delivery method	**N=188**	
Spontaneous Vaginal Delivery	129 (68.6)	36 (27.9)
Caesarean Section (CS)	47 (25)	13 (27.7)
Elective CS	12 (6.4)	4 (25.0)
Delivery status	N=165	
Complicated	75 (45.5)	25 (33.3)
Uncomplicated	90(54.5)	24(26.7)
HEV status positive	**N=49**	
Complicated	25 (51.0)	
Uncomplicated	24 (49.0)	
HEV status negative	N=116	
Complicated	50(43.1)	
Uncomplicated	66 (56.9)	
4. Swine production grps.	**N=447**	
Young pigs	253(56.6)	
Sows	103(23.1)	
Boars	91(20.3)	

*The numbers shown in the brackets are the percentages.

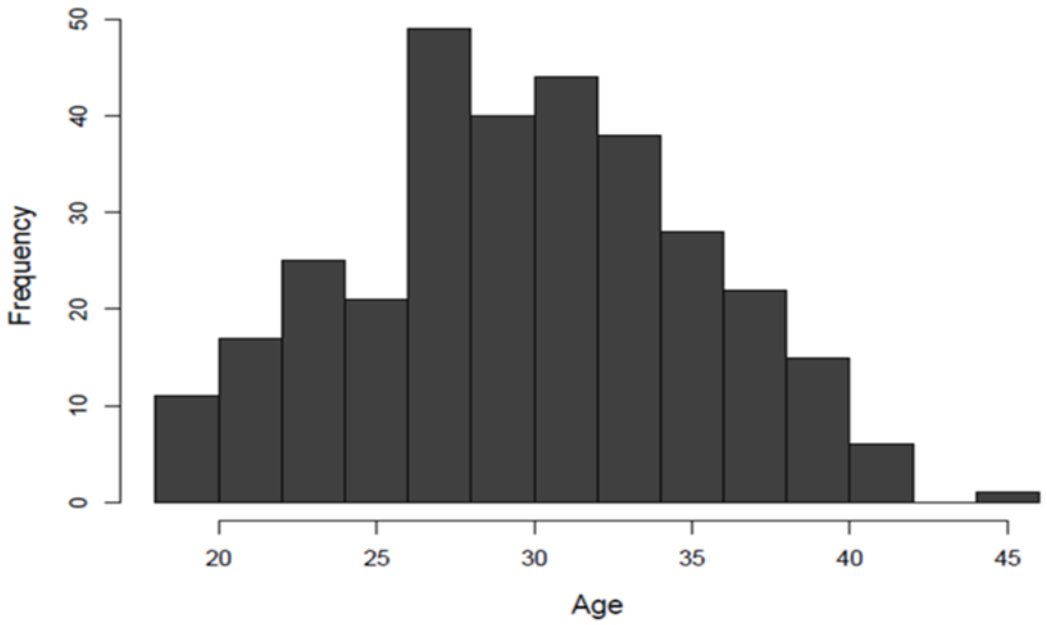

Figure 5: Age distribution of pregnant women, Sekondi Takoradi Metropolis, 2016

The ages of the pregnant women in their third trimester ranged from 18 to 45 years, with a mean age ± SD of 30.27 ± 5.425 years. The ages distribution of the pregnant women seropositive for HEV ranged from 19 to 42 years and their median and modal ages were 30 and 30.5 years, respectively. The age distribution of pregnant women seronegative for HEV ranged from 15 to 41 years and both their median and modal ages were 28 years.

4.2 Study 1 Prevalence of human HEV infection

Three hundred and sixty (360) pregnant women in their third trimester of pregnancy homesteads were sampled from 4 antenatal clinic location located in each sub metropolitan district (Figure 6).

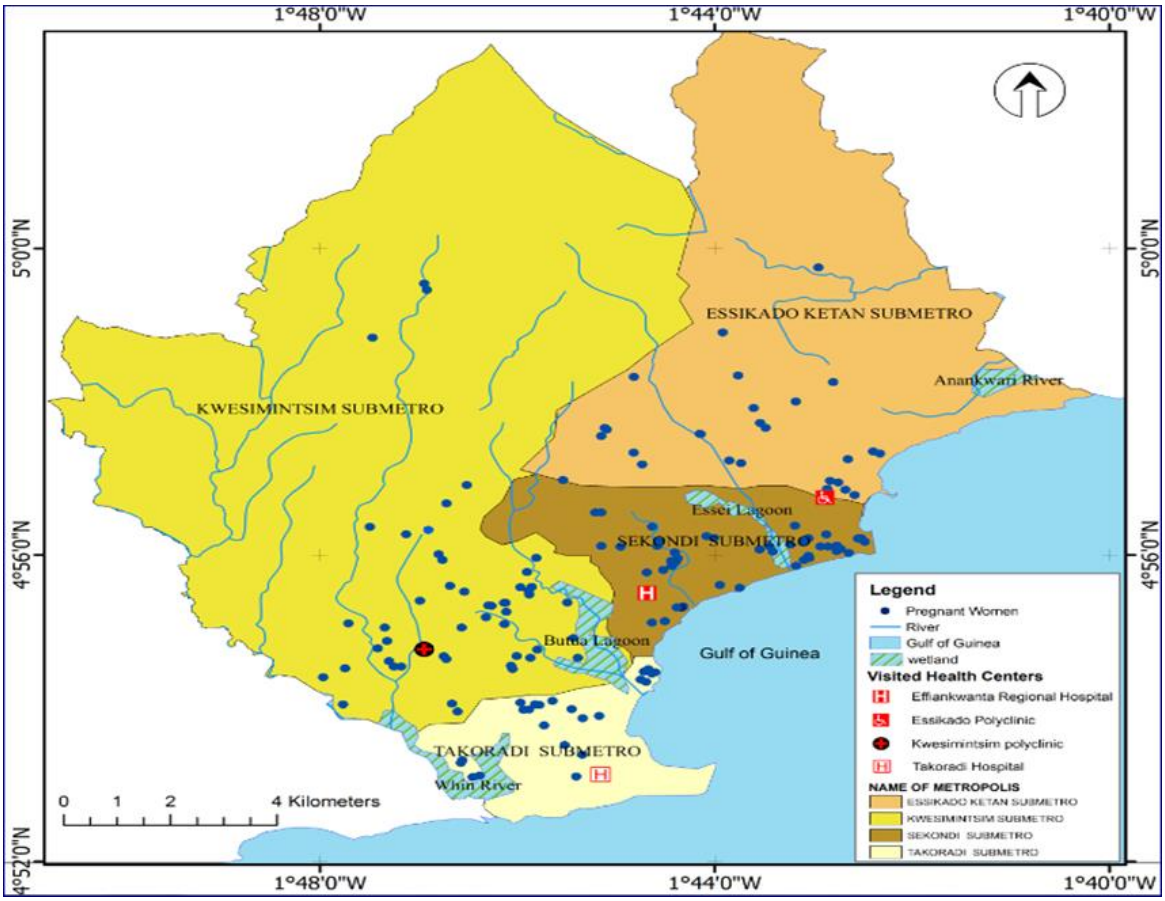

Figure 6: Distribution of homesteads of study participants, antenatal clinics and wetlands, Sekondi-Takoradi Metropolis, 2016.

Further analysis of the blood sera indicated the prevalence of recent human HEV infection (anti HEV IgM) and for previous HEV exposure (anti HEV IgG) were 22.5% (95% CI, 18.2 to 26.8) and 11.0%, respectively.

The first study determined the prevalence of acute HEV infection in apparently healthy pregnant women in their third trimester. The objective was important in view of the identified gaps in the surveillance data for the specific viral hepatitis E for pregnant women, in Ghana. For example, what is the prevalence of HEV infection in other parts of Ghana where the HEV infection has not been determined?

Anti-HEV IgM prevalence at the Sekondi-Takoradi Metropolis of the Western Region of Ghana is 22.5% (95% CI, 18.2 to 26.8). Pregnant women attending ANC at the Takoradi Hospital recorded the highest HEV seroprevalence of 28.6%, while Essikardo ANC attendees had the lowest prevalence of 12.0%.

The present study determined the prevalence of infections of HEV in the third-trimester of pregnancy and investigated spatial characteristics of the infection in the Sekondi-Takoradi Metropolis of Ghana. This is a cross-sectional study that assumed that the 699 pregnant women in their third trimester of pregnancy, who were selected for this study were healthy at the point of receiving regular antenatal medical service. To note, a random selection of 360 pregnant women in their third trimester were tested for anti-HEV IgM. The present study reported 22.5% (81 out of 360) for anti-HEV IgM compared to the previously reported 64.40% (29 out of 45) (Adjei *et al.,* 2009). Pregnant women in the third trimester accounted for 75.79% (119 out of 157 women) in the previous study, which makes comparisons of the two seroprevalence rate favourable. However, another plausible

reason for the disparity could be that as the current study targeted at specifically the third trimester of pregnancy a larger sample size and power underlies this report. Regional and geographical differences could also account for the differences in the reported seroprevalence across a country. The current study was conducted in the south-western part of the country, which is predominantly agricultural but currently one of the fastest growing locations in Ghana in terms of immigration and business development due to oil and gas find. This compares with the location of the previous study, however the former is a metropolitan and capital city located in the south-eastern part of Ghana where apparently the structure of the population is similar. Differences in seroprevalence can result from the strain of the virus responsible for infection. For instance, it has been noted that while the strain of the swine type viruses does not cause significant role in zoonotic infections in India (Khuroo *et al.,* 1980).

Foremost, an issue of discontent to most reviewers are the differences in the performance of test kits used in the determination of HEV infection. Without standardized kits and assays comparing seroprevalence rates across reports and countries become a challenge (Salines *et al.,* 2017). The current study procured assays that have reportedly out performed other competitive kits within Europe as reported by Baylis *et al.* (2015) and documented in WHO (2015). The assays have also been reported in 32 recent research publications (Mikrogen Germany, 2016). Moreso, is how results are computed within one test is important. In the current study, to safeguard internal validity all the borderline seroprevalence results for IgM test, seven cases in number, were removed from the study. The implication for this study is the dipping of the calculated sample size and power, and above all, the calculated seroprevalence rate. To note, the anti-HEV immunoglobulin M (IgM) was measured 22.5% (81 out of 360) as against approx. 24% (88 out of 368) if

borderline results were treated as positive or 22.0% (81 out of 368) if treated as negatives. Gageldonk-Lafeber (2016) in the study of Netherland, the borderline results were treated as if they were positives but analzed separately from the verified positive results.

Figure 7 is a map of Sekondi-Takoradi Metropolis showing the point locations, and features in the study area, indicative of the spatial characteristics and pattens of the dataset. This includes the distribution of the pregnant women: by their households and HEV infection status, the location of the four ANCs. The ANCs are located within the four public-funded hospitals. There are four wetlands in each of the four sub-metropolitan districts of the STM. The patterns identified is a random distribution of the households and their infection status. The northen and western parts of the metropolitis are greenbelts, not indicated. Each of the four districts could be analysed as separate geographical units at a finer resolution. To avoid cluttering, the domestic pig farms have not been shown on this map.

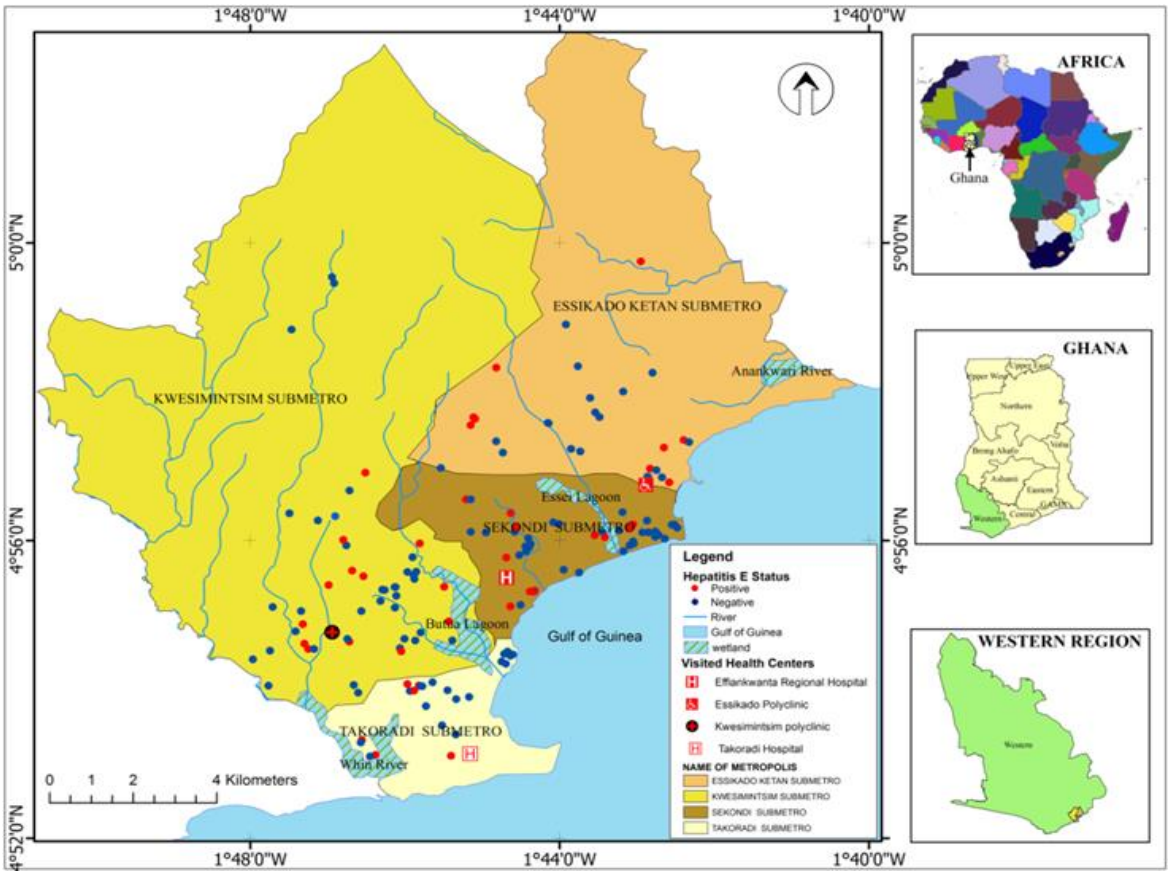

Figure 7: Distribution of pregnant women by HEV infection status in the Sekondi-Takoradi Metropolis, 2016.

The current study complements two other previous studies. Ofori-Asenso and Agyeman (2017) have indicated in a systematic review that two previous studies, until August 2016, were the only studies on HEV infection in pregnant women in Ghana (Agyei *et al.*, 2009; Bonney *et al.*, 2012). The current report is located in the Western Region of Ghana and to the best of our knowledge and experience, it is the first report on HEV in pregnant women. The seroprevalence rate herein reported in Ghana is lower than previously reported (approx. 65%) for the third trimester of pregnancy (Adjei *et al.*, 2009). Tsega and Hansson (1992) and Tsega and Krawczynski (1993) have reported higher seroprevalence levels during pregnancy too. However, the settings of both reported data is different. The reported seroprevalence in Accra the analysis used a relatively smaller

denomenetor while Tsega and Krawczynski (1993) reported of seroprevalence during a hepatitis E outbreak. The striking difference of both reports emanating from Ghana of HEV prevalence in pregnant women is the higher proportion of IgM found compared to IgG. The current and previous reports documented anti-HEV IgM of 22.5% and 64.40% compared to 11% and 35.6% for IgG, respectively. These contrasting dichotomies in the setting of Ghana should be considered in light of differences in the geographic, human activity, virologic, genotypic, zoonotic and area specifics in the epidemiology of HEV and HEV infections. For example, Khuroo *et al*. (2016) reported of HEV infection in Africa, in Nigeria, Ivory Coast, Liberia and Sudan under conditions of hyperendemic disease; these various reports vary by the seroprevalence between recent and earlier exposures of HEV.

In Figure 8, it is indicated that, no HEV RNA was detected in the human serum samples tested by real-time RT-PCR.

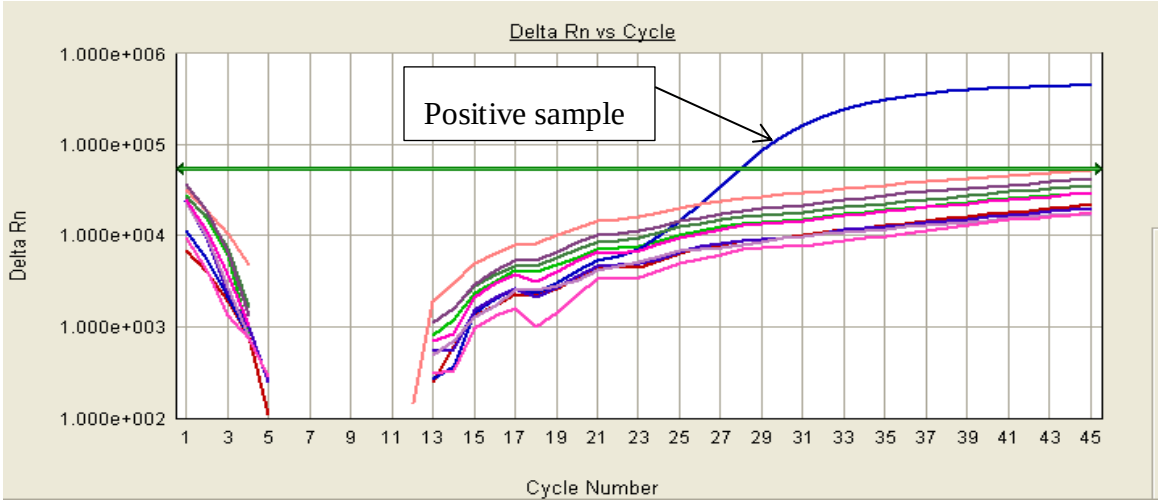

Figure 8: HEV real-time PCR Analysis of human serum samples, Sekondi-Takoradi Metropolis, 2016.

4.3 Study 2 Risk factors of infection with HEV

What sociodemographic, household, environmental and zoonotic factors determine the distribution of the infection in the study area? This section therefore discusses the identified factors pertaining to the current study and to other reports in Ghana, and also in other countries. This section of the study presents the identified sociodemographic and household risk factors of infection with HEV.

The median ages of the anti-HEV seropositive pregnant women and anti-HEV seronegative women were 31 and 30 years, and their modal ages 31 and 28 years, respectively. The median and modal ages of all of the pregnant women studied were 30 and 28 years, respectively. In a simple regression model, age is not a predictor of HEV infection {p = 0.401, 0.98, 95% CI 0.93 – 1.03) SE 0.024}. The mean age (± SD) of the seropositive pregnant women (29.37 ± 5.58 years) is not different significantly (P < 0.652) compared to the mean age of the seronegative pregnant women (30.61 ± 5.42 years). The ages of the pregnant women were not statistically different between the positive and negative women by HEV infection status (Figure 9).

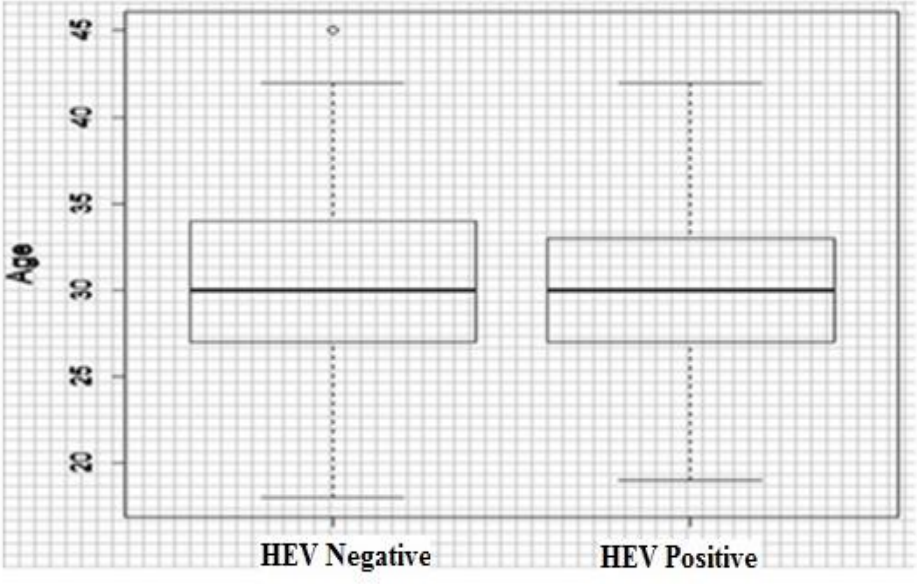

Figure 9: Box plot of age relative HEV infection status of pregnant women

The age variable and determination of infection with HEV. Pregnant women attending ANC at the Takoradi Hospital recorded the highest statistically significant HEV prevalence of 28.6%, while Essikardo ANC attendees had the lowest prevalence of 12.0%. Seropositity decreases significantly with age, from 36.7% among the 18-22 year-group to 17.1% in the 33-37 year-group and increases to 22.2% among the 38 years and > age-group. All the age-groups are predictive of HEV infection, except the 23-27 age-group (Table 3).

Table 3: The odds of infection with HEV and age groups of pregnant women

Variables	B	Women (n)	HEV-Positive [n (%)]	p-value	OR	95%CI(OR)
Age (years)				0.000		
18-22		30	11 (36.67)		1	
23-27	-.501	104	20 (19.23)	0.556	0.606	0.114-3.208
28-32	-1.903	131	32 (24.43)	0.014	0.149	0.033-0.680
33-37	-1.807	70	12 (17.14)	0.013	0.164	0.039-0.687
≥38	-1.562	9	2 (22.22)	0.072	0.210	0. 038-1.151

Pertaining to HEV seropositivity and age increasing age was a factor for decreasing levels in antibody levels in the current study. Seropositity decreases with age from 36.7% among the 23-27 year-group to 17.1% in the 33-37 year-group and the effect was statistically significant.; However, this finding contrasts with the study reported in Nigeria among pregnant women where HEV seropositity increased with age. The current study also contrasts with the study among general population in pig rearing areas in the Netherlands where increasing positivity with increased age among both sexes, from 10% in adolescents to 33% among those aged 50 and above, was reported. Several epidemiological studies not related to pregnancy (Divizia *et al*., 1999; Daniel *et al.*, 2004) have identified age of persons to determine HEV seroprevalence. In general, the prevalence increased with age supporting the assumption of a cumulative lifetime

exposure to HEV in the Netherlands and elsewhere, as well as a higher infection pressure in the past. Apart from age, a number of other factors potentially determined the presence of infection with HEV in pregnant women in the metropolis. Anti-HEV reactivity among women with no formal education (26.8%, 26 out of 97) was higher than that of their counterparts with middle/JHS (21.2%; 14 out of 66), secondary (27.0%, 10 out of 37), and tertiary (13.33%; 6 out of 45) level of education. Statistically significant association was found between education and HEV positivity (Table 4).

Table 4: Point estimates and standard errors of regression coefficients in a logistic regression model of socio-demographic and household factors influencing the presence of infection with HEV in pregnant women, STM, Ghana

Explanatory variables	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	0.64785	0.236231	2.74245	0.006418	0.18321	1.11250
Age	0.00110	0.011065	0.09993	0.920459	-0.02066	0.02287
Age group	-0.04727	0.062221	-0.75964	0.447995	-0.16965	0.07512
Marital status	-0.04628	0.046882	-0.98726	0.324243	-0.13849	0.04593
Education	-0.03575	0.016334	-2.18837	0.029317	-0.06787	-0.00362
Occupation	-0.00218	0.011632	-0.18709	0.851697	-0.02505	0.02070
District of household	-0.04358	0.021293	-2.04672	0.041447	-0.08546	-0.00173
Housing class	0.02339	0.030024	0.77916	0.436423	-0.03566	0.08245
Tap water	0.00422	0.048216	0.08757	0.930274	-0.09061	0.09906
Water closet	-0.10022	0.046967	-2.13387	0.033563	-0.19268	-0.00784

There was no statistical difference by strata of education, $P > 0.05$ (Table 4).

Table 5: Infection with HEV and educational level of pregnant women, STM, Ghana

Variables	N	%	P-Value	or	95%CI
Educ. (Level)	245				
None	97	26 (26.8)		1	
Middle/JHS	66	14 (21.2)	0.582	0.81	0.38- 1.69
Secondary/SHS	37	10 (27.0)	0.954	1.03	0.42-2.36
Tertiary	45	6 (13.3)	0.145	0.50	0.19-1.21

In the current study, a determinant not statistically associated with an increased risk for HEV seropositivity with the level of education. This finding contrasts with the Dutch

study by which low level of education was a risk factor for seropositivity. Indeed, the point estimate analysis gave education an overall statistically association as protective factor for infection with HEV which agrees with literature.

Occupation and marital status have no effect on HEV seropositivity are indicated how occupation determine infection with HEV in pregnant women in the STM (Table 6).

Table 6: Infection with HEV and occupation, marital status of pregnant women, STM

Variables	N	%	P-Value	OR	95%CI
Occupation	267				
Unemployed	31	6 (19.4)		1	
Trading	94	24 (25.5)	0.328	1.65	0.63- 4.87
Artisan	69	20 (28.9)	0.325	1.69	0.62- 5.14
Civil Servant	45	6 (13.3)	0.614	0.72	0.21- 2.47
Cleaner	28	4 (14.3)	0.654	0.73	0.17- 2.89
Marital Status	269				
Married	71	17(23.9)		1	
Never Married	198	43 (21.7)	0.988	1.500	0.52-1.88

For a unit of WC the odds ratio of having or not having a having infection with HEV increases by 54%. Alternatively, for every one unit of having WC, the log odds of having or not having infection with HEV increase by β1. This is the increase in seroprevalence of HEV of markers of recent or on-going infections and previous infections with HEV among women in their third-trimester of pregnancy.

The hepatitis E virus is a major cause of disease throughout the world but it is a poorly understood and underappreciated virus. The interplay of the host-immune factors, viral factors and genetic changes in genotypes could explain the pathogenesis of HEV-associated hepatitis. This could also explain why HEV epidemiology differs in pregnancy

from another geographic location; and in part elucidate the general confusion about the transmission and epidemiology of the virus. First, in developing countries large outbreaks are associated with genotype 1 that could result in high morbidity and deaths among pregnant women and infants. In developed countries where Gt-3 is the predominant viral genotype. Severe disease is not the feature and no epidemic has been reported. Rather sporadic (individual) disease are the features involving this globally-distributed genotype. In the current study Gt-3 contributed 35% to the HEV seroprevalence 19.1% among pregnant women in the area. While infection rates (seroactivity) could reach 21% in the general population in developed countries involving Gt-3 symptomatic cases could be few or altogether infections could progress without symptoms. An important viral factor is that to date, genotype 1 has been found only in humans, whereas genotype 3 has been found in both human and swine with close strain similarities to aid interspecies transmission.

However, HEV was positively associated with the districts of residence and not having a domestic water closet toilet system. The study found a significant negative association between seropositivity and ownership of WCs: 39.3% seropositivity in those without WCs versus 23.1% with WCs but not so was with having assess to domestic tapwater systems. Infection by HEV were different by submetros and districts as there was a significant effect of the district on prevalence of HEV. Other studies have reported similar results. Drinking of HEV contaminated water and lack of portable water in domestic toilet systems are known risk factors for HEV infection in developing countries, the latter which the current study amply demonstrated by its findings. Pertaining to the household factor of ownership of WCs, there is a significant effect between infection with HEV, ($P = 0.026$: OR 0.386 CI 0.167-0.893) however the association is negative: 40.0% HEV seropositivity of those without WCs versus 24.0% with WC. The odds of infection of a woman with a

WC in the household is decreased by almost 40% compared to a pregnant woman not having the set. Ownership of a WC set is a protective factor for HEV infection. However, no conclusions can be drawn about domestic tap water ownership because the effect is not significant.

The findings agree with other studies found in the literature. HEV genotypes 1 and 2 cause acute hepatitis only in humans and are more prevalent in poorer countries where there is limited access to portable water (Meng, 2009). This is so in particular according to Huang *et al.* (2004) HEV genotypes 3 infect both humans and swine where humans are in contact with infected swine.

Table 7: Infection with HEV and household factors of pregnant women, STM

Household factors	Pregnant women, N	HEV positives	P-Value	OR	95%CI
Access to Tap Water	N=133				
No	39 (29.3)	11 (28.2)	1		
Yes	94 (70.7)	31 (33.0)	0.386	1.21	0.27-1.09
Access to Water Closet	N=135				
No	60 (44.4)	24 (40.0)			
Yes	75 (55.6)	18 (24.0)	0.026	0.386	0.167-0.893

The findings of risk factors are similar to reports of several studies in which location were risk factors for HEV infection; whether it is by location in urban and local settings or use of WC toilet facilities or open defecationas were reported (Divizia *et al*., 1999; Lin *et al.* 2004; Dong *et al.*, 2007 and Junaid *et al.*, 2014). For example, the current study is consistent and complements the reports by Juneaid and coauthors emanating from Nigeria, where open defecation is a risk factor for infections with HEV.

Other studies show that the level of sanitation, geographical distribution of HEV genotypes, socioeconomic status, availability and access to drinking water are some of the

determinants that could predict whether disease will occur or not (Teshale *et al*., 2010; Pischke *et al.,* 2013; Riveiro-Barciela *et al.*, 2013).

Factors in the district of residence determines infection with HEV in pregnant women in the STM. The proportion of seropositive women infected with HEV was highest 42.9% (21 of 49) and 21.8% (22 out of 101) among pregnant women who live at Takoradi and Effia-Kwesimintsim submetros, respectively. Followed by those living at Sekondi and Essikado-Ketan submetros with 20.7% (18 out of 87) and 14.6% (13 out of 89), respectively. All the districts were significantly associated with HEV seropositivity at $P < 0.05$ (Table 8).

Table 8: HEV infection and district, housing classification of pregnant women

Household factors	**Pregnant women, N**	**HEV positives**	**P-Value**	**OR**	**95%CI**
Submetro/District	**N=326**		**0.007**		
Ketan-Essikado	101 (31.0)	22 (21.8)	0. 009	0.230	0.76-0.694
Sekondi	49 (15.0)	21 (42.9)	0.008	0.371	0.178-0.776
Takoradi	87 (26.7)	18 (20.7)	0.001	0.249	0.111-0.556
Effia-Kwesimintim	89 (27.3)	13 (14.6)	0.011	0.373	0.174-0.797
Residential classification	N=334				
First class	12 (3.6)	4 (33.3)	0.880	0.925	0.335-2.553
Second class	186 (55.7)	37 (19.9)	0.384	1.773	0.488-6.441
Third class	102 (30.5)	22 (21.6)	0.229	1.696	0.717-4.008
Fourth class	34 (10.2)	11 (32.4)	0.773	0.917	0.507-1.658

4.3.1 Modeling infection with HEV

From the logistic regression models, all the districts had significant effects on changes in the odds of being infected with HEV. The final and a statistical predictive equation that could be used to predict infection with HEV in the metropolis was developed. Model is well-fitting under binomial sampling. The residual means deviance is 1.08 (333.92/308).

4.3.2 Model diagnostics - visualizing the effects of Logistic Regression

The graphical model diagnostics by Geographical Analysis Machine (GAM) was applied to the data (Pfeiffer, 2008). The analysis tested for second-order effects: towards the determination of spatial independence of regression residuals. The scatter diagramme is a model diagnostic plot for heteroskedasity in the error terms of the regression model.In the GAM plot the error term is homoscedastic, the error remains the same over the range of observations and regardless of functional form (Figure 10).

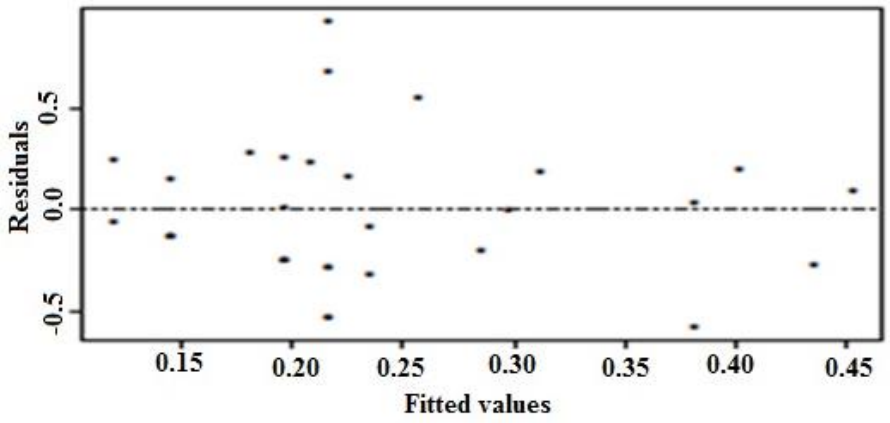

Figure 10: Model assessment for spatial heteroskedasity

The bubble plot compared households (HH) of pregnant women in relation to HEV status and effect size location in the STM. They are exploratory posting of the data values in space to check for significant trends in the paired dataset. The plot indicates the distribution of 168 cases, each plotted at their household locations. They are distributed homogenously throughout the study area, approximately 20 km in east-south-west extent and 19 km south north-west. The posting shows some hint of a South-West trend (Figure 11).

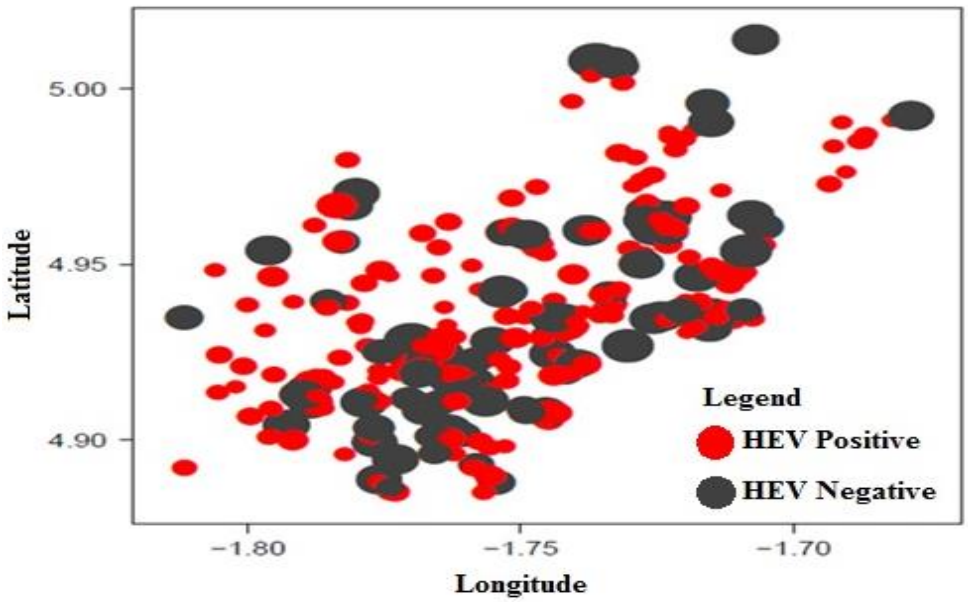

Figure 11: Model assessment for trends

The age-effect plot predicted the odds of infection with HEV and the values of odds vary along the x-axis (Figure 12). Constructed are 95% CI (bands) about the estimate that overlap. Overlapping bands can be significantly different at the 95% confidence bands (Fox, 2003). Adolescent pregnant women have a higher expected odds of infection than older women (0.37 vs 0.25): pregnant women of older maternal age had lower odds of recent HEV infections.

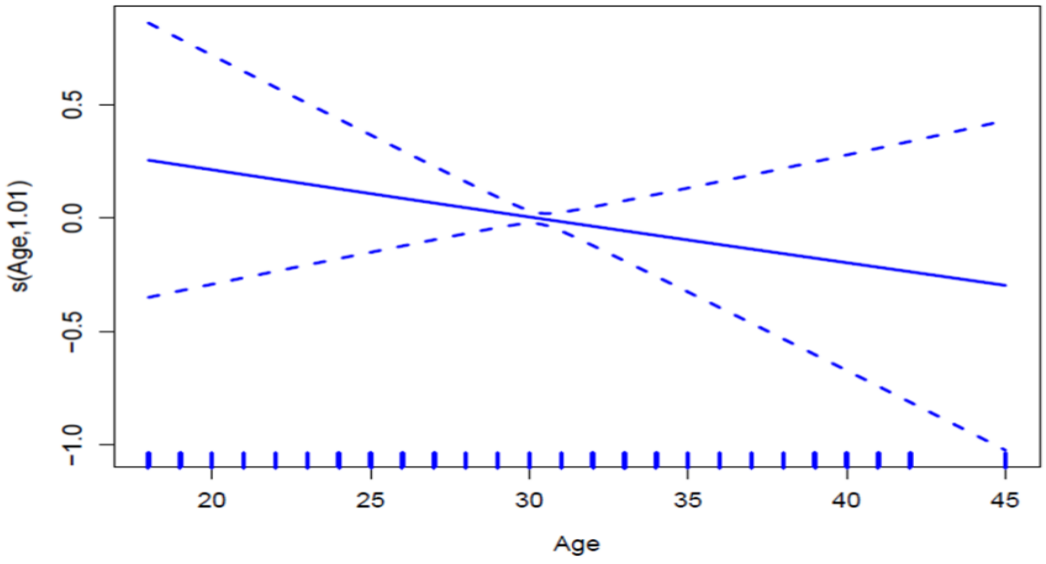

Figure 12: Simulated odds of HEV infection as a function of age

The current study developed an omni-directional variogram to average behavior in all directions. To model this, a non-directional plots was drawn that, indicates an isotropic variogram of infection with HEV (Figure 13). The limits were constructed by 95% of 999 Monte Carlo simulations. The Global Moran's Index (Moran's I) were: Moran's Index 0.004071; Expected Index 0.00302; Variance 0.000091; ZScore 0.742 and P = 0.458. These statistics are indicative of lack of spatial dependence in the dataset. A Moran's I of zero indicates the null hypothesis of no clustering, a positive Moran's I indicates positive spatial autocorrelation (i.e. clustering of areas of similar attribute values), while a negative coefficient indicates negative spatial autocorrelation (i.e. that neighbouring areas tend to have dissimilar attribute values).

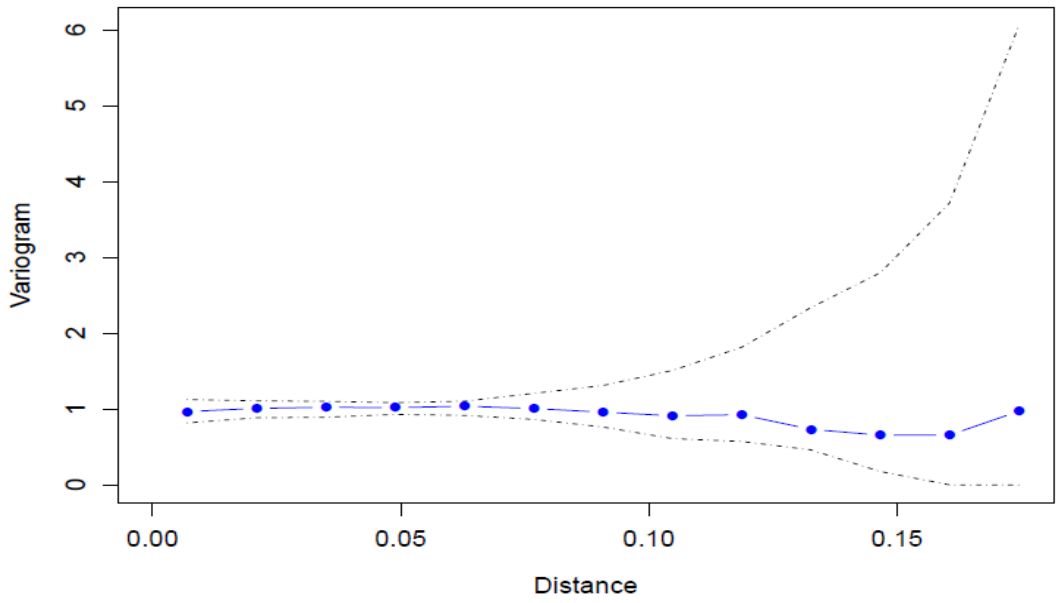

Figure 13: Isotropic variogram of infection with HEV

The resulting theoretical flat shape of the theoretical variogram function also suggest absence of spatial dependence. Graham *et al.* (2005) suggested that, an exponential shape increases with values of lag distance to reflect the presence of autocorrelation.

A directional variogram at varying degrees from zero was further constructed with HEV data to demonstrate to demonstrate anisotropy in the spatial distribution of HEV in the study area. The absence of spatial dependence in the data is further confirmed. The omni-directional model is consistently to the left of the variograms constructed at angles from 0º, 45º, 90º to-135º. This is an indication of the absence of geometric anisotropy (Figure 14).

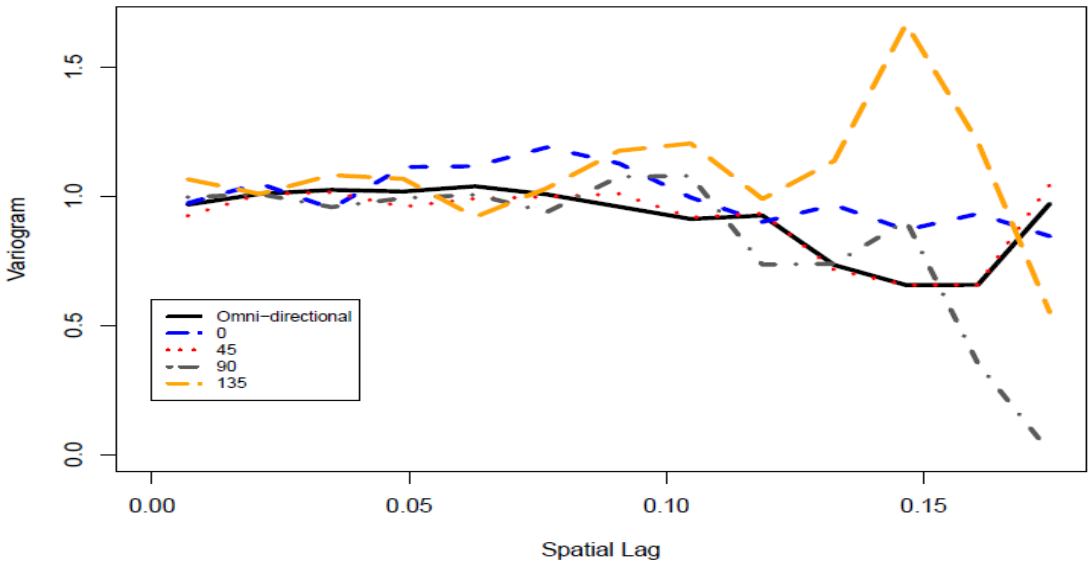

Figure 14: Anisotropic variogram of infection with HEV

Pertaining to model diagnostics, once the first-order effects have been accounted for, possible second-order effects were investigated, using geostatistical methods. According to Isaaks and Srivastava (1989), "Geostatistics offers a way of describing the spatial continuity of natural phenomena and provides adaptations of classical regression techniques to take advantage of this continuity." Ten years on, Olea (1999) defined the discipline as a "collection of numerical techniques that deal with the characterization of spatial attributes, employing primarily random models in a manner similar to the way in which time series analysis characterizes temporal data."

The above theoretical framework informed the current study to explore geospatial analysis of the geographical data collected in the course of the study. Hence, the study developed the diagnostic plot that, indicated homoscedasticity of the residual terms in our dataset. Thus, the model was assessed for heteroscedascity with good result, without which biased and misleading parameter estimates could be produced in the logic model. Deviance residuals were used to evaluate the Poisson model in this way since they should be approximately normally distributed. However, extra-Poisson variation (overdispersion) was observed in the dataset, with a deviance mean of 1.08. The diagnostics provided evidence overdispersion and lack of normality in the distribution of the deviance residuals, suggesting that the model should be reparameterized. However, it is a common phenomenon for biological data of the pregnant women to exhibit overdispersion.

However, geostatistical methods and 'numerical techniques' are optimal, only when data are normally distributed and stationary – i.e. mean and variance do not vary significantly in space. This is because significant deviations from normality and stationarity can cause problems in the interpretation of the result. In this thesis, the basic models of HEV infection and how the infection is likely predictable by other explanatory variables have been developed. However, because the validity of the overall model predictive capabilities depended on upholding the basic assumptions of logistic regression analysis, the subsequent steps of these assumptions needed evaluation: to observe and eliminate (1) 'unusual' data (i.e. individual data points that do not show the same relationships as the bulk of the data), and (2) to observe homosckedacity in the distribution and pattern of variance in the error terms, and (3) non-linearity.

This author is aware, however, that one thing that might have led to the observed overdispersion in the dataset is an abundance of zero counts from the three satellite

hospital on the pregnant women. The model is well-fitting since under binomial sampling (Figure 14) the residual means deviance is 1.08 (333.92/308). Figure 10 is model assessment for heteroscedascity. With a basic model developed the next step evaluated: (1) 'unusual' data (i.e. individual data points that do not show the same relationships as the bulk of the data), and (2) the distribution and pattern of variance in the error terms, and (3) non-linearity. The diagnostic plot shown in Figure 10 indicated that the residual terms were homoscedastic. Thus the model was assessed for heteroscedascity as shown in Figure 10 with good result. In logistic regression heteroskedasticity can produce biased and misleading parameter estimates. However, overdispersion was observed in the dataset, with a deviance mean of 1.08. It is a common phenomenon for biological data to exhibit overdispersion. However, one thing that might have led to the observed overdispersion in the dataset is an abundance of zero counts "Overdispersion happens for real; scientifically important reasons, and these reasons may throw doubt upon our ability to interpret the experiment in an unbiased way" (Cawley, 2007).

Geospatial analysis used variograms to investigate spatial depence pertaining to HEV infection and no relationship was found. During the previous and current decades, the variogram function has been applied for both health and social research, aside its previously known geological exploration applications (Berke, 2004). This thesis used the variogram shape to investigate and to draw inferences about spatial dependence of hepatitis E virus infection in pregnant women in the study area. Similarly, Graham *et al.* (2005) used the shape of the variogram to draw conclusions about the relative importance of spatial dependence relating to an epidemiological study. The omnidirectional variogram function was used by Meng *et al.* (2009) to assess spatial autocorrelation for household incomes in a Canadian neighbourhood study, and also prior to spatial

interpolation analysis. The current study found no evidence of spatial dependence in the data. However, Geremew *et al.* (2019) used geospatial analysis to draw conclusions of the spread measles in children in Ethiopia. However, in spatially dependent structures variogram modeling becomes an precious tool in the arsenal of geostatisticians to conduct interpolative surveys. Why not the biostatistician and field epidemiologists, especially in the current spread of zoonotic infections across the globe. The clarion call therefore for the One Health approach in investigating infections common to man and animals requires newer approaches for dealing with infections that are spread and cluster geographically.The find of HEV infection in swine in the current study that interfaces with humans is the reason: the need tofind newer methods in dealing with geographic data. However, variogram modeling deserves caution. Aretouyap *et al.* (2016) determined that even under spatial dependence the use of innapropriate variogram model can distort the results of an evaluation, assessment or prediction survey.

Infection with hepatitis E virus (HEV) in the Sekondi-Takoradi Metropolis of the Western Region showed distinct epidemiological patterns with regards to location. For this reason, any conclusion about a derived model would be incomplete without further interrogation about the role of space and evaluation of models against a set of assumptions and spatial influence. Autocorrelated data is the subject matter of geostatistics, and the Oxford American Dictionary defines autocorrelation as “the correlation between elements of a series and others from the same series separated from them by a given interval.”. In perspective, how does the phenomena of HEV infections among pregnant women vary in space? Interestingly, this query is also the definition of the subject of biostatistics, as offered by Deutsch (2002); the query, which this thesis amply investigated. In contrast, the publication of Divizia *et al.* (1999); Lin *et al.* (2004) and Dong *et al.* (2007) did not report on the spatial dimentions, albeit roles of geographical variables were the identified

having played roles in the distribution of HEV infection in respective study locations. This author opiniates that's duo-constraints why many epidemiologists would shy away from spatial analysis is not far fetched, which are money and time. (i) There are costs in assessing immerging software and skills versed in geostatistical analysis. (ii) The time spent and additional costs involved in collecting quality and adequate georeferenced data pertaining to particular research endeavor may be prohibitive. Nontheless, many researchers have also walked the path of the current thesis by investigating spatial dependence, albeit not related to hepatitis E (Diggle, 1990; Diggle, 2002; Graham *et al.*, 2005) and also the cited report of Geremew *et al.* (2019).

4.3.3 Zoonotic factors

4.3.3.1 Prevalence of swine HEV infection

The distribution of 20 swine farm locations contributing swine blood samples to this study is shown in Figure 15. The prevalence of previous exposure to swine HEV infection in swine was 4.5%. Swine HEV is negative by RT-PCR analysis (Figure 16).

This and the next section of the study present and discuss the identified zoonotic risk factors of infection with HEV. The results for zoonotic factors are at two levels: Potential risk factors of direct contact with HEV infected swine, as determined by the presence of swine HEV in the study area and indirectly estimating proximity to domestic pig farms. There were 9 positive cases (4.5%) out of the 200 swine tested. Four of the 20 domestic pig farms (25%) were found to be infected with HEV.

If the gel test reactions identified any bands in the swine HEV test runs sequencing of the products was possible for identification of the HEV genotype present in a sample. However further characterization required amplification of sequences from the ORF2

region or better full genome sequencing (Echevarria, 2014). In our particular study, a commercial assay was used; not all the primer sequences are made available.

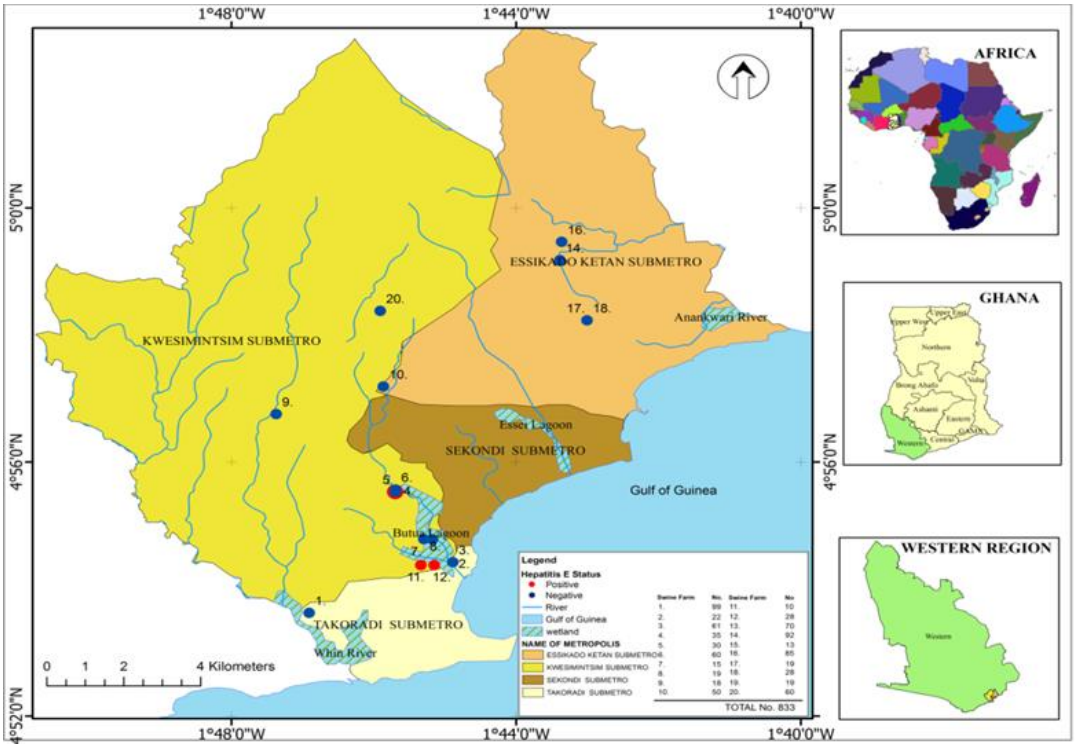

Figure 15: Distribution of 20 domestic pig farms investigated for infection with swine HEV in the Sekondi-Takoradi Metropolis, Ghana, 2016.

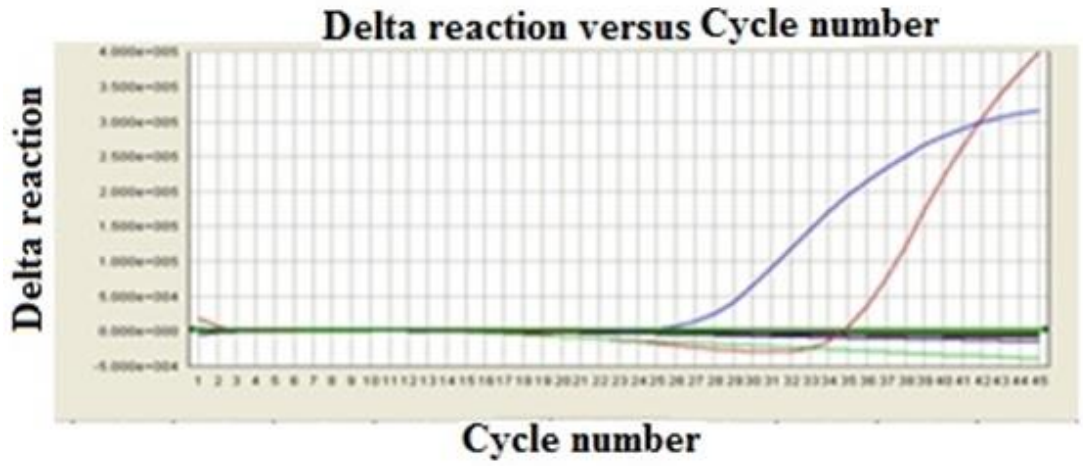

Figure 16: HEV real-time PCR Analysis of swine serum samples, Sekondi-Takoradi Metropolis, 2016.

N.B: The 2 curves are real-Time PCR signals generated for HEV-cDNA from a positive reference kindly donated by Robert Noch Institute, Germany.

In Table 10, it is shown that were no errors during amplification as in all the trial and test runs the Ct values of the positive control were < 33 (27.0-27.9).

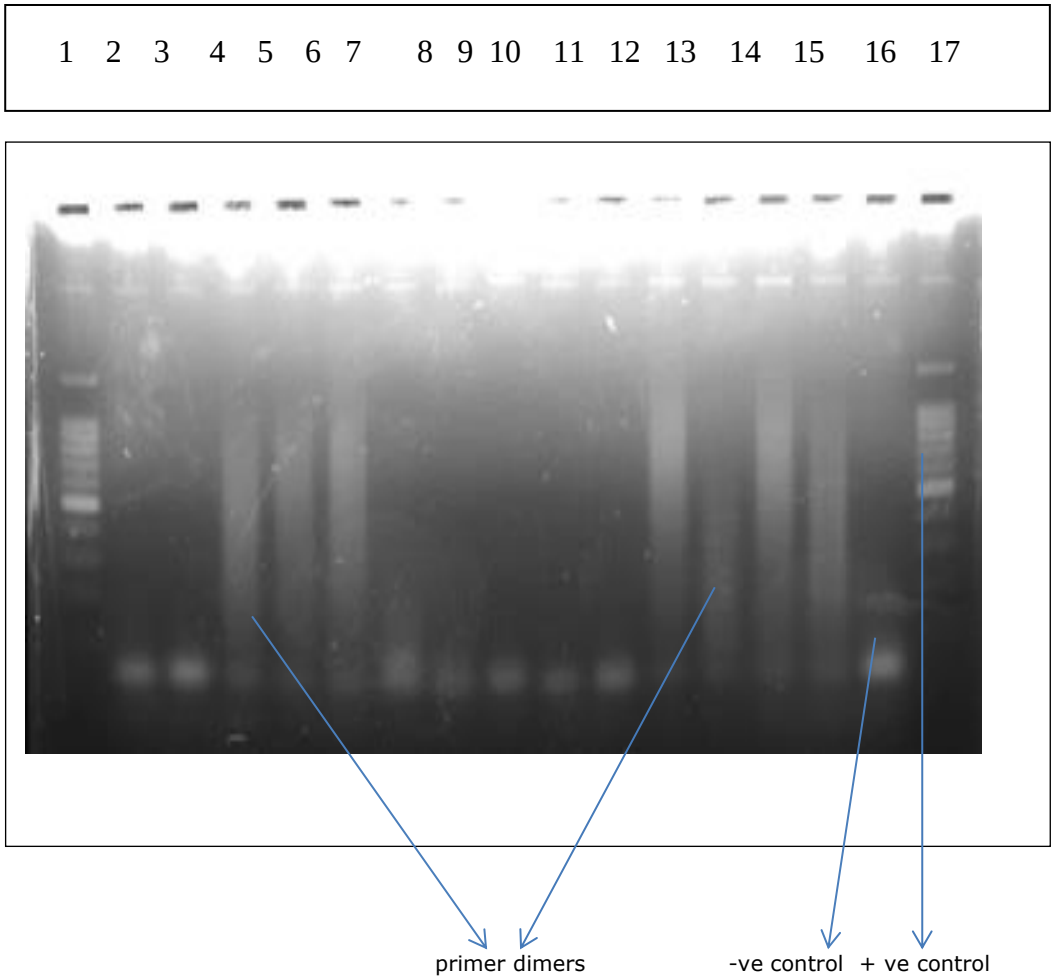

Figure 17: Agarose gel electrophoresis showing smeared PCR products during analysis of swine samples for HEV. Lanes 1 and 17; 100 bp ladder of molecular weight markers; lanes 2-13 13 serum specimens from swine serum; lane 16: negative control from saline.

As a guide to users of this assay, the manufacturers indicated that a BLAST search tool (www.ncbi.nih.gov/blast) shows that the selected primers and probes of the ampliCube HEV 2.0 is able to detect all the relevant HEV genotypes. The manufacturers attested that for analytical sensitivity, the limit of detection (LOD) of the ampliCube HEV 2.0, determined by probit analysis, is 36.13 IU/ml (95% CI: 24.80-78.16 IU/ml). For analytical

specificity none of the negative serum samples from patients suspected of having an HEV infection tested otherwise.

If the gel test reactions had identified any bands in the swine HEV test runs the presence of genomic swine HEV RNA would have been confirmed. Then sequencing of the products could have been possible to identify of the HEV genotype present in the sample. Genotype characterization required amplification of sequences from the ORF2 region or better full genome sequencing (Echevarria, 2014). In the current study, a commercial assay was used and as such not all the primer sequences were made available.

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It must be noted that as a limitation of the method, a negative HEV test result does not exclude the possibility of infection with HEV, which should always be considered in conjunction with clinical overview. This molecular diagnosis was based on amplification of sequences from the ORF1 and ORF3 region of HEV.

The same cannot be said of the swine strains prevalent across much of Europe and China where humans are sporadically infected and become ill from zoonotic these sources

(Pavio *et al.*, 2010). The author of the current study found anti-HEV immunoglobulin in swine (unpublished reports) for the first time in Ghana, which offers an opportunity for the determination of the circulation swine strains in Ghana. Without further research in that field we are unable to determine how swine HEV is transmitted to humans in Ghana; and also lose out in the general discourse and comparisons across countries and regions.

4.3.3.2 Exposure to contaminated environment of domestic pig farms as potential infectious sites for HEV

The hypothesis of swine as a source of transmission of infection with HEV to pregnant women needed validation. The basic assumption is that those nearer the farms endangers are more likely to get infected than those further away more frequent interaction and propensity for zoonotic transfer of infection. This is because one of every swine farm was infected with HEV.

In this study, the odds of infection with HEV or not: $\text{Log}(y/(1-y) = X_1B_1 + X_2B_2 \ldots .X_nB_n$. The coefficients of the explanatory variables are B_1 to B_n. The explanatory variables were entered in the logistic regression model (Stevens, 2009), where: [(X_1 = domestic pig farms: swfm_1 = 1, swfm_2 = 2, swfm_3 = 3, swfm_4 = 4, swfm_7 = 7, swfm_9 = 9, swfm_10 = 10, swfm_11 = 11, swfm_12 = 12, swfm_13 = 13, swfm_16 = 16, swfm_17 = 17, swfm_19 = 19, swfm_20 = 20). It should be noted that, only fourteen domestic pig farms out of 20 (Swfm_1…Swfm_20) were analyzed. Six domestic pig farms (Swfm_5, 6, 8, 14, 15 and 18) were removed from the analysis because of their closeness to each other, as it was assumed that close-by farms shared similar risks with closeby neighbours. The Euclidean distances of the homesteads of each of the 160 randomly selected from 330 pregnant women to the 14 swinefarms and to the centroids of the 4 wetlands. The itinerations were 2240 and 640 for the domestic pig farms and wetlands respectively. A total of 12 288 km for the 2 240 itinerations to the swinefarms of 5.5 km per initeration.

The sum of the minimum and maximum distances are 54.2 km and 541.7 km respectively, while the median is 70.1 $\pm$ 40.8 km. The nearest swinefarm is 0.34 km and the farthest farm in the neibourhood of a pregnant woman's household is 3.39 km.

Proximity to 28.6% (4 out of 14) of the domestic pig farms were risks for HEV infection. The farms were: Swine farm No. 2 (Swfm_2) (P = 0.031, odds ratio (OR) 0.397 95% confidence interval (CI) (0.172-0.916), Swfm_5 (P = 0.023, OR 1.29, 95% CI 1.04-1.61, Swfm_6 (P = 0.013, OR 0.507, 95% CI 0.366-0.888) and Swfm_13 (P = 0.046, OR 1.38, 95% CI 1.01-1.90) and Sw_20 (P = 0.034, OR 0.407, 95% CI 0.178-0.935).

Table 9: Logistic regression model of infection with HEV and proximity of domestic pig farms and wetlands of pregnant women, STM, Ghana

Summary Output					
		Coefficients	**Standard Error**	**t Stat**	**P-value**
Ser. No.	Intercept	0.762	0.305	2.50	0.014
1	Swfm 1	-0.108	0.066	-1.626	0.106
2	Swfm 2	0.589	0.286	2.059	0.042**
3	Swfm 3	0.2069	0.181	1.141	0.256
4	Swfm 4	-0.113	0.064	-1.759	0.081
5	Swfm 7	-0.916	0.403	-2.274	0.025**
6	Swfm 9	0.225	0.112	2.014	0.046**
7	Swfm 10	0.214	0.332	0.643	0.521
8	Swfm 11	0.720	0.436	1.652	0.101
9	Swfm 12	-0.453	0.461	-0.982	0.327
10	Swfm 13	1.595	1.155	1.382	0.169
11	Swfm 16	-1.022	0.895	-1.141	0.255
12	Swfm 17	-0.482	0.227	-2.129	0.035**
13	Swfm 19	0.300	0.161	1.857	0.065
14	Swfm 20	-0.762	0.426	-1.790	0.076

Significance (P<0.05) Back yard domestic pig farms (Sw_1…Sw_20) and wetlands (Wetland_1 to wetland_4: are the potential environmental risk locations. ** = statisticaly significant

N.B.: Fourteen domestic pig farms out of 20 were analyzed. Five domestic pig farms were removed because of their closeness to each other. It was assumed that close-by farms shared similar risks; therefore the six (Swfm_5, 6, 8, 14, 15 and 18) were removed from the analysis. (Sw_1…Sw_20). Wetlands (Wetland_1 to wetland_4: are the potential environmental risk locations.

However, the involvement of the zoonotic factor globally, as reported by Salines *et al.* (2017) and potentially in Africa (Kaba *et al.*, 2010; Salete de Paula *et al.*, 2013) is becoming evident with the reports of HEV find in swine. The zoonotic factor and together with the related apparently contaminated environment forms the subject matter for the second objective. Thus, two related potential sources of risks were considered: exposure to environmental and zoonotic sources of HEV. Pertaining to the environment nearness to wetlands was assessed as the risk factorfor HEV infection. For zoonotic factors, nearness to domestic pig farms, whether infected or not with HEV was the parameter of risk assessment.

During oro-faecal transmission (Bouwknegt and Teunis, 2010) it was presumed that the HEV infections in domestic swine holdings have spread to all swine on a particular farm. A recent report by Risalde *et al.* (2017) is consistent with the current study that non-viremic swine could remain a source of transmission of HEV to humans. In the cited report non-viremic wild boars were found by histopathochemical methods to have persistent HEV-antigens in their liver. Thus the report of Risalde supports the long-held hypothesis and the position of the current study that low viral titres may persist in the liver of non-viremic individual animals: a plausible source of contamination of animal food and human handlers and in-contacts on domestic pig farms.

The current study speculates that the Gt strain of HEV found in swine could be the source of transmission to local residents including the pregnant women. Rodriguez-Frias (2015), Salete de Paula (2013) and Gageldonk-Lafeber (2016) also reported a high prevalence of HEV antibodies in the household-raised pig population in Philippines, in Nigeria and in the Netherlands respectively. They suggested the potential risk of Gt 3 HEV infection

among local residents; they raised concerns over Gt 3 zoonotic transmissions because genotype 3 of HEV was predominant and known to be circulating in swine, which also agrees with the reports of Geng (2012). There were differences in but not with distance from residential address and occupation Whereas distance within 2 km to 4 km of the domestic pig farms was either a risk factor or not for seropositivity that, was not the case in the Dutch study where domestic pig farms within 1 km of residential address was not a risk factor for seropositivity. Of concern in the current study is the observation of the author of citings of swine pens in dry portions of wetlands in the study area, which facilitate the washing of potentially HEV contaminated faeces into the wetland waters.

Indeed, Gageldonk-Lafeber (2017) in yet another latter report from the Netherlands also raised similar concerns about infected swine excreting large amounts of HEV into the environment. Earlier reports from Nigeria by Salete de Paula (2013) and from the Philipines by Rodriguez-Frias (2015) also raised similar concerns regarding zoonotic transmission of HEV into the environment. Thus, the current study supports the generally held position that the citing of domestic pig farms in populated inner cities and rural farming locations is a risk factor and potential facilitator of environmental transmission of HEV to humans.

The results of the current study show that there are different public health risk levels of the domestic pig farms for infections with swine HEV. It is consistent with and could be explained by the different farming and hygiene level practices of the farms. These include but not limited to effluent disposal management practices into the wetlands environment and use of protective clothings as highlighted by Schielke *et al*. (2015). The finding is also supported by the results from reviews of international scientific literature. Salines *et al.* (2017) reported on the epidemiological characteristics of HEV shedding in swine

populations. The review by Salines reported high HEV prevalence estimates that, differed greatly from one study to another. Consistently, there are high variabilities between farms, suggesting the existence of multifactorial conditions related to infection and within-farm transmission of the virus. Farming practices, passive immunity and co-infection with immunosuppressive agents were identified as the main factors influencing HEV infection dynamics. We allude to the suggestions by Salines that further investigations are needed to clarify the different HEV infection patterns observed in pig herds as well as HEV transmission between farms. Indeed, the current study being an exploratory survey should create opportunities for other researchers to do indepth studies about farmed swine and transmission of HEV in the study area. Relevant surveillance programmes and control measures from farm to fork also have to be fostered to reduce the prevalence of contaminated pork products entering the food chain.

Swine infected with the Gt 3 HEV variants that, could cause chronic hepatitis in the infected immunocompromised, liver transplant patients and those with HIV. Saline (2017) affirms that, should vulnerable population groups be infected with HEV Gt 3 the eventual development of liver cirrhosis raises a major public health concern that cannot be simple wished away or discounted (Mirazo *et al.,* 2014). The current study also expresses caution. In a couple of years this decade will come to an end and humanity shall begin another third of the 21 century. Philosophically, international public health will wake up to face another imminent viral transmission source of liver cancer. Swine HEV shall that cause. Soo (2012) reported of a hepatitis E vaccine debuts, a rare Chinese biotech partnership that raises hopes for prevention of HEV infection. However, this hope falters because five years on, the world is still waiting for the commercial vaccine roll-out to benefit the vulnerable pregnant population.

Pertaining to distance to domestic pig farms and the risk of HEV infection proximity to 25% (5 out of 20) of the domestic pig farms were risk factors for HEV infection. In Nigeria, Junaid (2014) reported that animal handlers had high seroprevalence rate of 66.7% compared to the general population. Domestic pig farms had a three-fold likelihood of HEV infection than the unexposed according to a report from the Netherlands by van Gageldonk-Lafeber (2017). It also confirm the report by Di Bartolo *et al.* (2011) that swine contacts increases the occurrence of HEV infections. Even though our nested study is limited to a survey for HEV infection in swine and did not interrogate eating habits, in case-control studies (Yamatani *et al.,* 2004; Tomiyama *et al.,* 2009) have identified the eating of undercooked deer meat and pork with increased risk for HEV infection.

4.3.4 Exposure to wetlands as potential HEV infectious sites

In this study, Log(y/(1-y) = the odds of infection with HEV or not. The coefficients of the explanatory variables are B_1 to B_n. The explanatory variables entered in the logistic regression model (Stevens, 2009) were: X_1 to X_n (X = wetlands: X_1 = wetland_1 = 1, X_2 = wetland_2 = 2, and X_3 = wetland_3 = 3, X_4 = wetland_4 = 4).

The hypothesis of wetlands as sources of transmission of infection with HEV to pregnant women needed validation. The basic assumption is that those nearer them are more likely to be infected than those further apart.

Logit model of determinants for infection with HEV

Table 10: Logistic regression model of infection with HEV and proximity to wetlands by pregnant women, STM, Ghana

Wetlands	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Wetland_1	0.103	0.065	1.574	0.116	-0.026	0.232
Wetland_2	0.016	0.058	0.274	0.784	-0.097	0.129
Wetland_3	0.028	0.023	1.210	0.227	-0.017	0.072
Wetland_4	-0.014	0.016	-0.840	0.402	-0.046	0.018

Proximity to the 4 wetlands - For the wetland_1 to wetland_4, P > 0.05. No significant statistical association between seropositivity and proximity to the wetlands with regards to HEV infection was found. In Ghana where environmental sanitation is precarious it is worthy to note that transmission of viral particles from the environment to humans is likely. This is possible as the virus is relatively stable in the environment and have been recovered from sewage samples (La Rosa *et al.*, 2010). It is not also surprising that most of the pigpens sampled in the metropolis are located near some major gutters and solid-waste collection containers where effluent from the pens could drain.

4.4 HEV infection and outcomes of pregnancy

The outcomes of pregnancy are the diagnosis at delivery as recorded in ward discharge books as well as captured in the Effia Nkwanta Regional Hospital database. The total number diagnosis recorded at diacharge were 188 (mean 12.56 Std Dev 4.48). Deliverys by Spontaneous Vaginal Delivery (SVD) were 129 (68.6%), by Caesarean Section (CS) 47 (25%) and by Elective CS 12 (6.4%). By the status of the delivery of 165 deliverys, 75 were classified as complicated deliveries representing 45.5% while 90 (54.5%) were uncomplicated. Of 165 deliverys 49 were positive for HEV infection of which 25 (51.0%) were complicated deliverys, while 24 (49.0%) were not uncomplicated. Of 116 HEV negative pregnancies 50(43.1%) were complicated and 66 (56.9) were uncomplicated.

Table 11: Infection with HEV and diagnosis at delivery of pregnant women at Effia Nkwanta Regional Hospital, Ghana, 2016

Diagnosis	N	HEV positive	Diagnosis	N	HEV positive
Breech	4	2(50%)	Multiple Uterine Fibroid, *MUF	1	0
Normal Pregnancy	85	23(27.1)	Fresh Still Delivery	1	1(100.0)
Pregnancy Induced Hypertention, *PIH	9	2(22.2)	Malaria	1	1(100.0)
Fetal Distress	6	2(33.3)	Premature Membrane Rupture,*FMP	1	0
Post partum haemorrage, *PPH	9	1(11.1)	Placenta Previa	2	2(100.0)
Transverse Lie	9	2(22.2)	LAP	1	0
Pre-eclampsia/Hemolysis, Elevated Liver enzymes, and Low Platelet count, *HELLP *HELLP	6	3(50.0)	Diarrhoea with Dehydration, *DwD	1	1(100.0)
Anaemia	1	1(100.0)	Abruptio Placentae	2	1(50.0)
Previous CS	11	2(18.2)	Spontaneous Vaginal Delivery *SVD	54	36 (28.3)
Failed Induction of Labour, *FIoL	1	1(100.0)	Complications	50	26 (52.0)
Large Fetus	6	2(33.3)	Premature Membrane Rupture, *PMR	1	0
Prolonged Pregnancy	3	0	Mal presentation	8	1(12.5%)

N.B: the numbers in the brackets are the percentages

N.B.: *PMR - Premature Membrane Rupture; *FIoL - Failed Induction of Labour; *MUF – Multiple Uterine Fibroid; *CS – Caesarean Section; *DwD - Diarrhoea with Dehydration; *PIH – Pregnancy Induced Hypertention; Pre-eclampsia/*HELLP – Hemolysis, Elevated Liver enzymes, and Low Platelet count; *SVD versus AoMD - Spontaneous Vaginal Delivery; HEV infection - Anti-HEV IgM.

Table 12: Logistic regression model of HEV infections and complications by diagnoses at delivery of pregnant women, Effia Nkwanta Regional Hospital.

Terms	Women (n)	HEV-Positive Women [n (%)]	B	p-value	OR	95% Cl: $e^{(B)}$
Complications				0.034		
Breech	4	2(50)			1	
Normal Pregnancy	85	23(27.1)	-1.052	0.000	0.349	0.232-0.525
*PIH	9	2(22.2)	-1.253	0.118	0.286	0.076-1.068
Fetal Distress	6	2(33.3)	-0.693	0.423	0.500	0.120-2.078
*PPH	9	1(11.1)	-1.609	0.142	0.200	0.023-1.712
Transverse Lie	9	2(22.2)	-2.079	0.050	0.125	0.022-0.715
Pre-Eclampsia/*HELLP	6	3(50.0)	0.000	1.000	1.000	0.261-0.831
Previous CS	11	2(18.2)	-0.693	0.423	0.500	0.120-0.78
Large Fetus	6	2(33.3)	-.693	.423	0.500	0.120-0.78
*SVD and *AoMD	54	36 (28.3)	-0.674	0.005	0.510	0.318-0.818
*Positivity and Complications	50	26 (52.0)	0.212	0.029	1.236	1.021-1.496

N.B.: *PMR - Premature Membrane Rupture; *FIoL - Failed Induction of Labour; *MUF Multiple Uterine Fibroid; *DwD-Diarrhoea with Dehydration; *CS – Caesarean Section; *DwD - Diarrhoea with Dehydration; *PIH – Pregnancy Induced Hypertention; *HELLP – Hemolysis, Elevated Liver enzymes, and Low Platelet count; *SVD versus AoMD - Spontaneous Vaginal Delivery versus All other Modes of Delivery; HEV infection - Anti-HEV IgM.

In Table 11 is indicated an overall statistically significant association with complications at delivery p= 0.034. Of the 92 pregnant women in their third trimester who had complications at delivery 28 (30.4%) were positive for HEV infection. Twenty-two complications are listed in Table 10; however, no other factor had itself a significant association with HEV prevalence except normal pregnancy, which has a negative association, ($p < 0.000$: OR = 0.349 95% CI 0.232-0.525) with HEV positivity. The complications that have not attained statistical significance are transverse lie ($p > .050$: OR

0.125 95% CI 0.022-0.715), postpartum haemorrhage (PPH)P = 0.200 95% CI.0331.212, Previous CS P = 0.054 OR 0.222 95% CI 0.061-0.804, PIH P = 0.118OR 0.286 95% CI 0.076-1.068, Fetal distress-0.693P = 0.423OR 0.500 95% CI 0.120-2.078, Pre-eclampsia P = 1.000 OR 1.00095% CI 0.261 3.831 all have P > 0.05 and negatively correlated with HEV seropositivity.

The third objective of this thesis investigated the complications of HEV infection at delivery. The purpose was to identify the impact on pregnancy of infection with HEV during delivery. An overall effect on on diagnosis at delivery was found, P = 0.034 was. Twenty-two diagnoses were identified among the pregnant women during admissions however no other factor had itself a significant association with HEV prevalence except normal pregnancy, which has a negative association, (P < 0.000: OR = 0.349; 95% CI 0.232-0.525) with HEV positivity. The complications that have not attained statistical significance are transverse lie (p >.050: OR 0.125 95% CI 0.022-0.715), postpartum haemorrhage (PPH)P = 0.200 95% CI.0331.212, Previous CS P = 0.054 OR 0.222 95% CI 0.061-0.804, PIH P = 0.118 OR 0.286 95% CI 0.076-1.068, Fetal distress-0.693P = 0.423OR 0.500 95% CI 0.120-2.078, Pre-eclampsia P = 1.000 OR 1.00095% CI 0.261 3.831 all have P > 0.05 and negatively correlated with HEV seropositivity.

Preeclampsia and PPH, even though not of statistical significance are of clinical significance during pregnancy and ill-health related to inflammation and deserve further mention. As stated by Hammoud *et al.* (2014) severe preeclampsia induces liver dysfunction and is 5-7% prevalent in the second and third trimester of pregnancies in the USA. The current study found out that of the six cases of pre-eclampsia three (50%) were found to be HEV positive. Thus the clinical significance of HEV in preeclampsia should

not be ignored as the infection may be contributing clinically to cases of preeclampsia encountered in our healthcare institutions. The other pathology that deserve attention is PPH, which this author opinionates may be of clinical significance as coagulopathy is a feature of liver disease. Our study found that 11.1% (1 out 9) of the pregnant women who had PPH were also positive for HEV infection: Table 12. However, Sleisenger (2010) reported that coagulopathy is manifested mostly in patients with chronic liver disease and results from impairments in the clotting and fibrinolytic systems, as well as from reduced number and function of platelets. Northup and Caldwell (2013) hinted that there is the initial response of the practitioner to assume a high risk of bleeding that is due to coagulation protein deficiencies in patient population. Several reports in hemostasis during the past decade has revealed this is a misconception (Tripodi *et al.,* 2006; Sleisenger *et al.,* 2010). These authors showed that in the presence of adequate platelet counts and thrombomodulin, an endothelial-derived cofactor in the anticoagulant system, cirrhosis patients have a normal capacity to generate thrombin. Because with the find of swine infection in the catchment area of this study with HEV the risk of the development of chronic hepatitis and eventual liver cirrhosis cannot be discounted in the pregnant women who participated in current study. Such is also the view of other authors (Mirazo *et al.,* 2014).

In conclusion, most ALF patients have reached a whole body hemostatic balance, despite the highly abnormal traditional coagulation indexes. The author of the current study informed the clinicians about the preliminary results of the tests of acute infection of HEV in the pregnant women for follow-up on any development of active liver disease and potentially ALF. In ALF patients, according to Northup and Caldwell podcast interviews (www.gastro.org/cghpodcast) a single dose of recombinant activated factor {VIIrFVIIa

(40 μg/kg)} can facilitate performance of intracranial pressure monitor placement. However, continuous infusions of rFVIIa in ALF patients is not recommended because of the potential for thrombotic complications. There is the potential pitfall of use of prophylactic transfusion of fresh frozen plasma (FFP) or platelets in ALF without clinically evident bleeding.

A question of impact on infants of HEV infection in mothers arises; for example, whether it was safe for them to breastfeed was often asked during the course of this study. The risk of HEV transmission in breastmilk to the infant when the woman eventually develops active disease exists (Chaudhry *et al.,* 2015). However, according to these authors, breastfeeding is considered safe in asymptomatic women infected with HEV. Similarly, in the current study where about I in every 5 (22.5%) asymptomatic pregnant women were positive for anti-HEV antibodies in their blood, the mothers could brestfeed despite the likely presence of HEV RNA in the colostrum. In acute hepatic disease or an increased viral load mothers can potentially transmit HEV to the infant in breastmilk (Kuma *et al.,* 2001). Chibber *et al.* (2004) recommended that feeding formulas should rather be used, to avoid viral transmission from infected breast milk or lesions on the nipple.

There is a significant association between HEV seropositivity and mode of delivery of babies. Spontaneous vaginal delivery (SVD) and all other modes of delivery (AoMD), which included caeserian sections and vacuum sunctions, were the explanatory variables. (CS), p = 0.000, OR 0.380 95% Cl 0.258-0.561): 30.0% seropositivity in CS versus 27.6% in spontaneous vaginal delivery (SVD).

This research determined the seroprevalence of HEV in the blood of asymptomatic pregnant women as its first objective. This was to fill in the gaps identified in the Ghana

Health Service report (DCD, 2014). However, because HE infection is a late adverse disease the stated purpose of the objective was to detect the infection in the pregnant women early at delivery. This was to engender close observation and monitoring by their physicians, and that was largely achieved. WHO supports the choice of screening method for the detection of HEV infection in the study (WHO, 2017). According to the factsheet, where active hepatitis, either acute or chronic, is common and the viral etiology is unknown, the detection of specific IgM antibodies to the virus in blood is adequate. Velosa *et al.* (2013) provided another target for whom anti-HEV detection is adequate, which are immunocompetent individuals. For immucompromised individuals, as in the case of the study participants who are pregnant women, molecular methods (RT-PCR) should always be applied because seroconversion can be delayed in these patients. It should further be stressed that the participants in our study are of a cross-sectional study who are largely deemed healthy individuals. The purpose of the tests applied were not diagnostic but for research purpose (Drobeniuc *et al.*, 2010; Pischke *et al.*, 2010).

This study found no HEV RNA during the screening of blood samples in the asymptomatic pregnant women. It is not surprising though that this was the case. Even in severe active infections the virus is insidious and difficult to detect. When active infections of HEV develop much of the epidemiology is a discussion about the prevailing genotypes involved in the particular outbreak. To the knowledge and experience of this author no report of the circulating genotypes has been determined in Ghana to date. HEV infections are much a discussion of genotypes. Although severe liver disease among pregnant women, with high mortality, is the hallmark of epidemics of HEV-1 in Asia and Africa, there have been reports of severe hepatitis among pregnant women infected with HEV-3 in countries where locally acquired genotype 1 infections do not occur. In Ghana,

however, in the knowledge and experience of the author of this thesis there has not been any reported epidemic outbreak of HEV in the population, albeit in the pregnant population.

Outbreaks of clinical hepatitis E attributed to HEV-3 was recently reported in a pregnant woman in Portugal (Velosa *et al.,* 2013), which is a sporadic case. HEV-3-linked acute liver failure also occurred in a nonpregnant Spanish woman whose medical history was devoid of known risk factors for severe hepatitis E. At the time of that report the genotype identification tests conducted had not been reported (Lindemann *et al.,* 2010). The woman reported long-term use of hormonal contraceptives (norgestrel/ethinyl estradiol) and was found to have hepatic adenomas upon examination, which the authors speculated may reflect elevated estrogen levels, mimicking pregnancy, prior to the onset of hepatitis E (Lindemann *et el.,* 2010). The reason behind benign impacts of infection with HEV at delivery remains unclear. However, it might relate to the likely presence of and infection by genotype 3 (Gt3) strains, which are known to be less virulent HEV strains found in swine and also agents of human infection in the study area. Reports have identified farming practices, passive immunity and co-infection with immunosuppressive agents (Tettey and Adjei, 2009) as the main factors influencing the risk factor-linked HEV infection dynamics on domestic pig farms; thus transmitting infection to caregivers and pork eating populations on and outside the farm setting (Colson *et al.,* 2010; Moal *et al.,* 2014; Gerolami *et al.,* 2017).

Swine-related strains of HEV have been reported in swine in Cameroun, in the DRC and Nigeria (Chanasit, 2013; Kaba *et al.,* 2010; Salete de Paula *et al.,* 2013). There are similar findings in Southern India and Egypt, where no difference in HEV severity in pregnant

women compared to non-pregnant women were reported (Rasheeda *et al.,* 2008). It should however, be noted that zoonotic strains could lead to chronicity, fibrosis and finally to cirrhosis in untreated patients. To buttress the chronicity tendency inherent of zoonotic strains, so far no human genotype 1 and 2 strains of the virus has been reported to have caused chronic hepatitis and cirrhosis (Khuroo *et al.,* 1981). Another explanation of the benign characteristics of the findings is the apparent high levels of previous exposure to HEV in early childhood. This could bring about long-lasting immunity, upon re-exposure to HEV later in life due to potentially milder infection (Kamar *et al.,* 2014).

The find of pre-eclampsia of the relatedness among the women prior to delivery with infection by HEV is significant. Pre-eclampsia has a known association with hepatitis in pregnancy (Navaneethan *et al.,* 2008), which makes this finding also clinically significant. My personal observations in the wards compliment this finding. Pre-eclampsia is a rampant feature during my ward visits. Kumar *et al.* (2010) reported that poor maternal nutrition and the use of herbal medicines are associated with increased severity of cases of hepatitis during pregnancy. However, the current study has not investigated these factors.

The current study reports differences of outcomes across districts, which may be related to differences in virulent strains and viral load that the reports of Navaneethan (2012) and Pérez-Gracia *et al.* (2017) also support. Additionally, genetic factors - variation in human leukocyte antigen (HLA) alleles in different geographical regions, could explain differences in infections with the presence of putative risk factors (Navaneethan *et al.,* 2008). This is also true for the effects and role of different environmental risk factors varying from the place of location of residence (Stoszek *et al.,* 2008). The reason behind benign impacts of infection with HEV at delivery remains unclear. However, it might

relate to infection by genotype 3 (Gt3) strains, which are known to be less virulent strain in swine, in the study area. There is a yet to be published report of my find of HEV infection in swine in the study area. Swine-related strains of HEV have been reported in swine in Cameroun, in the DRC and Nigeria (Chanasit, 2013; Kaba *et al.,*2010; Salete de Paula *et al.,* 2013). There are similar findings in Southern India and Egypt, where no difference in HEV severity in pregnant women compared to non-pregnant women were reported (Rasheeda *et al.,* 2008).

It should however, be noted that zoonotic strains could lead to chronicity, fibrosis and finally to cirrhosis in untreated patients. To buttress the chronicity tendency inherent of zoonotic strains, so far no human genotype 1 and 2 strains of the virus has been reported to have caused chronic hepatitis and cirrhosis have not been reported (Khuroo *et al.,* 1980). Undoutedly, HEV Gt3 is an addition to the economic and public health burden of chronic diseases in Ghana and global (Rapoport, 2004). Another explanation of the benign characteristics of the find is the apparent high levels of previous exposure to HEV in early childhood. This could bring about long-lasting immunity, upon re-exposure to HEV later in life due to potentially milder infection (Kamar *et al.,* 2014).

Hepatitis E virus is an important cause of epidemic and sporadic hepatitis that is responsible for morbilities and mortalities among infected pregnant women. Hepatitis E during pregnancy is termed as a condition with late adverse effect because the severest effects are observed at the later stages when interventions are less effective. It is a paradox that world-wide only a handful of countries have classified hepatitis E as a reportable (scheduled) disease nor is screening a requirement during pregnancy. The third trimester could either be an increased risk to the survival of the pregnant woman, the pregnancy

itself or to the foetus. The various scenarios have been demonstrated by this study and that of Bonney *et al.* (2012).

Two, is the role of host immune factors. Although the severity of HEV-associated acute hepatitis is believed to rely on the status of the host's immune system, viral factors may also play important roles in the pathogenesis of the disease. Indeed, the genotype of HEV and intra-genotype variants may contribute to the pathogenesis of HEV-associated hepatitis (Smith *et al.,* 2015). Genotype 3 HEV variant infected patients showed more severe form of the viral hepatitis than genotype 3 HEV infected patients (Geng *et al.,* 2012). Indeed, genetic mutations could affect the virulence factors and antigenicity, which in turn could modify the apthogenesis and manifectation of HEV assocated diseases. Thus, the genetic changes in HEV genotypes may affect the effectiveness of virus transmission and, in turn, the severity of HEV-associated hepatitis. Because infections with HEV can be clinically inapparent or produce symptoms and signs of hepatitis of varying severity and occasional fatality. This is the scenario playing out and that was observed in the study area.

In summary the research identified innovative finds that make his study unique, which are the following: (i) This research is the first study of seroprevalence of HEV infections in the Western Region of Ghana. With the find of 22.5% in the Sekondi-Takoradi Metropolis of the in pregnant women in their third trimester. The study also investigated domestic pigs for the first timefou d of HEV infection on 25% of domestic pig farms in the Western of Ghana. This find is particularly important because of the known zoonotic potential of swine HEV, (ii) The recent infections with HEV seroprevanlence data of 22.5% in pregnant women in third trimester could be combined with other reports from the Greater

Accra and Central Regions to extrapolate estimates of the national HEV infection statistics, (iii) the find of HEV-risk factors at the household level, for example the lack of access to water closet facilities as a risk factor for HEV infection have implications for policy. This is because in the absence of vaccines to control the disease education as a strategy could to be used in dealing with HEV infections, (iv) the find of proximity factors have policy implications: to reduce distances between households and domestic pig farms to lower new infections of HEV in the general population and in particular among the pregnant women population, and(v) the find of association of recent infection HEV and complications uniquely provides the evidence that could help direct the efforts of care givers to lower the potential consequences HEV infections at the time of delivery: to implement measures to benefit the safety of both mother and child at delivery.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

From the findings of this study, the following conclusions are made:

i. Seroprevalence of recent infection with HEV in the third trimester of pregnancy is 22.5% in Sekondi-Takoradi Metropolis of Western Region, Ghana
ii. Seroprevalence of swine HEV infection is 25% on domestic pig farms in the Sekondi-Takoradi Metropolis of the Western Region, Ghana
iii. The absence of water closet toilets and proximity to domestic pig farms are associated risk factors for HEV infection, whilst proximity to wetlands is not an associated risk factor to HEV infection
iv. Complications at delivery are positively associated with recent infections of HEV.

5.2 Recommendations

i. The Ministry of Health and the Ghana Health Service at the regional and national levels should consider using the statistics on HEV infections in pregnant women emanating from this study. The purpose could be for the derivation of national and regional statistics on HEV and for education as a strategy to control the infection in the public and among pregnant women
ii. The Veterinary Public Health Units of the Ghana Veterinary Service at the regional and national levels should consider using the statistics on HEV infections in swine and pregnant women emanating from this study as a tool for education for farmers.

iii. Metropolitan, municipal and local government authorities (MMLGA) should increase support for access to water closet toilet facilities in households

iv. The MMLGAs should encourage location of domestic pig farms farther away from residential accomodations

v. Public health authorities should encourage public awareness creation about HEV infection in pregnancy In the absence of vaccine against HEV public education for prevention control potential of Swine HEV causing liver cancer in Ghana

vi. Research institutions should undertake research into infections of HEV and in particular swine-HEV because of liver cancer potential

vii. Pertaining to the impacts pregnant women should be the focus of future HEV research and vaccine development against HEV infection

viii. A refocus on HEV research as a One Health challenge because of the swine involvement and the only widely known cause of zoonotic hepatitis and human cancer reported in other countries

ix. Improved research skills for: dealing with maps, improved data/geospatial analysis, laboratory test protocols.

REFERENCES

Adjei, A., Tettey, Y., Aviyase, J. T., Adu-Gyamfi, C., Mingle, J. A. and Ayeh-Kumi, P. F. (2009). Hepatitis E virus infection among swine handlers in Accra, Ghana. *East African Medical Journal* 86: 359-363.

Aggarwal, R. and Krawczynski, K. (2000). Hepatitis E: an overview and recent advances in clinical laboratory research. *Journal of Gastroenterology and Hepatology* 15: 9-22.

Arankalle, V. A. and Chobe, L. P. (2003). Swine HEV infection in south India and phylogenetic analysis (1985-1999). *Journal of Medical Virology* 69: 391-396.

Aretouyap, Z., Nouch, P. N., Nouayou, R., Kemgang, F. E. G., Toko, A. D. P. and Asfahani, J. (2016). Lessening the adverse effect of the semivariogram model selection on an interpolative survey using kriging technique *Springer Plus* 5: 549-566.

Auchincloss, A. H., Gebreab, S. Y., Mair, C. and Diez Roux, A. V. (2012). A review of spatial methods in epidemiology, 2000-2010. *Annual Review of Public Health* 33: 107-122.

Baddeley, A. and Turner, R. (2000). Practical maximum pseudolikelihood for spatial point patterns. *Australian and New Zealand Journal of Statistics* 42(3): 283-322.

Balayan, M. S. (1983). Hepatitis E virus infection in Europe: Regional situation regarding laboratory diagnosis and epidemiology. *Clinical and Diagnostic Virology* 1: 1-9.

Baylis, S. A., Terao, E., Kay-Martin, O. and Hanschmann, B. (2015). Collaborative Study to Establish the 1st World Health Organization International Reference Panel for Hepatitis E Virus RNA Genotypes for Nucleic Acid Amplification Technique (NAT)-Based Assays. WHO/BS/2015.2265. [https://www. sciencedirect.com › science › article › pii] site visited on 09/08/2018.

Berke, O. (2004). Exploratory disease mapping: kriging the spatial risk function from regional count data. International. *Journal of Health Geographics* 3: 18-25.

Bonney, J. H., Kwame-Aryee, R. A., Obed, S., Tamatey, A. A., Barnor, J. S., Armah, N. B., Oppong, S.A. and Osei-Kwesi, M. (2012). Fatal hepatitis E viral infection in pregnant women in Ghana: *BMC Research Notes* 475: 478-503.

Bonney, J. H., Osei-Kwasi, M., Adiku, T. K., Barnor, J. S., Amesiya, R., Kubio, C., Ahadzie, L., Olschläger, S., Lelke, M., Becker-Ziaja, B., Pahlmann, M. and Günther, S. (2013). Hospital-based surveillance for viral hemorrhagic fevers and hepatitides in Ghana. *PLoS Neglected Tropical Diseases* 7(9): e2435.

Bouwknegt, M. and Teunis, P. F. (2010). Estimation of the Likelihood of Fecal-Oral HEVTransmission among Swine. *Risk Analysis* 31: 879-1035.

Cawley, M. J. (2007). *The R Book*. United Kingdom: John Wiley & Sons Ltd. Institute of Zoology, Zoological Society of London, London, UK. 522pp.

Chanasit, J. (2013). Hepatitis E virus genotype 3 strains in domestic pigs, Cameroun. *Journal Emerging Infectious Diseases* 19: 686-688.

Chaudhry, S. A., Verma, N. and Koren, G. (2015). Hepatitis E infection during pregnancy. *Canadian Family Physician* 61: 607-608.

Chibber, R. M., Usmani, M. A. and Al-Sibai, M. H. (2004). Should HEV infected mothers breast feed? *Archives of Gynecology and Obstetetrics* 270: 15–20.

Chobe, L. P. and Chadha, M. S. (1997). Detection of HEV RNA in faeces, by RT-PCR during the epidemics of hepatitis E in India (1976-1995). *Journal of Viral Hepatology* 4: 129-133.

Choi, C. and Ha, S. K. (2004). Development of nested RT-PCR for the detection of swine hepatitis E virus in formalin-fixed, paraffin-embedded tissues and comparison with in situ hybridization. *Journal of Virological Methods* 115: 67-71.

Choi, I. S., Kwon, H, J., Shin, N. R. and Yoo, H. S. (2003). Identification of swine hepatitis E virus (HEV) and prevalence of anti-HEV antibodies in swine and human populations in Korea. *Journal of Clinical Microbiology* 41: 3602-3608.

Clemente-Casares, P. and Pina, S. (2003). Hepatitis E virus epidemiology in industrialized countries. *Emerging Infectious Diseases* 9: 448-454.

Colson, P., Borentain, P., Queyriaux, B., Kaba. M., Moal. V., Gallian, P., Heyries, L., Raoult, D. and Gerolami, R. (2010). Pig liver sausage as a source of hepatitis E virus transmission to humans. *Journal of Infectious Diseases* 202: 825–834.

Cooper, B. S., White, L. J. and Siddiqui, M. R. (2017). Reactive and pre-emptive vaccination strategies to control hepatitis E infection in emergency and refugee settings: a modeling study. *Research* 12(9): 1401–1404.

Dalton, H. R., Fellows, H. J., Gane, E. J., Wong, P., Gerred, S., Schroeder, B., Croxson, M., C. and Garkavenko, O. (2007). Hepatitis E in New Zealand. *Journal of Gastroenterology and Hepatology* 22: 1236-1240.

Daniel, H. D., Warier, A., Abraham, P. and Sridharan, G. (2004). Age-wise exposure rates to hepatitis E virus in a southern Indian patient population without liver disease. *American Journal of Tropical Medicine Hygiene* 71: 675-678.

Di Bartolo, I., Ponterio, E., Castellini, L., Ostanello, F. and Ruggeri, F. M. (2011). Viral and antibody HEV prevalence in swine at slaughterhouse in Italy. *Veterinary Microbiology* 149: 330-338.

Diggle, P. (1990). A point process modelling approach to raised incidence of a rare phenomenon in the vicinity of a prespecified point. *Journal of the Royal Statistical Society* 153: 349–362.

Diggle, P., Moyeed, R., Rowlingson, B. and Thomson, M. (2002). Childhood malaria in the Gambia: A case study in model-based geostatistics. *Journal of the Royal Statistical Society* 51: 493–506.

Divizia, M., Gabrieli, R., Stefanoni, M. L., Renganathan, E., El Ghazzawi, E., Kader, O. A. and Degener, A. M. (1999). HAV and HEV infection in hospitalised hepatitis patients in Alexandria, Egypt. *European journal of epidemiology* 15(7): 603-609.

Doceul, V., Bagdassarian, E., Demange, A. and Pavio, N. (2016). Zoonotic Hepatitis E Virus: Classification, Animal Reservoirs and Transmission Routes. [http//:www.mdpi.com/journal/viruses] site visited on 10/08/2018.

Dong, C., Meng, J., Dai, X., Liang, J. H., Feagins, A. R., Meng, X. J. and Glass, G. E. (2011). Restricted enzooticity of hepatitis E virus genotypes 1 to 4 in the United States. *Journal of Clinical Microbiology* 49(12): 4164-4172.

Drebber, U., Odenthal, M., Aberie, S. W., Winkel, N., Wedemeyer, I., Hemberger, J., Holzmann, J. and Dienes, H. P. (2013). Hepatitis E in liver biopsies from patients with acute hepatitis of clinically unexplained origin. *Frontier in Physiology* 4(351): 1-5.

Elduma, A. H., Zein, M. M. A., Karlsson, M., Elkhidir, I. M. E. and Norder, H. (2016). A Single Lineage of Hepatitis E Virus Causes both Outbreaks and Sporadic Hepatitis in Sudan. *Viruses* 8: 273-283.

Emerson, S. and Purcell, R. (2004). Hepatitis E virus. *Review Medical Virology* 13: 145-154.

Feldt, T., Sarfo, F. S., Zoufaly, A., Phillips, R. O., Burchard, G., van Lunzen, J., Jochum, J., Chadwick, D., Awasom, C., Claussen, L., Drosten, C., Drexler, J. F. and Eis-Hübinger, A. M. (2013). Hepatitis E virus infections in HIV-infected patients in Ghana and Cameroon. *Journal of Clinical Virology* 58: 18-23.

Formiga-Cruz, M., Allard, A. K., Conden-Hansson, A. C., Henshilwood, K., Hernroth, B. E., Jofre, J., Lees, D. N., Lucena, F., Papapetropoulou, M., Rangdale, R.,Tsibouxi, E., Vantarakis, A. and Girones, R. (2002). Distribution of human virus contamination in shellfish from different growing areas in Greece, Spain, Sweden, and the United Kingdom. *Applied Environmental Microbiology* 68: 5990-5998.

Fox, J. (2003). Effect displays in R for generalised linear models. *Journal of Statistical Software* 8(15): 1–27.

Futurity Group (2016). Subject: PRO/EDR> Hepatitis E - Bangladesh: pregnancy, increased mortality, risk factors. From: [promed-edr@promedmail.org] site visited 15/01/2016.

Galiana, C., Fernández-Barredo, S., García, A., Gómez, M. and Pérez-Gracia, M. T. (2010). Occupational exposure to hepatitis E virus (HEV) in swine workers. *American Journal of Tropical Medicine and Hygiene* 78: 1012-1015.

Geng, J. B., Wang, M. R., Wang, L., Wang, J., Yang, Z. G., Cheng, Y. and Wang, M. (2012). Genetic characteristics and pathogenicity of human hepatitis E virus in Nanjing, China. *World Journal of Gastroenterology* 18(9): 965-971.

Geremew, T. T., Gezie, L. D. and Abejie, A. N. (2019). Geographical variation and associated factors of childhood measles vaccination in Ethiopia: a spatial and multilevel analysis. BMC Public Health 19:1194-1196.

Ghana Ministry of Health (2014). *Health Surveillance Annual Report, 2014*. 12pp.

Graham, S. L., Barling, K. S., Waghela, S., Scott, H. M. and Thompson, J. A. (2005). Spatial distribution of antibodies to Salmonella enterica serovar Typhimurium O antigens in bulk milk from Texas dairy herds. *Preventive Veterinary Medicine* 69(1-2): 1-162.

Guthmann, J. P., Klovstad, H. and Boccia, D. (2006). A large outbreak of hepatitis E among a displaced population in Darfur, Sudan 2004: the role of water treatment methods. *Clinical Infectious Diseases* 42: 1685–1691.

Hammoud, G. M. and Ibdah, J. A. (2014). Preeclampsia-induced Liver Dysfunction, HELLP syndrome, and acute fatty liver of pregnancy. *Clinical Liver Disease* 4: 69–73.

Hogan, M. C., Foreman, K. J. and Naghavi, M. (2010). Maternal mortality for 181 countries, 1980–2008: a systematic analysis of progress made towards Millennium Development Goal 5. *Lancet* 375(9726): 1609–1623.

Huang, G., Haqshenas, D., Guenette, K., Halbur, P. G., Schommer, S. K., Pierson, F. W., Toth, T. E. and Meng, X. J. (2004). Detection by reverse transcription-PCR and

genetic characterization of field isolates of swine hepatitis E virus from swine in different geographic regions of the United States. *Journal Clinical Microbiology* 40: 1326-1332.

Huang, R. T., Li, D. R., Wei, J., Huang, X. R., Yuan, X. T. and Tian, X. (1992). Isolation and identification of hepatitis E virus in Xinjiang, China. *Journal of General Virology* 73: 1143–1148.

Hussaini, S. H., Skidmore, S. J., Richardson, P., Sherratt, L. M., Cooper, B. T. and O'Grady, J. G. (1997). Severe hepatitis E infection during pregnancy. *Journal of Viral Hepatitis* 4: 51–54.

Isaaks, E. H. and Srivastava, R. M. (1989). *Applied Geostatistics.* Oxford University Press, New York. 561pp.

Janssen, I. (2012). Health care costs of physical inactivity in Canadian adults. *Applied Physiology, Nutrition, and Metabolism* 37(4): 803-806.

Jothikumar, N., Cromeans, T. L., Robertson, B. H., Meng, X. J. and Hill, V. R. (2006). A broadly reactive one-step real-time RT-PCR assay for rapid and sensitive detection of hepatitis E virus. *Journal of Virological Methods* 131: 65–71.

Junaid, S. A., Agina, S. E. and Abubakar, K. A. (2014). Epidemiology and associated risk factors of Hepatitis E virus infection in Plateau State, Nigeria. *Research and Treatment* 5: 15–26.

Kaba, M., Colson, P., Musongela, J. P., Tshilolo, L. and Davoust, B. (2010). Detection of hepatitis E virus of genotype 3 in a farm pig in Kinshasa (DRC). *Infection. Genetics and Evolution* 10: 1154–1157.

Karimuribo, E. D., Chenyambuga, S. W., Makene, V. W. and Mathias, S. (2011). Characteristics and production constraints of rural-based small-scale pig farming in Iringa region, Tanzania. *Livestock Research for Rural Development* 23(8): 172-181.

Katano, H., Kano, M., Nakamura, T., Kanno, T., Asanuma, H. and Sata, T. (2011). A novel real-time PCR system for simultaneous detection of human viruses in clinical samples from pregnant women with uncertain diagnoses. *Journal of Medical Virology* 83: 322–330.

Khuroo, M. S. (1980). Chronic liver disease after non-A, non-B hepatitis. *Lancet* 2: 860–861.

Khuroo, M. S. and Khuroo, M. S. (2016). Hepatitis E: An emerging global disease—From discovery towards control and cure. *Journal of Viral Hepatitis* 23: 68–79.

Khuroo, M. S. and Kamili, S. (2003). Aetiology, clinical course and outcome of sporadic acute viral hepatitis in pregnancy. *Journal of Viral Hepatitis* 10(1): 61-69.

Khuroo, M. S., Khuroo, M. S. and Khuroo, N. S. (2016). Hepatitis E: global impact, control and cure. *World Journal of Gastroentrology* 22: 7030-7045.

Khuroo, M. S., Saleem, M., Teli, M. R. and Sofi, M. A. (1980). Failure to detect chronic liver disease after epidemic non-A, non-B hepatitis. *Lancet* 2: 97–98.

Khuroo, M. S., Teli, M. R., Skidmore, S., Sofi, M. A. and Khuroo, M. I. (1981). Incidence and severity of viral hepatitis in pregnancy. *The American Journal of Medicine* 70(2): 252-255.

Kim, J. H., Kenrad, E. N., Panzner, U., Kasture, Y., Labrique, A. B. and Wierzba, T. F. (2014). A systematic review of the epidemiology of hepatitis E virus in Africa. *BMC Infectious Diseases* 14: 308-323.

Kumar, R. M., Uduman, S., Rana, S., Kochiyil, J. K., Usmani, A. and Thomas. L. (2010) Sero-prevalence and mother-to-infant transmission of hepatitis E virus among pregnant women in the United Arab Emirates. *European Journal of Obstetetrics, Gynecology and Reproductive Biology* 100(1): 9–15.

La Rosa, G., Pourshaban, M., Iaconelli, M., Vennarucci, V. S. and Muscillo, M. (2010). Molecular detection of hepatitis E virus in sewage samples. *Applied and Environmental Microbiology* 76(17): 5870-5873.

Lee, G. H., Tan, B. H., Teo, E. C. Y., Lim, S. G., Dan, Y. Y., Wee, A. and Purdy, M. A. (2016). Chronic infection with camelid hepatitis E virus in a liver transplant recipient who regularly consumes camel meat and milk. *Gastroenterology* 150(2): 355-357.

Lewis, H. O., Wichmann, O. and Duizer, E. (2010). Transmission routes and risk factors for autochthonous hepatitis E virus infection in Europe: a systematic review. *Epidemiology and Infection* 138: 145-166.

Lin, D. B., Lin, J. B., Chen, S. C., Yang, C. C., Chen, W. K. and Chen, C. J. (2004). Seroepidemiology of hepatitis E virus infection among preschool children in Taiwan. *Journal of Medical Virology* 74: 414-414.

Lindemann, M. L., Morales, J. G., Fernandez-Barredo, S., Dominguez, M. R., Garcia de la, H. F., Halfon, P. and Perez Gracia, M. T. (2010). Fulminant hepatitis E in a woman taking oral contraceptive medication. *American Journal of Tropical Medicine and Hygiene* 82: 12–15.

Liu, X., Saito, M., Sayama, Y., Suzuki, E., Malbas, F. F. Jr., Galang, H. O., Furuse, Y., Li, T., Suzuki, A. and Oshitani, H. (2015). Seroprevalence and molecular characteristics of hepatitis E virus in household-raised pig population in the Philippines. *BMC Veterinary Research* 11: 11-19.

Meng, X. (2009). Hepatitis E virus: animal reservoirs and zoonotic risk. *Veterinary Microbiology* 140: 256–265.

Meng, X. and Wiseman, B. (2009). Prevalence of Antibodies to Hepatitis E Virus in Veterinarians Working with Swine and in Normal Blood Donors in the United States and Other Countries. *Journal of Clinical Microbiology* 40: 117-122.

Mikrogen Germany (2016). List of Hepatitis E (HEV) Publications Floriansbogen. *Journal of Hepatology* 62(11):1464-1473.

Mirazo, S., Ramos, N., Mainardi, V., Gerona, S. and Arbiza, J. (2014). Transmission, diagnosis, and management of hepatitis E: an update. *Hepatic Medicine : Evidence and Research* 6: 45-59.

Moal, V., Gerolami, R. and Colson, P. (2012). First human case of co-infection with two different subtypes of hepatitis E virus. *Intervirology* 55: 484–487.

Modiyinji, A. F., Atsama, M. A., Monamele, G. C., Nola, M. and Njoum, R. (2018). High prevalence of hepatitis E in pigs suggests an animal reservoir in Cameroon. *The Journal of infection in Developing Countries*12 (08): 678-679.

Motte, A., Roquelaure, B., Galambrun, C., Bernard, F., Zandotti, C. and Colson, P. (2012). Hepatitis E in three immunocompromized children in southeastern France. *Journal of Clinical Virology* 53: 162–166.

Navaneethan, U., Al Mohajer, M. and Shata, M. T. (2008). Hepatitis E and pregnancy: understanding the pathogenesis. *Liver international* 28: 1190-1199.

Northup, P. G. and Caldwell, S. H. (2013). Coagulation in Liver Disease: A Guide for the Clinician. *Clinical Gastroenterology and Hepatology* 11: 1064–1074.

Obiri-Yeboah, D., Asante Awuku, Y., Adu. J., Pappoe, F., Obboh, E., Nsiah, P., Amoako-Sakyi, D. and Simpore, J. (2018). Sero-prevalence and risk factors for hepatitis

E virus infection among pregnant women in the Cape Coast Metropolis, Ghana. *PLoS One* 13(1): e0191685.

Ofori-Asenso, R. and Agyeman, A. A. (2017). Hepatitis E infection among Ghanaians: a systematic review. *Infectious Diseases of Poverty* 6: 29-36.

Ofosu-Appiah, L. H., Boney, J. H. K., Adiku, T., Barnor, J. and Odoom, J. K. (2016). Serological and Molecular evidence of hepatitis E infection among patients with suspected viral hepatitis seen at Korle-Bu Teaching Hospital, Accra. *Journal of Scientific Research and Reports* 10(1): 1-10.

Olea, R. A. (1999). *Geostatistics for Engineers and Earth Scientists*. Kluwer Academic Publishers, Boston Mass. 318pp.

Owolodun, O. A., Gerber, P. F., Giménez-Lirola, L. G., Kwaga, J. K. and Opriessnig, T. (2014). First report of hepatitis E virus circulation in domestic pigs in Nigeria. *The American Journal of Tropical Medicine and Hygiene* 91(4): 699-704.

Peng, J., Kuk, L. L. and Gary, M. I. (2002). An Introduction to Logistic Regression Analysis and Reporting. *Journal of Educational Research* 96(1): 3-14.

Pfeiffer, D., Robinson, T. P., Stevenson, M., Stevens, K. B., Rogers, D. J. and Clements, A. C. (2008). New York, NY, USA: Oxford University Press. *Spatial Analysis in Epidemiology* 2(1): 1756-3305.

Purdy, M. A. and Khudyakov, Y. E. (2010). Evolutionary History and Population Dynamics of Hepatitis E Virus. *PLoS ONE* 5(12): e14376.

Rapoport, J., Jacobs, P., Bell, N. R. and Klarenbach, S. (2004). Refining the measurement of the economic burden of chronic diseases in Canada. *Chronic Diseases and Injuries in Canada* 25(1): 13-21.

Reddick, K. L., Jhaveri, R., Ghandhi, M., James, A. H. and Swamy, G. K. (2011). Pregnancy outcomes associated with viral hepatitis. *Journal of Viral Hepatitis* 18(7): 394-398.

Risalde, M. A. Rivero-Juarez, A., Romero-Palomo, F., Friaz, M., Lopez-Lopez, P. and Cano-Terriza, D. (2017) Persistence of hepatitis E virus in the liver of non-viremic naturally infected wild boar. *PloS ONE* 12(11): e186858.

Rodriguez-Frias, F., Buti, M. and Hepatitis, E. (2015). Virus: New faces of an old infection. Saito. T. Li, Suzuki, A. and Oshitani, H.. Seroprevalence and molecular characteristics of hepatitis E virus in household-raised pig population in the Philippines. *BMC Veterinary Research* 11: 11-22.

Ruggeri, F. M., Di Barte, P. I., Ostanello, F. and Trevisani, M. (2013). Epidemiology of HEV infection in Animals in Hepatitis E Virus. *Springer Briefs in Food, Health, and Nutrition.* Springer, New York, NY. 84pp.

Salete de Paula, V., Wiele, M., Mbunkah, A. H., Daniel, A. M., Kingsley, M. T. and Schmidt-Chanasit, J. (2013). Hepatitis E Virus Genotype 3 Strains in Domestic Pigs, Cameroon. *Emerging Infectious Diseases* 19: 686-688.

Salines, M., Andraud, M. and Rose, N. (2017). From the epidemiology of hepatitis E virus (HEV) within the swine reservoir to public health risk mitigation strategies: a comprehensive review. *Veterinary Research* 48: 31-42.

Schielke, A., Ibrahim, V., Czogiel, I., Faber. M., Schrader. C., Dremsek, P., Ulrich, R.G. and Johne, R. (2015). Hepatitis E virus antibody prevalence in hunters from a district in Central Germany, a cross-sectional study providing evidence for the benefit of protective gloves during disembowelling of wild boars. *BMC Infectious Diseases* 15: 440-445.

Sleisenger, M. H., Feldman, M. and Friedman, L. S. (2010). Sleisenger and Fordtran's gastrointestinal and liver disease: pathophysiology, diagnosis, management. *Saunders/Elsevier*, Philadelphia. 2480pp.

Smith, D. B., Ijaz, S., Tedder, R. S., Hogema, B., Zaaijer, H. L., Izopet, J. and Simmonds, P. (2015). Variability and pathogenicity of hepatitis E virus genotype 3 variants. *Journal of General Virology* 96(11): 3255-3264.

Soo, B. P. (2012). Hepatitis E vaccine debuts: Success of Chinese biotech partnership raises hopes for prevention of overlooked diseases. *Nature* 491: 21–22.

Stoszek, S. K., Abdel-Hamid, M., Saleh, D. A. A., Kafrawy, S. E., Narooz, S., Hawash, Y. and Mikhail, N. (2008). High prevalence of hepatitis E antibodies in pregnant Egyptian women. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 100(2): 95-101.

Takahashi, M., Nishizawa, M. and Okamoto, H. (2003). Polyphyletic Strains of Hepatitis E Virus Are Responsible for Sporadic Cases of Acute Hepatitis in Japan. *Journal of Clinical Microbiology* 40: 3209-3218.

Teshale, E. H., Hu, D. J. and Holmberg, S. D. (2010). The two faces of hepatitis E virus. *Clinical Infectious Diseases* 51: 328–334.

Tettey, Y. and Adjei, A. (2009). Hepatitis E infection is highly prevalent among pregnant women in Accra, Ghana. *Virology Journal* 6: 10-23.

Tettey, Y., Adjei, A., Ayivase, J., Adu-Gyamfi, C., Mingle, J., Nartey, E. and Kumar, G. (2009). Serological evidence of hepatitis E virus infection among volunteer blood donors at the Accra area blood transfusion center, Accra, Ghana. *Journal of Ghana Science Association* 13(2): 64–73.

Tomiyama, D. and Inoue, E. (2009). Serological evidence of infection with hepatitis E virus among wild Yezo-deer, Cervus nippon yesoensis, in Hokkaido, Japan. *Journal of Viral Hematology* 16: 524–528.

Tripodi, A., Primignani, M., Chantarangkul, V., Clerici, M., Dell'Era, A,. Fabris, F., Salerno. F. and Mannucci, P. M. (2006). Thrombin generation in patients with cirrhosis: the role of platelets. *Hepatology* 44: 440–445.

Tyagi, S. and Surjit, M. (2004). The ORF3 protein of hepatitis E virus interacts with liver-specific alpha1-microglobulin and its precursor alpha1-microglobulin/bikunin

precursor (AMBP) and expedites their export from the hepatocyte. *Journal of Biological Chemistry* 279: 2930-3019.

Velosa, W., Vivek, M. and Munoz, S. J. (2013). Fulminant hepatitis E in a pregnant woman. *GE Jornal Português de Gastrenterologia* 20(5): 210-214.

Wang, X. and Chibber, R. M. (2004). Should HEV infected mothers breast feed? *Achieves of Gynecological Obstetrics* 270: 15-20.

Webster, R. and Oliver, M. A. (2001). *Geostatistics for Environmental Scientists*, John Wiley and Sons, Ltd., Chichester, England. 271pp.

WHO (2010). The Global Prevalence of Hepatitis E Virus Infection and Susceptibility: A Systematic Review. Immunization, Vaccines and Biologicals. Geneva, Switzerland. 308pp.

WHO (2013). Hepatitis E outbreak in Tanzania. Global Dispatch. [http://www. theglobaldispatch.com/hepatitis-e-outbreak-in-Tanzania - 22258/] Site visited on 24/2/2017.

WHO (2014). Waterborne outbreaks of hepatitis E: recognition, investigation and control. *Technical report.* [http://www.who.int/ hiv/topics/ hepatitis/en/] Site visited on 8/9/2019.

WHO (2015). First World Health Organization International Reference Panel for Hepatitis E Virus RNA Genotypes for Nucleic Acid Amplification Technique (NAT)-Based Assays. WHO/BS/2015.2265. [https://apps.who.int › iris › handle] site visited on 15/07/2017.

Wibawa, I., Suryadarma, I. G. A., Tsuda, F., Matsumoto, Y., Ninomiya, M., Takahashi, M. and Okamoto, H. (2007). Identification of genotype 4 hepatitis E virus strains from a patient with acute hepatitis E and farm pigs in Bali, Indonesia. *Journal of Medical Virology* 79(8): 1138-1146.

Wu, T., Zhu, F. C. and Huang, S. J. (2012). Safety of the hepatitis E vaccine for pregnant women, a preliminary analysis. *Hepatology* 55(6): 2038-2043.

Zhang, J., Ge, S. X., Huang, G. Y., Li, S. W., He, Z. Q., Wang, Y. B., Zheng, Y. J., Gu, Y., Ng, M. H. and Xia, N. S. (2004). Significance of serological markers and virological marker for hepatitis E in rhesus monkey model. *Zhonghua Gan Zang Bing Za Zhi* 12: 7-10.

Zhang, S., Wang, J., Yuan, Q., Ge, S., Zhang, J., Xia, N. and Tian, D. (2011). Clinical characteristics and risk factors of sporadic Hepatitis E in central China. *Virology Journal* 8: 152-579.

Zhao, C., Ma, Z., Harrison, T. J., Feng, R., Zhang, C., Qiao, Z. and Wang, Y. (2009). A novel genotype of hepatitis E virus prevalent among farmed rabbits in China. *Journal of Medical Virology* 81(8): 1371-1379.

APPENDICES

Appendix 1: Questionnaire

2 Questionnaires

SECTION B: SOCIO-DEMOGRAPHICS OF RESPONDENT

B1a. What is your name?¡(Name on folder, to be coded due to ethical considerations.) (Wo fr1 wu d1n?)

B1b Respondent Code¡(001¡K)

B1c Has blood sample been taken? 1. Yes 2. No 3. Not yet

B1d Where will a sample be taken 1. ANC 2. Labour ward. 3. Other¡

B1e. If no, please indicate why blood sample will not be taken¡

B2. What is your date of delivery? ¡(See folder)

(1dzi nfe ahen?)

B3. For how many months have you been pregnant?.....1. 7 months 2. 8 months 3. 9 months.(See folder)

(Inyins1nee abosom ahen nye yi?)

2

B4. What is your highest level of education? (Please tick („Ï) as appropriate) (Ask respondent) (Nwomasua p1np1ndo hen na edur?)

Informal /

Primary/Koranic school (Kanee)

Middle/JSS (Mfinimfini)

O-Level/SSS

A-Level

Technical/Vocational

Graduate

Postgraduate

B.5 What is your occupation?

None (Hwee)

Entrepreneur/Self-employed

(Ebue wo edwuma)

Civil servant (Aban dwumay1nyi)

B6. Marital status

1.Married (4warifo)

2. Separated (Aware ntsetsewmu)

3. Divorced (Egyai Awar)

4. Widowed (Kunafo)

5. Not married (Sigyanyi)

B7. Do you have any children of your own yet? a) Yes b) No

(Ew4 woankasa wo mba?)

B8. If yes, how many children do you have?.....

(S1 nyo a, wo mbaa y1 ahy1n)

B9. Temperature reading.....

B10. What are your complaints, if any? (Please tick („Ï) as appropriate) (See folder/ ask respondent, where necessary)

B10.1

High fever if B9 is above 380C

B10.2

Headache (Etsir pai)

B10.3

Loss of appetite (Wokon ndo edziben)

B10.4

Diarrhea (Wo hy1m hwe)

B10.5

Abdominal pains

B10.6

Throat pain (Wo memim y1y1)

B10.7

Vomiting (Wo fi)

3

B10.8

Cough (Wo po w4w)

B10.9

Yellowing of eyes (Wo enyi ay1 yellow)

B10.10

What is her bodyweight/height in Kg and meters? KG/M to enable BMI calculation

B10.11

Is she anaemic? (Will confirm with Hb level determination at the time of blood collection?)

1. Yes 2. No

B10.12

Has liver function test been conducted?

B10.13

If yes, was the result positive? Describe the result in C14, below.

B.11. How long have you stayed in this

household?.....

(Mmfi ay1n na atsena efia aha)

B 12. Do you have animals in your household? a) Yes b) No

(Wo w4 mbowa w4 wo fie?)

B 13. Which animals do you have in your household? Please, write their numbers in the lines.

(ben mbowa na 4w4 wo fie)

1. Sheep (Oguan) ¡K¡K¡K 2.Goats (Aponkye)¡K¡K¡K 3.Birds (Anoma)¡K¡K¡K 4.Pigs (Preko)¡K¡K¡K..

5. Cows (Nentwi)¡K¡K.. Any other

The next questions are for participants who have pigs in household.

B14. How long have you had pigs in the household? 1. Under one year 2. 1-3 years 3.4-6 years 4 Above 6 years

B14.1 Do you work with the pigs? a) Yes b) No

(Ene mpreko y1 edwuma?)

B14.2 If yes, how long have you worked with pigs? 1. Under one year 2. 1-3 years 3.4-6 years 4 Above 6 years

B 15. If yes, how frequent do you consider your work with the pigs to be? a) rarely b) frequent c) very frequent.

(S1 nyo a, mp1n ay1n na edzi w4n y1 edwuma?)

(If attendance to swine is twice a day that is very frequent, once a day is frequent and not attending to the animals on some days should be assessed as rarely).

B 16. Have you been assisting the sows during delivery of piglets? (farrowing) a) Yes b) No

(Wo boa preko no ew4 w4n awomu?)

B 17. Are you involved in the feeding of swine? a) Yes b) No

(Woka wo na woma w4n edzib1n?)

B 18. Are you involved in the cleaning of barns? a) Yes b) No

(Woka wo na yesiesie won dabi1?)

B 19. Are you involved in slaughter of swine? a) Yes b) No

(Woka won a yeku preko no?)

B 20. Do you have access to pipe-borne water for household use? a) Yes b) No
(Wo ny1 nsupapa y1 adzi w4 fie?)
4
B. 21. How often are the pipes opened? 1. Once every week 2.Twice every week 3. Throughout the week
(Mp1n dodow ay4n na pipe no bue)
4. Other, specify.
B 22. What is your source of water during the periods of water scarcity 1. Buying 2. Ground water 3. Bore hole.
(Mmbr1 a nsu ewo11y1 dzin no, eb1n nsu na edzi y1 edzi?)
B 23. Do you have a practice of hand washing with soap and or sanitizer while in the house? a) Yes b) No. (Wo taa dzi samina ne nsu wowor wo nsa?)
B 24. Do you have a practice of hand washing with soap and or sanitizer while outside the house? a) Yes b) No
(Wo taa dzi samina ne nsu wowor wo nsa?)
B 25. How often do you wash your hands in a day? 1. Once 2. Twice 3. Trice 4. More than three times
(Mp1n dodow ahyen na 1wowor wo nsa)
B 26.Do you eat pork? (khebab) a) Yes b) No
(Wo dzi preko y1 tutuw?)
B27. Do you have access to flash toilet in your household? a) Yes b) No
(Wo w4 ndabri tsiafi 1w4 wofie?)
B 28. If no, Where do you go to toilet?
(S1 dabi a, 1hy1n fa na egye wo nan?)
B 29. Have you heard of hepatitis E? a) Yes b) No
(Wo atse hepatitis E nnka?)
B 30. If yes, do you know how one acquires the disease? a) Yes b) No
(S1 enyo a, wonim kwan a obi ny1 yareba no?)
B 31. Is HEV sexually transmitted? a) Yes b) No
(Ana HEV y1 ndamu yareba?)
B 32. Is HEV acquired through blood transfusion? a) Yes b) No
(Ana HEV y1 nye bri a wotwi mbogya?)
B 33. Is HEV acquired through eating roasted meat? a) Yes b) No
(Ana HEV y1 nye abiri a edzi nam a yetutuw?)
B 34. Is HEV acquired through eating raw meat? a) Yes b) No
(Ana HEV y1 nye abiri a edzi nam amun?)

B 35. Is HEV acquired through eating blood meal? a) Yes b) No

(Ana HEV y1 ny3 abiri a edzi mbogya edziban?)

B 36. Have you ever had jaundice? a) Yes b) No

(Ana wony1 jaundice da?)

B 37. Have you ever observed signs of jaundice in any of your household members a) Yes b) No

(Ana wo awun s1kyreni a 4kyer1 jaundice w4 wo fie a?)

B 38. Do you have a health insurance number? a) Yes b) No

(Ana wo w4 apomudzen b1nbo ?)

B 39. If yes, have you renewed your health insurance? a) Yes b) No

(S1 eyo a, ana wo apomudzen banbo ahy1 mu kena?)

B39.1 Has the woman ever had surgery? 1. Yes 2. No

5

B 40. Has the woman ever had blood transfusion? 1. Yes 2. No

B 41. If yes, what date did the blood transfusion take place?.....

B 42. Has the women ever travelled to a HEV-endemic region of the world? 1. Yes 2. No

B 43. Indicate the GPS Reading of respondent¡¦s residence

(To be completed by Research Assistant)

SECTION C: COMPLICATIONS OF PREGNANCY (Refer folder)

C1. Has the women developed pre-eclampsia? 1. Yes 2. No

C2. Has the women experienced preterm labour (PTL)? 1. Yes 2. No

C3 Has the woman experienced preterm delivery (PTB)? 1. Yes 2. No

C4. Has there been a still-delivery? 1. Yes 2. No

C5. Has there been a live-delivery? 1. Yes 2. No

C6. If yes, what is the baby¡¦s weight at delivery?

C6.1 Babies Folder Number¡

C7. Has the woman developed gestational diabetes mellitus (GDM)? 1. Yes 2. No

C7.1. Any other type of diabetes before pregnancy? 1. Yes 2. No

C8. Has the woman developed intrauterine growth restriction (IUGR)?

C9. Has the woman developed cholestasis? 1. Yes 2. No

C10. Has the woman died? 1. Yes 2. No

C11. If yes, what is the cause of death? (Refer to autopsy report)

C12. How many times has Sulfadoxine Pyrimethamine (SP) since she became pregnant?

1) Once 2) Twice 3) Thrice (Tick as appropriate)

(Please Thank the Interviewee. Kindly request her to see the PI with the Filled Questionnaire and Folder to enable completion of C13-C19).

C13. Hepatitis B +ve or ¡Vve;

C14. HIV +ve or ¡Vve;

C15. Syphilis +ve or ¡Vve;

C16. HCV+ve or ¡Vve; C18.

C17. STI +ve or ¡Vve;

C18. HEV +ve or ¡Vve.

C19. Mention/describe any other complications, and also on any of the above in Section C, where necessary.

Appendix 2: Ethical approval

NOGUCHI MEMORIAL INSTITUTE FOR MEDICAL RESEARCH
Established 1979
A Constituent of the College of Health Sciences
University of Ghana

INSTITUTIONAL REVIEW BOARD

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Ghana

My Ref. No: DF/22
Your Ref. No:

2nd March, 2016

ETHICAL CLEARANCE

FEDERALWIDE ASSURANCE FWA 00001824 **IRB 00001276**

NMIMR-IRB CPN 088/15-16 **IORG 0000908**

On 2nd March 2016, the Noguchi Memorial Institute for Medical Research (NMIMR) Institutional Review Board (IRB) at a full board meeting reviewed and approved your protocol titled:

TITLE OF PROTOCOL : **Epidemiology of hepatitis E virus infection in pregnant women at healthcare facilities at Secondi Takoradi Metropolis, Ghana**

PRINCIPAL INVESTIGATOR : **Dr. Reuben M. K. Tetteh**

Please note that a final review report must be submitted to the Board at the completion of the study. Your research records may be audited at any time during or after the implementation.

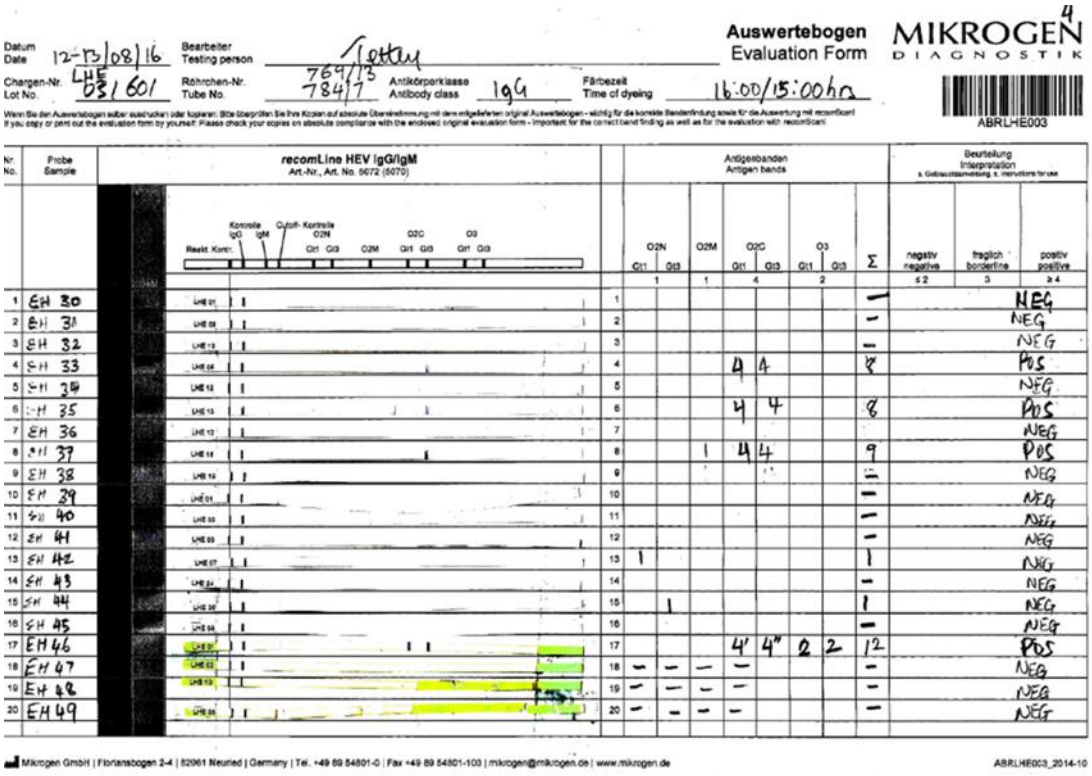
Any modification of this research project must be submitted to the IRB for review and approval prior to implementation.

Please report all serious adverse events related to this study to NMIMR-IRB within seven days verbally and fourteen days in writing.

This certificate is valid till 1st March, 2017. You are to submit annual reports for continuing review.

Signature of Chair: .....
Mrs. Chris Dadzie
(NMIMR – IRB, Chair)

Appendix 3: Sample of Evaluation sheet of pasted strips from capture ELISA analysis (Mikrogen, Germany)



Auswertebogen
Evaluation Form

MIKROGEN DIAGNOSTIK

ABRLHE003

Datum / Date: 12-13/08/16 — Bearbeiter / Testing person: [illegible] — Färbezeit / Time of dyeing: 16:00/15:00hrs

Chargen-Nr. / Lot No.: LHE 031/601 — Röhrchen-Nr. / Tube No.: 769/13, 784/17 — Antikörperklasse / Antibody class: IgG

recomLine HEV IgG/IgM
Art.-Nr., Art. No. 5072 (5070)

Nr. No.	Probe Sample	O2N G11	O2N G13	O2M	O2C G11	O2C G13	O3 G11	O3 G13	Σ	negativ negative ≤2	fraglich borderline 3	positiv positive ≥4
		1		1	4		2					
1	EH 30								–			NEG
2	EH 31								–			NEG
3	EH 32								–			NEG
4	EH 33				4	4			8			POS
5	EH 34											NEG
6	EH 35				4	4			8			POS
7	EH 36											NEG
8	EH 37			1	4	4			9			POS
9	EH 38								–			NEG
10	EH 39								–			NEG
11	EH 40								–			NEG
12	EH 41								–			NEG
13	EH 42	1							1			NEG
14	EH 43								–			NEG
15	EH 44		1						1			NEG
16	EH 45								–			NEG
17	EH 46				4'	4"	2	2	12			POS
18	EH 47	–	–	–	–				–			NEG
19	EH 48	–	–	–	–				–			NEG
20	EH 49	–	–	–	–				–			NEG

Mikrogen GmbH | Floriansbogen 2-4 | 82061 Neuried | Germany | Tel. +49 89 54801-0 | Fax +49 89 54801-100 | mikrogen@mikrogen.de | www.mikrogen.de

ABRLHE003_2014-10

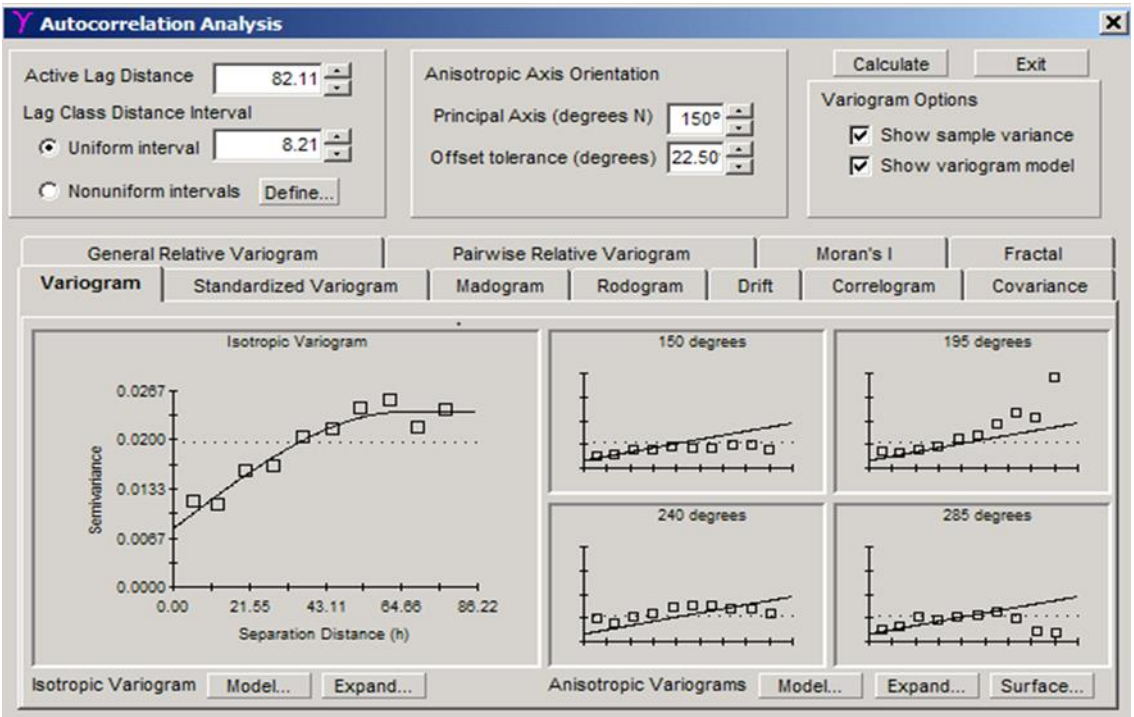
Appendix 4: RNA concentration

RNA Sample	Conc1 ng/µl	Conc2 ng/µl	Purity1 A260	Purity2 A260
EN130	51.2	48	1.29	1.199
EN107	53.6	51.8	1.341	1.295
T035	42.3	46.6	1.058	1.166
EN020	78.5	75.8	1.964	1.894
EN205	75.9	69.6	1.898	1.715
K004	41.2	44.6	1.029	1.116
E047	53.7	53.9	1.343	1.347
EN011	49	37.6	1.225	0.939
EN024	74.3	72.9	1.857	1.823
EN120	58.3	55	1.457	1.374
EN070	25.7	27.1	0.644	0.677
T041	40.1	41.3	1.003	1.033
T012	29.6	29.2	0.741	0.729
K052	49.7	52.8	1.242	1.321
EN089	59.9	62.9	1.498	1.571
EN084	47.2	50.3	1.18	1.258
T014	37.5	33.4	0.937	0.834
EN011	49	37.6	1.225	0.939

Appendix 5: Summary statistics of RNA concentration and purity

Runs	N Statistic	Range Statistic	Minimum Statistic	Maximum Statistic	Mean Statistic	Std. Deviation Statistic	Variance Statistic
Concentration run 1, µl/l	18	52.8	25.7	78.5	50.928	14.6741	215.330
Concentration run 2, µl/l	18	48.7	27.1	75.8	49.467	14.2404	202.789
Purity run 1, A 260	18	1.320	0.644	1.964	1.27400	0.366853	0.135
Purity run 2, A 260	18	1.217	0.677	1.894	1.23500	0.354066	0.125

Appendix 6: Graphic User Interface of the GS+ programme for Autocorrelation analysis (Gamma Design_10, Michigan, USA)



Appendix 7: Swine coordinates used for parameter estimations

No	Code	Sw_pop	N	W
1.	Swfm_1	99	4.89372	-1.78180
2.	Swfm_2	22	4.90701	-1.74816
3.	Swfm_3	61	4.90701	-1.74816
4.	Swfm_4	35	4.92550	-1.76164
5.	Swfm_5	30	4.92550	-1.76164
6.	Swfm_6	60	4.92610	-1.76157
7.	Swfm_7	15	4.91301	-1.75296
8.	Swfm_8	19	4.91307	-1.75496
9.	Swfm_9	18	4.94588	-1.78953
10.	Swfm_10	50	4.95314	-1.76445
11.	Swfm_11	10	4.90625	-1.75565
12.	Swfm_12	28	4.90625	-1.75253
13.	Swfm_13	70	4.98665	-1.72313
14.	Swfm_14	92	4.98630	-1.72310
15.	Swfm_15	13	4.98608	-1.72314
16.	Swfm_16	85	4.99107	-1.72266
17.	Swfm_17	19	4.97047	-1.71675
18.	Swfm_18	28	4.97047	-1.71675
19.	Swfm_19	19	4.96057	-1.73732
20.	Swfm_20	60	4.97298	-1.76515