

Walden University

College of Health Sciences

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Walden University
2017

Abstract

Hepatitis E: Determinants of Severe Symptomatic Disease in Displaced Populations of

South Sudan

by

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MPH, Makerere University Kampala, 2006

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Dissertation Proposal Submitted in Partial Fulfillment

of the Requirements for the Degree of

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Abstract

Hepatitis E virus (HEV) has over the last three decades emerged as a cause of outbreaks in displaced populations. Effective deployment of an HEV intervention toolkit that includes HEV vaccines requires epidemiological characterization of HEV trends in vulnerable populations. The study purpose is to describe the epidemiology of HEV and identify factors for severe HEV disease in displaced populations of South Sudan. The agent-host-environment model was used. A nested retrospective cohort study was used with a sample of 14,404 cases for the descriptive case-series and 4,810 cases for the retrospective cohort. Data analyses included cumulative incidence and mortality rates, SatScan® space-time analysis, correlation and simple linear regression, odds ratio, and logistic regression. Sustained HEV transmission occurred from 2012 to 2017 with rising transmission in the rainy season but no significant correlation between precipitation and HEV cases. The median outbreak duration was 1 year 11 months. The outbreaks were attributed to HEV genotype 1 subtype 1e with the risk of HEV disease and death (as cases and deaths per 10,000) being higher in males (591 versus (vs) 23), adults (18-59 years) (367 vs 14), and elderly (60+ years) (353 vs 22). The factors associated with severe HEV disease include (a) altered mental status (adjusted Odds Ratio [aOR] = 640.24, 95% CI: 209.35– 1958.02), (b) death (aOR 28.06, 95% CI: 14.77-53.29), (c) pregnancy (aOR 16.90, 95% CI: 9.54-29.94), (d) illness onset in rainy season (aOR 0.33, 95% CI: 0.23-0.46). The implications for positive social change entail using present findings to guide clinical screening of HEV cases and to inform the effective deployment of the HEV intervention toolkit, including HEV vaccines that reduce the impact of HEV in displaced populations.

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Dedication

“Everything is possible for him who believes” Mark 9:23

Completing this dissertation after six years can only be attributed to divine guidance, wisdom, perseverance, and strength. As I dedicate this thesis to the almighty God, I pray that his infinite glory continues to manifest in work of my hands. Finally, I dedicate this work to my parents, wife, children, siblings, and friends for their prayers, support, guidance, and encouragement that kept me strong, determined, and motivated.

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“Trust in the LORD with all your heart and lean not on your own understanding; in all your ways acknowledge him, and he will make your paths straight” Prov. 3:5-6

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Chapter 1: Introduction to the Study

Hepatitis E virus (HEV) is a leading cause of enterically transmitted acute viral hepatitis, with most outbreaks reported from Africa and Asia (Rein, Stevens, Theaker, Wittenborn, & Wiersma, 2012a; J. H. Kim et al., 2014; Hakim et al., 2017). In these settings, HEV stands out for causing large and protracted outbreaks. These outbreaks are characterized by high morbidity and mortality in pregnant women and occur in populations with inadequate access to safe drinking water and sanitation (Isaacson, Frean, He, Seriwatana, & Innis, 2000; E. H. Teshale, Howard, et al., 2010a; Rein et al., 2012a; Ahmed et al., 2013a; Thomson et al., 2013). The occurrence of enterically transmitted HEV, therefore, reflects a mismatch between the epidemiology of the disease and the interventions required to interrupt transmission in high-risk populations. Timely deployment of HEV vaccines alongside other conventional interventions for HEV prevention and control requires a thorough understanding of the epidemiology of the HEV disease in high-risk populations.

In 2005, global estimates showed that enterically transmitted HEV accounted for 70,000 deaths, with the follow up 2013 estimates showing that these had reduced to 50,000 deaths (Rein et al., 2012a; Stanaway et al., 2016). However, significant transmission continues in sub-Saharan Africa and South Asia where HEV constitutes a considerable proportion of acute viral hepatitis deaths (Stanaway et al., 2016). Consequently, outbreaks of HEV are increasingly reported in sub-Saharan Africa with vulnerable populations like refugees and internally displaced persons (IDP) being among the most affected (Isaacson et al., 2000; Guthmann et al., 2006; Boccia et al., 2006a; E.

H. Teshale, Howard, et al., 2010a; Ahmed et al., 2013a; Thomson et al., 2013; Browne et al., 2015; Azman et al., 2017; MSF USA, 2017; Anonymous, 2017a; MSF, 2017; WHO, 2017). The risk of HEV spread and developing adverse outcomes is potentially high in displaced populations with less than ideal living conditions.

HEV outbreaks in displaced populations continue to register consistently high attack rates. During the 2007 HEV outbreak among internally displaced persons in Northern Uganda, the overall symptomatic attack rate was estimated at 25%, with pregnant women having the highest attack rate of 81% (E. H. Teshale, Howard, et al., 2010a). This outbreak lasted more than two years, but published data was only available from 2007 to 2009 (E. H. Teshale, Howard, et al., 2010a). In South Sudan, Thomson et al. (2013) reported an overall attack rate of 7.4% among refugees in Maban county. The outbreak lasted more than three years, though published data was only available from 2012 to 2015 (Thomson et al., 2013). A 2015 serosurvey conducted in displaced populations living in the United Nations protection of civilian (PoC) camps in Juba, South Sudan showed a high HEV IgG seroprevalence of 71% (Azman et al., 2017). These findings suggest significant levels of HEV vulnerability in displaced populations.

The current response to HEV outbreaks in developing countries entails improving access to safe drinking water, sanitation, and personal and environmental hygiene in high-risk populations (E. H. Teshale, Howard, et al., 2010a; Ahmed et al., 2013b; Thomson et al., 2013; WHO, 2014). However, these interventions have not appeared to stop many outbreaks, resulting in protracted epidemics with high morbidity and mortality (Boccia et al., 2006b; E. H. Teshale, Howard, et al., 2010a; Thomson et al., 2013; Patel,

Kamili, & Teshale, 2015). The control of HEV outbreaks remains challenging for several reasons. Hepatitis E virus has a long incubation period of over one month. There is, therefore, a significant time lag for initiating and targeting recommended control measures to appropriate locations and populations where active transmission is occurring (Hakim et al., 2017; Kamar, Dalton, Abravanel, & Izopet, 2014). Besides, HEV currently has no definitive treatment, so most of the patient care is symptomatic (WHO, 2014). This, therefore, allows accumulation of a reservoir pool of infectious individuals who in vulnerable populations perpetuate transmission to next susceptible host through the fecal-oral route. Reversing the current trend of HEV especially in displaced populations calls for detailed characterization of outbreaks and augmenting the existing interventions for HEV control.

The 2015 World Health Organization (WHO) HEV position paper recommends the complementary use of Hecolin®, HEV 239 vaccine alongside conventional interventions to optimize HEV outbreak response in vulnerable populations. The primary objective of responding to humanitarian crises involving displaced populations entails the rapid deployment of lifesaving public health interventions to prevent excess morbidity, mortality, and disability (WHO, 2009). While the equitable complementary use of the new HEV vaccine would accelerate progress towards attaining this objective, high-risk groups should be identified and targeted using evidence-based and well-timed interventions to mitigate the risk of HEV (WHO, 2015b). Consequently, epidemiological information on the timing, periodicity, and duration of HEV outbreaks is critical for optimum deployment of the HEV intervention toolkit that includes HEV vaccines.

Ultimately, epidemiological data establishing the overall impact of HEV should inform the implementation of complementary public health interventions in vulnerable populations.

This present study, therefore, sought to document the timing, periodicity, and duration of HEV outbreaks in South Sudan. Also, the study assessed the determinants of severe HEV disease and the overall impact of HEV disease in relation to other causes of morbidity and mortality in displaced populations of South Sudan. The positive social change implications include updated epidemiological data that describes periodicity, magnitude, and distribution of HEV in displaced populations. Additionally, the findings are expected to inform the timely and well-targeted deployment of the HEV intervention toolkit that includes HEV vaccines to mitigate the impact of HEV in displaced populations. The factors associated with severe HEV disease will be used to improve clinical screening of cases for early initiation of supportive care to prevent adverse outcomes. These interventions will ultimately contribute to reduced HEV morbidity, mortality, and disability in displaced populations of South Sudan. These findings can also be used to plan HEV interventions among similar populations in Africa.

Chapter 1 presents the clinical and epidemiological characteristics of HEV. A description of the present study and its implications for social change then follows. A summary of the related literature is then presented in the background section, followed by a description of the current knowledge gap and how the present study intended to address this gap. The problem statement includes the research gap and evidence from the literature to show that the problem is relevant. This chapter also includes sections on the

study purpose, the research questions and hypotheses, the theoretical framework, nature of the survey, conceptual definitions, assumptions, scope, delimitations and limitations, study significance, and ends with a summary of the chapter.

Background

Hepatitis E virus was first identified in 1983 and is a major cause of enterically transmitted acute viral hepatitis. Possible outbreaks with a unique HEV epidemiological profile were first identified at the end of the 18th century in France (Balayan et al., 1983; Teo, 2012). The HEV infective for humans belongs to one serotype and four genotypes (1, 2, 3, and 4) (Kamar et al., 2014; J. H. Kim et al., 2014). Large HEV outbreaks are currently exclusive to developing countries where sub-optimal access to safe water and sanitation favors sustained transmission of waterborne HEV genotypes 1 and 2 (Rein et al., 2012a; J.H. Kim et al., 2014; Kamar et al., 2014; Hakim et al., 2017). HEV disease runs a largely benign course with jaundice as the hallmark for clinical disease, though genotype 1 outbreaks in Asia and sub-Saharan Africa cause high mortality in pregnancy (E. H. Teshale, Howard, et al., 2010a; Teo, 2012; Thomson et al., 2013; Chaudhry, Verma, & Koren, 2015; Khaskheli, Baloch, Sheeba, & Baloch, 2015). Globally, there are two epidemiological patterns of HEV transmission. The waterborne genotypes 1 and 2 predominantly occur in developing countries while the zoonotic genotypes 3, and 4 cause sporadic cases in developed countries (Kamar et al., 2014, Said et al., 2014; Mor et al., 2015; Schielke et al., 2015a; Tholen, Schinkel, Molenkamp, & Ang, 2016; Hakim et al., 2017)).

The HEV genotypes 1 and 2 infections predominantly occur in developing countries where an estimated 20 million cases occur every year (Rein et al., 2012a). Results from the 2013 global burden of disease study showed a decline in HEV mortality globally though transmission in sub-Saharan Africa and Asia remains substantial (Stanaway et al., 2016). The study showed that deaths due to HEV globally declined from 52,000 in 1990 to 50,000 deaths in 2013 (Stanaway et al., 2016). The age standardized HEV deaths rates (deaths per 100,000) for East and Central sub-Saharan Africa were estimated at 20.4 and 25.3 respectively (Stanaway et al., 2016). These HEV mortality rates are high when compared to other regions and therefore suggest substantial transmission in sub-Saharan Africa.

Consistent with these trends are the increasing reports of HEV in displaced populations of sub-Saharan Africa over the last three decades. Several countries including Namibia, Uganda, South Sudan, Sudan, Ethiopia, Kenya, and Lake Chad basin involving Niger, Nigeria, and Chad have reported HEV outbreaks in displaced populations (van Cuyck-Gandré et al., 1997; Isaacson et al., 2000; E. H. Teshale, Grytdal, et al., 2010; Boccia et al., 2006b; E. H. Teshale, Howard, et al., 2010a; Thomson et al., 2013; Ahmed et al., 2013b; Cummings et al., 2014; Gerbi et al., 2015a; Browne et al., 2015; WHO, 2016b; Hakim et al., 2017; MSF USA, 2017; Anonymous, 2017a; MSF, 2017; WHO, 2017). In South Sudan, HEV transmission in displaced populations has been reported every year since 2012 (Thomson et al., 2013; WHO, 2016b; Azman et al., 2017). The agent, host, and environmental factors that are yet to be determined are responsible for the increasing reports of HEV outbreaks in displaced populations.

In the present study, we used the host-agent-environment model to identify the factors associated with severe symptomatic HEV disease in displaced populations (McLeroy, Bibeau, Steckler, & Glanz, 1988). Several factors are believed to influence the risk of symptomatic severe HEV disease. These factors include the agent genotype, sub-genotype, and sequence polymorphism; host demographic and behavioral characteristics; and environmental factors (Inoue et al., 2009; Browne et al., 2015; Gad et al., 2012; Rein et al., 2012a; Nguyen et al., 2012; Labrique et al., 2013a; Joon, Rao, Shenoy, & Baliga, 2015; Thomson et al., 2013). Application of this knowledge to inform public health response requires an understanding of host, agent, and environmental attributes and is critical for selecting appropriate public health interventions to prevent and control HEV disease. Epidemiological studies on Hepatitis E in Africa have largely used descriptive case-series, cross-sectional surveys, and analytical studies (case-control and case-cohort) approaches to characterize HEV in affected and high-risk populations.

In the present study, we used case-series data from multiple disease outbreaks in displaced populations spanning a five-year period in South Sudan. This approach has the advantage of establishing a consistent pattern of when outbreaks start and allows the cumulative incidence by season, age, and gender to be determined. Also, the study assessed the overall public health impact of HEV disease in relation to other common illness in displaced populations. The findings from the present study will inform policy decisions for HEV prevention and control in displaced populations. In the same way, the findings will inform updates to the clinical case screening criteria and will facilitate effective timing of well-targeted interventions for HEV prevention and control in displaced populations.

Problem Statement

Hepatitis E virus (HEV) is a major cause of acute viral hepatitis that is estimated to cause at least 50,000 deaths globally every year (Stanaway et al., 2016). In sub-Saharan Africa, significant transmission continues against the backdrop of declining global HEV burden (Stanaway et al., 2016). Predominantly waterborne spread of HEV occurs in Asia and sub-Saharan Africa where access to safe drinking water and sanitation is sub-optimal (Rein et al., 2012a; J. H. Kim et al., 2014; Kamar et al., 2014; Hakim et al., 2017). Consequently, over 35,000 HEV cases and at least 650 deaths have been reported in Africa in the last three decades (J.H. Kim et al., 2014). In the last three decades, reports of HEV outbreaks in displaced populations of sub-Saharan Africa have increased. Several countries including Namibia, Uganda, South Sudan, Sudan, Ethiopia, Kenya, and Lake Chad basin involving Niger, Nigeria, and Chad have reported HEV outbreaks in displaced populations (van Cuyck-Gandré et al., 1997; Isaacson et al., 2000; E. H. Teshale, Grytdal, et al., 2010; Boccia et al., 2006b; E. H. Teshale, Howard, et al., 2010a; Thomson et al., 2013; Ahmed et al., 2013b; Cummings et al., 2014; Gerbi et al., 2015a; Browne et al., 2015; WHO, 2016b; Hakim et al., 2017; MSF USA, 2017; Azman et al., 2017; Anonymous, 2017a; MSF, 2017; WHO, 2017). In South Sudan, HEV transmission has occurred in displaced populations since 2012 (Thomson et al., 2013; WHO, 2016b; Azman et al., 2017). The current response to HEV transmission entails improving access to safe drinking water, adequate sanitation, and proper personal and environmental hygiene (Gerbi et al., 2015b; E. H. Teshale, Howard, et al., 2010b; Thomson et al., 2013; WHO, 2016b).

Given the long incubation period and absence of a cure for HEV, timely containment of outbreaks has remained a distant objective with most epidemics persisting for several incubations cycles thus increasing the risk of severe disease and mortality. (Gerbi et al., 2015b; E. H. Teshale, Howard, et al., 2010b; Thomson et al., 2013; WHO, 2016b). In 2015, WHO recommended the complementary use of the new HEV 239 vaccine optimize conventional interventions for outbreak control (WHO, 2015b). However, the paucity of detailed HEV epidemiological data on the timing, periodicity, magnitude, duration, case distribution, and factors for severe HEV disease in displaced populations precludes the effective use of HEV 239 vaccine to augment the existing interventions (WHO, 2015b).

Purpose of the Study

The purpose of this nested retrospective cohort study was to determine the periodicity, timing, duration, magnitude (morbidity and mortality), epidemiological distribution, and impact of HEV outbreaks. Also, the study assessed the factors associated with severe symptomatic HEV disease. The study used data from the South Sudan Ministry of Health integrated disease surveillance and response (IDSR) and the Early Warning Alert and Response Network (EWARS) database. The databases have standardized reporting tools with predefined variables for describing acute jaundice syndrome cases. The database included all confirmed and suspect HEV cases linked to confirmed outbreaks in South Sudan. The present study only included HEV cases identified in displaced populations of South Sudan from 2012 to early 2017. The current study used variables from the Ministry of Health standardized acute jaundice syndrome database. The Early Warning Alert and Response Network (EWARS) dataset was used to

determine the impact of HEV in relation to the other common diseases and conditions in displaced populations.

The independent variables in this study included the demographic, clinical, and epidemiological characteristics of HEV cases. The independent demographic variables included: age, gender, displacement status as either internally displaced person (IDP), refugee, or host community, and residence by camp, village, and county. The independent clinical variables included: the date of consultation at the health facility, the presenting clinical signs and symptoms, the presence of co-morbidities, pregnancy in females, and outcome information as alive or died. The independent epidemiological variables included: the date (season) of illness onset, history of travel to other displaced populations camp, and history of contact with other jaundiced persons. The dependent variable in this study was the occurrence of severe symptomatic HEV disease. The WHO recommends that all HEV cases at high-risk of severe symptomatic HEV disease or those clinical and/or laboratory evidence of severe HEV disease be admitted and managed as inpatients for close monitoring and early initiation of supportive care (WHO, 2014). In the present study, all HEV cases admitted to designated HEV treatment centers were considered to be at-risk or to have severe symptomatic HEV disease. Consequently, severe symptomatic HEV disease was any HEV case admitted for inpatient supportive care in a designated HEV treatment center. Hence, the hospitalization criteria at designated treatment centers included changes in mental status, hypoglycemia, unexplained bleeding, severe nausea, vomiting, generalized weakness and severe

lethargy, pregnancy (especially in the third trimester), positive malaria test, and evidence of bacterial infection (WHO, 2014).

Research Questions and Hypotheses

The research questions and hypotheses of the study were as follows:

Question 1: What is the timing, periodicity, magnitude (morbidity and mortality), laboratory characterization, and duration of HEV outbreaks in South Sudan, and how are the cases distributed by person, time, and place?

H_0 1: The timing, periodicity, duration, and magnitude, and laboratory characterization of symptomatic HEV disease do not differ by place, person, and time.

H_1 1: The timing, periodicity, duration, magnitude, and laboratory characterization of symptomatic HEV disease differs by place, person, and time.

Question 2: What is the overall impact of symptomatic HEV disease in relation to the other causes of morbidity and mortality in displaced populations?

H_0 2: Symptomatic HEV disease is not a significant cause of morbidity and mortality in relation to the other diseases and conditions in displaced populations.

H_1 2: Symptomatic HEV disease is a significant cause of morbidity and mortality in relation to the other diseases and conditions in displaced populations.

Question 3: Is there a significant association between demographic, clinical, and epidemiological characteristics and the occurrence of severe symptomatic HEV disease?

H_0 3: There is no significant association between demographic, clinical, and epidemiologic characteristics and the occurrence of severe symptomatic HEV disease.

H_1 3: There is a significant association between demographic, clinical, and epidemiologic characteristics and the occurrence of severe symptomatic HEV disease.

Theoretical Framework

The host-agent-environment model was used to elucidate the epidemiology of HEV in displaced populations of South Sudan (McLeroy et al., 1988). The model posits that disease may result from transmission of an infectious agent from its environment or animal reservoir through an appropriate portal of entry on the susceptible host, given a favorable environment that allows the agent to infect the host (McLeroy et al., 1988). A combination of factors in the host, agent, reservoir, and environment are critical to the model. These factors determine if exposure of a host to an agent will result in infection and if the infection of a host by an agent in a favorable environment will lead to symptomatic and/or severe disease in a susceptible host (Aggarwal, 2011a). Under the theoretical framework, I present the host, agent, and environmental attributes that favor symptomatic and severe HEV disease.

Symptomatic HEV disease and/or the occurrence of adverse clinical outcomes depends on the presence of HEV of genotypes 1, 2, 3, and 4 that are pathogenic to humans (Kamar et al., 2014). Only HEV genotypes 1 and 2 are known to cause large outbreaks and severe disease especially in pregnant women and patients with chronic liver disease (Gad et al., 2012; Rein et al., 2012a). There are several modes of HEV

transmission from an infected host, environmental source, and/or animal reservoir to a susceptible host. The transmission occurs through drinking fecally contaminated water or eating improperly cooked infected meat, or through person-to-person transmission, or transfusion with contaminated blood. The transmission of HEV may consequently result in symptomatic disease in a susceptible host (Kamar et al., 2014).

Host susceptibility is a major determinant for disease exposure, infection, and manifestation of symptomatic disease (Centers for Disease Control, 2012). At the host level, the risk of exposure to disease causing agents is influenced by demographic factors and behaviors (Howard et al., 2010; Li, Xue, & Chen, 2013; Junaid, Agina, & Abubakar, 2014). The host factors for HEV include availability and use of personal hygiene, sanitation, and use of safe water (Li et al., 2013; Junaid et al., 2014). Hepatitis E outbreaks have been linked to host behaviors concerning proper personal hygiene, sanitation, and use of safe drinking water (Howard et al., 2010; Li et al., 2013; Junaid et al., 2014). Demographic predilections for HEV infection and symptomatic disease include being a child, young adult male, or being pregnant (E. H. Teshale, Howard, et al., 2010b; Kamar et al., 2014; Patel et al., 2015). Host response to new infections is also influenced by the presence of co-infections like schistosomiasis, Hepatitis B virus, Hepatitis C virus, HIV, and chronic liver disease (Kamar et al., 2014; J.-H. Kim et al., 2014). In the same way, vaccination with safe and effective vaccines reduces host susceptibility to HEV infection and symptomatic disease (Zhu et al., 2010; WHO, 2015b).

A favorable environment is critical to the survival of the pathogen and facilitates transmission to a susceptible host (Centers for Disease Control, 2012). In developing countries, HEV genotypes 1 and 2 thrive in contaminated water sources from where they are ingested by the susceptible host (Howard et al., 2010; Kamar et al., 2014). The risk of contamination for unprotected water sources is high during the rainy season, floods, or other water stressed time-periods like the acute phase following population displacement (E. H. Teshale, Howard, et al., 2010a; Thomson et al., 2013). The risk of HEV infection is therefore high for internally displaced and refugee populations with inadequate access to safe water and sanitation, and poor hygiene (Browne et al., 2015; E. H. Teshale, Howard, et al., 2010b; Thomson et al., 2013).

Zoonotic transmission is the predominant mode of HEV transmission in developing countries. In these settings, domestic and wild animal reservoirs like pigs, deer, and wild boar are the source of HEV genotypes 3 and 4 for susceptible hosts (Kamar et al., 2014; B.-S. Kim et al., 2015a; Schielke et al., 2015a). The risk of infection by HEV genotypes 3 and 4 is high for individuals exposed to source animals through occupational or recreational activities (Kamar et al., 2014; B. S. Kim et al., 2015a; Schielke et al., 2015a).

Since the occurrence of severe symptomatic HEV disease is influenced by a combination of agent, host, and environmental factors the host-agent-environmental model, therefore, provides an appropriate framework for identifying the determinants of severe symptomatic HEV disease. In the same way, a combination of environmental, agent, and host factors influences the distribution of HEV disease by time (season),

person, and place. The theoretical framework, therefore, provided the basis for using a retrospective cohort nested within a descriptive case series to answer the research questions.

Nature of the Study

The study used quantitative techniques to document the timing, periodicity (seasonality), duration, magnitude, and distribution of HEV cases. Also, the study assessed the overall impact of symptomatic HEV disease in relation to the other causes of morbidity and mortality in displaced populations. The study also sought to identify the factors for severe symptomatic HEV disease in displaced populations of South Sudan.

More specifically, a retrospective cohort study nested within a descriptive case-series was used to determine the timing, periodicity, duration, magnitude, and distribution of HEV cases. The study also assessed the overall impact of symptomatic HEV disease and evaluated factors for severe symptomatic HEV diseases in displaced populations.

The study used secondary data from the South Sudan Ministry of Health integrated disease surveillance and response (IDSR) database. The database uses standardized reporting forms that include standardized demographic, clinical, and epidemiological variables used to characterize acute jaundice syndrome cases. The study included all the confirmed and suspect HEV cases linked to confirmed outbreaks in displaced populations of South Sudan from 2012 to early 2017. The South Sudan Ministry of Health IDSR database contained all the Hepatitis E case-based data from these outbreaks. Data from the Ministry of Health Early Warning Alert and Response

Network (EWARS) dataset on morbidity and mortality was used to determine the impact of HEV in relation to other diseases and conditions in displaced populations.

The rationale for using a retrospective cohort study nested within a descriptive case-series stems from the fact that the South Sudan Ministry of Health maintains a database of cases with symptomatic HEV disease since 2012. I used this HEV case data for the descriptive case-series that characterized symptomatic HEV disease with respect to timing, periodicity, duration of outbreaks, magnitude, and overall impact. The same database allowed the identification of clinical, epidemiological, and demographic characteristics associated with severe symptomatic HEV disease. Since the same database identifies a proportion of symptomatic HEV cases that suffered from severe symptomatic HEV disease, a retrospective cohort study was undertaken to determine the demographic, clinical, and epidemiological factors for severe symptomatic HEV disease. The demographic variables in the study included: age, gender, displacement status as either IDP or refugee, and residence. Socioeconomic factors like race, wealth, and education are important covariates in similar settings. However, the present study did not consider these factors for several reasons:

1. Race, wealth, and education were not variables in the Ministry of Health database used for the present study.
2. Race was homogeneous in the study population and was therefore not considered a covariate in the current study.

3. While education is a covariate for HEV infection and disease, it was not included in the present study since it was not listed as a variable in the Ministry of Health database (Labrique et al., 2013a; Junaid et al., 2014; Yoon et al., 2014).
4. Since the present study focused on displaced populations that at the time of the outbreak had lost all their investments and source of livelihood, and barely had the minimum required for survival, wealth too, was not considered as a covariate in the present study.

Clinical information included the date of consultation at the health facility, presenting clinical symptoms and signs, the presence of co-morbidities, pregnancy or being postpartum in females, and outcome information as alive or died. The epidemiological information included: the date (season) of onset of illness, travel to other displaced populations camps, and contact with other jaundiced persons. The WHO recommends that all HEV cases at high-risk of severe symptomatic HEV disease or those clinical and/or laboratory evidence of severe HEV disease be admitted and managed as inpatients for close monitoring and early initiation of supportive care (WHO, 2014). A case of severe symptomatic HEV disease was any HEV case admitted for inpatient care in a designated HEV treatment center.

The study population included confirmed and suspect cases (linked to confirmed HEV outbreaks) in children and adults with symptomatic HEV disease that were identified at designated treatment centers in displaced populations and recorded in the Ministry of Health HEV database from 2012 to early 2017. The analysis of data from the study excluded all acute jaundice syndrome cases that were determined to have acute

viral hepatitis due to the other hepatitises besides HEV and lacked laboratory evidence of HEV infection. The minimum sample size for the retrospective cohort was calculated using OpenEpi, an online open source software for epidemiologic statistics (A.G. Dean, Sullivan, & Soe, 2015). The inputs into the software included (a) a two sided confidence interval of 95%, (b) power for study of 80%, (c) ratio of exposed to unexposed as 1:1, (d) proportion of outcome (mortality) from HEV disease as 4% (WHO, 2015b), and (e) the least extreme relative risk or hazard ratio to be detected as 2. The minimum sample size for the retrospective cohort as calculated by the software was 1,372 symptomatic HEV cases.

The minimum sample size for the descriptive case-series was calculated using OpenEpi, an online open source software for epidemiologic statistics (A.G. Dean et al., 2015). The inputs into the software included (a) the populations size (for finite population correction factor) (N) and for this we used the software value of 1,000,000 that is used for large populations (displaced populations in South Sudan are over 2 million), (b) the hypothesized prevalence of HEV in displaced population and for this we used 50% that maximizes the sample size, (c) confidence limits of 5%, and (d) a design effect of 1 since this was not a cluster survey. The minimum sample size for the descriptive case series as calculated by the software was 384 HEV cases for a confidence level of 95%.

A data abstraction tool was designed and used to abstract data for all symptomatic probable and confirmed HEV cases entered into the Ministry of Health database from 2012 to 2017. To document HEV transmission hotspots, seasonal peaks, duration and magnitude of outbreaks, the cumulative incidence of symptomatic HEV disease and

mortality were computed by person, place, and time. The cumulative incidence was calculated by dividing the number of symptomatic HEV cases in a selected group by the size of the population at risk at the beginning of a specified period. The HEV proportional mortality rate was calculated to determine the relative importance of HEV as a major cause of death in displaced populations. The HEV proportional mortality is critical for documenting the overall impact of HEV infection in relation to other causes of mortality in displaced populations. The retrospective space-time permutation scan statistic that does not require population-at-risk data was used to identify outbreak clusters of space-time clustering and to determine if they are statistically significant (Kulldorff, Heffernan, Hartman, Assunção, & Mostashari, 2005). For every geographical location under surveillance, the statistic can detect one day or multi-day outbreaks, with the capacity to identify both rapidly rising and slowly emerging outbreaks (Kulldorff et al., 2005). To adjust for the underlying populations in each of the displaced population locations, follow up analysis was undertaken to identify clusters of spatial or space-time clustering, a Poisson model-based statistical software, SatScanTM, was used (Kulldorf, 2016). Descriptive analyses entailed running frequencies, measures of central tendency and dispersion, chi-square test for independence, and odds ratio. Stepwise logistic regression analysis was undertaken to identify determinants of severe symptomatic HEV disease.

Operational Definitions

There were several definitions used in this study.

Acute jaundice syndrome: Acute onset of jaundice and severe illness in the absence of

any known precipitating factors or acute onset of jaundice, with or without fever, and absence of any known precipitating factors (Cannolly, 2005; WHO, 2014).

Jaundice: Yellow discoloration of the sclera of the eye, other mucous membranes, and skin (Aggarwal, 2011b).

Integrated disease surveillance and response (IDSR): A strategy by the World Health Organization Regional Office for Africa to improve epidemiologic surveillance and response to communicable diseases and other events of public health importance in the African region. It entails integrating all activities for detection, laboratory testing, investigation, and response to public health emergencies of national and international concern (WHO, 2010a).

Early Warning Alert and Response Network (EWARN): An adjunct to the national disease surveillance system that is set up to make up for disease surveillance and response needs in crisis affected populations.

Suspect case: A suspect HEV case is one with acute onset of jaundice in the absence of any known precipitating factors with or without dark urine, anorexia, malaise, extreme fatigue, or right upper quadrant tenderness (WHO, 2014). The South Sudan Ministry of Health uses this WHO case definition.

Confirmed case: Any person that has a serum sample positive for HEV immunoglobulin (Ig) M or IgG antibodies, or by reverse transcriptase Polymerase Chain Reaction (PCR) regardless of whether they meet the criteria for a suspect HEV case (WHO, 2014). The South Sudan Ministry of Health uses this WHO case definition.

Incidence: The number of new cases of a specified disease reported over a given period (Cannolly, 2005).

Case fatality rate (CFR): The percentage of persons diagnosed as having a specified disease who die as a result of that disease within a given period, usually expressed as a percentage (cases per 100) (Cannolly, 2005).

Attack rate: The cumulative incidence of cases (persons meeting case definition since the onset of the outbreak) in a group observed over a period during an outbreak (Cannolly, 2005).

Genotype: The genetic constitution of an organism like a virus (Cannolly, 2005).

Encephalopathy: Compromised performance of the brain resulting from infections, liver damage, anoxia, or kidney failure (Sahai & Kiran, 2015).

Coagulopathy: A bleeding disorder that results from reduced ability of the liver and other organs to coagulate blood (Sahai & Kiran, 2015).

Coma: Altered mental condition due to compromised performance of the brain resulting from infections, liver damage, anoxia, or kidney failure (Sahai & Kiran, 2015).

Immunoglobulin (Ig): A sterile preparation of concentrated antibodies recovered from pooled human plasma.

Immunoglobulin (Ig) M: These are the first circulating antibodies to appear in response to a foreign antigen in the body. They signify recent exposure to an infectious agent.

Immunoglobulin (Ig)G: These are the most abundant of the circulating antibodies that protect against bacteria, viruses, and toxins circulating in blood and lymph (Cannolly, 2005). They signify both recent and past exposure to an infectious agent.

Severe symptomatic Hepatitis E virus disease: The WHO recommends that all HEV cases at high-risk of severe symptomatic HEV disease or those clinical and/or laboratory evidence of severe HEV disease be admitted and managed as inpatients for close monitoring and early initiation of supportive care (WHO, 2014). In the present study, all HEV cases admitted to designated HEV treatment centers were considered to be at-risk or to have had severe symptomatic HEV disease. Consequently, a case of severe symptomatic HEV disease was any HEV case admitted for inpatient supportive care in a designated HEV treatment center. Hence, the hospitalization criteria at designated treatment centers included changes in mental status, hypoglycemia, unexplained bleeding, severe nausea, vomiting, generalized weakness and severe lethargy, pregnancy (especially in the third trimester), positive malaria test, and evidence of bacterial infection (WHO, 2014).

Displacement status: This was defined as either being an internally displaced person (IDP) for South Sudanese nationals who were forced out of their villages of residence, or refugees for foreign nationals that were displaced in refugee camps in South Sudan, or host communities being indigenous residents of locations hosting camps for refugees or internally displaced populations.

Residence: This refers to the address of abode (defined by camp, village, payam, and county) for a case at the time the HEV disease symptoms started.

Co-morbidities: The simultaneous occurrence of one or more diseases with HEV as the primary disease in an individual.

Date (season) of illness onset: This is the date (season) the clinical symptoms commence for any given patient that meets the case definition for the disease in question.

Travel to other displaced population camps: This refers to the history of travel by a case from their place of abode to another location where displaced people reside within the six weeks preceding the onset of illness.

Clinical illness outcome: This refers to the clinical outcome of the patient as dead or alive as indicated in the case line list at designated HEV treatment centers.

Contact with other jaundiced persons: This entails direct physical exposure or contact with soiled contaminated linen or sharing the same room with a symptomatic HEV case.

Treatment as an outpatient: A patient that was not admitted for inpatient supportive care but received treatment at the designated treatment centers after presenting with HEV compatible signs and symptoms, epidemiological linkage or laboratory confirmation.

Treatment as an inpatient: A patient with HEV compatible signs and symptoms with epidemiological linkage or HEV laboratory confirmation that received treatment while admitted at the designated clinic due to clinical evidence of ongoing or eminent severe HEV disease.

Presenting clinical signs and symptoms: This refers to individuals whose signs and symptoms were consistent with the case definition for HEV.

Completeness of reporting routine weekly data: This is an indicator used to assess the performance of a surveillance system. As part of the IDSR strategy, completeness of reporting routine weekly data is the proportion of the total reporting units that submit their weekly reports in any given week. For good representativeness, the completeness should be 80% and above (WHO, 2010a).

Target population: Refers to a collection of individuals or units about who or which inferences are desired (Miquel, 2014).

Season: Concerning the time periods for the onset of the rain and dry season in South Sudan, the rain season was any period from April to October while the dry season was defined as any period from November to March.

Study population: Refers to the actual population from which sample units are drawn and to which the study findings are generalized (Miquel, 2014).

HEV intervention toolkit: This includes a set of interventions for eliminating or controlling the source of HEV infection through improving access to safe drinking water, proper sanitation, and observing personal and food hygiene. The kit also includes strategies for identifying high-risk groups and protecting them from infection. Besides, the toolkit is critical for prevention of deaths through prompt diagnosis and treatment of cases. A new strategy entails the use of safe and effective HEV vaccines to prevent disease in vulnerable groups before and during outbreaks (WHO, 2014).

Assumptions

In this study, secondary data from the Ministry of Health was used to answer the research questions. Completeness of reporting routine weekly data refers to the

proportion of reporting units that submit their reports in any given reporting epidemiological week. Since the completeness in reporting was above 80%, the records included in the database were representative of the total population of HEV patients. HEV disease is largely a mild or asymptomatic and self-limiting illness, and therefore many of these patients most likely did not seek care at designated health facilities. However, as part of the behavioral change communication during the outbreak, symptomatic cases are encouraged to seek medical attention at the nearest health facility. It is, therefore, assumed that these messages penetrated deep into at-risk communities and that many of the mildly ill HEV cases sought care at the designated health facilities where they received treatment and were line listed. Additionally, since all the variables included in the present study were limited to the fields in the acute jaundice syndrome database, it is assumed that the healthcare workers completed all the fields accurately.

Scope and Delimitations

The study included HEV cases that presented to the health facilities during the outbreak. The cases presenting to the healthcare facilities are likely to be less than the actual cases at the community level. This discrepancy relates to the nature of HEV disease that is largely an asymptomatic or mildly symptomatic self-limiting illness. The asymptomatic HEV cases will therefore never come to the health facility, and many of the mildly ill patients will never make it to the healthcare facility due to poor health seeking behavior or inadequate access to healthcare, a scenario that is more common in developing countries especially during humanitarian crises. This difference, therefore, raises the possibility of healthcare access bias, a common type of selection bias in health

facility-based studies. Given these observations, the cases included in the present study can only be said to represent moderate and/or severely ill HEV cases that presented to the designated treatment centers during the outbreak period and were therefore eventually entered into the acute jaundice syndrome database. The accuracy of the information collected on each of the cases entered into the database may be influenced by several factors including the techniques used by the clinicians to interview cases and record the data and the reduced ability of the patients to recall exposures given the long incubation period of HEV. These put together will result in nondifferential misclassification bias, which typically reduces the ability to detect significant differences between comparison groups. Given this study context, the findings from the present study are generalizable to patients with moderate or severe HEV disease that presented to designated treatment centers in outbreak locations within displaced populations of South Sudan and possibly to other displaced populations in sub-Saharan Africa.

Significance

Given the current challenges in improving access to safe water, sanitation, and proper personal and environmental hygiene to prevent and control HEV outbreaks in displaced populations, WHO currently recommends the complementary use of HEV vaccines to improve the overall response to HEV outbreaks. However, deployment of HEV vaccines should be guided by epidemiological data to allow timely and well-targeted deployment of comprehensive interventions including vaccines for HEV control. The present study, therefore, provided epidemiological data required for effective and rapid implementation of the HEV intervention toolkit that includes HEV vaccines to

mitigate the risk of HEV disease in displaced populations of South Sudan. Overall, there is a high likelihood that implementing these interventions together could ultimately reduce the duration of outbreaks and the risk of severe HEV disease and ultimately prevent excess morbidity, mortality, and disability in displaced populations, and thus contribute to a positive social change.

Summary

Hepatitis E virus remains a significant public health threat in sub-Saharan Africa where it is estimated to have caused 50,000 deaths globally in 2013 (Stanaway et al., 2016). Large HEV outbreaks are currently limited to endemic locations in sub-Saharan Africa and Asia. In these settings, suboptimal access to safe drinking water and poor sanitation and hygiene practices drive transmission (Guthmann et al., 2006; Sailaja et al., 2009; Rein et al., 2012a; Thomson et al., 2013; Murthy, Khan, Kiran, & Hakeem, 2014; Hakim et al., 2017; MSF USA, 2017; Azman et al., 2017). Global trends show that HEV mortality declined from 52,000 deaths in 1990 to 50,000 in 2013 though reports of HEV outbreaks in displaced populations of sub-Saharan Africa have increased in the recent years (Isaacson et al., 2000; E. H. Teshale, Howard, et al., 2010a; Boccia et al., 2006b; Thomson et al., 2013; Ahmed et al., 2013b; Browne et al., 2015; Stanaway et al., 2016; MSF USA, 2017; Azman et al., 2017; Anonymous, 2017a; MSF, 2017; WHO, 2017). Displaced populations are vulnerable and may suffer high morbidity and mortality rates from disease outbreaks especially during the acute phase of a humanitarian crisis. Hepatitis E outbreaks in displaced populations are typically protracted thus highlighting the need to augment the current HEV intervention toolkit. In a move to optimize the

strategy for HEV prevention and control, WHO recommended the complementary use of HEV vaccines alongside conventional water, sanitation, and hygiene (WASH) interventions (WHO, 2015b). Through this strategy, vaccines are administered to reduce the HEV susceptible population in the immediate term as WASH interventions are rolled out to address the underlying risk factors in the medium and long term. However, effective deployment of HEV vaccines requires detailed HEV epidemiological data on the periodicity, timing, duration, magnitude, and distribution of HEV in vulnerable populations. Also, identifying high-risk groups for severe symptomatic HEV disease is critical for a targeted response to prevent adverse outcomes. The present study, therefore, set out to determine the timing, periodicity, duration, magnitude and distribution of HEV, and the factors associated with severe symptomatic HEV disease in displaced populations. The study findings would allow timely and well-targeted complementary interventions including HEV vaccines for prevention and control of HEV. The host-agent-environment model was used to identify factors for severe symptomatic HEV disease. The study used data from the Ministry of Health IDSR database for the period 2012 to early 2017. The implications for positive social change included a better understanding of the epidemiology of HEV disease in displaced populations for effective deployment of HEV interventions including vaccination to prevent excess morbidity and mortality from HEV in displaced populations.

Chapter 2, sets out with the literature search strategy followed by a detailed description of the theoretical basis for the present study. A review of literature related to the independent and dependent variables is then presented, including related studies that

address the association between independent and dependent variables. The chapter ends with a synopsis of the current body of knowledge and gaps and how the present study addressed them.

Chapter 2: Literature Review

Introduction

Hepatitis E virus is the leading cause for enterically transmitted acute viral hepatitis in sub-Saharan Africa with an increasingly high number of reports in displaced populations. In the last three decades, at least nine countries have reported HEV outbreaks in displaced populations of sub-Saharan Africa (Ahmed et al., 2013b; Boccia et al., 2006a; E. H. Teshale, Howard, et al., 2010a; Thomson et al., 2013; Browne et al., 2015; Hakim et al., 2017; MSF USA, 2017; Azman et al., 2017; Anonymous, 2017a; MSF, 2017; WHO, 2017). The current HEV outbreak response interventions include improving access to safe drinking water, sanitation, and personal and environmental hygiene (WHO, 2015b). However, these interventions have not been effective in securing timely outbreak containment especially in displaced populations (E. H. Teshale, Howard, et al., 2010b; Thomson et al., 2013; WHO, 2015b). The primary objective of responding to humanitarian crises entails the rapid deployment of life-saving public health interventions to prevent excess morbidity, mortality, and disability (WHO, 2009). Equitable use of the new HEV vaccine would accelerate progress towards attaining this objective. However, high-risk populations should be identified to allow prioritization of vaccine needs to mitigate the risk of HEV disease. Besides, epidemiological data on the timing, periodicity, duration, magnitude, and distribution of HEV outbreaks should inform the optimal deployment of HEV interventions including HEV vaccines. Ultimately, data elucidating the overall HEV impact in displaced populations should justify the implementation of complementary interventions for HEV control. The present

study, therefore, sought to document the timing, periodicity, duration, magnitude, and distribution of HEV; identify factors for severe symptomatic HEV disease; and determine the overall impact of HEV disease in displaced populations of South Sudan.

This chapter presents the strategy used to retrieve articles related to the current study. A detailed description of the theoretical basis for the current study then follows. This section will describe the origin of the theory, its application in related research studies, and how it was used to provide answers to the research questions in the present study. Guided by a model as suggested by Creswell (2009), the literature related to the main variables in the current study will then be presented. The model proposes five components (a) an introduction, (b) presentation of literature related to independent variables, (c) a review of studies related to dependent variables, (d) review of studies that address both the independent and dependent variables, and (e) concluding with a summary that highlights gaps in current literature to justify why additional research is needed and how the present study will address the gaps (Creswell, 2009).

Literature Search Strategy

The literature search was implemented to establish the state of knowledge on HEV in displaced populations and other vulnerable groups in South Sudan, sub-Saharan Africa, Asia, and developed countries. This information was eventually used to define the research gap that motivated the present study. Hence, as part of the literature review, the following databases, accessed through the Walden University Library were used: PubMed, Cinahl+Medline, ProQuest Health and Medical Complete; and Science Direct. For the articles that were not available in full text, this researcher used the “Find an Exact

Article tab on the Walden University homepage. The tab allows one to search by journal title and once the journal is displayed, access to the full-text version of the article through the databases listed under the selected journal. I used Walden University's Document Delivery Service (DDS) for the rest of the articles that were not available from the Walden University Library databases. The other search engines used were Google Scholar and the World Health Organization's Global Information Full Text (GIFT). I restricted the search to articles published after December 2012 for most of the review. A few articles from earlier years were included to capture significant microbiological, clinical, epidemiological information, and seminal findings on hepatitis E, its prevention, and control in displaced populations. The search also included non-peer reviewed sources from the WHO, the United Nations Office for the Coordination of Humanitarian Affairs (OCHA), Relief Web, Medecins Sans Frontiers (MSF), and the Ministry of Health in South Sudan. To ensure an exhaustive review since January 2013, the researcher examined the reference sections of all the articles downloaded and the "related articles" and "cited by" features to identify additional articles related to the present study.

Search terms included 'viral hepatitis', 'acute viral hepatitis', 'hepatitis E', 'hepatitis E virus', 'Hepatitis E antibody', 'enterically transmitted non a non b hepatitis', 'hepatitis E incidence', 'hepatitis E attack rates', 'hepatitis E prevalence', 'hepatitis E burden', 'risk factors', 'hepatitis E microbiology', 'hepatitis E epidemiology', 'hepatitis e laboratory testing', 'hepatitis E genetic polymorphism', 'hepatitis E severe disease', 'hepatitis E vaccines', 'hepatitis E prevention', and 'hepatitis E control'. More specifically, I used the following terms for the magnitude of hepatitis E: 'hepatitis E

prevalence'; 'hepatitis E burden'; 'hepatitis E epidemiology'; 'hepatitis E incidence'; 'hepatitis E attack rate'; and 'acute viral hepatitis burden.' The following terms were used for the interventions to control hepatitis E: 'hepatitis E risk factors'; 'hepatitis E vaccines'; 'immunogenicity'; and safety, efficacy, and effectiveness.' The search terms were combined using the Boolean terms "OR", "AND", and "NOT".

Theoretical Foundation

Ecological models are suitable for understanding infectious disease transmission dynamics since they incorporate interactions between the pathogen, host, and the environment to inform disease control efforts (Smith et al., 2005). The ecological theory, therefore, provides an appropriate framework for describing the epidemiology of HEV disease to inform comprehensive interventions for HEV control. The WHO (2015b) position paper on HEV vaccines refers to the complementary use of HEV vaccines alongside other interventions for improving personal hygiene, access to safe drinking water, and sanitation to target high-risk populations during outbreaks. These recommendations incorporate the ecological perspective in disease control by emphasizing the role of a conducive environment in supporting the sustainable execution of health behavior, which ultimately reduces the risk of disease at individual, organizational, and population level (McLeroy et al., 1988; WHO, 2015b).

While many ecological models exist for use in social sciences, the host-agent-environment or epidemiologic triad is more commonly used in public health and is most appropriate for infectious diseases, which typically are caused by a single agent (McLeroy et al., 1988; Centers for Disease Control, 2012). The tenets of the theory speak

to the fact that health events like infectious diseases do not occur randomly in populations (Centers for Disease Control, 2012). Consequently, the risk of disease transmission will depend on the presence of a susceptible host and an infectious agent in an environment that favors disease transmission (McLeroy et al., 1988; Centers for Disease Control, 2012). The model is therefore useful for targeting preventive interventions to either reduce host susceptibility, maintaining a protective environment and/or eliminating the agent (Centers for Disease Control, 2012). The model also allows infectious diseases to be prevented through interventions that keep the agent outside the environment and/or those that protect the host from the agent (McLeroy et al., 1988; Centers for Disease Control, 2012). Also, the effectiveness of such interventions can be optimized if implemented before the transmission season and targeted to groups at risk of symptomatic and severe disease.

Ecological models of health behavior have their roots in biological sciences that focus on the interrelationships between organisms and their environments (McLeroy et al., 1988). Consequently, their current application in behavioral sciences and public health focuses on the nature of the interaction between people and their social, physical, and cultural environment (McLeroy et al., 1988). Ecological psychologists like Kurt Lewin undertook seminal work on ecological models that confirmed the significant influence that the outside environment has on individuals and their behavior (Lewin, 1951). To further emphasize the role of the environment in explaining behavior, Urie Bronfenbrenner in his 'systems theory' suggested three levels of environmental influence and interaction. The 'microsystems' is the first level of interaction of individuals in

families or respective work groups. The ‘mesosystems’ constitute the second tier of interaction with family, school, and workplaces. The third level of influence is the ‘ecosystems’, which encompass culture, politics, and economics (Bronfenbrenner, 1981). In related work on ecological models designed to guide behavioral interventions, McLeroy (1988) described five sources of influence on behavior including intrapersonal factors, interpersonal processes and primary groups, organizational factors, community factors, and public policy. This work created the foundation for using ecological theories to inform interventions targeting the multiple levels of influence on disease prevention and health promotion. The primacy of the environment is rooted in the Ottawa Charter for health promotion that emphasizes the role of the environment and policies in supporting the execution of health behaviors by motivated and educated individuals (WHO, 1986).

A variant of ecological models of health behavior that is more applicable to infectious disease causation, transmission, and public health prevention is the host-agent-environmental model (Terris, 1986; McLeroy et al., 1988; Centers for Disease Control, 2012). The model has its roots in research on the origins of disease by Louis Pasteur and Robert Koch that laid the foundation for the germ theory of disease causation and transmission (Karamanou, Panayiotakopoulos, Tsoucalas, Kousoulis, & Androutsos, 2012). Disease causation theories have evolved over the years, giving rise to models like the epidemiologic triad or the host-agent-environment model and Rothman’s causal pies that explain how infectious and noninfectious diseases are caused (Rothman, 1976; McLeroy et al., 1988; Gordis, 2008; Centers for Disease Control, 2012).

The following description focuses on the host-agent-environment model as the theoretical basis for transmission and control of infectious diseases like Hepatitis E Virus. Based on the host-agent-environment model, a disease may result from the transmission of an infectious agent from its reservoir to a susceptible host given a favorable environment that allows the agent to infect the susceptible host (Gordis, 2004; CDC, 2011). A combination of factors in the host, agent, and environment play a critical role in determining if infection of a host by an agent in a favorable environment will result in asymptomatic, mild, or severe disease in the host (Rothman, 1976; Gordis, 2008).

The Infectious Agent

As stated in the germ theory of infectious disease causation and transmission, an agent must be present for an infectious disease to occur (Gordis, 2008; Karamanou et al., 2012). However, several virulence and pathogenic characteristics of the infectious organism are critical if disease symptoms are to manifest. Hepatitis E virus causes hepatitis E disease in humans. HEV is a nonenveloped, single-stranded RNA virus that belongs to the genus *Hepevirus* in the *Hepeviridae* family (Kamar et al., 2014). The HEV strains infective for humans belong to one serotype and four genotypes (1, 2, 3, and 4). Genotypes 1 and 2 mainly infect humans and are acquired from contaminated water via the fecal-oral route (Kamar et al., 2014; J. H. Kim et al., 2014). Large outbreaks and sporadic cases of HEV in developing countries are attributed to genotypes 1 and 2 (Rein, Stevens, Theaker, Wittenborn, & Wiersma, 2012b; Elduma, Zein, Karlsson, Elkhidir, & Norder, 2016; Kamar et al., 2014; Hakim et al., 2017). On the other hand, genotypes 3

and 4 are more prevalent in developed countries, rarely cause outbreaks, and are zoonotically acquired from pigs, wild boar, and deer (Kamar et al., 2014).

The Susceptible Human Host

The spectrum of HEV disease spans from an illness that in most of the patients is asymptomatic or mildly symptomatic, to severe disease that is fatal. The symptomatic disease presents as fever, jaundice, abdominal pain, dark yellow urine, pale stools, and sometimes body itching occurs (Kaba, Moal, Gérolami, & Colson, 2013; Kamar et al., 2014). Though symptomatic HEV disease rates are low, rates of up to 42% have been reported during active outbreaks in developing countries (Gerbi et al., 2015a). Overall, rates of symptomatic HEV disease are lower in developing (40%) than developed countries (75%) (Kamar et al., 2014). Severe and potentially life-threatening fulminant acute liver disease occurs in pregnancy, young children, the elderly, patients with pre-existing liver disease and those who are immunosuppressed (Thomson et al., 2013; Marano et al., 2015; Browne et al., 2015). The occurrence of severe HEV disease has been demonstrated consistently in pregnancy with rates of 11-70% (Kumar, Beniwal, Kar, Sharma, & Murthy, 2004; Sahai & Kiran, 2015). The other co-infections associated with an increased risk of HEV disease include Hepatitis B Virus, Hepatitis C Virus, HIV, and schistosomiasis (J. H. Kim et al., 2014). Given the high infectious disease burden in sub-Saharan Africa, their impact on the risk of HEV infection should be more diverse, extensive, and expected to vary depending on the prevailing infectious disease epidemiology. In developing countries where genotypes 1 and 2 are the predominant cause of infection, the risk of infection is high in children although most of these

infections tend to be anicteric (Ekanem, Ikobah, Okpara, & Udo, 2015; Patel et al., 2015). In the same way, the risk of HEV disease is higher in adolescents and young adults during outbreaks of genotype 1 and 2 in developing countries (Thomson et al., 2013; J. H. Kim et al., 2014; Kamar et al., 2014; Cummings et al., 2014; Marano et al., 2015; Gerbi et al., 2015a).

Most outbreaks in developing countries are attributed to fecal-oral transmission of genotypes 1 and 2 from contaminated water sources (Bile et al., 1994; Howard et al., 2010; Kamar et al., 2014). Person-to-person transmission of HEV occurs but its overall contribution to disease outbreaks is believed to be minimal (Kamar et al., 2014; E. H. Teshale, Grytdal, et al., 2010). Hepatitis E virus infection shows a male preponderance that most probably relates to cultural, occupational, and/or recreational gender-specific practices that increase the risk of exposure and gender-based predispositions (Cummings et al., 2014; Kamar et al., 2014; Gerbi et al., 2015a). Vaccination significantly attenuates the risk of HEV infection. Clinical trials involving the HEV vaccine, Hecolin® reported an efficacy of 100% with no safety concerns after two doses of the vaccine (Zhu et al., 2010; WHO, 2015b). However, vaccine effectiveness and coverage studies are required to assess the performance of the vaccine in community settings. The HEV 239 vaccine is not WHO prequalified but based on its efficacy and safety profile, WHO recommends its use to complement outbreaks (WHO, 2015b). Personal behaviors like handwashing practices, treatment of drinking water, and sanitation also influence the risk of HEV infection and disease at the individual level (Guthmann et al., 2006; Howard et al., 2010; Kamar et al., 2014).

Environmental Determinants for HEV Occurrence

Environmental factors play a significant role in the transmission of Hepatitis E virus. The virus can resist and survive harsh environmental conditions and has been isolated from unprotected contaminated water sources during disease outbreaks (Howard et al., 2010; Kamar et al., 2014). The survival and transmission of HEV increases during the rainy season, especially in areas with flooding (E. H. Teshale, Howard, et al., 2010a; Thomson et al., 2013; Browne et al., 2015). Several factors including poor sanitation, contamination of unprotected water sources, and poor personal hygiene enhance the risk of HEV transmission in these settings. Factors critical for its spread include the socioeconomic status and population density that in turn affect access to safe water and sanitation facilities. Socioeconomic conditions that lead to overcrowding in poorly planned settlements and poor access to health services increase the risk of HEV infection (Browne et al., 2015; E. H. Teshale, Howard, et al., 2010a; Thomson et al., 2013). In developing countries, HEV genotypes 3 and 4 are the predominant genotypes. The risk of HEV transmission in these settings is associated with consumption of raw or undercooked pork products or game meat (Kamar et al., 2014; Said et al., 2014; Krumbholz et al., 2014; Cossaboom et al., 2016; Guillois et al., 2016).

The rationale for using this theory derives from the germ theory of disease causation and transmission that emphasizes the presence of an infectious agent, HEV for infection and symptomatic disease to manifest in a susceptible host. Besides, the theory recognizes the interaction between the infectious agent in a favorable environment that

facilitates transmission of the agent to a susceptible host. These disease transmission dynamics are consistent with ecologic approaches of disease causation, which emphasize the interaction between the host and agent in a favorable environment. For an organism like HEV that has adapted to survive in susceptible hosts and harsh environmental conditions, the model, therefore, becomes a suitable guide to initiate interventions for reducing host susceptibility, eliminating the agent, and assuring a favorable environment to mitigate the risk of disease.

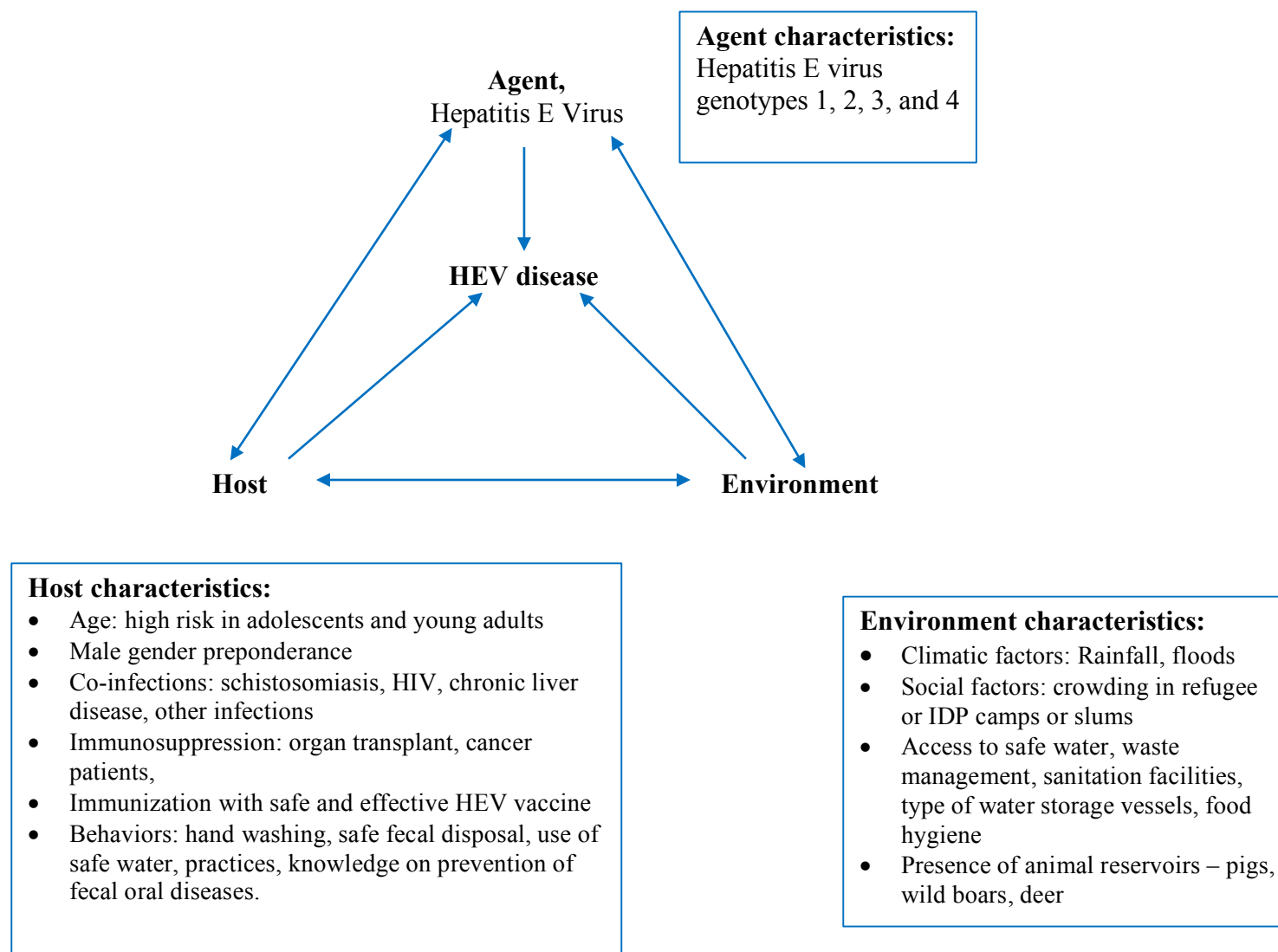


Figure 1 Factors for Hepatitis E disease based on agent-host-environment model.

The host-agent-environmental model offers a platform for identifying populations at risk for severe symptomatic HEV disease. While the control of HEV has primarily focused on improving access to safe drinking water, sanitation, and improving environmental and personal hygiene, interventions have not been as effective in securing timely outbreak containment in displaced populations. Thus, HEV outbreaks in developing countries have been protracted lasting three to five incubation cycles in displaced populations where adverse outcomes are worst in pregnant women (E. H. Teshale, Howard, et al., 2010b; Thomson et al., 2013). However, there is optimism that the complementary use of HEV 239 vaccine, Hecolin® alongside interventions to improve hygiene, access to safe drinking water, and sanitation will effectively reverse the current HEV trend. Effective use of the HEV vaccine, however, requires epidemiological data that identify groups at risk for symptomatic and severe HEV disease. Equally important are epidemiological data elucidating the timing, seasonality, and duration of outbreaks as this will allow timely and targeted preventive interventions including HEV vaccination. Guided by the host-agent-environment model, the present study sought to identify high-risk groups for severe symptomatic HEV disease. The findings from the present study will inform targeted deployment of the HEV 239 vaccine alongside other interventions to reduce the risk of HEV disease and adverse outcomes. Also, the findings from the present study will inform timely deployment preventive interventions during the pre-transmission season.

Hepatitis E Virus Disease in Humans

Hepatitis refers to the inflammation of the liver commonly caused by infections, heavy alcohol use, or exposure to toxic agents or medicines (CDC, 2016). Acute hepatitis usually follows viral, parasitic, or bacterial infections with some of the common infective agents being hepatitis A, B, C, and E, yellow fever, dengue virus, and leptospirosis (WHO, 2014). These infections typically cause acute hepatitis with symptoms that are clinically indistinguishable among biological agents. Hepatitis presents with variable degrees of progressive liver decompensation that manifests as yellowing of the skin and mucous membranes (jaundice)(WHO, 2014). As part of disease and outbreak surveillance, these illnesses constitute a syndrome, ‘acute jaundice syndrome’. The syndrome includes jaundice of acute onset with or with or without fever and absence of precipitating factors (WHO, 2014). Consequently, confirmation of a specific etiological agent usually requires definitive laboratory testing.

Hepatitis E virus disease has a wide spectrum of clinical presentation in humans that varies depending on several factors including age, gender, the prevalent HEV genotypes in a geographical setting, the presence of comorbidities like chronic liver disease, and physiological conditions like pregnancy. Symptomatic disease follows a long incubation period of 2-10 weeks after exposure to HEV, with most infections occurring within 5-6 weeks or 40 days (CDC, 2015; WHO, 2016a). Epidemiological findings from two of the initial volunteer studies on HEV showed that the anicteric phase of HEV disease starts 30-36 days after ingestion of the virus (Balayan et al., 1983; Chauhan, Jameel, Dilawari, Chawla, & al, 1993). Overall, HEV causes a mild, short, and self-

limiting illness that for most immunocompetent patients resolves within 2-6 weeks (WHO, 2016a). Hence for the majority of immunocompetent infected persons, HEV causes an asymptomatic or mildly symptomatic illness lasting a few weeks (CDC, 2015; WHO, 2014). The initial volunteer studies showed that the first symptoms are nonspecific and include general weakness, epigastric abdominal pain, nausea, vomiting, loss of appetite, dark urine, and liver enlargement (Balayan et al., 1983; Chauhan et al., 1993). This presentation has been reported in cases detected as part of epidemic and sporadic transmission in endemic sub-Saharan Africa and Asia (Shujun Zhang et al., 2011; Goumba, Konamna, & Komas, 2011; Murthy et al., 2014; Chakrabarti et al., 2016). The other symptoms reported during the anicteric phase include mild fever, chills, and headache (CDC, 2015, 2016).

Just like other hepatotropic viruses, jaundice, the hallmark of symptomatic HEV disease, appears 38-40 days after exposure to the virus and persists for 60-120 days (Balayan et al., 1983; Chauhan et al., 1993; CDC, 2015). The jaundice is often associated with enlargement of the liver and spleen, passing of dark urine and pale stools, and generalized itching in some of the patients (Goumba et al., 2011; Murthy et al., 2014; CDC, 2015). Symptomatic HEV disease as seen from jaundice is estimated to occur in 40% of patients with HEV genotypes 1 and 2 infections in developing countries (Kamar et al., 2014). On the other hand, 75% of HEV infections for genotypes 3 and 4 present with symptomatic infections in developed countries (Kamar et al., 2014). A community survey undertaken during the 2008 HEV outbreak in Northern Uganda showed that icteric HEV disease varied from 18.9% in areas with active transmission to 30.1% in

areas where transmission had peaked (E. H. Teshale, Howard, et al., 2010a). Icteric HEV disease is overestimated in hospital-based serosurveys with rates of over 70% reported in endemic locations in Asia and Africa (Goumba et al., 2011; Murthy et al., 2014; Chakrabarti et al., 2016). During the 2014 HEV outbreak in Bangui, Central Africa Republic, a cross-sectional survey involving 11 health facilities estimated icteric HEV disease at 93% (Goumba et al., 2011). Corresponding health facility-based surveys in South India reported icteric HEV disease rates of 79-81% (Murthy et al., 2014; Chakrabarti et al., 2016).

In endemic locations of sub-Saharan Africa and Asia, symptomatic HEV disease occurs more commonly in adolescents and young adults aged 15-40 years. In a survey undertaken during the 2008 HEV epidemic in Northern Uganda, the prevalence of icteric HEV disease increased with age with the highest prevalence of 33.6% and 37.3% reported in persons aged 15-44 years and 45 years or more respectively (E. H. Teshale, Howard, et al., 2010a). During the same outbreak, a prospective acute jaundice syndrome surveillance system was set up to determine the causes of acute jaundice syndrome and the proportion attributable to HEV. The prevalence of acute jaundice syndrome due to HEV was highest (86%) among pregnant women in the 15-49-year age group (Gerbi et al., 2015a). In North Eastern Uganda where HEV eventually spread after ravaging Northern Uganda, HEV cases were reported from 2009 to 2012 in four districts with young adults aged 20-29 years being the most affected (Cummings et al., 2014). Similar trends were reported during the HEV outbreak of 2013 among the refugees in Maban, South Sudan where adults 18-59 years were the most affected (Thomson et al., 2013).

HEV infection rates are high in children in developing countries but tend to be anicteric and therefore are underdiagnosed. During the 2008 HEV outbreak in Northern Uganda, icteric HEV disease was lowest in children under 2 years who registered an attack rate of 2% (E. H. Teshale, Howard, et al., 2010a). A serosurvey undertaken among children during the outbreak showed that one-third of the children aged 0-15 years had evidence of recent HEV infection with over half of the children aged 11-15 years showing evidence of recent HEV infection (Patel et al., 2015). In the same way, icteric HEV disease was lowest in children below 10 years of age during the 2009 HEV outbreak in North Eastern Uganda (Cummings et al., 2014). A community survey conducted in 2012 among children 1-18 years in Cross Rivers state, Nigeria, showed that the overall HEV prevalence increased with age (Ekanem et al., 2015). Also, the overall seroprevalence of 7.7% and a median age of 9 years for HEV positive cases (Ekanem et al., 2015).

The distribution of HEV cases in developed countries differs from the pattern in developing countries. The adults, both middle-aged and older adults and males constitute the bulk of HEV cases in developed countries. A national HEV seroprevalence survey in Israel demonstrated increasing HEV prevalence by age with persons 60 years of age and above having the highest prevalence of 37.5% (Mor et al., 2015). Corresponding serosurveys in countries like Germany, Korea, England, and Wales have reported HEV seroprevalence rates of 23-60% in middle-aged and older adults aged 50 years and above (Krumbholz et al., 2014; Yoon et al., 2014; B. S. Kim et al., 2015a).

The risk of HEV infection is also reported to be higher in males when compared to females, with a larger proportion of males affected in developed countries. This distribution is more likely related to the greater risk of zoonotic infection among the males in the developed countries (Goumba et al., 2011; Cummings et al., 2014; Krumbholz et al., 2014; Yoon et al., 2014; B. S. Kim et al., 2015b; Chakrabarti et al., 2016). A few reports have however reported higher rates of HEV disease in females (E. H. Teshale, Howard, et al., 2010a; Thomson et al., 2013; Mor et al., 2015). These gender-based variations for the risk of HEV disease highlight the risk of exposure to the source of infection in the settings where the infection occurs.

At the other end of the HEV clinical spectrum, severe disease is characterized by acute liver failure that manifests with mental changes, restlessness, unexplained bleeding, persistent vomiting, and loss of consciousness (Kamar et al., 2014). The risk of severe HEV disease is high in pregnant females with the risk of death increasing progressively from the first to the third trimester. HEV pregnancy-related complications are exclusive to HEV genotype 1 infections in developing countries in Asia and sub-Saharan Africa. Excess mortality in jaundiced pregnant women is unique to HEV and has been used to identify early outbreaks dating back to the 18th century (Teo, 2012). In sub-Saharan Africa and Asia where sporadic and epidemic transmission of HEV has been reported, the admission of increasing numbers of pregnant females with adverse outcomes following acute hepatitis is often used to suspect the emergence of HEV. Over the years, increasing evidence shows that HEV genotype 1 infections lead to severe disease in pregnancy with adverse maternal and fetal outcomes.

During the 2004 HEV outbreak in Western Darfur, epidemiological studies to elucidate the impact of HEV in pregnancy showed that mortality varied from 8.2% at the community level to 31% in pregnant women admitted to hospital with HEV disease (Boccia et al., 2006a). To further highlight the risk of severe HEV disease in pregnancy, a cross-sectional survey undertaken during the 2008 HEV epidemic in Northern Uganda showed that the symptomatic HEV attack rate in pregnancy was 81% with a correspondingly high mortality rate of 6.9% (E. H. Teshale, Howard, et al., 2010a). In the same way, case series data from the 2012 HEV outbreak among refugees in Maban county of South Sudan showed that the risk of death in pregnant women with jaundice was five times higher when compared to their non-pregnant counterparts aged 15-59 years (Browne et al., 2015). In Asia, pregnancy-associated HEV disease rates of up to 27% were reported from a hospital-based prospective study in India (Kumar et al., 2004). Similarly, as part of a prospective pregnancy surveillance project in northwest Bangladesh, hepatitis-like illness attributable to HEV was estimated to account for 9.8% of pregnancy-related deaths (Alain B. Labrique et al., 2012). Projections from this study showed that over 10,500 HEV pregnancy-related deaths were occurring in south Asia annually (Alain B. Labrique et al., 2012). Corresponding estimates of the 2005 global burden of HEV study showed that the probability of death following symptomatic HEV disease in pregnancy was 19.8%, which was higher when compared to 1.9% for symptomatic HEV disease in their nonpregnant counterparts (Rein et al., 2012a). The adverse outcomes of HEV disease in pregnancy were estimated to result in 3,000 still births annually (Rein et al., 2012a). HEV related severe disease can be as high as 30% in

pregnancy (Kumar et al., 2004). Some of the common complications of severe HEV disease in pregnancy include hepatic coma and disseminated intravascular coagulation and have been reported to occur in 49% and 64% of cases respectively (Khaskheli et al., 2015). The cause of severe HEV disease during pregnancy has not been established but is suspected to be related in part to the hormonal-mediated regulation immunological factors (Salam et al., 2013). Overall, these studies show that the risk of severe HEV disease is high in pregnant women living in endemic locations in Asia and Africa. The risk is potentially greater in vulnerable displaced populations. Additional data on severe HEV disease in pregnant women living in displaced camps will justify the deployment of targeted preventive interventions to avert the risk.

Severe HEV disease is associated with poor prognosis and follows infection with HEV genotypes 1, 2, 3 and 4 in patients with preexisting chronic liver disease. In India, mortality rates of up to 70% have been reported in HEV cases with preexisting chronic liver disease (Acharya et al., 2007). Chronic HEV infection occurs in HEV genotypes 3 infections in developed countries but not been documented in HEV genotypes 1 and 2 cases in developing countries (Aggarwal, 2011a; Kamar et al., 2014). Chronic infection commonly occurs in solid organ transplant patients that are taking immunosuppressants, HIV patients or patients on chemotherapy for cancer (Aggarwal, 2011a; Kamar et al., 2014).

Besides the liver, HEV is also known to affect several other organs including the central and peripheral nervous system, the kidneys, pancreas, and blood cells. Several neurological syndromes are associated with HEV-associated neurological damage leading

to Guillain-Barre syndrome, Bell's palsy, neuralgic amyotrophy, acute transverse myelitis, and acute meningoencephalitis (Aggarwal, 2011a; Kamar et al., 2014). These neurological complications have occurred following infection with all four HEV genotypes (Aggarwal, 2011a; Kamar et al., 2014). Besides neurological damage, HEV genotypes 1 and 3 have been reported to cause damage to the glomerulus in the kidneys leading to membranoproliferative and membranous glomerulonephritis and thus compromising the renal function (Aggarwal, 2011a; Kamar et al., 2014). Pancreatic injury has also been reported in HEV genotype 1 infections and manifests as acute pancreatitis (Aggarwal, 2011a; Kamar et al., 2014). The common HEV related hematological disorders include thrombocytopenia and aplastic anemia that result in bleeding manifestations and varying degrees of congestive heart failure (Aggarwal, 2011a; Kamar et al., 2014).

The Epidemiology of Hepatitis E Virus Disease in Humans

Hepatitis E virus disease is caused by hepatitis E virus, a spherical and non-enveloped virus with a positive sense, single-stranded 7.2-kilobase RNA genome with three open reading frames (Kamar et al., 2014). Hepatitis E virus belongs to the genus *Hepevirus* and *Hepeviridae* family. The HEV strains pathogenic to humans belong to a single serotype and four genotypes 1 to 4 [HEV1, HEV2, HEV3, HEV4] and 24 subgenotypes including 5 for HEV1, 2 for HEV2, 10 for HEV3, and 7 for HEV4 (Kamar et al., 2014). The geographical distribution of HEV genotypes and subgenotypes is distinct. In the developing countries of Asia and Africa, where access to safe drinking water and sanitation is suboptimal, both infected humans and contaminated water sources

serve as reservoirs for HEV genotypes 1 and 2 in endemic populations. HEV genotype 1 is most widely distributed with sporadic and outbreak cases occurring in endemic areas in Asia and Africa. South America has also reported indigenous cases of HEV genotype 1 (WHO, 2015b). Travelers returning from endemic locations in Asia and Africa constitute the other high-risk group for HEV genotype 1 infections (WHO, 2010b, 2015b). Three HEV genotypes 1, 2, and 3 occur in sub-Saharan Africa but HEV genotype 1 accounts for most sporadic and outbreak cases (E. H. Teshale, Howard, et al., 2010a; Delarocque-Astagneau et al., 2012; E. Teshale & Ward, 2015; J. H. Kim et al., 2014). In Egypt, a study involving acute hepatitis cases attending infectious disease hospitals in Cairo isolated HEV genotype 1 (Delarocque-Astagneau et al., 2012). Among refugee and internally displaced populations in Sudan, South Sudan, and Uganda, HEV genotype 1 has been the sole cause of acute jaundice syndrome (E. H. Teshale, Howard, et al., 2010a; E. Teshale & Ward, 2015; Elduma et al., 2016). The distribution of HEV subgenotypes within endemic areas in Africa and Asia is distinct. Asia is endemic for HEV subgenotypes 1a, 1b, and 1c while sub-Saharan Africa is endemic for subgenotypes 1d and 1e (Lu, Li, & Hagedorn, 2006; Purdy & Khudyakov, 2011).

Endemic locations in Asia and Africa where HEV genotypes 1 and 2 are predominant, contaminated water sources and human beings are the sole reservoir for the virus. Thus, most of the transmission of HEV genotypes 1 and 2 is fecal-oral with water contaminated with fecal matter being the main common vehicle of transmission in the developing countries. Evidence of fecal-oral transmission of HEV derives from several studies with one of the earlier studies involving the isolation of HEV from the stools of a

volunteer who ingested pooled fecal extracts derived from outbreak cases (Balayan et al., 1983). During the 1991 HEV outbreak in Kanpur, India, the source of the outbreak was contaminated municipal water from the Ganges (Naik, Aggarwal, Salunke, & Mehrotra, 1992). In 2005, a large outbreak of HEV occurred in Hyderabad India with most cases reported from locations that were supplied by water pipes that crossed open sewer lines (Sailaja et al., 2009). Additional evidence of fecal-oral transmission with contaminated water as the common vehicle derives from recent HEV outbreaks in refugees and displaced populations (E. Teshale & Ward, 2015). During the 2007 HEV outbreak among internally displaced populations in Northern Uganda, HEV RNA was isolated from water sources in the camps (Howard et al., 2010). These findings underscore the significance of poor access to safe drinking water and sanitation as drivers of sporadic and epidemic transmission reported in HEV endemic locations in Africa and Asia.

In the developed countries, autochthonous HEV infection is mainly attributed to genotypes 3 and 4 that are zoonotically acquired with the source animals being domestic pigs, wild boar, and deer (Kamar et al., 2014). HEV genotype 3 has a worldwide distribution while HEV genotype 4 cases have been reported in China, Japan, and Europe (Kamar et al., 2014). In a serosurvey conducted in areas with high pig densities, the prevalence of HEV genotype 3 increased with age from 7.5% of persons 18-39 years to 23% of persons 50 years and older (Krumbholz et al., 2014). During the same survey, the risk of HEV infection was more significant for 18-39-year-olds that were exposed to pigs in comparison to their counterparts who were not exposed to pigs (Krumbholz et al., 2014). Consumption of undercooked meat products from these source animals is

responsible for most sporadic cases of HEV genotype 3 and 4 in Europe. In England and Wales, a case control study was conducted to test the hypothesis that increasing HEV infections were related to consumption of pork products (Said et al., 2014). The study showed that consumption of pork pie [odds ratio (OR) 6.33, 95% confidence interval (CI) 1.41-28.48], sausages [OR 7.59, 95% CI 1.81-31.84], and ham [OR 10.98, 95% CI 1.84-65.35] were associated with HEV infection (Said et al., 2014). In France, 17 cases of mostly asymptomatic infection occurred after a wedding and were attributed to consumption of spit-roasted pork (relative risk (RR) 1.69, 95% CI 1.04-2.73) (Guillois et al., 2016). In West Africa, a cross-sectional survey conducted in Ouagadougou, Burkina Faso showed that occupational exposure to pork products increased the risk of HEV infection when compared to the general population (OR 3.46, 95% CI 2.85-4.21) (Kuan Abdoulaye Traoré et al., 2015). While zoonotic transmission remains a major mode of autochthonous transmission for genotypes 3 and 4 in the developed countries, there is no evidence to show that zoonotic transmission of the two genotypes is a major risk factor for HEV transmission in developing countries in Asia and Africa.

Besides the oral-fecal and zoonotic modes of transmission, HEV infection occurs following person-to-person, vertical, and transfusion-related transmission. Person-to-person transmission is suspected to play a significant role during HEV outbreaks in which multiple cases occur in the same household with overlapping incubation periods. The findings from a survey conducted during a large HEV outbreak in Northern Uganda in 2007 suggested that person-to-person transmission played a significant role (E. H. Teshale, Grytdal, et al., 2010). During the outbreak, sustained transmission occurred

amidst interventions for improving access to safe drinking water. As such, high secondary attack rates of up to 25% were registered, a trend attributed to attending funerals, sharing hand washing containers before eating food, and the low frequency of handwashing after defecation. The possibility of zoonotic transmission was ruled out after serum samples collected from domestic animals tested negative for HEV infection (E. H. Teshale, Grytdal, et al., 2010).

Several case series have demonstrated evidence of vertical transmission of HEV. In a study of eight babies whose mothers infected with HEV in the third trimester, HEV RNA was isolated from the cord blood of 5 (63%) neonates thus demonstrating vertical transmission of HEV (M. S. Khuroo, Kamali, & Jameel, 1995). In the same way, a hospital-based prospective case series showed evidence of vertical transmission in 6 (33%) of 18 pregnant mothers who were infected with HEV in the third trimester (Kumar et al., 2004). As part of a facility based case series, hepatitis E virus was isolated from one of three babies born to HEV infected mothers (Bonney et al., 2012).

Transfusion-related transmission of all four genotypes of HEV occurs in both developed and developing countries, although its role in outbreaks is limited. The risk of transfusion transmission derives from the fact that hepatitis E viremia starts approximately one week before symptom onset and persists for at least three weeks (Chauhan et al., 1993). Consequently, exposure to blood or blood products derived from viremic patients is a known risk factor for HEV infection. In a retrospective review of blood donations collected in southern England, 0.94% of them were found to be viremic for HEV (Hewitt et al., 2014). Additionally, the viremic donations had a high HEV viral

load but, the majority were seronegative for HEV IgM and IgG thus increasing the risk of undetected transfusion related HEV transmission. Subsequently, 42% of the recipients of these products were infected with HEV (Hewitt et al., 2014). A similar study in the Netherlands showed 17 (0.32%) HEV viremic donations from 2011 to 2012 (Slot et al., 2013). A lower HEV RNA detection rate of 0.045% was reported in a similar blood products study in France (Gallian et al., 2014). A much higher risk of transfusion related HEV transmission has been reported in Ouagadougou, Burkina Faso, where 1.9% of donations had evidence of recent HEV infection by way of IgM (Kuan A. Traoré et al., 2016).

The Magnitude and Distribution of Hepatitis E Virus Disease

Seroprevalence data on HEV disease is limited, especially in endemic locations of Asia and Africa. However, substantial serological survey data is available and has shaped the current epidemiological description of the disease (Rein et al., 2012a). Nevertheless, the comparability of findings derived from varying commercial assays is constrained by the absence of a gold standard for HEV serological testing (Abravanel et al., 2013; Pas et al., 2013; Norder et al., 2016). Despite this limitation, these findings constitute the current state of knowledge and continue to be used to inform clinical and public health decisions for HEV control. The 2005 Global Burden of HEV infection and disease used data derived from these studies (Rein et al., 2012a). The findings from this study showed that the seroprevalence of HEV was lowest in North Africa and the Middle East, where except for Egypt, the peak seroprevalence rate was 15%. In Asia, South Asia South and East Asia reported the highest peak prevalence of at least 25% while the other regions

posted a prevalence of 15-25% (Rein et al., 2012a). The survey showed a higher seroprevalence of more than 50% for individuals older than 5 years of age in Egypt (Rein et al., 2012a). Children and young adults aged 5-20 years were the most affected in all the regions in Africa and Asia (Rein et al., 2012a). The annual incidence increased from 0.5-1.0% in persons 0-15 years to 1.0-1.4% in the 15-20-year-olds but reduced after that to 0.2% in persons older than 30 years (Rein et al., 2012a). The overall annual burden of HEV genotypes 1 and 2 was estimated at 20 million incident infections, 3.4 million symptomatic cases, 70,000 deaths, and 3,000 stillbirths (Rein et al., 2012a).

The 2013 global burden of disease study showed that while mortality from HEV had declined globally, significant HEV mortality continues in sub-Saharan Africa and Asia (Stanaway et al., 2016). The study showed that the proportion of acute viral hepatitis mortality attributed to HEV declined from 0.058% in 1990 to 0.034% in 2013 (Stanaway et al., 2016). The age-standardized HEV mortality rates (cases per 100,000) for east, central, and west sub-Saharan Africa were 20.4, 25.3, and 35 respectively (Stanaway et al., 2016). Globally, the HEV deaths declined from an estimated 52,000 deaths in 1990 to 50,000 deaths in 2013 (Stanaway et al., 2016). The 2013 global burden of disease study lacked corresponding risk estimates for HEV disease in vulnerable groups like displaced populations.

Consistent with the findings from the 2013 global burden of disease study, significant HEV transmission continues in sub-Saharan Africa. Most HEV cases in Africa occurred as part of large outbreaks with the highest attack rates reported in displaced populations where access to safe drinking water and sanitation are usually suboptimal

during the acute crisis phase. The 2007 HEV outbreak in northern Uganda, the largest ever recorded, occurred in internally displaced populations. During the epidemic, the symptomatic attack rate was estimated at 25% and varied by location depending on the outbreak phase. The symptomatic attack rate was lowest in children under two years at 6.9% and was highest in pregnant women at 80.7% (E. H. Teshale, Howard, et al., 2010a). A serosurvey undertaken during this outbreak showed that while children had low symptomatic attack rates, the overall HEV IgG seroprevalence was 25.4% for children 1-15 years and reached highs of 31% in children 0-5 years (Patel et al., 2015). In the same way, the prevalence of recent infection in children under 15 years was 36.6% (Patel et al., 2015). These findings show that the risk of HEV infection in children living in endemic locations is high though most of these occur without overt clinical manifestations.

In North Eastern Uganda, the HEV outbreak that occurred from 2009 to 2012 registered a lower symptomatic attack rate of 14 cases per 10,000, and as seen in other outbreaks in similar settings, the epidemic showed a preponderance for males and persons aged 20-29 years of age (Cummings et al., 2014). The outbreaks of HEV reported among IDPs in Darfur in 2004, and the refugee camps in Maban, South Sudan in 2013 registered higher attack rates of 3.3% and 7.4% respectively (Guthmann et al., 2006; Thomson et al., 2013). The attack rates were highest in persons aged 15-45 years and 18-59 years (Guthmann et al., 2006; Thomson et al., 2013).

The 2015 serosurvey undertaken in internally displaced populations residing in the United Nations protection of civilians' camps in Juba South Sudan showed that HEV

remains a significant public health problem in displaced populations (Azman et al., 2017). The age-adjusted seroprevalence of HEV Ig G from this study was 71%, 95%CI 63-78% while evidence of recent infection as seen from HEV IgM was estimated at 4.45% (Azman et al., 2017). These were findings in a displaced population with no clinical evidence of symptomatic HEV disease.

In the Lake Chad basin, Borno state in Nigeria, Diffa region in Niger, and Salamat region in Chad have reported an increasing number of HEV cases (WHO, 2017). The protracted civil strife in the basin left thousands displaced with inadequate access to safe drinking water and sanitation since 2016 (Anonymous, 2017a; MSF, 2017; MSF USA, 2017; WHO, 2017). In Diffa region, Niger, the initial cases occurred in December 2016 in a population of at least 240,000 refugees and IDPs with poor access to safe drinking water and sanitation (MSF USA, 2017). By 29 June 2017, at least 1,096 HEV cases including 167 confirmed HEV cases and 34 HEV deaths (25 deaths in pregnant women) had been reported in Diffa, Bosso, and N'Guigmi regions, Niger (MSF USA, 2017; WHO, 2017). In Chad, HEV cases originated from Salamat and Aboudeia regions since September 2016 (MSF, 2017; WHO, 2017). Hence by 30 June 2017, the districts of Aboudeia, Amtiman North, Amtiman South, and Moraye in Salamat region had registered 1,631 HEV cases including 98 HEV confirmed cases, at least 64 hospitalized HEV cases, and 18 HEV deaths (MSF, 2017; WHO, 2017).

The recent HEV cases in Nigeria were first reported on 3 May 2017 in Damasak among displaced populations. Epidemiological data from the outbreak showed that by 30 June 2017, Mobbar, Monguno, and Ngala Local Government Areas had registered 146

HEV cases, including 22 confirmed HEV cases (Anonymous, 2017a; WHO, 2017).

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Outside outbreak settings, data from serosurveys suggest that the prevalence of HEV is high in Africa and Asia. The data collected from 2010 to 2012 as part of the prospective acute jaundice syndrome surveillance program in Northern Uganda showed that the prevalence of IgG in this population was 42% with the highest seroprevalence registered in persons 15-49 years (Gerbi et al., 2015a). A correspondingly high IgG seroprevalence of 37.8% was recorded as part of the acute febrile illness surveillance program in Kibera, Kenya (Furukawa et al., 2016). A serosurvey was undertaken from 2011 to 2012 to assess HEV transmission during the interepidemic period for areas in Darfur and Northern Kordofan that were previously affected by HEV outbreaks (Elduma et al., 2016). The study showed evidence of recent infection, with HEV IgM seroprevalence rates of up to 25% (Elduma et al., 2016). In Zambia, a community survey involving children and adults reported a high HEV IgG seroprevalence of 42% in adults and 16% in the children (Jacobs et al., 2014). The HEV seroprevalence was significantly higher in HIV seropositive individuals (71%) than in HIV seronegative individuals (28%) (Jacobs et al., 2014). The study reported a significant association between HEV and HIV infection, OR 6.2, 95%CI (2.2-18) (Jacobs et al., 2014). In a similar cross-sectional study undertaken among HIV infected men and women in Malawi, the HEV IgG was reported

as 16.5% (Taha et al., 2015). However, in contrast to the findings from Jacobs et al. (2014), the study reported a significantly higher HEV seroprevalence of 20.2% in HIV negative individuals when compared to the prevalence of 12.9% in HIV infected individuals (Taha et al., 2015).

A serosurvey conducted in two rural villages in Egypt reported high HEV seroprevalence in all age groups, in a huge contrast to seroprevalence findings from all the other parts of Africa. During the survey, the overall seroprevalence was 67.7 %, 95%CI 66.7-68.6% (Fix et al., 2000). Individuals in the second decade of life registered the highest seroprevalence of 75.6%. The HEV seroprevalence increased with age from 36.2% in children under five years to 64.7% in children 5-9 years of age (Fix et al., 2000). The seroprevalence of HEV was significantly higher in the males when compared to the females (Fix et al., 2000).

Hepatitis E seroprevalence studies in West Africa have reported high HEV disease burden. In a cross-sectional study undertaken in Plateau state, Nigeria, the HEV IgG seroprevalence was reported at 42.7% with a significantly higher seroprevalence of 66.7% in animal handlers (Junaid et al., 2014). In Burkina Faso, the HEV IgG seroprevalence was 39%, 95%CI 36.5-41.5% and registered a steady increase with age (Kuan A. Traoré et al., 2016). A correspondingly higher seroprevalence was reported in pork butchers (76%) when compared to the general population (47.8%) in Ouagadougou (Kuan Abdoulaye Traoré et al., 2015). The serological survey findings from Burkina Faso suggest zoonotic transmission as a significant mode of HEV spread in the populations studied.

In Asia, the burden of HEV is high but comparable to the trends in Africa with both sporadic and epidemic disease known to occur. Contaminated water sources and a lesser extent, zoonotic exposure, account for most of the transmission in Asia. Hepatitis E virus is endemic in China and is one of the leading causes of morbidity and mortality in high-risk populations. Findings from a national survey showed a high HEV seroprevalence of 23.46%, 95%CI (18.4-28.5%) (Jia et al., 2014). The same study showed that the prevalence of HEV was low in children but increased with age (Jia et al., 2014). Persons 15-60 years were the most affected (Jia et al., 2014). The high-risk groups included persons 15-60 years, farmers, and individuals living in areas where waterborne and zoonotic transmission were more likely to occur (Jia et al., 2014). Recent data from the health information system (HIS) in China, estimated the HEV incidence at 4 cases per 100,000 population with the highest transmission in the first quarter of the year and among persons 40-60 years of age (Liu et al., 2016).

In India, HEV is a major cause of enterically transmitted acute viral hepatitis. Seroprevalence of HEV has varied from 10.54% in a two-year hospital-based acute viral hepatitis case series in Mangalore to 20.9% among healthy individuals during an outbreak in North India and 41.8% in a hospital based study in West Bengal India (Chandra, Ojha, Chatterjee, & Chattopadhyay, 2014; Majumdar, Singh, Goyal, Chawla, & Ratho, 2015; Shenoy, Baliga, Joon, & Rao, 2015). The prevalence of HEV was highest in young adults 10-30 years and 21-40 years respectively (Chandra et al., 2014; Shenoy et al., 2015).

The high-income Asian Pacific countries like Korea have reported a comparatively lower prevalence of HEV prevalence than India. A nationwide HEV seroprevalence survey in South Korea reported a prevalence of 5.9% with the prevalence being significantly higher in males, older adults, and those with less education who resided in rural coastal areas (Yoon et al., 2014). A related survey showed that zoonotic transmission of HEV genotype 3 from domestic pigs resulted in a high HEV seroprevalence of up to 33% in slaughterhouse workers (B. S. Kim et al., 2015a).

pigs (B.-S. Kim et al., 2015a).

The prevalence of HEV is even lower in Europe and North America, and large outbreaks are not common. Autochthonous transmission of HEV that is attributable to zoonotic exposures or international travel to endemic locations occurs in developed countries. Nonetheless, HEV seroprevalence is much lower in developed countries and is associated with a higher frequency of asymptomatic HEV disease when compared to developing countries. In the United States, data from the National Health and Nutrition Examination Survey (NHANES) from 2009 to 2010 showed that the prevalence of HEV IgG declined from 10% for the period 1988-1994 to 6% in 2009-2010 (E. H. Teshale et al., 2015). The drop in HEV prevalence was more significant for US natives than foreign nationals (E. H. Teshale et al., 2015). As part of a laboratory surveillance project for acute viral hepatitis, the Centers for Disease Control and Prevention tested samples for HEV from 2005-2012 (Drobeniuc et al., 2013). The positivity rate for HEV IgG was 17% with non-travelers and older individuals having significantly higher proportions of anicteric infections when compared to travelers (Drobeniuc et al., 2013). In the same

way, all HEV cases in non-travelers were solid organ transplant patients and had HEV genotype 3 while genotypes 1 and 4 were isolated from the travelers (Drobeniuc et al., 2013). These findings reaffirm the declining HEV trend in the United States with solid organ transplants and international accounting for most of the ongoing low-grade local transmission.

Like in the United States, low-grade local transmission has been reported in Europe and is linked to occupational, recreational, and zoonotic exposure to animal reservoirs. Using a nationally representative sample, Faber et al. (2012) reported a prevalence of HEV IgG of 16.8%, 95%CI 15.6-17.9% in Germany. The survey also showed that the prevalence of HEV IgG increased with age with a peak prevalence of 26% in persons 60-64 years (Faber et al., 2012). The mean incidence of HEV was 3.9, 95% CI 3.6-4.2 per 1,000 population (Faber et al., 2012). The HEV transmission studies in Europe show that magnitude of HEV is high in areas with a high risk of zoonotic transmission. These include areas with high pig densities and those where hunting of game reservoirs occurs (Krumbholz et al., 2014; Schielke et al., 2015b). In Israel, a cross-sectional survey using a nationally representative sample reported an HEV IgG prevalence of 10.6%, 95% CI 8.4-13% (Mor et al., 2015). The seroprevalence increased with age reaching a peak of 37.5% in persons older than 60 years (Mor et al., 2015). The seroprevalence was significantly higher in persons born in Asia and Africa when compared to the natives of Israel (Mor et al., 2015). In Italy, Lanini et al. (2016) reported an HEV IgG prevalence of 5.4%. The seroprevalence of HEV IgG increased with age and was significantly higher in persons born outside Italy and in men having sex with men

(Lanini et al., 2015). These findings suggest that anal contact between men may enhance the risk of HEV transmission.

Overall, the burden of HEV is highest in sub-Saharan Africa and Asia, and big HEV outbreaks are exclusive to these two continents. Fecal-oral spread accounts for most of the cases in sub-Saharan Africa and Asia where access to safe drinking water and sanitation is sub-optimal. Outbreaks of HEV genotypes 1 and 2 in the developing world are seasonal with the majority starting during the rainy or monsoon season. Floods have also been reported to increase the risk of HEV outbreaks. An emerging trend in Africa in the recent years entails the increasing reports of HEV outbreaks among displaced populations living in refugee or IDP camps. South Sudan has experienced protracted outbreaks in displaced populations from 2012 to 2017. Effective targeting of comprehensive HEV interventions including the use of vaccines requires further characterization of HEV outbreaks in displaced populations to identify high-risk groups, the timing of disease outbreak onset, and peak transmission of HEV.

Factors for Symptomatic and Severe HEV Disease

Hepatitis E virus causes clinical disease that is indistinguishable from other viral hepatitis but stands out for causing severe disease. Severe HEV disease is characterized by fulminant acute hepatic failure and neurological symptoms in pregnant women and patients with pre-existing liver disease (Khaskheli et al., 2015; Sahai & Kiran, 2015). While the determinants of severe HEV disease are not well-known, studies have proposed three categories of factors, namely: host, viral, and environmental, aligned to

the host-agent-environment model (McLeroy et al., 1988; Aggarwal, 2011a; Centers for Disease Control, 2012).

Host Factors for Symptomatic and Severe HEV Disease

Acute jaundice syndrome with fulminant acute hepatic failure in pregnancy is most of the time suggestive of hepatitis E virus disease. This presentation is unique to HEV genotype 1 in endemic locations in Africa and Asia. The risk of severe HEV infection is high in pregnancy and is more likely to be fatal in the third trimester (Gad et al., 2012). Rein et al. (2012) reported a 20% risk of death from symptomatic HEV disease in pregnancy in comparison to 2% risk for nonpregnant cases with symptomatic HEV disease. Symptomatic HEV disease in pregnancy is associated with adverse perinatal outcomes and is estimated to cause at least 3,000 stillbirths annually (Rein et al., 2012a). During the HEV outbreak in Northern Uganda, the highest symptomatic HEV attack rate of 81% and mortality rate of 6.9% were reported in pregnant women (E. H. Teshale, Howard, et al., 2010a). Thomson et al. (2013) reported a mortality risk for HEV disease that was five times higher in pregnant women when compared to their nonpregnant counterparts aged 18-59 years. These findings suggest that pregnancy is a major determinant of severe symptomatic HEV disease in endemic locations in Africa and Asia. Collaborated findings in displaced populations would justify targeted interventions including vaccination to mitigate the risk of severe HEV disease.

Demographic characteristics like age and sex are major determinants of symptomatic and severe HEV disease. The potential for symptomatic HEV disease in developing countries typically increases with age reaching a peak in adolescents and

young adults aged 15-39 years with the risk being higher in males when compared to females. In a case control study for HEV risk factors in Bangladesh, 70% of the cases were older than 15 years of age and female gender was protective, though not significantly (Labrique et al., 2013b). In a study conducted to profile HEV cases in a tertiary hospital in Southern India, young adults 21-30 years were the most affected and constituted 33% of the patients enrolled into the study (Chakrabarti et al., 2016). A seroprevalence survey in Nigeria reported increasing HEV prevalence with age with a peak incidence of 51% in persons older than 60 years (Junaid et al., 2014). Also, the study showed that the prevalence of HEV was higher in males when compared to females (50% versus 45%) though this did not reach statistical significance (Junaid et al., 2014). A meta-analysis of primary risk factors for HEV infection showed that the risk of HEV infection was higher in males when compared to females, OR, 1.67, 95% CI 1.46-1.92 (Li et al., 2013). The overall global epidemiology of HEV shows an overall male preponderance with a higher ratio of males than females affected in the developed (3:1) when compared to the developing (2:1) countries (Mohammad S Khuroo, Khuroo, & Khuroo, 2016). In the developed countries where HEV genotypes 3 and 4 are more prevalent, middle aged adults 40-60 years are the most affected with severe disease more likely to occur in males as opposed to females (Kmush, Nelson, & Labrique, 2015). In these settings, most infections follow recreational, occupational, and/or zoonotic exposure to reservoir animals (Krumbholz et al., 2014; Schielke et al., 2015b). The identification of demographic factors for symptomatic and severe HEV disease is

essential to launching targeted interventions including vaccination for HEV prevention and control in vulnerable populations like displaced populations.

Pre-existing chronic liver disease from other viral hepatitis increases the risk HEV infection and severe disease that typically has a poor prognosis and a higher risk of death (Acharya et al., 2007; Gad et al., 2012). Severe HEV disease is common in patients with co-infections like HIV, Hepatitis A virus, Hepatitis C virus, Hepatitis B virus (Gad et al., 2012; Jacobs et al., 2014; Shenoy et al., 2015). In principle, however, any disease that causes inflammation of the liver could potentially exacerbate the severity of HEV disease. The list of co-infections with the potential to cause severe HEV disease will possibly vary depending on the local epidemiological patterns.

The state of the host immune response is a major determinant of symptomatic and severe HEV disease. While HEV disease is a largely mild and asymptomatic illness, symptomatic and chronic HEV infections have been associated with immunosuppressants in solid organ transplant patients and cancer chemotherapy (Aggarwal, 2011a; Kamar et al., 2014). Additional research is required to fully understand the role of HIV on the risk of symptomatic and severe disease since conflicting findings have been reported from previous studies (Jacobs et al., 2014; Taha et al., 2015). For HEV endemic areas in Africa and Asia where malnutrition is highly prevalent, the risk of severe HEV disease is potentially high though the paucity of data limits the current understanding of the role of acute malnutrition on severe HEV disease. In the same way, locations in sub-Saharan Africa that are heavily endemic for Hepatitis B infection constitute a priority group for

preventive HEV interventions given the risk of adverse outcomes following dual infection by the two viruses.

Viral Factors for Symptomatic and Severe HEV Disease

Viral factors play a significant role in symptomatic and severe HEV disease. These factors range from dose of inoculum, genotypic and sub-genotypic variations in HEV strains. Animal studies have demonstrated a correlation between the HEV inoculum and the degree of liver injury and hence severe disease (Aggarwal, 2011a). In the same way, fulminant hepatitis due to HEV is exclusive epidemics caused by HEV genotype 1 and is associated with high symptomatic attack rates and mortality rates especially in pregnant women (Gad et al., 2012; Rein et al., 2012a). Sub-genotypic variations are known to contribute to a higher risk of HEV infectivity and severe symptomatic disease. These changes result from mutations attributed to insertion of human ribosomal sequences or substitution of nucleotides or domains coding for the capsid protein (Inoue et al., 2009; Nguyen et al., 2012).

Environmental Determinants of Symptomatic and Severe HEV Disease

In endemic locations of sub-Saharan Africa and Asia, where enterically transmitted HEV genotypes 1 and 2 are predominant, environmental factors influence exposure timing, dosing, and may, therefore, contribute to the varied pattern of illness.

The onset of the rains is known to increase the risk of HEV outbreaks in endemic areas in sub-Saharan Africa and Asia. The rains in these settings are associated with flooding and contamination of open water sources thus increasing the risk of transmission especially in densely populated communities or displaced populations. A 2-year

prospective cohort study conducted in rural Bangladesh showed that the incidence of HEV was higher during the rainy season (72.4, 95% CI 47.3-106.1 per 1,000 person-years) when compared to the dry season (60.3, 95%CI: 44.6-79.7 per 1,000 person-years) (Labrique et al., 2010). A similar trend was reported from a two-year hospital-based cross-sectional study in Mangalore, India (Joon et al., 2015). During the study, peak HEV transmission occurred at the beginning of the rainy season (Joon et al., 2015). Case-series data from outbreaks in displaced populations of Ethiopia, South Sudan, and Uganda show that HEV onset and peak transmission coincide with the rainy season (Browne et al., 2015; E. H. Teshale, Howard, et al., 2010a; Thomson et al., 2013). These studies, however, fell short of documenting the long-term seasonality, duration, and detailed epidemiological characterization of HEV outbreaks in displaced populations.

Hygiene and sanitation factors influence the risk and dose of infection and therefore the risk of disease and severe illness following HEV infection by genotypes 1 and 2. Since water is the principal reservoir for HEV genotypes 1 and 2, access to ample amounts of safe drinking water is a major determinant for HEV infection. Unsafe water sources are major drivers of the risk of HEV transmission in Africa and Asia. During the 2007 HEV outbreak in Northern Uganda, HEV was isolated from surface water sources of drinking water (Howard et al., 2010). During the same outbreak, the risk of HEV transmission associated with hygiene practices was highlighted after the HEV was isolated from the communal hand rinse (Howard et al., 2010). The study identified washing hands in a shared basin as a risk factor for symptomatic HEV disease (Adjusted odds ratio [AOR], 1.9, 95% CI: 1.07-3.38) (Howard et al., 2010). In the same way, the

practice of using wide-mouthed containers to store drinking water that increases the risk of drinking water contamination was associated with a higher risk of HEV disease (AOR 2.83, 95% CI 1.16-6.94) (Howard et al., 2010). In a cross-sectional study undertaken in Plateau state, Nigeria, persons who were using river water as the source of drinking water were significantly more likely to be infected with HEV (OR 3.6, 95% CI 1.6-7.8) (Junaid et al., 2014). In the same study, open defecation, a practice that increases the chances of contaminating unprotected water sources like rivers, was significantly associated HEV infection (OR 2.9, 95% CI 1.4-5.7) (Junaid et al., 2014). Several other practices are known to enhance the risk of HEV infection. These practices include dinning out, unboiled drinking water, failure to wash hands before meals, eating contaminated food, inability to use latrines, contact with jaundiced cases, recent urban travel, and contact with animals (Junaid et al., 2014; Labrique et al., 2013a; Li et al., 2013). These findings show that inadequate access to safe drinking water and sanitation continue to be the principal drivers for outbreaks of HEV genotypes 1 and 2 in Africa and Asia. Their contribution to the risk of transmission varies in space and time. Specific investigations are therefore warranted to identify the drivers in each outbreak setting to allow targeted response.

Summary and Conclusions

Hepatitis E virus (HEV) is a major cause of acute viral hepatitis worldwide that is estimated to cause 50,000 annual deaths globally (Faber et al., 2012; Rein et al., 2012b; Mor et al., 2015; Lanini et al., 2015; Stanaway et al., 2016; Hakim et al., 2017). While the initial studies suggested the disease was indigenous to developing countries in Africa

and Asia, evidence of autochthonous transmission in developing countries is now substantial (Faber et al., 2012; Lanini et al., 2015; Mor et al., 2015; E. H. Teshale et al., 2015). The epidemiology of the disease in the two settings is distinct with big outbreaks of HEV genotypes 1 and 2 only known to occur in developing countries (E. H. Teshale, Howard, et al., 2010a; Delarocque-Astagneau et al., 2012; J.-H. Kim et al., 2014; E. Teshale & Ward, 2015). Sporadic transmission occurs in the developed countries and is predominantly related to zoonotic exposure following consumption of raw or undercooked pork products or game meat (Faber et al., 2012; Krumbholz et al., 2014; Schielke et al., 2015b). A more recent trend entails the increasing reports of HEV in displaced populations of Africa (Isaacson et al., 2000; Boccia et al., 2006a; E. H. Teshale, Howard, et al., 2010a; Ahmed et al., 2013a; Thomson et al., 2013; Browne et al., 2015; MSF USA, 2017; Azman et al., 2017; MSF USA, 2017; MSF, 2017; Anonymous, 2017a; WHO, 2017). The protracted nature of outbreaks in displaced populations highlights the need to augment the traditional interventions for HEV control with new tools like vaccination. However, current paucity of epidemiological data in these settings precludes the effective use of HEV vaccines to complement the existing interventions for HEV prevention and control.

The present study employs the host-agent-environment model to assess the determinants of severe HEV disease in displaced populations (McLeroy et al., 1988). A combination of factors aligned to the agent-host-environment model influence the risk of severe HEV disease (Inoue et al., 2009; Browne et al., 2015; Gad et al., 2012; Rein et al., 2012a; Nguyen et al., 2012; Labrique et al., 2013a; Joon et al., 2015; Thomson et al.,

2013). There are four genotypes, HEV 1, 2, 3, and 4 that are known to infect humans. However, severe disease in pregnancy remains exclusive to HEV genotype 1 (Alain B. Labrique et al., 2012; Kamar et al., 2014; Rein et al., 2012a). Severe HEV disease has also been reported in patients with pre-existing chronic liver disease (Kamar et al., 2014). Studies have also shown that sub-genotypic differences and genetic polymorphism are associated with severe HEV disease (Inoue et al., 2009; Nguyen et al., 2012). In applying this knowledge to the prevention and control of HEV, an understanding of the agent attributes is critical for selecting appropriate public health interventions to prevent and control HEV disease. Also, timely deployment of HEV vaccines alongside other conventional interventions for HEV prevention requires a thorough understanding of the epidemiology of the HEV disease in the population of interest. Studies in endemic locations in sub-Saharan Africa and Asia have shown that HEV outbreaks are more likely to occur in the rainy season and/or in association with flooding (Inoue et al., 2009; Browne et al., 2015; Gad et al., 2012; Rein et al., 2012a; Nguyen et al., 2012; Labrique et al., 2013a; Joon et al., 2015; Thomson et al., 2013).

The present study used case-series data from multiple disease outbreaks in displaced populations spanning a five-year period. This approach has the advantage of establishing the trends, magnitude, and distribution of HEV in high-risk populations. Also, the study sought to determine the overall public health impact of HEV disease by comparing the morbidity and mortality of HEV to other common diseases and conditions in displaced populations. The information from the present study will enhance prioritization of HEV prevention and control activities as part of the overall humanitarian

response in displaced populations. In the same way, the finding from the present study will inform effective timing and targeting of HEV prevention and control interventions in displaced populations.

Chapter 3 presents the research design used to explore the literature gaps identified in chapter 2. The methodology that includes the definition of the target and study populations; and description of the study sample and sampling procedures then follows. This chapter also includes a description of the study dataset, the instruments, and variables. The chapter ends with a description of the data collection methods and data analysis plan followed by a description of threats to validity, ethical procedures, and a summary of the chapter.

Chapter 3: Research Methods

Introduction

The purpose of this quantitative nested retrospective cohort study was to determine the timing, periodicity, duration, magnitude, and distribution of HEV in displaced populations. The study also sought to determine the overall impact of symptomatic HEV disease and the factors associated with severe symptomatic HEV disease in displaced populations of South Sudan. The study used data from the South Sudan Ministry of Health IDSR and EWARS datasets for the period 2012 to early 2017. This chapter describes how the study was organized to answer the research questions. The major sections of this chapter include the study design and rationale, methodology, threats to validity, ethical procedures, and a summary of the chapter. The research design and rationale section includes the study design, the independent, and dependent variables. The methodology section presents information on the study population, sampling methods and procedures, archival data, instrumentation and materials, the operationalization of variables, and data analysis plan. The section on threats to validity describes the major threats to internal, external, construct or statistical validity and the corresponding remedial measures in the present study. The ethical procedures section describes the ethical issues addressed during research planning, data collection and analysis; and presentation of recommendations for the current study. The section also presents the procedures for securing ethical clearance for the study. The chapter ends with a summary of the study design and methodology.

Research Design and Rationale

The dependent variable in this study was the occurrence of severe symptomatic HEV disease. WHO recommends that all HEV cases at high-risk of severe symptomatic HEV disease or those clinical and/or laboratory evidence of severe HEV disease be admitted and managed as inpatients for close monitoring and early initiation of supportive care (WHO, 2014). (WHO, 2014). In the present study, all HEV cases admitted to designated HEV treatment centers were considered to be at-risk or to have severe symptomatic HEV disease. Consequently, severe symptomatic HEV disease included any HEV case admitted for inpatient supportive care in a designated HEV treatment center.

The independent variables included demographic, clinical, and epidemiological characteristics. The demographic variables in the study included: age, gender, displacement status as either IDP, refugee, or host community, and residence by camp, village, and county. Clinical variables included the date consultation at the health facility, presenting clinical symptoms and signs, the presence of co-morbidities and their identities, pregnancy in females, and outcome information as alive or died. The epidemiological variables included: the date (season) of onset of illness, travel to other displaced populations camp, and contact with other jaundiced persons.

The present study was a quantitative, observational, retrospective cohort nested within a descriptive case series. The rationale for using a retrospective cohort study nested within a case series derives from the fact that the Ministry of Health has maintained a database of cases with symptomatic HEV disease since 2012. The present

study used data from this database to execute a descriptive case series that characterized symptomatic HEV disease in terms of timing, periodicity, outbreak duration, magnitude, overall impact. Since the same database identified a proportion of symptomatic HEV cases that developed severe symptomatic HEV disease a retrospective cohort study was undertaken to determine the demographic, clinical, and epidemiological determinants associated with severe symptomatic HEV disease.

The retrospective cohort design allows the association between a risk factor and a disease to be determined and is useful for studying rare events like severe symptomatic HEV disease (Centers for Disease Control, 2012; Gordis, 2008). The advantage of using a retrospective cohort nested within a descriptive case-series derives from the fact that all the exposure and outcome data has been previously captured into the database and will, therefore, save time and resources that would be required to follow up and collect outcome data on a cohort of cases exposed to HEV. Most of the studies that assessed the risk of severe HEV disease within the context of ongoing outbreaks used descriptive case-series (Browne et al., 2015; E. H. Teshale, Howard, et al., 2010a; Thomson et al., 2013). Cross-sectional study designs have mostly been used to identify risk factors for HEV exposure (Jacobs et al., 2014; Jia et al., 2014; Mor et al., 2015; Taha et al., 2015). In the present study, we used a retrospective cohort study design that is robust and the resulting findings can be used to contribute to at least two of the criteria, the strength of association and temporality, for establishing causality (Lucas & McMichael, 2005; Gordis, 2008; Centers for Disease Control, 2012).

Methodology

The study methodology presents the study population, the sampling strategy and procedures, archival data, instrumentation and materials, operationalization of variables, and the data analysis plan.

Population

The generalization of findings from sample surveys requires a clear understanding of the target and study population. The target population includes individuals or units to whom or which study findings are inferred (Miquel, 2014). On the other hand, the study population is the entire collection of individuals from which a sample is drawn and to which a researcher can extrapolate and generalize the study findings (Miquel, 2014). In the present study, the target population included all patients in displaced populations of South Sudan that had HEV disease during the period 2012 to 2017. However, since HEV disease is largely an asymptomatic or mildly symptomatic illness, as seen in previous HEV outbreaks, it is expected that many of the asymptomatic and mildly symptomatic HEV cases did not seek care at the designated treatment centers in the outbreak areas. Thus, if the Ministry of Health IDSR acute jaundice syndrome database includes cases seen at the designated treatment centers, then it captured more of the cases with symptomatic HEV disease warranting evaluation than asymptomatic or mildly symptomatic HEV cases. Therefore, the study population for the present study will include cases with symptomatic HEV disease that presented at designated treatment centers in displaced populations of South Sudan during the period 2012 to 2017.

Sampling and Sampling Procedures

The descriptive case-series study included all confirmed and suspect symptomatic HEV cases linked to confirmed outbreaks. These cases were identified at designated treatment centers in displaced populations of South Sudan from 2012 to early 2017. The routine Ministry of Health IDSR surveillance system defines priority diseases and conditions including HEV. A suspect case of HEV is a person having jaundice (yellow discoloration of the sclera) with or without any of the following symptoms, malaise, anorexia, abdominal pains, joint pains, fever or history of fever, and headache (WHO, 2014). A confirmed HEV case is defined as a case in whom definite laboratory evidence of current (IgM and/or PCR positive) or recent and/or past (IgG positive) infection is present, whether or not clinical signs or symptoms are present (WHO, 2014). The sampling frame for the present study, therefore, included all confirmed and suspect symptomatic HEV cases linked to confirmed outbreaks that presented at designated treatment centers in displaced populations of South Sudan. These cases were eventually entered into the Ministry of Health IDSR acute jaundice syndrome database. The Ministry of Health Early Warning Alert and Response Network (EWARS) dataset was used to demonstrate the impact of HEV in displaced populations. To achieve this, morbidity and mortality due to HEV were determined and compared to other causes of morbidity and mortality in mortality in displaced populations of South Sudan.

The analysis of data for the present study excluded all acute jaundice syndrome cases determined to have acute viral hepatitis due to the other viral hepatitises besides HEV and who had no laboratory evidence of HEV infection. Acute jaundice syndrome is

a syndromic diagnosis that is used in surveillance to detect several distinct infections though clinically indistinguishable and characterized by yellowing of the skin and mucous membranes (jaundice). Jaundice results from acute hepatitis and usually follows viral, parasitic, or bacterial infections like hepatitis A, B, C, and E, yellow fever, dengue virus, and leptospirosis (WHO, 2014). The present study excluded cases from the source database that tested positive for any of the other causes of acute jaundice syndrome besides HEV and had no laboratory evidence of HEV infection.

The minimum sample size for the retrospective cohort was calculated using OpenEpi, an online open source software for epidemiologic statistics (A.G. Dean et al., 2015). The inputs into the software included (a) a two sided confidence interval of 95%, (b) study power of 80%, (c) ratio of exposed to unexposed as 1:1, (d) proportion of outcome (mortality) from HEV disease as 4% (WHO, 2015b) and, (e) the least extreme relative risk or hazard ratio to be detected as 2. The minimum sample size for the retrospective cohort, as calculated by the software, was 1,372 symptomatic HEV cases (Table 1). In the same way, the minimum sample size for the descriptive case-series was calculated using OpenEpi (A.G. Dean et al., 2015). The inputs into the software were (a) the populations size (for finite population correction factor) (N) and for this we used the software value of 1,000,000 that is used for large populations (displaced populations in South Sudan are over 2 million), (b) the hypothesized prevalence of HEV in displaced population and for this we used 50% that maximizes the sample size; (c) confidence limits of 5% and, (d) a design effect of 1 since this was not a cluster survey. The minimum sample size for the descriptive case series as calculated by the software was

384 HEV cases for a confidence level of 95% and 1,512 for a confidence level of 99.99%

(Table 2).

Table 1

Sample Size Calculation for The Retrospective Cohort Study

Sample Size: X-Sectional, Cohort, & Randomized Clinical Trials			
Two-sided significance level(1-alpha):	95		
Power(1-beta, % chance of detecting):	80		
Ratio of sample size, Unexposed/Exposed:	1		
Percent of Unexposed with Outcome:	4		
Percent of Exposed with Outcome:	7.7		
Odds Ratio:	2		
Risk/Prevalence Ratio:	1.9		
Risk/Prevalence difference:	3.7		
	Kelsey	Fleiss	Fleiss with CC
Sample Size - Exposed	634	633	686
Sample Size-Nonexposed	634	633	686
Total sample size:	1268	1266	1372
References			
Kelsey et al., Methods in Observational Epidemiology 2nd Edition, Table 12-15			
Fleiss, Statistical Methods for Rates and Proportions, formulas 3.18 &3.19			
CC = continuity correction			
Results are rounded up to the nearest integer.			
Print from the browser menu or select, copy, and paste to other programs.			
 Results from OpenEpi, Version 3, open source calculator--SSCohort			
Print from the browser with ctrl-P			
or select text to copy and paste to other programs.			

Table 2

Sample Size Calculation for The Descriptive Case-Series Study

Start	Enter	Results	Examples	Help
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Sample Size for Frequency in a Population

Population size(for finite population correction factor or fpc)(N): 1000000
Hypothesized % frequency of outcome factor in the population (p): 50%+/-5
Confidence limits as % of 100(absolute +/- %)(d): 5%
Design effect (for cluster surveys-DEFF): 1

Sample Size(n) for Various Confidence Levels

ConfidenceLevel(%)	Sample Size
95%	384
80%	165
90%	271
97%	471
99%	664
99.9%	1082
99.99%	1512

Equation

Sample size $n = [DEFF * Np(1-p)] / [(d^2 / Z^2_{1-\alpha/2} * (N-1) + p * (1-p)]$

Results from OpenEpi, Version 3, open source calculator--SSPropor
Print from the browser with ctrl-P
or select text to copy and paste to other programs.

Archival data

South Sudan has been implementing the integrated disease surveillance and response (IDSR) strategy since 2006. The strategy was developed by the WHO Regional Office for Africa with the overall aim of improving capacities for disease surveillance and response in the African region. It entails integrating all activities for detection, laboratory

testing, reporting, outbreak investigation, and response to public health emergencies of national and international concern (WHO, 2010a). As part of the strategy, priority diseases have been identified by countries based on the local, regional, and global epidemiological disease risks and the requirements under the international health regulations of 2005 (IHR (2005) (WHO, 2008, 2010a). These priority diseases are prioritized for surveillance with guidelines and reporting tools being developed to facilitate case detection, case reporting, case investigation, and outbreak response. All epidemic prone diseases like acute jaundice syndrome are immediately notifiable and are reported using a standardized line list with a defined set of variables. Rapid response teams at national and sub-national levels are mandated to investigate and respond to emerging suspect outbreaks. When investigations confirm HEV as the cause of acute jaundice syndrome in an area, all subsequent acute jaundice syndrome cases are classified as probable HEV cases if they are linked epidemiologically to the confirmed cases. Regular sample testing continues during the progression of the outbreak to monitor the etiology and to determine if the outbreak transmission has ceased. All confirmed and suspect HEV cases linked to confirmed outbreaks that present at designated treatment centers are line listed. Line listing entails initially entering these cases into paper-based case line lists to capture demographic, clinical, and epidemiological variables. The health facilities then transmit the paper-based line lists to the County Health Department, where they are entered into a Microsoft Excel acute jaundice syndrome database. The counties then send the excel line lists to the state and national level Ministry of Health by email. Data cleaning occurs at the county, state, and national level Ministry of Health. This case-

based data is regularly analyzed and used to inform outbreak response decisions. The national Ministry of Health maintains a database for all the disease outbreak line lists submitted from states with ongoing outbreaks.

The Early Warning Alert and Response Network (EWARS) was set up to support the disease surveillance and outbreak response functions within conflict-affected areas. The 2013 South Sudan crisis incapacitated the health infrastructure in these areas (WHO, 2009). Reporting through EWARS is managed through a network of 55 partner-supported sentinel sites (WHO, 2009). These sites support the early warning functions for outbreak detection, investigation, and outbreak response as well as mortality surveillance (WHO, 2009). The ensuing data inform humanitarian response efforts that are intended to keep both the crude and under five mortality rates below the emergency thresholds (WHO, 2009). EWARS uses a web-based, and mobile device enabled software for accurate data capturing, collation, analysis, and transfer to the county, state, and national level in real-time (WHO, 2015a). The EWARS web-based reporting application is simple to use, flexible to adapt, is easily deployable and has several modules for reporting of aggregate weekly priority diseases data, alert reporting, verification, and risk assessment, outbreak case-based line listing, mortality, and nutritional surveillance (WHO, 2015a). The EWARS electronic reporting platform was introduced in September 2015 to support reporting of EWARS data from the conflict affected areas in South Sudan, and by June 2017, arrangements were underway to roll it out to support surveillance and response functions countrywide (Wamala, Haskew, & Ebrahim, 2017). EWARS is currently used for case-based reporting of cholera cases but has not been used to report HEV case-based

data from outbreak sites. The present study used the National Ministry of Health IDSR and EWARS datasets for the period 2012 to early 2017. A formal request to secure and use the datasets for academic purposes was written and addressed to the Director General Planning, budgeting, monitoring, evaluation, and research; the Officer mandated to authorize the regulated release of the data for public health and academic purposes (Appendix D).

Precipitation data for South Sudan.

South Sudan has five meteorological stations, Malakal, Wau, Juba, Raja, and El Renk that provide observational precipitation data for the respective sites. However, the displaced population sites for the present study were located in Awerial, Maban, Rubkona, Pariang, Nyirol, and Mayom. These sites had no observed historical precipitation data since they lack meteorological stations. However, using Global Positioning System (GPS) coordinates (longitude and latitude) for each of the respective displaced population sites and with the support of the Climate Hazard Group Infrared Precipitation with Station (CHIRPS) satellite rainfall estimates (as precipitation per month) for the study period (2012-2017) were obtained.

Instrumentation and Materials

The present study used data from the Ministry of Health IDSR and EWARS datasets. Designated HEV treatment centers and EWARS sentinel sites capture the integrated disease surveillance and EWARS case-based data using standardized Ministry of Health paper-based and/or electronic reporting tools. The WHO Regional Office for Africa designs reporting templates for adaptation by countries to suit local contexts.

Standardized reporting templates adapted by the South Sudan IDSR program are therefore used to obtain the Ministry of Health IDSR and EWARS datasets. To ensure reliability and accuracy of entries at health facility level, the Ministry of Health conducts regular training on integrated disease surveillance and response. IDSR tool use is a module in these trainings. Also, during outbreaks, national and state rapid response teams provide on the job orientations to health facility staff on filling out the tools. All filled reporting tools are submitted to the county health department and eventually to the state and national level Ministry of Health where the data managers check for accuracy, consistency, and completeness of the entries into the database. There are no published studies on the reliability and validity of the data derived from IDSR and EWARS reporting templates for aggregate and case-based data during outbreaks in the African region. However, there is reasonable evidence to suggest that the data is reliable since it is collected using standardized and simplified instruments and there are efforts to train health workers as part of outbreak preparedness and response on the proper use of the tools. Also, data cleaning takes place at the different levels of the reporting hierarchy to ensure that the data reported is complete, consistent, and accurate. Furthermore, efforts were undertaken by the Ministry of Health with support from WHO to roll out electronic EWARS reporting. EWARS electronic reporting limits errors at data entry by using reporting templates that use pre-coded entries, dropdown lists, and minimal free text data entries. Since data is only entered once at the point of data generation, this eliminates multiple levels of data entry thus minimizing the errors.

The data cleaning entailed consistency checks including frequency checks on all the variables in the de-identified data obtained from the Ministry of Health. The aim was to identify and rectify remnants of typos, errors, and missing information. These procedures were undertaken to ensure internal consistency and accuracy of the data for the present study. Further checks were conducted to ensure there were no remnant case identifiers. Password-protected computers were used to secure all data for the current study. The long-term plan entailed preserving this data for at least five years. The Ministry of Health received the research findings and a copy of the dataset. The researcher retained a copy of the same dataset for at least five years after which, the files will be erased from the computer hard drive.

Study Variables

Although studies have suggested several factors for severe symptomatic HEV disease, the variables presented in this section are limited to those relevant to answering the research questions, especially the variables available in the Ministry of Health IDSR and EWARS datasets.

Dependent variable.

The dependent variable in this study was the occurrence of severe symptomatic HEV disease. The WHO recommends that all HEV cases at high-risk of severe symptomatic HEV disease or those clinical and/or laboratory evidence of severe HEV disease be admitted and managed as inpatients for close monitoring and early initiation of supportive care (WHO, 2014). In the present study, all HEV cases admitted to designated HEV treatment centers were considered to be at-risk or to have severe symptomatic HEV

disease. Consequently, severe symptomatic HEV disease entailed any HEV case admitted for inpatient supportive care in a designated HEV treatment center. Severe HEV disease was a dichotomous variable that took on any of two values 1 for Yes/presence of severe HEV disease and 2 for No/absence severe HEV disease.

Independent variables. These were categorized into demographic, clinical, and epidemiological variables.

Demographic variables. These included age, gender, displacement status, and residence.

Age. This refers to the age of the case at the time of illness onset measured in years. The age was measured both as a continuous and categorical variable with the following categories (a) 0-4 years (Cat 1), (b) 5-11 years (Cat 2), (c) 12-17 years (Cat 3), (d) 18-59 years (Cat 4), and (e) 60+years (Cat 15). These are the age categories used in displaced population datasets (International Organization for Migration (IOM) South Sudan, 2017; UNHCR, 2017). These age groups were used in the analysis to derive age-specific cumulative incidence rates and for computing the age-specific risk of symptomatic HEV disease with 0-4 years as the reference category.

Gender. This refers to the physiological and biological characteristics of the case, measured as a dichotomous variable with values Cat 1 = male and Cat 2 = female.

Displacement status. This refers to the type of settlement the case was residing at the time of becoming sick. Displacement status was measured as a categorical variable with values Cat 1 – refugee; Cat 2 – internally displaced population (IDP); and Cat 3 – Host community.

Residence. This was the patient's place of abode at the time they became ill and as captured in the database at the time the case sought care at the designated treatment center. The residence was captured as a nominal variable using the name of the camp, village, payam (a South Sudanese fourth level administrative division), and county.

Clinical variables. These included the date of consultation, presenting clinical symptoms that are also part of the case definition for HEV disease, the presence of any comorbidity; for females if they were pregnant or postpartum; and the final clinical outcome.

Date of consultation. This is the date the patient first presented to the designated treatment center with an illness whose clinical signs and symptoms were consistent with HEV disease. This was measured as a continuous variable.

Presenting clinical symptoms. This was defined as the manifestation of clinical signs and symptoms consistent with the case definition for HEV disease and these included: jaundice of acute onset; fever; dark urine; anorexia; malaise; fatigue; abdominal pains; mental status; and any form of unexplained bleeding. These signs and symptoms were measured as categorical variables with values Cat 1 – Present; Cat 2 – not present.

Altered mental status is a common clinical presentation in HEV patients with acute fulminating hepatitis. Mental status was coded as follows: 'normal' for a score of 15 on the Glasgow coma scale; or 'altered' for a score of 3-14 on the Glasgow coma scale (Teasdale & Jennett, 1974; Anonymous, 2011). The Glasgow coma scale rates the level of consciousness on three criteria, (a) eye opening (spontaneous – 4; to verbal command – 3; to pain – 2; none – 1), (b) best motor response (obeys verbal command – 6; localizes

painful stimuli – 5; flexion withdrawal from painful stimuli – 4; decorticate response to painful stimuli – 3; decerebrate response to painful stimuli – 2; and none – 1), and (c) best verbal response (oriented conversation – 5; disoriented conversation – 4; inappropriate words – 3; incomprehensible words – 3; incomprehensible sounds – 2; none – 1) (Anonymous, 2011; Teasdale & Jennett, 1974).

The presence of any comorbidity. This refers to the simultaneous occurrence of one or more diseases with a primary disease (HEV). This was measured as a categorical variable with values Cat 1 – for the presence of any comorbidity; and Cat 2 – for no detectable comorbidity.

Pregnancy in females. This was defined as physiological or clinical evidence of pregnancy in female diagnosed with HEV disease. For purposes of the current study, this category included females who were pregnant or within 42 days after delivery at the time the HEV illness started. This was measured as a categorical variable with values Cat 1 – Pregnant; and Cat 2 – not pregnant.

Final clinical outcome. This was determined as the clinical outcome of the patient after being treated on supportive treatment by the healthcare workers and was measured as a categorical variable with values Cat 1 – for those who died; and Cat 2 – for those who recovered from the disease.

Epidemiological variables. These included the date the disease symptoms started, history of travel to any other displaced persons' camp, and contact with another jaundiced patient.

Date of onset of illness. This is the date the HEV disease symptoms started based on the patient's recollection. This was measured as a calendar date, a continuous variable. The date of onset was also transformed into the epidemiological week of onset as derived from the epidemiological calendars for the epidemiological years of the study. It is also known that the rainy season in South Sudan starts in May and ends in October (Anonymous, 2017b). Therefore, the date of onset was transformed into a variable with Category 1 corresponding to the wet season for dates of onset from May to October; while Category 2 corresponds to the dry season for all the dates of onset from November to April.

Travel. This was defined as travel to other displaced population camps especially those with acute jaundice syndrome cases. Travel that occurred within six weeks before the onset of HEV disease symptoms was considered. This was measured as a categorical variable with values Cat 1 – history of travel to a displaced persons' camp within six weeks before the onset of illness, and Cat 2 – no travel history to a displaced persons' camp in the six weeks before the HEV disease symptoms started.

History of contact. This was defined as contact with other jaundiced person or persons in the six weeks before the HEV disease symptoms started. This was measured as a categorical variable with values Cat 1 – positive history of exposure/contact with another jaundiced person in the six weeks before the onset of HEV disease symptoms, and Cat 2 – no history of contact with another jaundiced person in the six weeks before the onset of HEV disease symptoms.

Laboratory tests for HEV. Since acute jaundice syndrome is a syndromic case definition that includes many other etiologies besides HEV, blood samples were obtained from suspect HEV cases for laboratory testing to identify and characterize the etiological agent. Several laboratories supported the testing of acute jaundice syndrome samples. These laboratories included the Centers for Disease Control (CDC)-Kenya Medical Research Institute (KEMRI) laboratory in Nairobi, Kenya; the Uganda Virus Research Institute (UVRI); CDC-Atlanta; and MRC-University of Glasgow Center for Virus Research, Scotland (UK). The UVRI and KEMRI laboratories conducted a range of tests that included reverse transcription–polymerase chain reaction (rt-PCR) for HEV, Yellow fever, and other viral hemorrhagic fevers. The MRC-University of Glasgow Center for Virus Research performed the genetic sequencing and genotyping of HEV.

Additional variables for the descriptive analyses. Further descriptive analyses were conducted to elucidate the impact of HEV disease in displaced populations. The variables are included in the EWARS dataset for aggregate weekly reporting. The variables from this dataset included ‘the total cases by priority disease’, ‘the number of patient consultations by site’, ‘the age-group categorized as under-fives and five years and above’, ‘the reporting health facility’, and ‘the epidemiological week of reporting.’ The diseases included as part of the weekly EWARS health facility reporting include cholera, acute watery diarrhoea, acute bloody diarrhoea, measles, meningitis, viral hemorrhagic fever, yellow fever, relapsing fever, acute jaundice syndrome, respiratory tract infection, malaria, neonatal tetanus, Guinea worm, and acute flaccid paralysis. The integrated disease surveillance and response strategy as recommended by the WHO

regional office for Africa standardizes case definitions for each of these priority diseases to ensure a uniform criterion for case detection and reporting (WHO, 2010a). Health facilities undertake mortality surveillance as part of EWARS within the displaced populations of South Sudan. The designated EWARS sites report case-based mortality data on a weekly basis. Reporting of deaths is part of the prospective mortality surveillance that uses standard reporting templates. The EWARS case-based mortality reporting template includes the following variables:

Origin. Refers to the name of the block and camp where the deceased resided at the time when they passed away. The origin was captured as a nominal variable using the name of the camp and block of residence

Date of death. Refers to the date the deceased was pronounced dead by the facility or community health worker in the camp. This was measured as a continuous variable.

Age in years. Refers to the age in years at the time when the deceased died and was measured both as a continuous variable and as a categorical variable with the following categories: Category (Cat) 1 - <5 years; and Cat 2 – ≥5 years.

Gender. Refers to the physiological and biological characteristics of the case and was measured as a categorical variable with values Cat 1 – male and Cat 2 – female

The immediate cause of death. Refers to all those diseases, morbid conditions, or injuries that either resulted in or contributed to death and the circumstances of the accident or violence which produced any such injuries (Miquel, 2014). The immediate

cause of death was captured as the nominal variable with the name of the disease as listed in the priority disease list.

The underlying cause of death. Refers to the disease or injury that initiated the train of events leading to death or the circumstances of the accident or violence that produced the fatal injury (Miquel, 2014). The underlying cause of death was captured as the nominal variable with the name of the disease as listed in the priority disease list. I deleted the other variables on the EWARS mortality case-based reporting template that are not relevant to the present study.

Precipitation data. Satellite precipitation data for Awerial, Maban, Rubkona, Pariang, Nyirol, and Mayom) was obtained with support from CHIRPS for each of the months of period 2012 to early 2017. The amount of rainfall was estimated in millimeters per month for each of the respective sites. Rainfall was therefore measured as a continuous variable with the units being precipitation in millimeters per month per site.

Data collection method

The researcher designed a data abstraction tool to obtain data from the Ministry of Health IDSR and EWARS datasets. The abstraction tool included all the variables relevant to the present study (see appendix A and B).

Data Analysis plan

The current study conducted descriptive, bivariate, and multivariate analyses using Microsoft Excel version 15.27 and IBM SPSS Statistics version 24. After securing the data, the researcher cleaned it to remove errors and inconsistent entries. The data cleaning process entailed running frequencies on variables of interest to identify errors

and inconsistencies. After data clean-up, the researcher applied variable codes to the variables of interest using the ‘recode’ function in SPSS. Missing values were identified and coded as missing. The researcher deleted all variables that were not relevant to the present study. Some of the existing variables were manipulated during analysis to create new variables to improve data presentation and analysis.

Research questions and hypotheses. The research questions and hypotheses of the present study are as follows:

Question 1: What is the timing, periodicity, magnitude (morbidity and mortality), laboratory characterization, and duration of HEV outbreaks in South Sudan and how are the cases distributed by person, time, and place?

H_0 1: The timing, periodicity, duration, magnitude, and laboratory characterization of symptomatic HEV disease do not differ by place, person, and time.

H_1 2: The timing, periodicity, duration, magnitude, and laboratory characterization of symptomatic HEV disease differs by place, person, and time.

Question 2: What is the overall impact of symptomatic HEV disease in relation to the other causes of morbidity and mortality in displaced populations?

H_0 2: Symptomatic HEV disease is not a significant cause of morbidity and mortality in relation to the other diseases and conditions in displaced populations.

H_1 2: Symptomatic HEV disease is a significant cause of morbidity and mortality in relation to the other diseases and conditions in displaced populations.

Question 3: Is there a significant association between demographic, clinical, and epidemiological characteristics and the occurrence of severe symptomatic HEV disease?

H_0 3: There is no significant association between demographic, clinical, and epidemiologic characteristics and the occurrence of severe symptomatic HEV disease.

H_1 3: There is a significant association between demographic, clinical, and epidemiologic characteristics and the occurrence of severe symptomatic HEV disease.

The researcher conducted separate analyses for each of the research questions. The analyses performed were therefore categorized as descriptive for research questions number one and two, and bivariate and multivariate analyses were employed for research question number three.

Descriptive analysis. Descriptive analysis was undertaken to organize and summarize the data for the present study. The descriptive analysis started with the total number of cases and deaths due to HEV including the case fatality rate (CFR). The CFR was derived as the proportion of total HEV cases that died during the study period, expressed as a percentage. The duration of each HEV outbreak was determined from the time difference in days or weeks between the date of onset of the initial case to the date that corresponds to twice the maximum incubation period after the last outbreak case died or was discharged from a designated treatment center. The data was summarized further by determining measures of central tendency and dispersion for continuous variables like age, date of consultation, and date of illness onset. The measures of central tendency included the mode, median, and arithmetic mean. The mode is the most frequent

observation in a dataset while the median is the observation located in the middle of the observations in the dataset (Centers for Disease Control, 2012). For datasets where the total observations have an even number, the median is calculated using the formula $N/2$, where N is the total number of observations in the dataset. When the total observations in the dataset have an odd number, then the median is calculated using the formula $(N+1)/2$. The arithmetic mean is calculated using equation 1.

Equation 1: $\bar{x} = \sum_i^n Xi / n$,

where $\sum_i^n Xi$ is the sum of the observations, and n is the total population (Centers for Disease Control, 2012).

The interquartile range represents the central distribution of a dataset and is calculated as the difference between the third quartile and the first quartile. The interquartile range includes 50% of the observations in the dataset leaving one-quarter on either side of the distribution (Centers for Disease Control, 2012). To determine the interquartile range, the observations in the dataset were arranged in increasing order to facilitate the delineation of positions for the first (Q_1) and third (Q_3) quartile in the dataset using Equations 1.1 and 1.2. Once the values of the 1st and 3rd quartiles were determined from Equations 1.1 and 1.2, then the interquartile range was determined by subtracting the value of the first quartile (Q_1) from the value of the third quartile (Q_3) (Centers for Disease Control, 2012).

Equation 1.1: $Q_1 = \frac{(n+1)}{4}$ and Equation 1.2: $Q_3 = \frac{3(n+1)}{4}$

The standard deviation is one of the measures of dispersion and measures the dispersion from the arithmetic mean. The formula for the standard deviation is shown in equation 2.

Equation 2: $S = \sqrt{\sum_i^n (X_i - \bar{x})^2 / n}$, where X_i is the value of the observation i and \bar{x} is the mean (Centers for Disease Control, 2012).

For the categorical variables, frequency distribution analysis was performed using equation 3

Equation 3: $\frac{X_i}{n} \times 100$, where x is the number of observations in category i and n is the population under study (Centers for Disease Control, 2012).

To document HEV transmission hotspots, seasonal peaks, and duration of outbreaks, the cumulative incidence of symptomatic HEV disease and mortality were computed by place and time. The cumulative incidence was defined as the number of new cases occurring during a given period divided by the total population at risk at the beginning of the specified period (Centers for Disease Control, 2012). Equation 4 was used.

Equation 4:

$$\text{Cumulative incidence} = \frac{\text{new cases during a given period}}{\text{population at risk at the beginning of the same period}} \times 10^n.$$

Therefore, for the present study, the cumulative incidence was computed by dividing the number of symptomatic HEV cases in a selected group by the population at risk at the beginning of a specified period. The displaced populations demographic statistics for the affected refugee and internally displaced population camps were obtained from the

respective data portals or the United Nations High Commission for refugees (UNHCR) and International Organization for Migration websites for South Sudan (International Organization for Migration (IOM) South Sudan, 2017; UNHCR, 2017).

Correlation and simple linear regression analyses were undertaken to assess the association between HEV cases and the precipitation (millimeters per month) for each of the displaced population sites. The respective measures of association were Pearson correlation (r) and R square. Scatter plots were presented to illustrate the relationship between precipitation and HEV cases in each of the displaced population camps.

To identify outbreak clusters of space-time clustering and to determine if they were statistically significant, we used the well-validated retrospective space-time permutation scan statistic included in the SatScan software package, a platform developed specifically for outbreak detection (Kulldorff et al., 2005). For every geographical location under surveillance, the statistic can detect one day or multi-day outbreaks including both the ability to detect both rapidly rising and slowly emerging outbreaks (Kulldorff et al., 2005). Across the entire dataset, the statistic compares expected versus observed case counts inside and outside mobile scanning windows to detect events that are least likely to have occurred by chance. Expected case counts are determined using data aggregated across the entire data set, both before and after the detected event and do not require underlying population estimates (Kulldorff et al., 2005). However, to adjust for the underlying populations in each of the displaced population locations, I also performed follow up analysis using Poisson models to identify clusters of spatial or space-time clustering significance (Kulldorf, 2016). To

document the overall impact of HEV disease in relation to other causes of mortality in displaced populations, the HEV cause-specific mortality rate was computed to determine the relative importance of HEV as a major cause of mortality in displaced populations. To achieve this, the proportional mortality for the top five causes of mortality in displaced populations was computed from the displaced persons' mortality database. The proportional mortality was calculated as the number of deaths from a specific cause during a given time interval divided by the total number of deaths from all causes in the same interval (Centers for Disease Control, 2012). Equation 5 was used

Equation 5: *Proportional mortality* =

$$\frac{\text{number of deaths due to a specific cause during a given time interval}}{\text{the total number of deaths from all causes during the same interval}} \times 10^n.$$

Bivariate analysis. As the first step for identifying factors for severe symptomatic HEV disease, the chi-square test for independence (χ^2) and the odds ratio was used.

Chi-square test for independence. This is a non-parametric test that is used to assess the relationship between two categorical variables. The chi-square test for independence was computed using equation 5.

Equation 6: $X^2 = \sum \frac{(F_o - F_e)^2}{F_e}$, where F_o stands for the observed frequencies and F_e the expected frequencies (Centers for Disease Control, 2012).

Fishers exact test was used when the sample size was small giving values of five or less in one of the two by two table cells (Centers for Disease Control, 2012). The use of the chi-square test for independence is bound by several assumptions including the

requirement that data is categorical, a large sample size, and independence of measures with categories created being mutually exclusive with no overlaps and have a theoretical basis for the study (Centers for Disease Control, 2012).

Odds ratio. The odds ratio is a measure of association in analytical studies that is used to quantify the association between an outcome and exposure. The odds ratio is defined as the ratio of the odds of exposure in the cases to the odds of exposure in the controls (Centers for Disease Control, 2012). The odds ratio was calculated using equation 7.

Equation 7: *Odds ratio* = $\frac{ad}{bc}$, where

a = number of persons with disease and with exposure of interest

b = number of persons without disease, but with exposure of interest

c = number of persons with disease, and without exposure of interest

d = number of persons without disease and lacking the exposure of interest

a+c = total number of persons with disease (cases)

b+d = total number of persons without disease (controls)

Equation 8 shows how the confidence level is calculated

Equation 8: $(e^{c_1} | e^{c_2})$, where

$$C_1 = \ln(\widehat{OR}) - Z_{1-\alpha/2} \sqrt{\frac{1}{a} + \frac{1}{b} + \frac{1}{c} + \frac{1}{d}} \text{ and}$$

$$C_2 = \ln(\widehat{OR}) - Z_{1-\alpha/2} \sqrt{\frac{1}{a} + \frac{1}{b} + \frac{1}{c} + \frac{1}{d}}$$

The odds ratio was considered as statistically significant if the confidence interval did not include the value of one (the null). The significance level of $p < 0.05$ was used to identify significant variables for inclusion in the multivariate analysis.

Multivariate analysis. Stepwise logistic regression was undertaken to identify factors for severe symptomatic HEV disease. The approach is used to determine the independent effects of an independent variable on a dependent variable after adjusting for other covariates in the model. Logistic regression applies to studies with a categorical outcome variable is categorical and not normally distributed. The logit transformation is used to transform the outcome variable into a linear distribution and yields values between 0 and 1 that correspond to the odds or probability of disease. For example, the logit transformation for the likelihood of developing disease y given exposure x was written as seen in equation 9

$$\text{Equation 9: } P(y \setminus x) = \ln \left[\frac{P(y \setminus x)}{1 - P(y \setminus x)} \right] = \alpha + \beta_x,$$

The adjusted odds ratio of developing disease y given exposure x will equal to the inverse of the natural log of β (Andrew G. Dean, Sullivan, & Soe, 2010). The use of logistic regression is bound by several assumptions including the independence of errors or cases, linearity of the relationship between the independent variables and, the logit transformed outcome variable. Logistic regression does not assume a linear relationship between the dependent and independent variables. However, the relationship between continuous independent variables and their log odds should be linear (A.G. Dean et al., 2015).

Building the final model. The stepwise approach was used to build the final model as follows. All the variables that were significant at $p < 0.05$ during the bivariate analysis

were entered into the model and run. In the following phase, all the variables from the bivariate analysis that were significant at the $p=0.25$ level were identified and added to the model one at a time with only the significant ones ($p < 0.05$) being left in the final model.

Threats to Validity

Internal validity refers to the degree to which the observed changes in the dependent variable are influenced by changes in the independent variable and not any other extraneous factors (Frankfort-Nachmias & Nachmias, 2008). Internal validity of findings may be affected by confounding variables. These can be controlled through multivariate analysis to determine the independent effects of the independent variables. However, one limitation of studies using secondary data with a defined set of variables relates to the fact that multivariate logistic regression does not adjust for other confounders that are not included in the model. In the present study, therefore, confounding variables that were not included in the model could not be ruled out.

Temporal antecedence is a major criterion for causality and hence a major determinant of internal validity (Frankfort-Nachmias & Nachmias, 2008). For the present retrospective cohort study, the temporal association between severe symptomatic HEV disease and exposure could be established since the outcome developed later during the illness. Since this was a two group comparison study design, history, maturation, testing, and statistical regression were not significant threats to internal validity in the study (Frankfort-Nachmias & Nachmias, 2008). The biased selection of cases was not a threat either since this was a retrospective cohort of cases with HEV disease hence the selection

of cases into comparison groups was not necessary. Instrumentation played an insignificant role since the case definition did not change during the outbreaks and the ongoing training of clinicians on IDSR ensure that the case definition is applied uniformly. In the same way, experimental decay can only be said to have played an insignificant role since the study used secondary data.

External validity is the representativeness of the study findings in the study and target populations (Frankfort-Nachmias & Nachmias, 2008). Healthcare access bias, a type of selection bias was a major threat to external validity in the present study. Since HEV disease is largely asymptomatic and given the poor health seeking behavior by patients in Africa, many of the asymptomatic or mildly ill HEV cases can be assumed not to have made it to designated treatment centers. Consequently, many mildly symptomatic cases were therefore not included in the Ministry of Health IDSR acute jaundice syndrome database. The cases represented in the database therefore largely include HEV patients with moderate or severe HEV disease that sought care at the designated treatment centers in displaced populations of South Sudan and therefore constitute the study population. The cases at the treatment centers were, therefore, fewer when compared to the target population of all the patients with clinical evidence of HEV disease in displaced populations in South Sudan. Interaction of effect testing had no impact on external validity since there was no pre-testing. In the same way, interaction effects of selection biases and the experimental treatment will play no role since the study intends to use secondary data. The other threats to external validity like reactive effects of

experimental arrangements and multiple-treatment interference will equally have no impact on external validity since we intend to use secondary data.

There were no major threats to construct validity since the host-agent-environment model used as the basis for the current study has been as the basis for identifying determinants of symptomatic and severe disease in related infectious diseases. While I included all the relevant variable that were available in the database into the final model, the fact that there are other covariates that were not included in the model raises the issue of statistical inclusion validity in the present study.

Ethical Procedures

As part of the ethical requirements for conducting biomedical research, I submitted the research proposal to the Institutional Review Board (IRB) of Walden University. The proposal was also presented to the South Sudan Ministry of Health Research and Ethics committee to ensure it adhered to all in-country ethical requirements for biomedical research (Appendix D). Appendix D along with the dissertation proposal were submitted to the South Sudan Ministry of Health Research and Ethics Committee. The South Sudan Ministry of Health Research and Ethics Committee issued the research ethical approval letter on 21 February 2017 (Appendix E). The Walden University IRB issued the corresponding ethical approval on 7 March 2017 (Appendix F). Only aggregate statistics were presented to comply with the confidentiality requirements for handling personal biomedical data. The researcher kept all data for the current study on a secured, password-protected personal computer. The long-term plan entailed storing secured data for five years. At the time of sharing the study findings; the dataset was

shared with the Ministry of Health. The researcher will retain a copy of the same dataset for at least five years after which, he will erase the files from the computer hard drive.

The final report was published to contribute to the ongoing scientific dialogue on Hepatitis E. Also, the final report was shared with the South Sudan Ministry of Health to inform policy decisions on Hepatitis E control in displaced populations of South Sudan.

Summary

The present study used a retrospective cohort design nested within a descriptive case series study to determine the timing, periodicity, duration, magnitude, and distribution of HEV by person, place, and time. The study also assessed the overall impact of symptomatic HEV disease and identified the factors for severe symptomatic HEV disease in displaced populations of South Sudan. Severe symptomatic HEV disease was used as the dependent variable while the independent variables were categorized into demographic, clinical, and epidemiologic factors. The demographic factors included age, gender, displacement status, and residence. The clinical variables included the date of consultation, presenting clinical symptoms, the presence of any comorbidity, pregnancy in females, and final clinical outcome. The epidemiological variables included the date of illness onset, travel to other displaced persons' camps, and contact with other jaundiced persons. The minimum sample size for the retrospective cohort study was 1,372 symptomatic HEV cases and 384 symptomatic HEV cases for the descriptive case-series study.

The present study used data from the South Sudan Ministry of Health IDSR acute jaundice syndrome database and the EWARS internally displaced populations mortality

database for the period 2012 to early 2017. All statistical analyses were conducted using Microsoft Excel version 15.27 and IBM SPSS statistics version 24. The data analysis included both descriptive and analytical approaches. The descriptive analyses included measures of central tendency and dispersion, frequency distribution, cumulative incidence, and proportional mortality. Bivariate analysis was undertaken and included the chi-square test for independence and the odds ratio including the confidence interval. Stepwise logistic regression was conducted as part of the multivariate analysis to identify factors for severe symptomatic HEV disease.

The researcher submitted the proposal for review and approval by the Institutional Review Board of Walden University (Appendix F) and the South Sudan Ministry of Health Ethics Review Board (Appendix E). Also, the researcher sought for permission from the South Sudan Ministry of Health to access and use the acute jaundice syndrome and the EWARS internally displaced persons' mortality database (Appendix D).

The next chapter presents the data collection process and study findings. The study findings section includes a description of the study participants followed by the presentation of results for each of the research questions. The chapter ends with a summary of the salient research findings.

Chapter 4: Results

Introduction

The purpose of this quantitative nested retrospective cohort study was to describe the timing, periodicity (seasonality), duration, magnitude and distribution of HEV by person, time, and place. Also, the study assessed the overall impact of symptomatic HEV disease and evaluated the factors for severe symptomatic HEV diseases in displaced populations of South Sudan. The research questions and hypotheses of the study were as follows:

Question 1: What is the timing, periodicity, magnitude (morbidity and mortality), laboratory characterization, and duration of HEV outbreaks in South Sudan and how are the cases distributed by person, time, and place?

H_0 1: The timing, periodicity, duration, magnitude, and laboratory characterization of symptomatic HEV disease do not differ by place, person, and time.

H_1 2: The timing, periodicity, duration, magnitude, and laboratory characterization of symptomatic HEV disease differs by place, person, and time.

Question 2: What is the overall impact of symptomatic HEV disease in relation to the other causes of morbidity and mortality in displaced populations?

H_0 2: Symptomatic HEV disease is not a significant cause of morbidity and mortality in relation to the other diseases and conditions in displaced populations.

H_1 2: Symptomatic HEV disease is a significant cause of morbidity and mortality in

relation to the other diseases and conditions in displaced populations.

Question 3: Is there a significant association between demographic, clinical, and epidemiological characteristics and the occurrence of severe symptomatic HEV disease?

H_0 3: There is no significant association between demographic, clinical, and epidemiologic characteristics and the occurrence of severe symptomatic HEV disease.

H_1 3: There is a significant association between demographic, clinical, and epidemiologic characteristics and the occurrence of severe symptomatic HEV disease.

This chapter describes the data collection process and study results. The data collection process entailed obtaining ethical approval from the South Sudan Ministry of Health Research (Appendix E) and Ethics Committee and the Walden IRB (Appendix F) to conduct the study by obtaining and analyzing the requisite datasets. The analysis initially entailed descriptive analyses to characterize the study population. The analyses tailored to the study research questions then followed. The study findings are presented starting with the description of the study participants followed by the systematic presentation of results for each of the research questions. The chapter ends with a summary description of the main study findings.

Data Collection

Prior the data collection process, I applied to the South Sudan Ministry of Health Research and Ethics Committee and subsequently to Walden University IRB to review the research proposal for compliance with ethical standards for conducting biomedical

research. The South Sudan Ministry of Health granted ethical approval to conduct the study on 21 February 2017. The researcher then submitted the Ministry of Health Research Approval Letter along with the application to Walden University IRB. Walden University IRB approval was eventually obtained on 7 March 2017 under approval number 03-07-17-0297498. The data obtained from the Ministry of Health Emergency Preparedness and Response Department consisted of three datasets for the period 2012 to March 2017. The first dataset was HEV case-based line list epidemiological and laboratory data collected as part of routine disease outbreak notification, investigation, and response. The Ministry of Health (MoH) gathered this data from all the displaced populations and contiguous host communities where HEV cases were investigated and confirmed during the study period. The second dataset included mortality data collected from the prospective mortality surveillance system for the internally displaced populations for the period 2013 to early 2017. The third dataset included morbidity data collected as part of the early warning alert and response network (EWARN) in internally displaced populations for the period 2013 to early 2017.

HEV Case-based Epidemiological Dataset

During the period 2012 to early 2017, the MoH gathered HEV case-based data from nine displaced population sites. These sites included four refugee camps (Doro, Batil, and Jamam/Gendrassa in Upper Nile state and Yida in Unity state) and five IDP camps (Bentiu Protection of Civilian Camp [PoC], Bentiu Town, and Mayom in Unity state; Lankien in Jonglei state; and Mingkaman in Lakes state). During this period, a total of 14,404 HEV cases were reported from the nine displaced population sites as shown in

Table 3. The total number of HEV cases in the study cases fell within the minimum sample size of 384 cases for the descriptive case series study.

Table 3

Distribution of HEV Cases by Place and Gender, 2012 to 2017

Location	Male	Female	Missing	Total
Mayom	40	45		85
Batil	2,702	2,666	105	5,473
Bentiu PoC	1,878	1,368	4	3,250
Bentiu Town	61	45		106
Doro	637	556	4	1,197
	1,605	1,798	41	3,444
Jamam/Gendrassa				
Lankien	14	13		27
Mingkaman	65	76	4	145
Yida	347	330		677
Total	7,349	6,897	158	14,404

Since the MoH captured the data over a duration of five years, the researcher assessed the nine HEV case-based datasets for completeness and consistency of capturing data on the study variables. The demographic variables used in the assessment included age, gender, displacement status, and residence. The clinical variables included the consultation date, admission date, discharge date, the date of death, fever, yellow eyes, vomiting, diarrhoea, nausea/loss of appetite, headache, epigastric pain, skin itching, the presence of coinfections, mental status, and unexplained bleeding. The epidemiological variables included travel to an affected area and contact with a case. The MoH consistently captured data on these study variables for six displaced population sites, namely, Bentiu PoC, Bentiu Town, Doro, Mayom, Lankien, and Mingkaman. The total number of cases from these six displaced population sites was 4,810 cases of HEV, which

falls within the minimum study sample size of 1,372 cases for the retrospective cohort study. Due to the varying versions of the HEV case-based line listing forms, three sites including Yida, Batil, and Jamam/Gendrassa had HEV case-based data sets that were lacking in most of the clinical and epidemiological variables and were therefore excluded from the assessments to identify determinants of severe HEV disease.

To be able to address the research questions adequately, the researcher derived additional variables from the existing HEV case-based datasets. The '*delay in seeking care*' was derived from the number of days between the admission date and the self-reported disease symptom onset date. The '*admission duration*' was derived from the number of days between the discharge date or date of death and the date of admission. To assess the effects of seasonality on the risk of symptomatic and severe HEV disease, a new variable, 'seasonality' was derived. The researcher calculated 'seasonality' from the date of illness onset. The reference category for 'seasonality' was 'the dry season' for case illness onset dates that occurred between November and April, while 'the rainy season' was the second category for case illness onset dates that occurred between May and October. The researcher coded 'altered mental condition' as normal for a score of 15 on the Glasgow coma scale; or as abnormal for a score of 3-14 on the Glasgow coma scale (Teasdale & Jennett, 1974; Anonymous, 2011). During the data collection process, it also emerged that the displaced population datasets are categorized as 0-4 years, 5-11 years, 12-17 years, 18-59 years, and 60+years (International Organization for Migration (IOM) South Sudan, 2017; UNHCR, 2017). These categories were eventually used in the

analysis to derive age-specific cumulative incidence and for deriving the age-specific risk of severe HEV disease with 0-4 years as the reference group.

As part of the data cleaning to ensure consistency in responses on all the demographic, clinical, and epidemiological variables, the researcher ran frequency distributions. For all variables, the reference category was coded as '0' while the second category was coded as '1'. The other categorical variables were coded using serial numbers with '0' as the reference category and serial numbers from '1' onwards assigned to correspond to each of the additional response categories. The researcher coded unknown responses on each of the study variables as missing ('2') categories.

Internally Displaced Mortality Dataset

This dataset included all deaths reported as part of the prospective mortality surveillance system in the internally displaced persons' camps from 2013 to March 2017. The principal variables in this dataset that were relevant to the present study included the camp of residence at the time of death, the date of death, age in years categorized as under-fives and five years and above, gender, the immediate cause of death, and the underlying cause of mortality. This dataset included 4,440 deaths reported from 12 internally displaced persons' sites from December 2013 to March 2017. Table 4 shows the distribution of deaths by IDP site from December 2013 to March 2017. The data cleaning process entailed running of frequencies to assess and update variable coding to ensure consistency of responses on variables for the five years of the dataset. For purposes of analyzing the causes of death, the 'underlying causes of death' variable was

used to assess the relative impact of HEV as a cause of mortality in relation to other causes of deaths among IDPs for the period December 2013 to March 2017.

Table 4

Mortality in Internally Displaced Persons by site, December 2013 to March 2017

IDP site	2013	2014	2015	2016	2017	Total
Agok		3				3
Akobo			42	68	22	132
Bentiu	5	343	944	733	104	2129
Bor	2	123	9			134
Juba 3	2	127	217	184	35	565
Kodok		1			3	4
Malakal		262	238	150	15	665
Melut		58	39	63		160
Mingkaman	8	142	47	25		222
Tongping	11	255		3		269
Wau PoC				20	10	30
Wau Shiluk		14	61	46	2	123
Missing		4				4
Total	28	1,332	1,597	1,292	191	4,440

Internally Displaced Morbidity Dataset

This dataset included aggregated data on causes of morbidity reported as part of the early warning alert and response network (EWARN) surveillance system in the internally displaced persons' camps from 2013 to March 2017. The principal dataset variables relevant to the present study included 'total cases by disease', 'the number of patient consultations by site', 'the age-group' categorized as under-fives and five years and above, 'the reporting health facility', and 'the epidemiological week of reporting'. Table 5 shows the number of cases by priority disease and the total consultations for every year from December 2013 to March 2017. The data cleaning process entailed

running of frequencies to assess and update variable coding to ensure all responses were uniform and consistent for all variables and records entered during the five years of the dataset.

Table 5

Cases of Morbidity in Internally Displaced Persons by site, December 2013 to March 2017

Year	Malaria	Acute Respiratory Infection (ARI)	Acute Watery Diarrhoea (AWD)	Acute Bloody Diarrhoea (ABD)	Measles	Hepatitis E Virus (HEV)	Gunshot wounds (GSW)	Cholera	Total consultations
2013	3,797	-	1,061	165	-	-	-	1	8,330
2014	236,294	95,728	88,405	16,846	1,597	293	186	6,293	1,137,184
2015	486,547	265,089	141,556	20,897	981	3,476	14	358	1,550,441
2016	436,458	322,303	136,462	17,633	4,819	8,011	1,168	2,447	1,632,422
2017	35,613	67,418	23,365	2,027	208	129	120	497	251,174
Total	1,198,709	750,538	390,849	57,568	7,605	11,909	1,488	9,596	4,579,551

Precipitation Dataset

Satellite precipitation data were obtained for each of the displaced population sites, Batil, Bentiou, Doro, Gendrassa, Lankien, Mayom, Mingkaman, and Yida for the period 2012 to 2017. Table 6 shows the mean precipitation in millimeters per month for the period 2012 to 2017. For most sites, the rain started rising in April reaching a peak in July and August and declining after that (Table 6).

Findings

In this section, the results are presented starting with the descriptive statistics that characterize each of the three datasets used in the study. An evaluation of statistical assumptions and presentation of the statistical analysis findings for each of the three research questions then follow.

Table 6

Mean precipitation (mm per month) by month and camp site for 2012-2017

Camp/month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Batil	-	-	2	13	120	96	143	161	113	167	8	1
Bentiu PoC	0	0	6	18	101	151	159	194	170	80	4	0
Doro	0	0	2	19	123	111	149	162	121	163	7	1
Gendrassa	0	0	2	18	125	102	147	158	115	162	8	2
Lankien	0	0	8	45	138	154	176	170	134	80	6	1
Mayom	-	1	9	19	100	151	174	201	150	90	5	-
Mingkaman	1	3	29	99	128	122	144	121	133	125	36	4
Yida	-	-	5	13	78	135	151	183	184	77	3	-

Descriptive analyses

HEV case-based data. A total of 14,404 cases including 571 deaths (Case Fatality Rate [CFR] 3.96%) of HEV were reported from nine displaced population camps of South Sudan from 2012 to early 2017. During this period, Batil refugee camp reported the highest number of HEV cases, followed by Jamam/Gendrassa refugee camp with 5,473 cases and 3,444 cases respectively (Table 7). The number of HEV cases increased from 3,270 cases in 2012 reaching the highest peak of 7,376 cases in 2013 with the majority of the cases occurring in Batil, Jamam/Gendrassa, and Yida refugee camps. The HEV trends reached the second peak of 2,260 cases in 2015 with the majority of the cases reported from Bentiu PoC (Table 7).

Table 7

HEV Cases by Year and Site, 2012 to 2017

Site/camp	2012	2013	2014	2015	2016	2017	Total
Mayom				10	49	26	85
Batil	1911	3562					5473
Bentiu PoC			19	2189	924	118	3250
Bentiu Town				10	96		106
Doro	4	1107	70	16			1197
Jamam/Gendrassa	1276	2168					3444
Lankien				24	3		27
Mingkaman			132	13			145
Yida	79	539	59				677
Total	3270	7376	280	2262	1072	144	14404

Regarding mortality, the MoH reported 571 HEV deaths between 2012 and March 2017. Bentiu PoC recorded 276 HEV deaths, the highest recorded during the study period (Table 8). The highest number of fatalities occurred in 2015 with Bentiu PoC reporting 235 HEV deaths in the same year (Table 8).

The HEV CFR ranged from 1.5% in Yida to 34% in Mayom with a median of 4.2% (Table 8). The overall HEV case fatality rate was 4.0% in displaced populations from 2012 to 2017 (Table 8). During this period, the HEV CFR increased from 2.8% in 2012 to the highest peak of 10.6% in 2015, the year when Bentiu PoC and Mayom posted the highest HEV case fatality rates (Table 8).

Table 8

HEV Deaths and Case Fatality Rates (%) by Year and Site, 2012 to 2017

Site/camp	2012 n (CFR)	2013 n (CFR)	2014 n (CFR)	2015 n (CFR)	2016 n (CFR)	2017 n (CFR)	Total n (CFR)
Mayom				2 (20.0)	22 (44.9)	5 (19.2)	29 (34.1)
Batil	62 (3.2)	80 (2.2)					142 (2.6)
Bentiu PoC			9 (47.4)	235 (10.1)	32 (3.5)		276 (8.5)
Bentiu Town					17 (17.7)		17 (16.0)
Doro	1 (25.0)	26 (2.3)	1 (1.4)				28 (2.3)
Jamam/Gendrassa	24 (1.9)	29 (1.3)					53 (1.5)
Lankien				2 (8.3)			2 (7.4)
Mingkaman			5 (3.8)	1 (8.3)			6 (4.2)
Yida	5 (6.3)	13 (2.4)					18 (2.7)
Total n (CFR)	92 (2.8)	148 (2.0)	15 (5.4)	240 (10.6)	71 (6.6)	5 (3.5)	571 (3.96)

n=number of deaths; CFR measured as percentage of cases that died

With regards to HEV case distribution by gender, males constituted 51% of the total HEV cases reported in displaced populations between 2012 and 2017 (Table 9).

Table 9

HEV Case Distribution by Gender and Site, 2012 to 2017

Location	Male n (%)	Female n (%)	Missing n (%)	Total n (%)
Mayom	40 (47)	45 (53)	0 (0)	85 (100)
Batil	2,702 (49)	2,666 (49)	105 (2)	5,473 (100)
Bentiu PoC	1,878 (58)	1,368 (42)	4 (0.1)	3,250 (100)
Bentiu Town	61 (58)	45 (42)	0 (0)	106 (100)
Doro	637 (53)	556 (46)	4 (0.3)	1,197 (100)
Jamam/Gendrassa	1,605 (47)	1,798 (52)	41 (1.2)	3,444 (100)
Lankien	14 (52)	13 (48)	0 (0)	27 (100)
Mingkaman	65 (45)	76 (52)	4 (2.8)	145 (100)
Yida	347 (51)	330 (49)	0 (0)	677 (100)
Total	7,349 (51)	6,897 (48)	158 (1.1)	14,404 (100)

As seen from Table 10, HEV deaths were evenly distributed by gender with males constituting 50.3% of the total deaths registered during the study period.

Table 10

Distribution of HEV Deaths by Gender and Site, 2012 to 2017

Site/camp	Male n (%)	Female n (%)	Missing n (%)	Grand Total n (%)
Mayom	16 (55.2)	13 (44.8)		29 (100)
Batil	61 (43.0)	81 (57.0)		142 (100)
Bentiu PoC	159 (57.6)	117 (42.4)		276 (100)
Bentiu Town	12 (70.6)	5 (29.4)		17 (100)
Doro	7 (25)	21 (75.0)		28 (100)
Jamam/Gendrassa	22 (41.5)	31 (58.5)		53 (100)
Lankien	0 (0)	2 (100)		2 (100)
Mingkaman	2 (33.3)	3 (50)	1 (16.7)	6 (100)
Yida	8 (44.4)	10 (55.6)		18 (100)
Grand Total	287 (50.3)	283 (49.6)	1 (0.2)	571 (100)

HEV case data was analyzed to determine the distribution of cases by age. As seen in table 11, children under five years constituted 15% of the total cases, 5-11-year-olds accounted for 20% of the cases, 12-17-year-olds (12%), adults 18-59 years (50%), and the elderly aged 60 years and above constituted 3% of the total HEV cases.

Table 11

Distribution of HEV Cases by Age and Site, 2012 to 2017

Site/camp	0-4yrs n (%)	5-11yrs n (%)	12-17yrs n (%)	18-59yrs n (%)	60+yrs n (%)	Grand Total n (%)
Mayom	16 (19)	23 (27)	15 (18)	26 (31)	4 (5)	84 (100)
Batil	444 (8)	687 (13)	575 (11)	3571 (65)	196 (4)	5473 (100)
Bentiu PoC	1048 (32)	1188 (37)	287 (9)	656 (20)	56 (2)	3235 (100)
Bentiu Town	36 (34)	49 (46)	5 (5)	15 (14)	1 (1)	106 (100)
Doro	124 (10)	233 (20)	184 (15)	616 (52)	31 (3)	1188 (100)
Jamam/Gendrassa	350 (10)	542 (16)	477 (14)	1945 (56)	130 (4)	3444 (100)

Site/camp	0-4yrs n (%)	5-11yrs n (%)	12-17yrs n (%)	18-59yrs n (%)	60+yrs n (%)	Grand Total n (%)
Lankien	4 (15)	2 (7)	3 (11)	18 (67)	0 (0)	27 (100)
Mingkaman	18 (13)	34 (24)	31 (22)	58 (41)	1 (1)	142 (100)
Yida	154 (23)	181 (27)	78 (12)	256 (38)	8 (1)	677 (100)
Grand Total	2194 (15)	2939 (20)	1655 (12)	7161 (50)	427 (3)	14376 (100)

The researcher analyzed HEV case data for the distribution of HEV deaths by age.

As seen in table 12, children under-five years constituted 22% of the total cases, 5-11-year-olds accounted for 19% of the cases, 12-17-year-olds (8%), adults 18-59 years (48%), and the elderly aged 60 years and above constituted 5% of the total HEV deaths.

Table 12

Distribution of HEV Deaths by Age and Site, 2012 to 2017

Site/camp	0-4yrs n (%)	5-11yrs n (%)	12-17yrs n (%)	18-59yrs n (%)	60+yrs n (%)	Grand Total n (%)
Mayom	3 (10)	4 (14)	7 (24)	12 (41)	3 (10)	29 (100)
Batil	4 (3)	1 (1)	10 (7)	117 (82)	10 (7)	142 (100)
Bentiu PoC	108 (39)	92 (33)	22 (8)	49 (18)	5 (2)	276 (100)
Bentiu Town	6 (35)	5 (29)	0 (0)	6 (35)	0 (0)	17 (100)
Doro	0 (0)	0 (0)	2 (7)	26 (93)	0 (0)	28 (100)
Jamam/Gendrassa	1 (2)	1 (2)	1 (2)	42 (79)	8 (15)	53 (100)
Lankien	0 (0)	0 (0)	0 (0)	2 (100)	0 (0)	2 (100)
Mingkaman	1 (17)	1 (17)	1 (17)	3 (50)	0 (0)	6 (100)
Yida	0 (0)	2 (11)	0 (0)	16 (89)	0 (0)	18 (100)
Grand Total	123 (22)	106 (19)	43 (8)	273 (48)	26 (5)	571 (100)

Internally displaced populations (IDP) mortality dataset. From December 2013 to March 2017, a total of 4,718 deaths (all causes) occurred in 12 IDP camps (Table 13). Bentiu PoC accounted for 2,386 (50.6%) of the total deaths (all causes) reported from December 2013 to March 2017. On the other hand, most of these deaths occurred in 2015 with 1,804 (38%) of the deaths occurring during the year (Table 13).

Table 13

Distribution of Deaths (All causes) in IDPs, December 2013 to March 2017

Site/camp	2013 n (%)	2014 n (%)	2015 n (%)	2016 n (%)	2017 n (%)	Total n (%)
Missing	0 (0)	4 (100)	0 (0)	0 (0)	0 (0)	4 (100)
Agok	0 (0)	3 (100)	0 (0)	0 (0)	0 (0)	3 (100)
Akobo	0 (0)	0 (0)	42 (32)	68 (52)	22 (17)	132 (100)
Bentiu	5 (0.2)	351 (15)	1151 (48)	775 (32)	104 (4)	2386 (100)
Bor	2 (1.5)	123 (92)	9 (7)	0 (0)	0 (0)	134 (100)
Juba 3	2 (0.4)	127 (22)	217 (38)	184 (33)	35 (6)	565 (100)
Kodok	0 (0)	1 (25)	0 (0)	0 (0)	3 (75)	4 (100)
Malakal	0 (0)	262 (39)	238 (36)	150 (23)	15 (2)	665 (100)
Melut	0 (0)	58 (36)	39 (24)	63 (39)	0 (0)	160 (100)
Mingkaman	8 (4)	142 (64)	47 (21)	25 (11)	0 (0)	222 (100)
Rumbek	0 (0)	0 (0)	0 (0)	21 (100)	0 (0)	21 (100)
Tongping	11 (4)	255 (95)	0 (0)	3 (1)	0 (0)	269 (100)
Wau PoC	0 (0)	0 (0)	0 (0)	20 (67)	10 (33)	30 (100)
Wau Shiluk	0 (0)	14 (11)	61 (50)	46 (37)	2 (2)	123 (100)
Total	28 (1)	1340 (28)	1804 (38)	1355 (29)	191 (4)	4718 (100)

The researcher analyzed the distribution of deaths in the IDPs by age for the period 2013 to 2017. During this period, most deaths among the IDPs occurred in children below five years; that accounted for 2,013 (43%) of the deaths (Table 14). Adults 18-59 years accounted for 1,360 (29%) of the deaths during this period (Table 14).

Table 14

Age Distribution of Deaths (All causes) in IDPs, December 2013 to March 2017

Site/camp	0-4yrs n (%)	5-11yrs n (%)	12-17yrs n (%)	18-59yrs n (%)	60+yrs n (%)	Missing n (%)	Total n (%)
(missing)	1 (25)	0 (0)	0 (0)	1 (25)	1 (25)	1 (25)	4 (100)
Agok	0 (0)	0 (0)	0 (0)	2 (67)	1 (33)	0 (0)	3 (100)
Akobo	61 (46)	13 (10)	2 (2)	47 (36)	9 (7)	0 (0)	132 (100)
Bentiu	1089 (46)	256 (8)	77 (3)	569 (25)	371 (17)	24 (1)	2386 (100)
Bor	62 946)	2 (1)	0 (0)	17 (13)	6 (4)	47 (35)	134 (100)

Site/camp	0-4yrs n (%)	5-11yrs n (%)	12-17yrs n (%)	18-59yrs n (%)	60+yrs n (%)	Missing n (%)	Total n (%)
Juba 3	227 (40)	21 (4)	5 (1)	250 (44)	62 (11)	0 (0)	565 (100)
Kodok	2 (50)	1 (25)	0 (0)	0 (0)	1 (25)	0 (0)	4 (100)
Malakal	204 (31)	41 (6)	29 (4)	231 (35)	131 (20)	29 (4)	665 (100)
Melut	51 (32)	7 (4)	9 (6)	62 (39)	29 (18)	2 (1)	160 (100)
Mingkaman	105 (47)	25 (11)	6 (3)	51 (23)	29 (13)	6 (3)	222 (100)
Rumbek	4 (19)	2 (10)	1 (5)	11 (52)	3 (14)	0 (0)	21 (100)
Tongping	159 (59)	21 (8)	5 (2)	70 (26)	9 (3)	5 (2)	269 (100)
Wau PoC	17 (57)	1 (3)	1 (3)	8 (27)	3 (10)	0 (0)	30 (100)
Wau Shiluk	31 (25)	1 (1)	5 (4)	41 (33)	45 (37)	0 (0)	123 (100)
Grand Total	2,013 (43)	391 (8)	140 (3)	1360 (29)	700 (15)	114 (2)	4718 (100)

The study assessed the distribution of deaths in IDPs by gender for the period 2013 to 2017. As seen from Table 15, most deaths in the IDPs occurred in males, who accounted for 2,462 (52%) of the total deaths. All the IDP camps had this pattern of HEV death distribution by gender during the study period (Table 15).

Table 15

Gender Distribution of Deaths (All causes) in IDPs, December 2013 to March 2017

Site/camp	Female n (%)	Male n (%)	Missing n (%)	Total n (%)
(Missing)	2 (50)	1 (25)	1 (25)	4 (100)
Agok	1 (33)	2 (67)	0 (0)	3 (100)
Akobo	59 (45)	72 (55)	1 (1)	132 (100)
Bentiu	1157 (48)	1219 (51)	10 (0.4)	2386 (100)
Bor	50 (37)	37 (28)	47 (35)	134 (100)
Juba 3	255 (45)	309 (55)	1 (0.2)	565 (100)
Kodok	0 (0)	4 (100)	0 (0)	4 (100)
Malakal	239 (36)	378 (57)	48 (7)	665 (100)
Melut	66 (41)	82 (51)	12 (8)	160 (100)
Mingkaman	82 (37)	109 (49)	31 (14)	222 (100)
Rumbek	8 (38)	13 (62)	0 (0)	21 (100)
Tongping	108 (40)	148 (55)	13 (5)	269 (100)
Wau PoC	11 (37)	19 (63)	0 (0)	30 (100)
Wau Shiluk	44 (36)	69 (56)	10 (8)	123 (100)

Site/camp	Female n (%)	Male n (%)	Missing n (%)	Total n (%)
Total	2082 (44)	2462 (52)	174 (4)	4718 (100)

We assessed the top causes of mortality among the IDPs for the period 2013 to 2017. During this period, the top causes of mortality among the IDPs (all age groups) included malaria, Tuberculosis, HIV/AIDS, pneumonia, medical complications of acute malnutrition, and HEV. (Table 16).

Table 16

Distribution of Top Causes of Mortality in IDPs (all age-groups), December 2013 to March 2017

Cause of death	2017	2016	2015	2014	2013	Total
Malaria	16	111	284	70	1	482
TB/HIV/AIDS	35	175	151	96		457
Pneumonia	14	93	116	101	3	327
SAM	14	98	118	97		327
HEV		49	238	14	1	302
Perinatal death	12	74	99	97	2	284
Acute watery diarrhoea	11	47	78	139	6	281
Sepsis	7	33	45	33		118
Measles		12	12	89	3	116
Gunshot wound	2	23	25	49	4	103
Others	80	640	638	555	8	1921
Total	191	1,355	1,804	1,340	28	4,718

In children under five years of age, the top causes of mortality in the IDPs included malaria, perinatal complications, severe acute malnutrition (SAM), pneumonia, acute watery diarrhoea, and HEV (Table 17).

Table 17

Distribution of Top Causes of Mortality in IDPs (Under-fives), Dec 2013 to Mar 2017

Cause of death	2017	2016	2015	2014	2013	Total
Malaria	11	56	192	29		288
Perinatal death	12	73	99	97	2	283
SAM	11	82	106	78		277
Pneumonia	8	67	91	76	3	245
Acute watery diarrhoea	8	21	46	96	2	173
HEV		15	97	2		114
Measles		12	12	79	3	106
Sepsis	2	16	24	16		58
TB/HIV/AIDS	2	19	15	11		47
Neonatal sepsis		12	13	13		38
Others	3	39	77	75	2	196
Total	61	483	838	619	15	2,016

IDP morbidity dataset. During the period starting December 2013 to March 2017, a total of 4,579,551 consultations were reported (Table 18). During this period, the top causes of morbidity among the IDPs included malaria 1,198,709 (26.2%), acute respiratory tract infections 750,538 (16.4%), and acute watery diarrhoea 390,849 (8.5%) (Table 18).

Table 18

Distribution of Top Causes of Morbidity in IDPs, December 2013 to March 2017

Year	Malaria	Acute Respiratory Infections	Acute watery diarrhoea	Acute Bloody Diarrhoea	Measles	Acute Jaundice Syndrome [HEV]	Gunshot wound	Cholera	Consultations
2013	3,797	-	1,061	165	-	-	-	1	8,330
2014	236,294	95,728	88,405	16,846	1,597	293	186	6,293	1,137,184
2015	486,547	265,089	141,556	20,897	981	3,476	14	358	1,550,441
2016	436,458	322,303	136,462	17,633	4,819	8,011	1,168	2,447	1,632,422

Year	Malaria	Acute Respiratory Infections	Acute watery diarrhoea	Acute Bloody Diarrhoea	Measles	Acute Jaundice Syndrome [HEV]	Gunshot wound	Cholera	Consultations
2017	35,613	67,418	23,365	2,027	208	129	120	497	251,174
Total	1,198,709	750,538	390,849	57,568	7,605	11,909	1,488	9,596	4,579,551

Research Question 1

What is the timing, periodicity, magnitude (morbidity and mortality), laboratory characterization, and duration of HEV outbreaks in South Sudan and how are the cases distributed by person, time, and place?

To answer this research question, I conducted multi-year (2012-2017) trend analyses of HEV cases in relation to the rainfall pattern and seasonality for South Sudan. The analysis is critical for elucidating the periodicity and seasonality of HEV disease outbreaks. Then, for each of the HEV outbreaks registered from 2012 to 2017, I computed the outbreak duration from the outbreak end date and onset dates. Subsequently, I derived the median, first quartile, third quartile, the minimum, maximum, and interquartile range for the outbreak duration in days and weeks. I used the retrospective space-time permutation scan statistic to detect local outbreaks from 2012 to 2017. To document the risk and impact of HEV in displaced populations, I computed cumulative incidence and mortality rates by place, person, and time. This section presents the results of these analyses.

Timing and periodicity of HEV outbreaks. From 2012 to early 2017, HEV cases were reported every week in displaced populations of South Sudan, a trend that is

consistent with endemic transmission of the disease. During this period, there were four discernible peaks of transmission with the initial peak of 610 cases occurring during the last two months (September and October) of the 2012 rainy season (Figure 2). The transmission in two refugee camps (Batil and Jamam/Gendrassa) in Maban county, Upper Nile state accounted for most cases registered during the peaks. From November 2012, the cases continued rising reaching the highest peak of 2,744 cases in January of 2013. Batil and Jamam/Gendrassa contributed the majority of the cases with additional cases reported from Doro refugee camp in Maban county, Upper Nile state, and Yida refugee camp in Pariang county, Unity state. The number of HEV cases declined steadily from February 2013 to December 2013 (Figure 2). There was low-grade transmission in 2014 attributed to declining and sporadic transmission in Doro and Yida refugee camps. However, during the same year (2014), new HEV outbreaks were reported in Mingkaman IDP settlement (starting January 2014) and eventually to Bentiu Protection of Civilians (POC) IDP camp (starting October 2014). In 2015, there were two peaks with the first peak of 187 cases occurring in January 2015 and mainly attributed to increasing transmission in Bentiu PoC IDP camp (Figure 2).

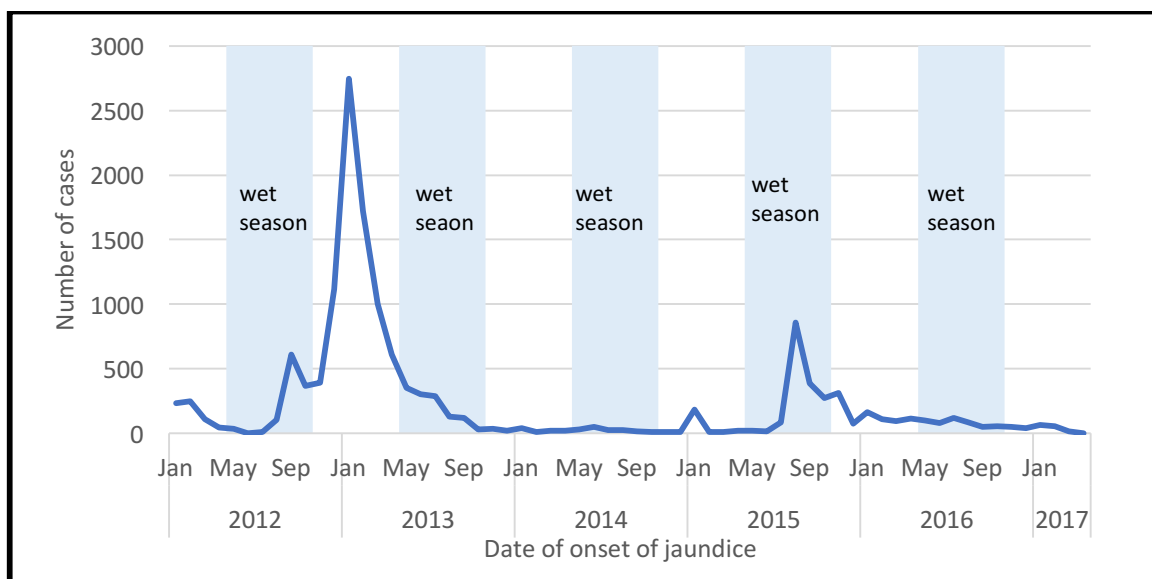


Figure 2 Seasonality of Hepatitis E in Displaced Populations of South Sudan, 2012-2017.

This peak occurred during the dry season of 2015. The second peak of 856 cases occurred in August 2015. This peak took place in the wet season with most cases originating from Bentiu PoC and to a lesser extent from Lankien IDPs and Mingkaman IDP settlement. The transmission of HEV in Bentiu declined from September 2015 with sustained and low-grade transmission persisting during the whole of 2016 and the first four months of 2017. Also, there was sporadic transmission from Mayom during the whole of 2016 and the first four months of 2017 (Figure 2).

Trends of HEV and precipitation by place. To further assess the seasonality of HEV, the researcher analyzed the rainfall and HEV case trends for each of the eight displaced population sites. As seen from Figures 3 to 10, HEV cases started rising or attained peak transmission during months that coincided with precipitation peaks in each of the displaced population sites, Batil, Doro, Bentiu, and Gendrassa. However, Batil,

Gendrassa, Mayom, and Yida also registered HEV transmission peaks in the dry season (Figures 3 to 10).

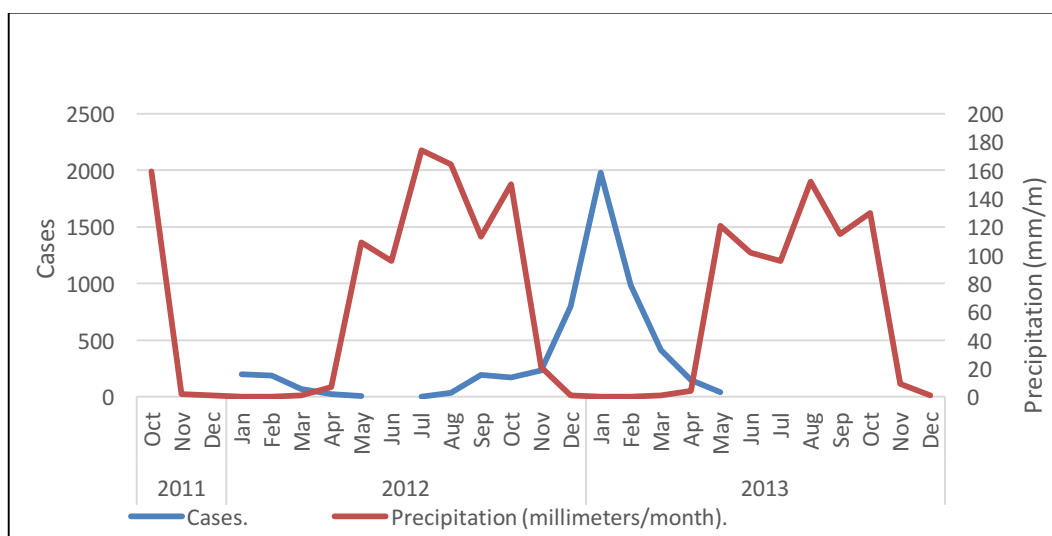


Figure 3 HEV cases and Precipitation trends, Batil, 2011-2013.

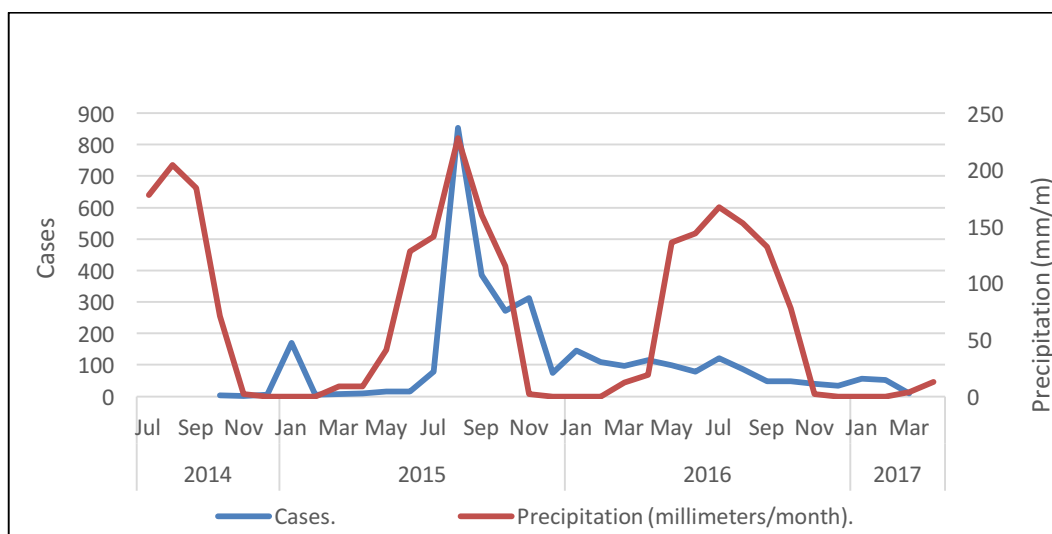


Figure 4 HEV cases and Precipitation trends, Bentiu, 2014-2017.



Scatter plots for the correlation between precipitation and HEV cases showed that only Bentiu PoC had a weak and significant relationship between precipitation and HEV cases ($r=0.52$; $p=0.03$) (Figure 11 and Table 19).

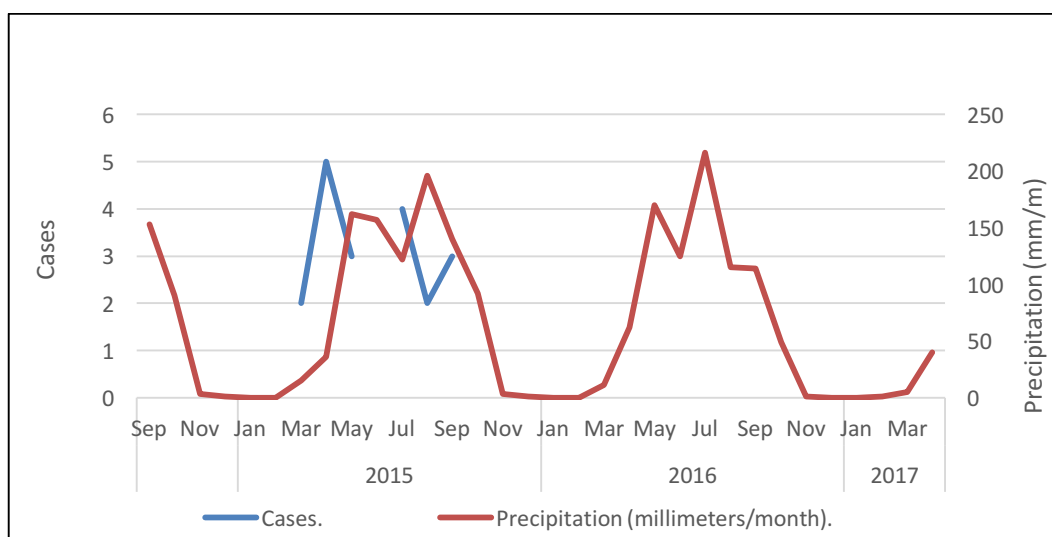


Figure 7 HEV cases and Precipitation trends, Lankien, 2014-2015.

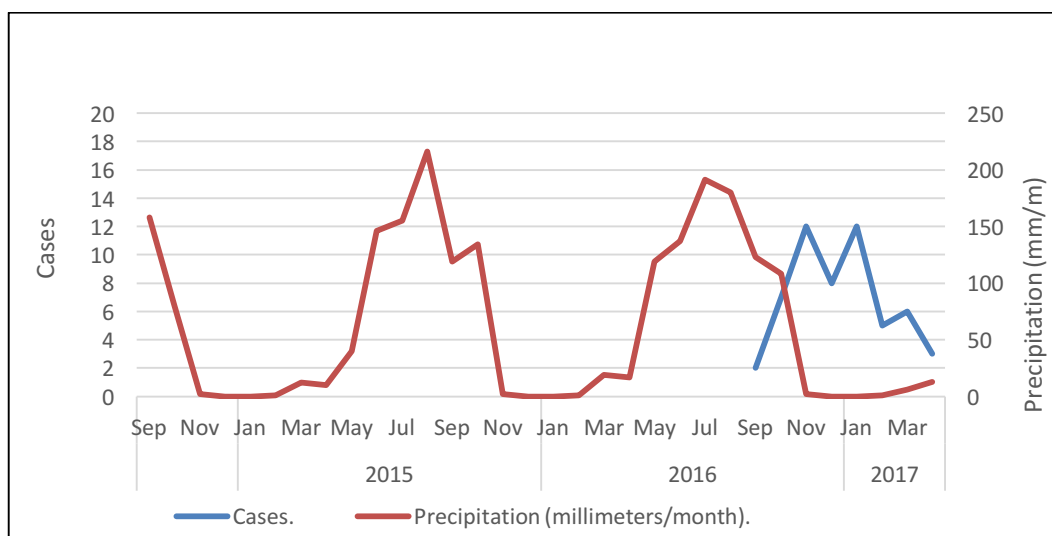


Figure 8 HEV cases and Precipitation trends, Mayom, 2016-2017.

Overall, the strength of association between precipitation and HEV cases as seen from the Pearson correlation (r) and the R square was low or insignificant for each of the eight displace population sites (Figure 11 and Table 19).

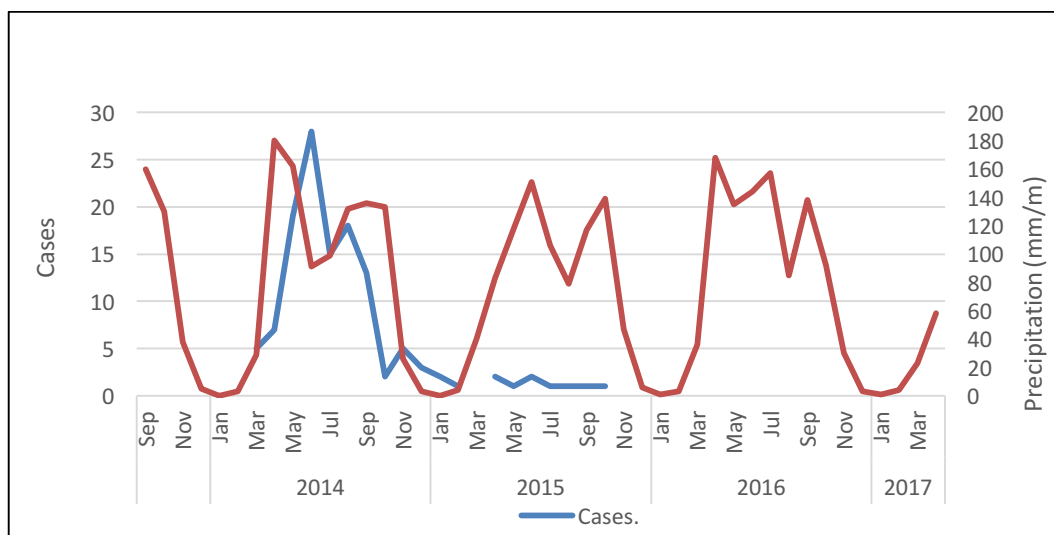


Figure 9 HEV cases and Precipitation trends, Mingkaman, 2014-2015.

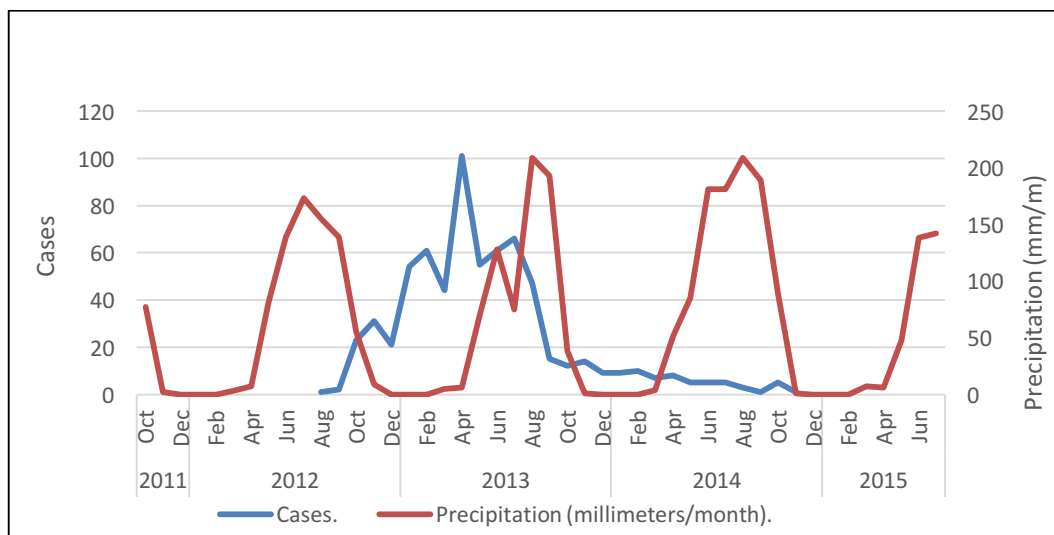


Figure 10 HEV cases and Precipitation trends, Yida, 2012-2014.

The duration of HEV outbreaks. During the period January 2012 to April 2017, a total of eight outbreaks of HEV were reported in displaced populations of South Sudan (Table 20). Of the eight outbreaks reported during this period, 4 (50%) occurred in refugee camps (Batil, Doro, Jamam/Gendrassa, and Yida) while the rest occurred in IDP camps (Mingkaman settlement, Bentiu PoC, Lankien, and Mayom) (Table 20).

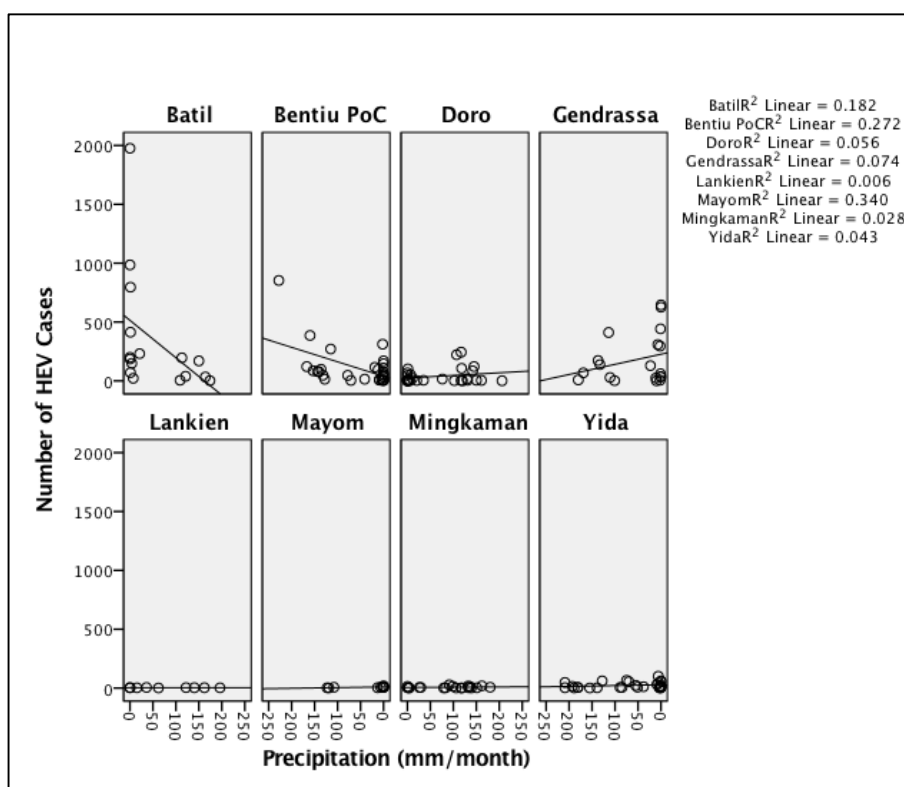


Figure 11 Scatter plots for HEV cases and precipitation by camp, 2012-2017.

Table 19

Correlation and simple linear regression analyses for HEV cases and precipitation by camp, 2012 to 2017.

No.	Camp	Pierson correlation (r)	R square	2-tailed p-value
1	Batil	-0.43	0.18	0.1
2	Bentiu PoC	0.52	0.27	0.03***
3	Doro	0.24	0.05	0.20
4	Gendrassa	-0.27	0.07	0.26
5	Lankien	-0.78	0.006	0.84
6	Mayom	-0.58	0.34	0.60
7	Mingkaman	0.17	0.03	0.48
8	Yida	-0.21	0.04	0.28
9	Overall	-0.083	0.0069	0.29

During this period, the epidemiological week of outbreak onset varied from 1- 40 with a median of six and a mean of 13. The duration of the outbreak ranged from 47 weeks in Mayom to 142 weeks in Yida with a median duration of 96.5 weeks and interquartile range of 62.25 weeks.

Table 20

HEV Outbreaks in Displaced Populations and Their Duration, 2012 to 2017

Outbreak site/ summary measure	Cases	Deaths (CFR%)	Outbreak onset		End of outbreak		Outbreak duration (weeks)
			Week	Year	Week	Year	
Batil	5,473	142 (2.6)	1	2012	21	2013	73
Jamam/Gendrassa	3,444	53 (1.5)	1	2012	52	2013	104
Yida	677	18 (2.7)	10	2012	48	2014	142
Doro	1,169	25 (2.1)	36	2012	21	2015	141
Mingkaman	127	5 (3.9)	2	2014	39	2015	89
Bentiu PoC	3,163	291 (9.2)	40	2014	15	2017*	132
Lankien	26	2 (7.7)	1	2015	17	2016	69
Mayom	56	25 (44.6)	20	2016	15	2017	47

*outbreak was still ongoing at the time of obtaining this data.

Identifying clusters of HEV cases, 2012- 2017. To detect outbreak clusters of HEV during the period 2012 to early 2017, the researcher initially used the retrospective space-time permutation scan statistic that does not require population-at-risk data. For every geographical location under surveillance, the statistic can detect one day or multi-day outbreaks with the ability to identify both rapidly rising and slowly emerging outbreaks. To adjust for the underlying populations in each of the displaced population locations, follow up analysis was undertaken to identify clusters of spatial or space-time clustering, the Poisson model-based statistical software, SatScanTM, was used (Kulldorf, 2016). As seen from table 21, four county-level clusters were detected using the non-Poisson adjusted retrospective space-time permutation scan statistic. The most likely cluster as seen from the likelihood statistic of 585.45, occurred in Bentiu PoC from 10 August 2015 to 6 September 2015 and was comprised of 808 cases compared to the expected 202 cases. There were three additional HEV clusters detected in Pariang (Yida), Awerial (Mingkaman), and Nyirol (Lankien). Table 21 presents the respective dates they occurred, the observed and expected cases, and the SatScan test statistic. Figure 12 is a map of South Sudan visualizing these clusters. Results from the follow up Poisson-adjusted spatial analysis with population adjustment showed that the most likely HEV outbreak clusters occurred in Maban (Batil, Jamam/Gendrassa, and Doro) with a likelihood statistic of 13,016 ($p < 0.001$). The other HEV outbreak cluster occurred in Rubkona (Bentiu PoC) with a likelihood statistic of 4.2513 ($p = 0.0046$).

Table 21

Retrospective Space-Time Analysis for Clusters with High Rates Using the Space-Time Permutation Model

Location	Cluster time frame		Number of cases in cluster		Test statistic	p value
	Start date	End date	Observed	Expected		
Rubkona (Bentiu PoC)	10-Aug-15	06-Sep-15	849	203	585.45	<0.001
Pariang (Yida)	17-Jun-13	14-Jul-13	70	4	142.15	<0.001
Awerial (Mingkaman)	26-May-14	22-Jun-14	28	0.6	80.1	<0.001
Nyirol (Lankien)	30-Mar-15	26-Apr-15	5	0.029	5	<0.001

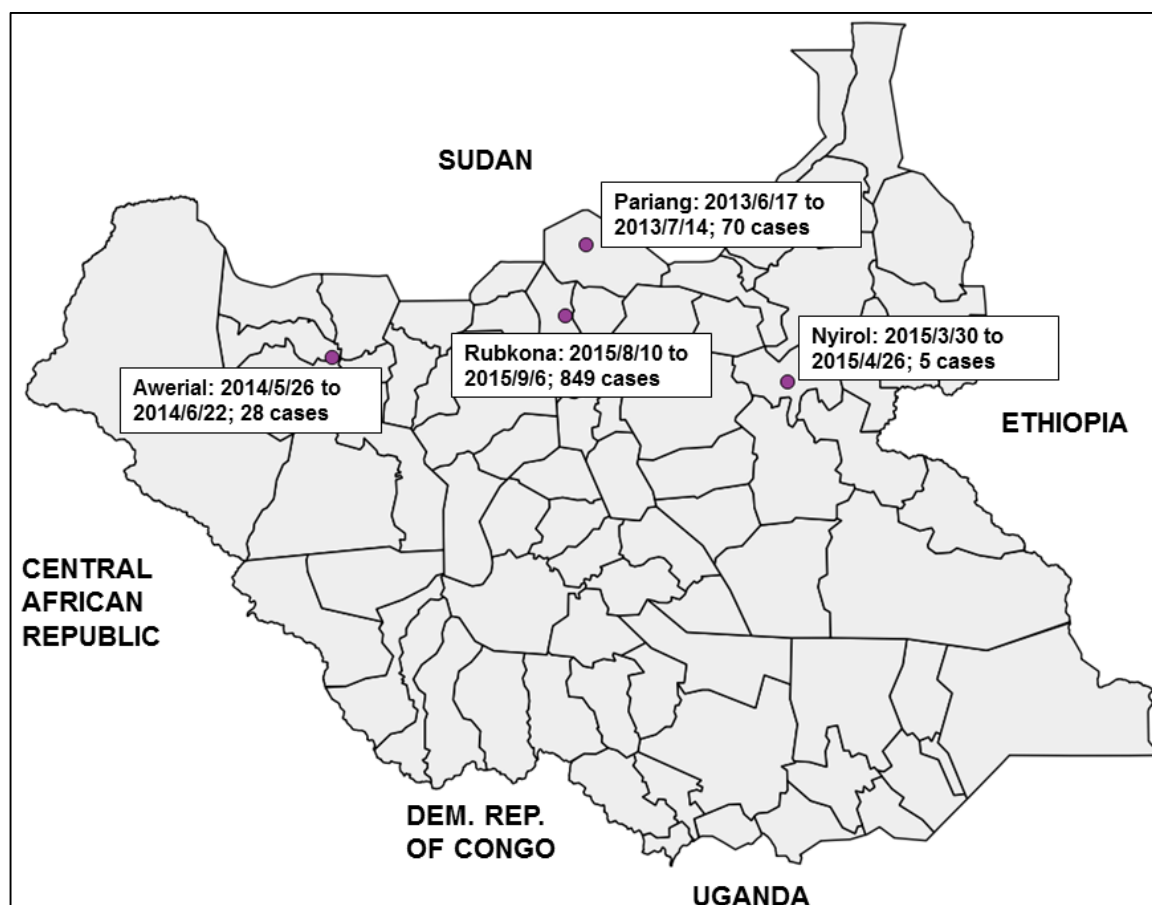


Figure 12 HEV Case Clusters Based on the SatScan Test Statistic, 2012-2017.

HEV cumulative incidence and mortality by place. To determine the overall risk of infection and death from HEV, we calculated the cumulative incidence and mortality by year for each of the affected displaced population camps for the period 2012-2017.

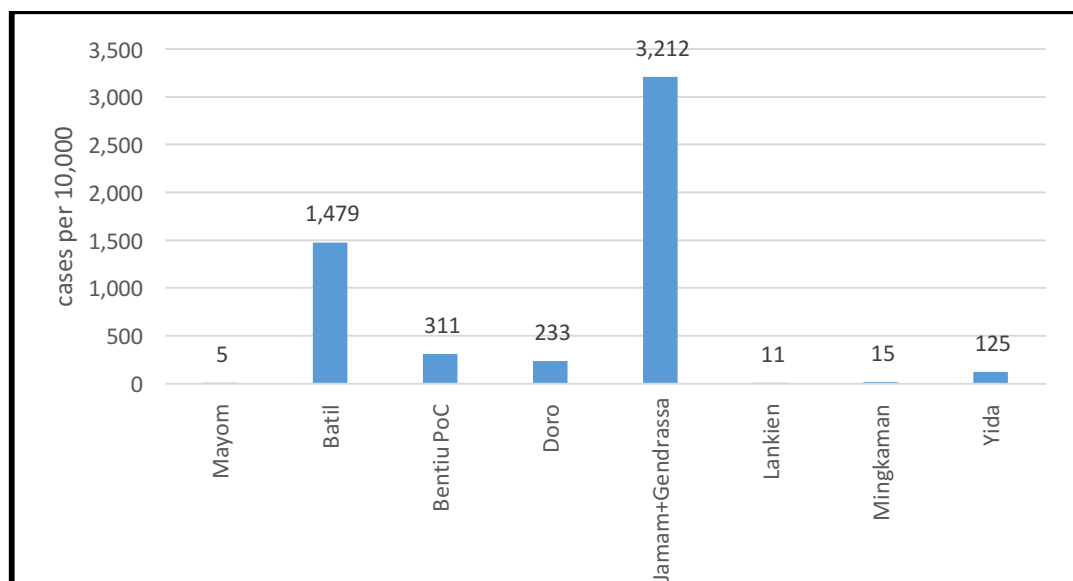


Figure 13 HEV Cumulative Incidence (Cases per 10,000) by Site, 2012-2017.

As seen from figure 20, the cumulative incidence (cases per 10,000) of HEV increased from 360.5 in 2012 reaching the highest peak of 424.6 in 2013 when most of the transmission occurred in Jamam/Gendrassa, Batil, and Doro refugee camps (Figures 14 and 15). The second but lower peak of transmission occurred in 2015 with an overall cumulative incidence of 56 and most of the transmission occurring in Bentiu PoC, Lankien, and Doro (Figures 17 and 20). In 2014, most HEV transmission took place in Mingkaman and Bentiu PoC while in 2016 most transmission occurred in Lankien and Bentiu PoC (Figures 16 and 18). By the time of obtaining data for the present study, there were two ongoing HEV outbreaks in Bentiu PoC and Mayom (Figure 19).

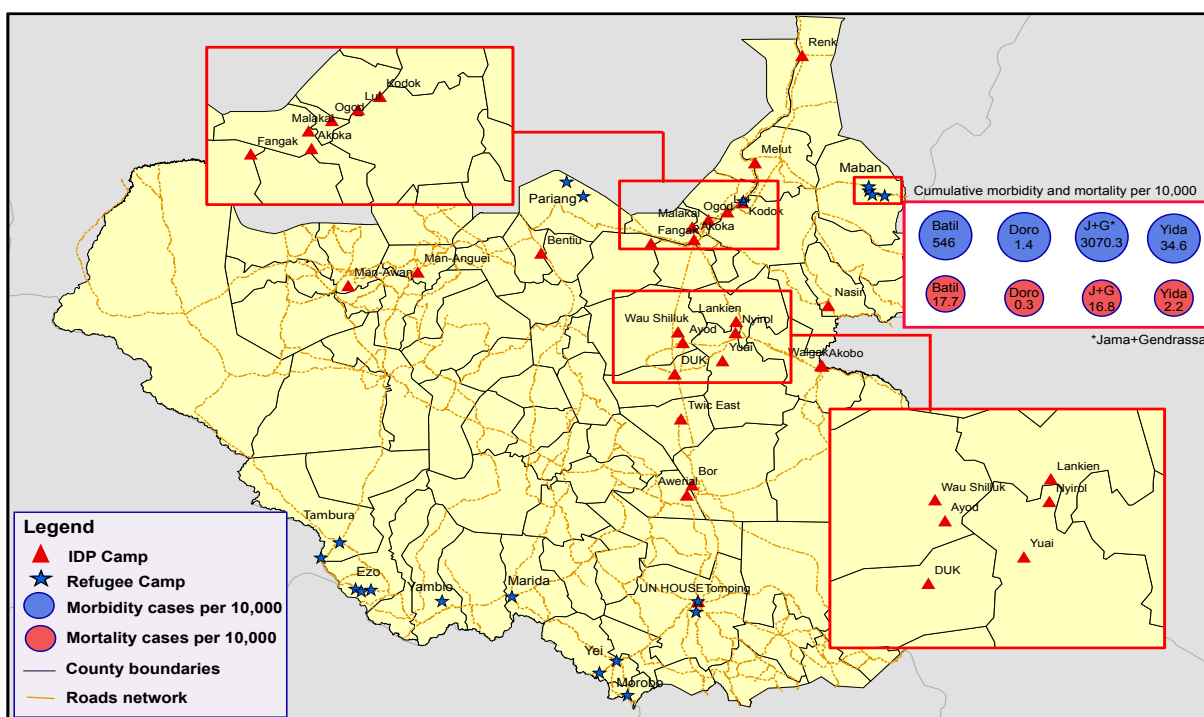


Figure 14 HEV Cumulative Incidence and Mortality by Site, 2012

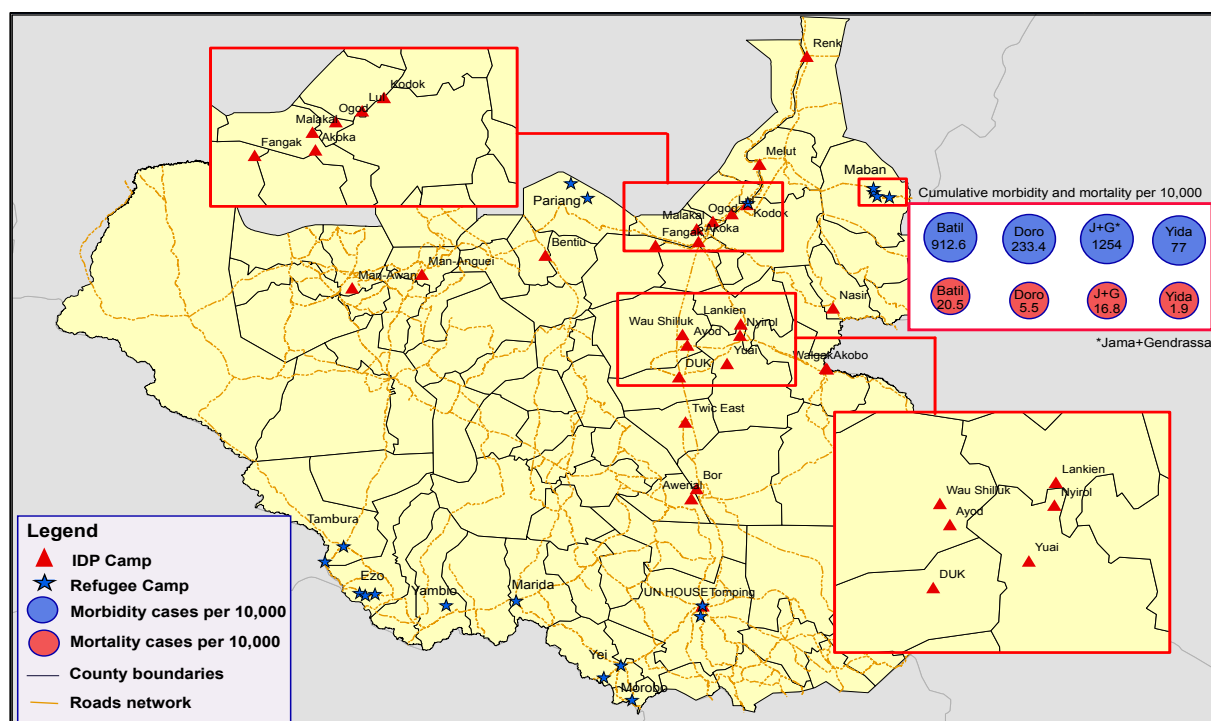


Figure 15 HEV Cumulative Incidence and Mortality by Site, 2013.

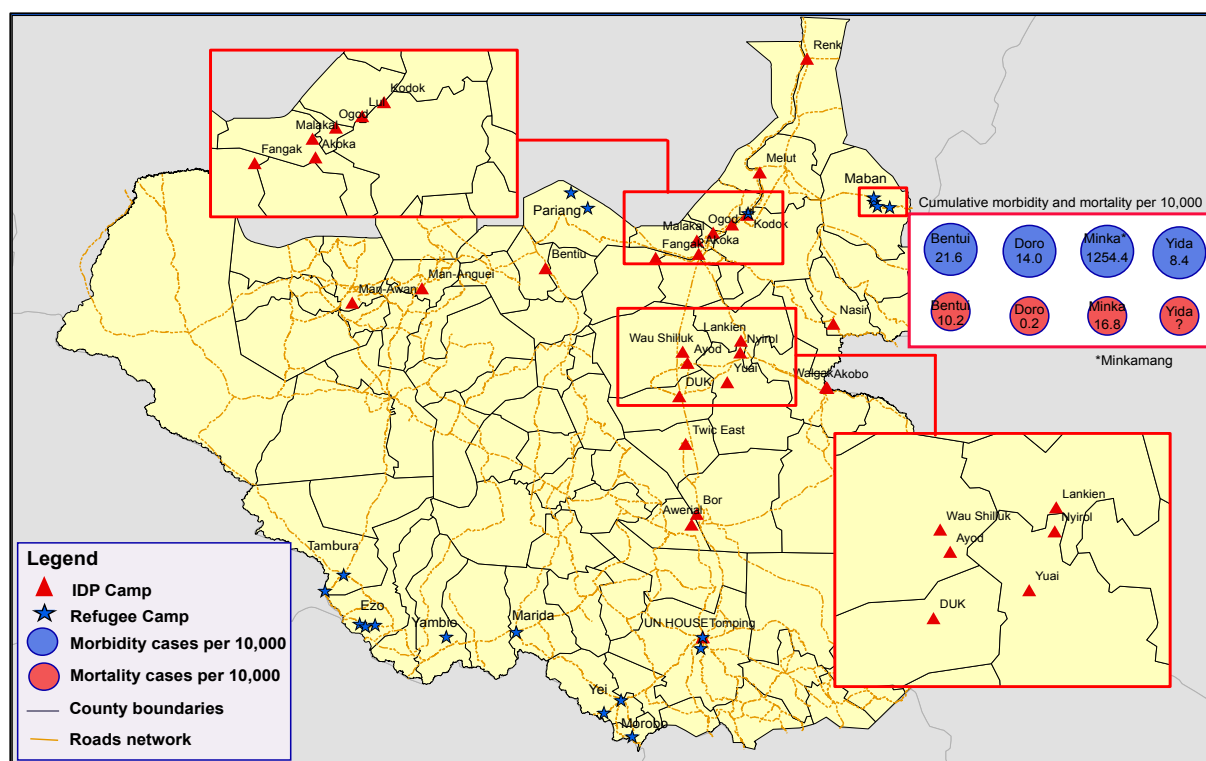


Figure 16 HEV Cumulative Incidence and Mortality by Site, 2014.

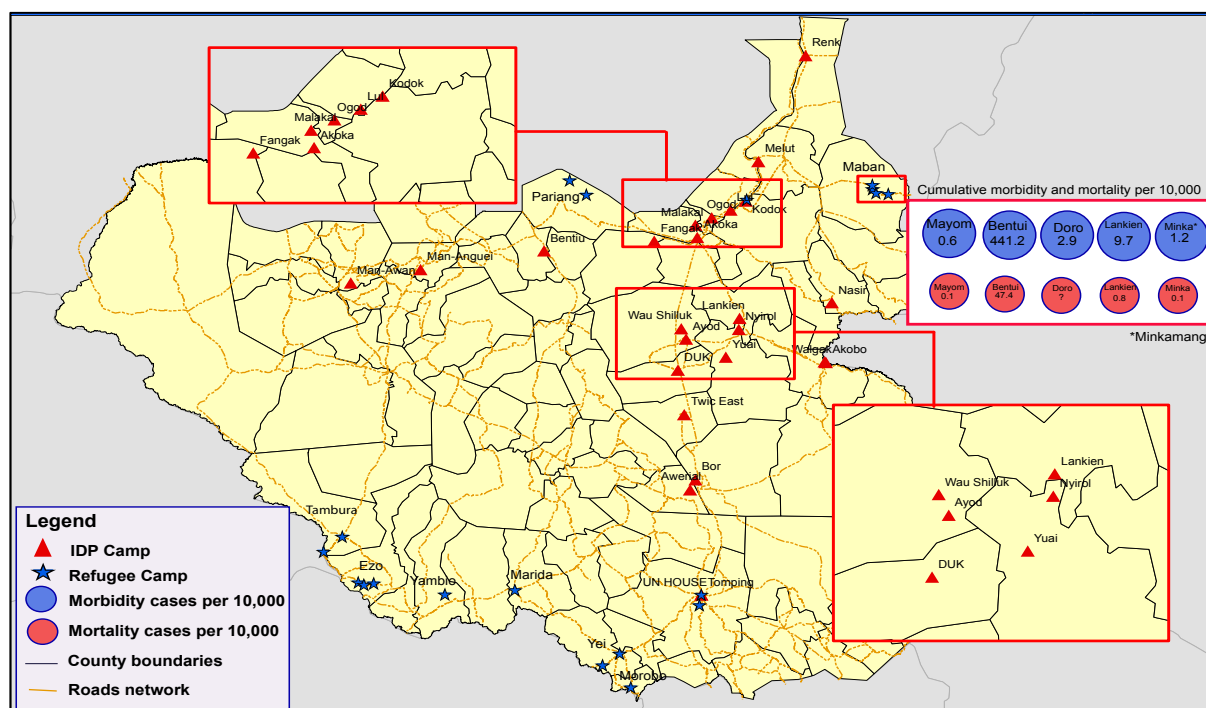


Figure 17 HEV Cumulative Incidence and Mortality by Site, 2015.

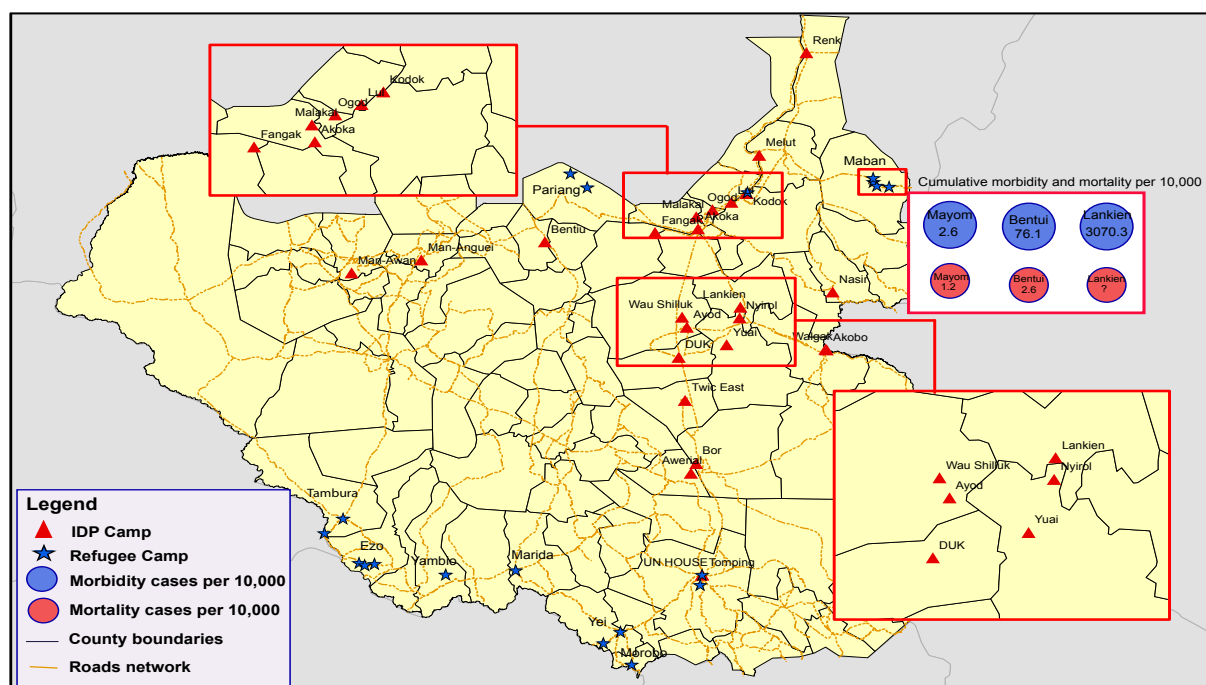


Figure 18 HEV Cumulative Incidence and Mortality by Site, 2016.

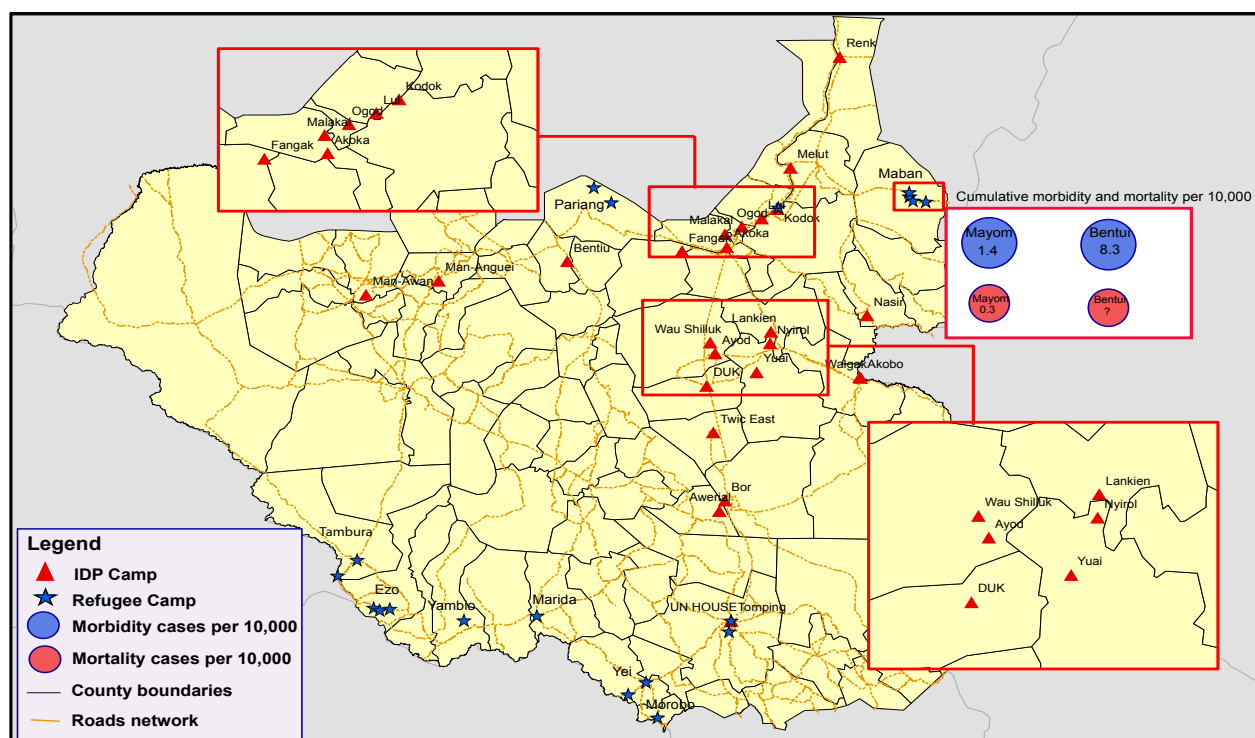


Figure 19 HEV Cumulative Incidence and Mortality by Site, 2017.

Regarding the overall risk of HEV disease by site, the cumulative incidence during the period 2012- early 2017 was highest in Jamam/Gendrassa refugee camp followed by Batil refugee camp, Bentiu PoC IDP, Doro refugee camp and Yida refugee camp (Figure 13).

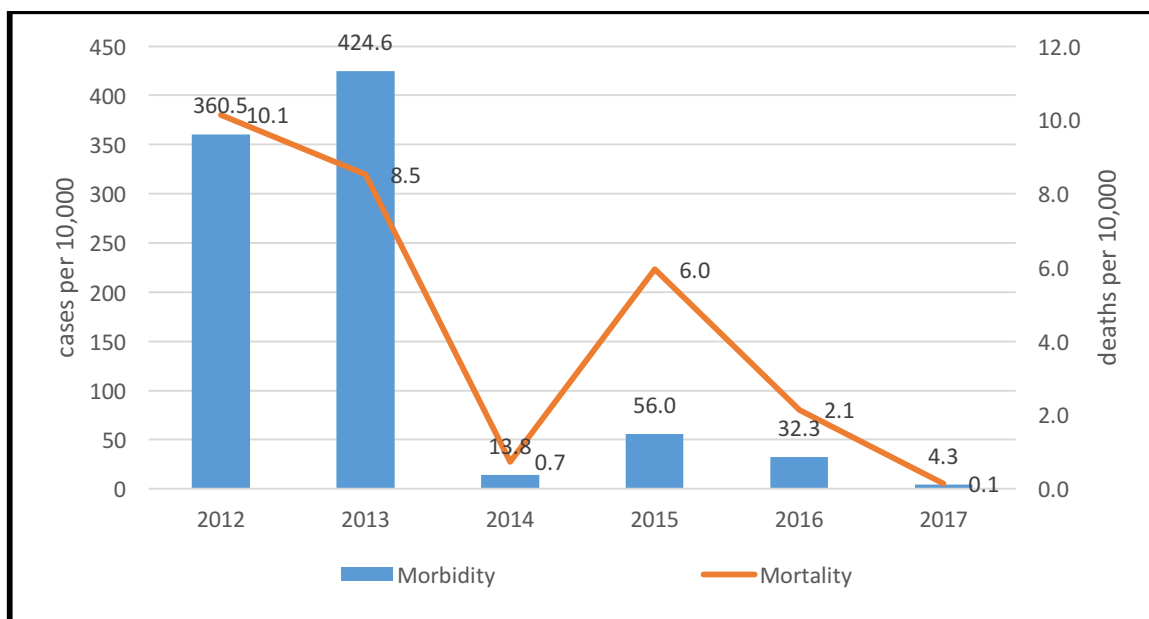


Figure 20 HEV Morbidity and Mortality Trends in Displaced Populations, 2012-2017.

With regards to the distribution of HEV mortality during 2012- early 2017, mortality was highest in 2012 and 2013 when the HEV mortality (deaths per 10,000) rates were 10.1 and 8.5 respectively with most deaths occurring in Jamam/Gendrassa and Batil (Figures 14 and 20). The third but lower peak of mortality (deaths per 10,000) of 6 occurred in 2015 with most of the deaths occurring in Bentiu PoC (Figures 17 and 20). Concerning the overall risk of HEV death by site, HEV mortality (deaths per 10,000) was highest in Jamam/Gendrassa refugee camp followed by Batil refugee camp and Bentiu

PoC with HEV mortality rates of 49, 38.4, and 26.4 deaths per 10,000 respectively (Figure 21).

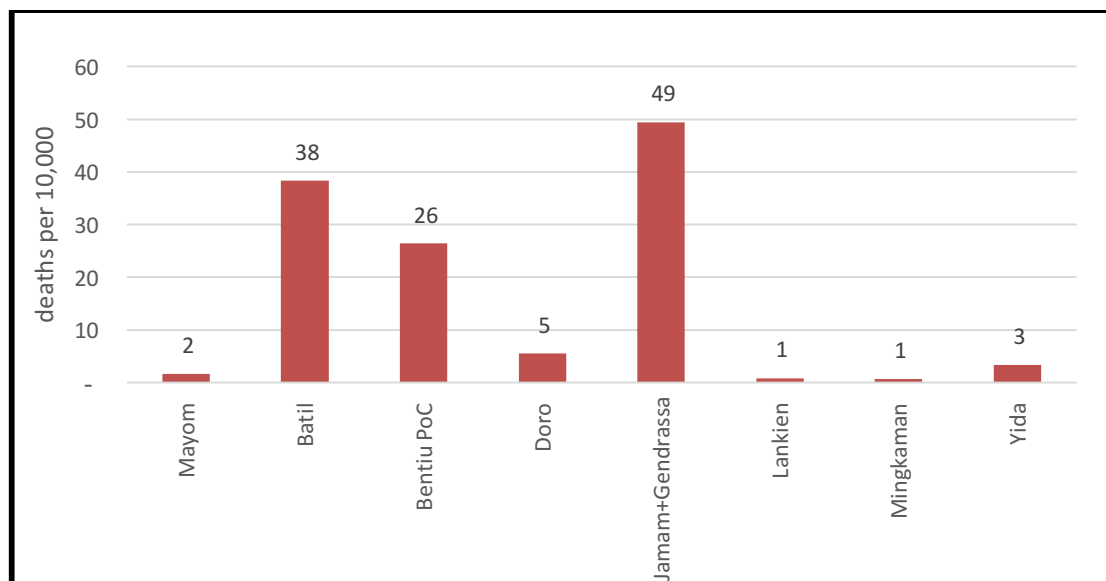


Figure 21 HEV Mortality (Deaths per 10,000) by Site, 2012-2017.

HEV cumulative incidence and mortality by gender. Table 22 shows the cumulative incidence of HEV by gender and year from 2012-2017. Apart from 2012 when the gender specific HEV incidence was higher in females than males, for the rest of the years from 2013 to 2017, the HEV gender-specific incidence was higher in males than females (Table 22). The overall cumulative incidence (cases per 10,000) from 2012-2017 was higher in males (590.8) than females (487.3) (Table 22).

Table 22

Cumulative Incidence of HEV Cases per 10,000 by Gender and Year, 2012-2017

Camp/site	2012		2013		2014		2015		2016		2017		Grand Total	
	F	M	F	M	F	M	F	M	F	M	F	M	F	M
Mayom							0.5	0.6	2.8	2.4	1.3	1.4	4.7	4.4
Batil	546.3	522.5	857.9	934.9									1381.1	1438.2
Bentiu PoC					12.2	33.4	343.8	561.9	54.7	103.1	5.3	12.0	304.7	525.9
Doro	2.1	0.7	217.4	249.0	11.0	17.0	1.1	4.4					246.1	292.6
Jamam/ Gendrassa	3267.8	2798.5	1285.4	1192.1									2724.9	2453.5
Lankien							11.0	8.7	0.9	1.5			11.9	10.2
Mingkaman					15.5	20.2	1.4	1.0					15.0	18.6
Yida	30.0	40.2	66.9	89.6	8.5	8.3							110.0	143.3
Grand Total	359.9	348.4	394.0	447.0	9.5	12.0	45.7	67.7	25.0	40.3	3.1	5.7	487.3	590.8

As seen from table 23, the HEV deaths per 10,000 were higher in males (23.1)

when compared to females (20). Table 23 shows the distribution of HEV deaths by site, year, and gender.

Table 23

HEV Mortality (Deaths per 10,000) by Gender and Year, 2012-2017.

Camp/site	2012		2013		2014		2015		2016		2017		Total	
	F	M	F	M	F	M	F	M	F	M	F	M	F	M
Mayom								0.2	1.0	1.3	0.3	0.2	1.4	1.8
Batil	20.9	14.5	22.2	18.7									42.0	32.5
Bentiu PoC					4.1	18.0	36.2	61.4	2.2	3.2			26.1	44.5
Doro	0.7		7.9	3.0	0.4								9.3	3.2
Jamam/ Gendrassa	57.5	58.0	21.9	11.6									47.0	33.6
Lankien							1.8						1.8	
Mingkaman					0.7	0.3		0.3					0.6	0.6
Yida	1.6	2.9	2.1	1.6									3.3	3.3
Grand Total	11.1	9.2	9.9	7.0	0.4	0.7	4.7	7.4	1.7	2.6	0.2	0.1	20.0	23.1

HEV cumulative incidence and mortality by age. As seen from Table 24, HEV cases were presented by site and age for the period 2012 to 2017. The overall age-specific cumulative HEV cumulative incidence (cases per 10,000) was highest among the 18-59

and was 367.3. The age-specific cumulative incidence as cases per 10,000 was 353.2 in the elderly 60+years (Table 24).

Table 24

HEV Cumulative Incidence (Cases per 10,000) by Age and Year, 2012-2017.

Camp/site	0-4yrs	5-11yrs	12-17yrs	18-59yrs	60+yrs	Total
Mayom	3.1	5.2	7.3	3.9	11.3	4.5
Batil	510.7	682.7	1,098.6	2,453.3	1,157.7	1,360.1
Bentiu PoC	370.4	485.0	252.4	178.0	285.9	314.2
Doro	102.1	180.0	275.2	349.6	271.5	235.1
Jamam/Gendrassa	855.7	1,248.0	2,142.9	2,903.9	2,103.6	1,916.0
Lankien	5.9	3.4	11.0	20.3		10.9
Mingkaman	8.8	19.6	32.5	25.8	6.7	19.9
Yida	90.8	92.7	72.1	120.0	67.7	96.9
Grand Total	147.5	211.6	239.3	367.3	353.2	255.0

Table 25 shows the gender specific mortality by camp/ site from 2012 to 2017. Mortality as deaths per 10,000 was highest in the 60+ years followed by the 18-59-year age-group with mortality rates of 21.5 and 14.0 respectively. Table 25 shows the mortality rates for the other age-groups.

Table 25

HEV Mortality (Deaths per 10,000) by Age and Year, 2012-2017.

Camp/site	0-4yrs	5-11yrs	12-17yrs	18-59yrs	60+yrs	Total
Mayom	0.6	0.9	3.4	1.8	8.5	1.6
Batil	4.6	1.0	19.1	80.4	59.1	35.3
Bentiu PoC	38.2	37.6	19.3	13.3	25.5	26.8
Doro			3.0	14.8		5.5
Jamam/Gendrassa	2.4	2.3	4.5	62.7	129.4	29.5
Lankien				2.3		0.8
Mingkaman	0.5	0.6	1.0	1.3		0.8
Yida		1.0		7.5		2.6
Grand Total	8.3	7.6	6.2	14.0	21.5	10.1

HEV incidence by age, sex, and place. As seen from figures 22-29, the risk of HEV disease in Mayom, Batil, Doro, Gendrassa, Lankien, and Mingkaman increased with age even after stratifying by sex. The most affected age groups being 18-59 years and 60+years (Figures 22-29). However, in Mingkaman, females aged 12-17 years were the most affected when compared to females in the other age groups (Figure 28). In Bentiu, males and females 5-11 years were significantly more affected (Figure 25).

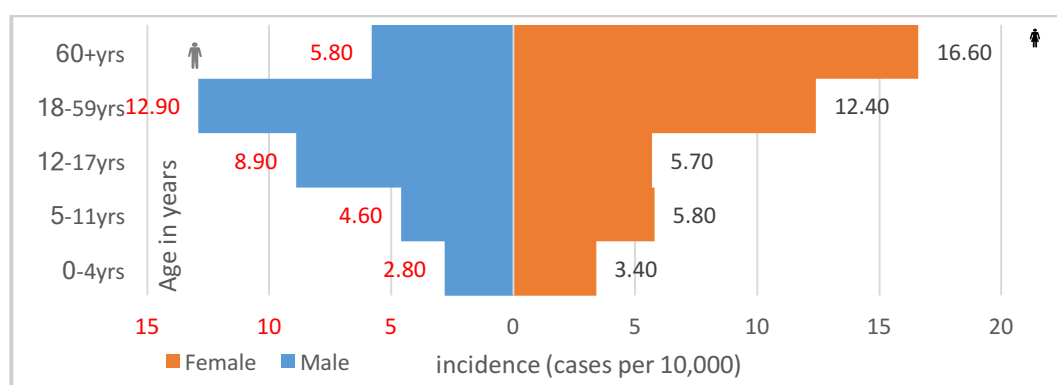


Figure 22 HEV Case Distribution by Age and Sex, Mayom, 2015-2017

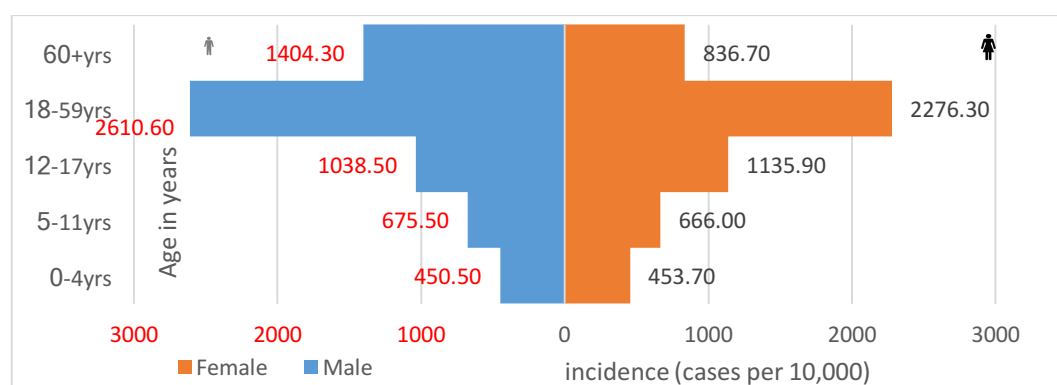


Figure 23 HEV Case Distribution by Age and Sex, Batil, 2012-2013

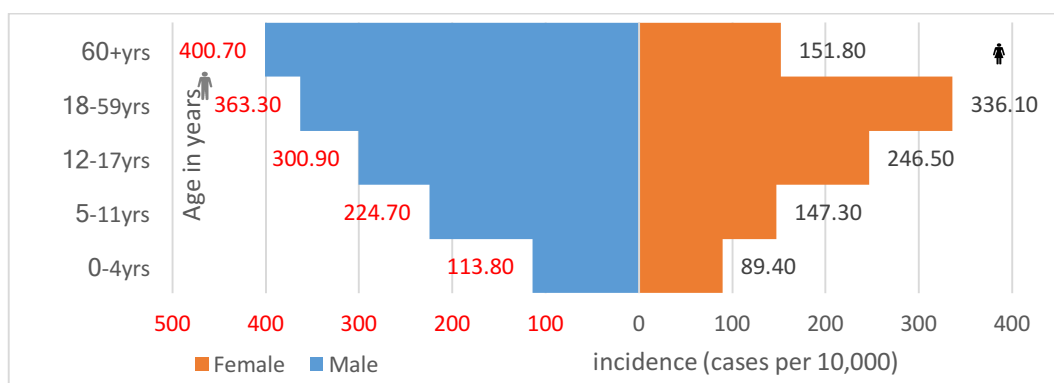


Figure 24 HEV Case Distribution by Age and Sex, Doro, 2012-2015

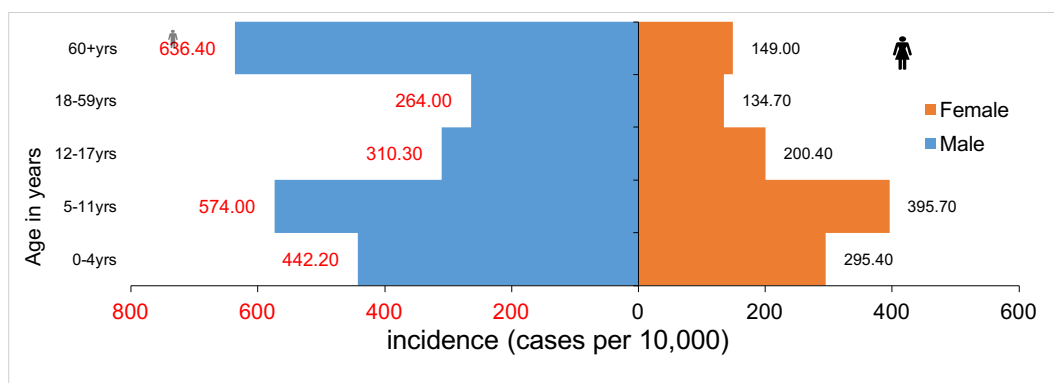


Figure 25 HEV Case Distribution by Age and Sex, Bentiu, 2012-2015

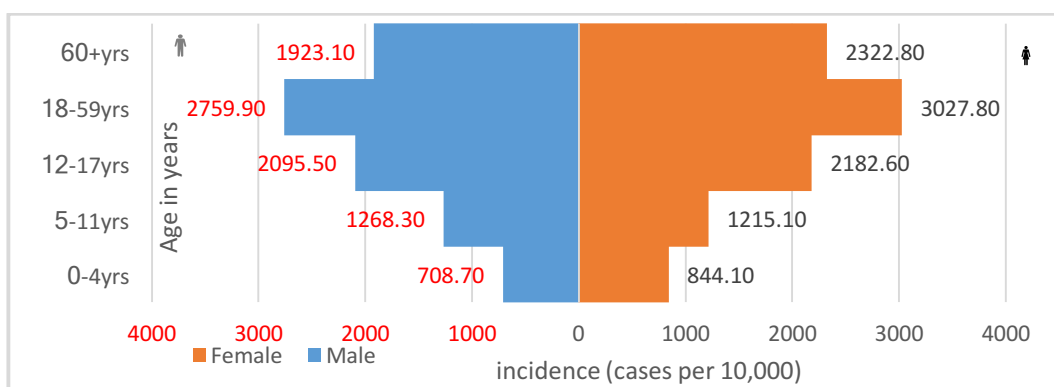


Figure 26 HEV Case Distribution by Age and Sex, Gendrassa, 2012-2013

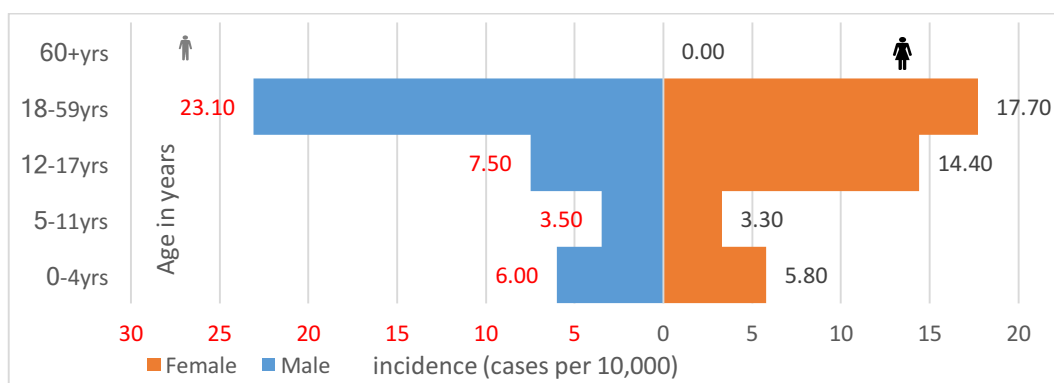


Figure 27 HEV Case Distribution by Age and Sex, Lankien, 2015-2016

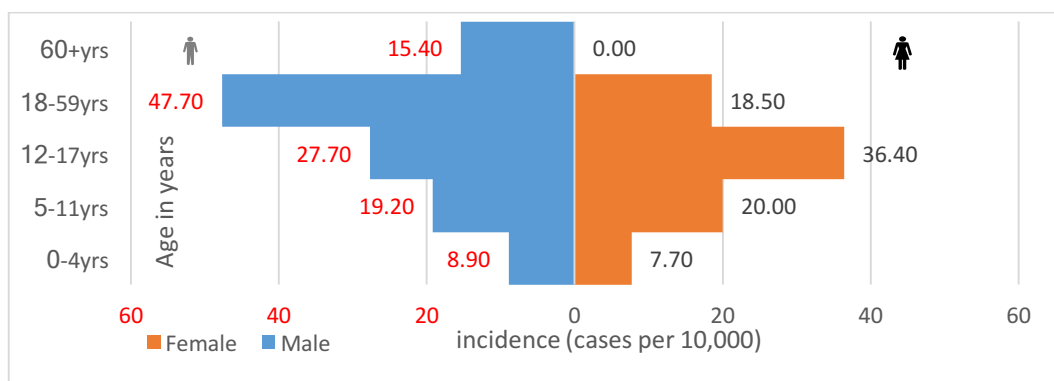


Figure 28 HEV Case Distribution by Age and Sex, Mingkaman, 2014-2015

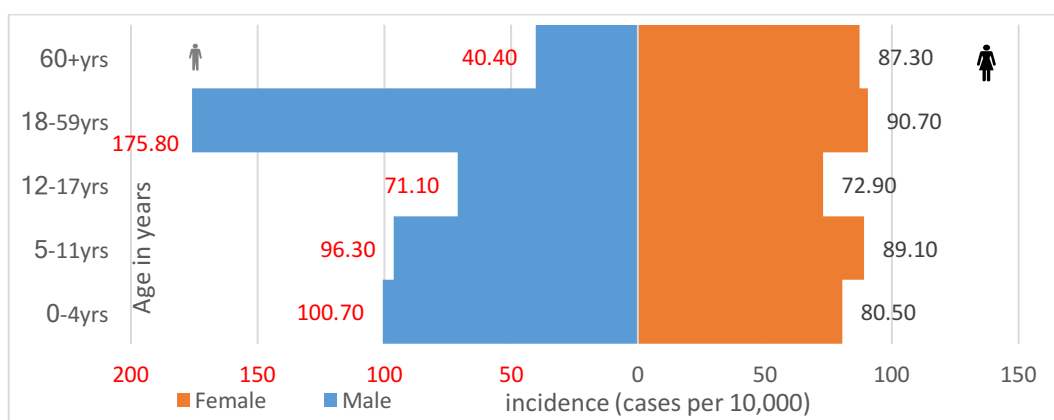


Figure 29 HEV Case Distribution by Age and Sex, Yida, 2012-2014

Laboratory characterization of HEV. Since acute jaundice syndrome is a syndromic case definition that includes many other etiologies besides HEV, blood samples were obtained from suspect HEV cases for laboratory testing to identify and characterize the etiological agent. From January 2012 to April 2017, eight outbreaks of HEV were confirmed positive by rt-PCR in Batil, Doro, Jamam/Gendrassa, and Yida refugee camps, while in the internally displaced populations, Mingkaman, Bentiu PoC, Lankien, and Mayom confirmed HEV cases. The HEV samples from the 2016 outbreak in Bentiu PoC were subjected to genetic sequencing and the results showed they belonged to HEV genotype 1. Further typing of the sequenced samples showed HEV genotype 1 subgenotype 1e was responsible for the South Sudan outbreak. The sequenced South Sudan samples were closely related to the samples collected from Uganda and Chad.

Research Question 2

What is the overall impact of symptomatic HEV disease in relation to the other causes of morbidity and mortality in displaced populations?

To answer this research question, I used the internally displaced populations (IDP) dataset for the period 2013 to early 2017 to determine the relative contribution of HEV disease towards the overall disease occurrence in the IDPs. The proportionate morbidity and mortality rates were computed to determine the top causes of illness and death for all ages and the top causes in the under-fives.

Morbidity disease trends in the IDPs, 2013-2017. Figure 31 shows the top causes of morbidity in IDPs by year during the period 2013 to early 2017. In 2014 and 2017, HEV was the sixth cause of morbidity in this population and accounted for 0.03% and 0.05% of the total consultations respectively (Figure 31).

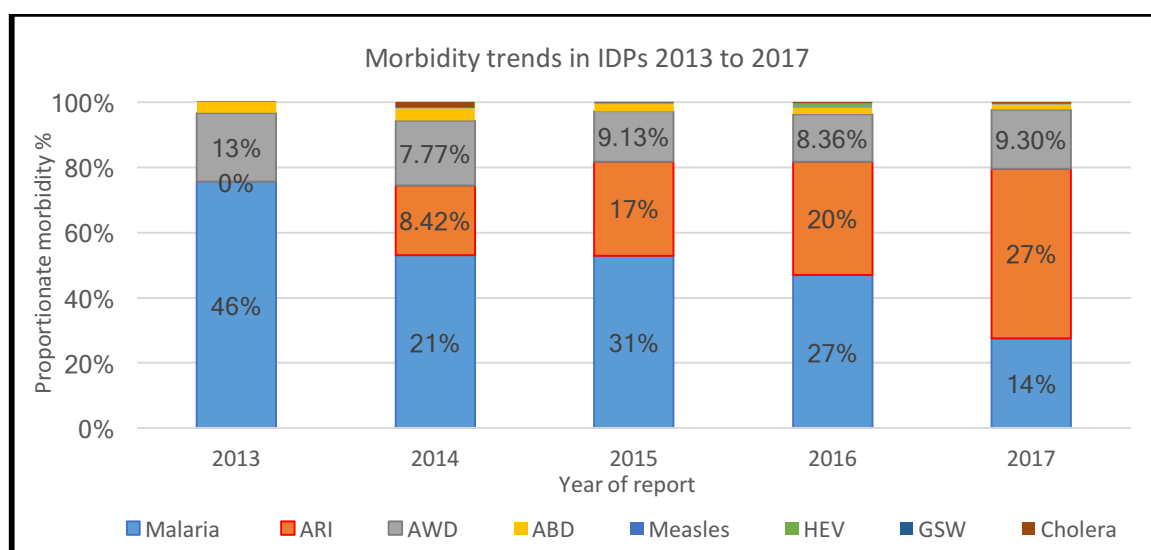


Figure 31 Morbidity Disease Trends in the IDPs, 2013-2017.

Then in 2015 and 2016, HEV was the fifth cause of morbidity in IDPs and accounted for 0.22% and 0.49% of the total consultations respectively (Figure 31).

Overall, HEV accounted for 0.26% of the consultations in IDPs during the period 2013 to 2017 thus making it the fifth cause of morbidity (Figure 32).

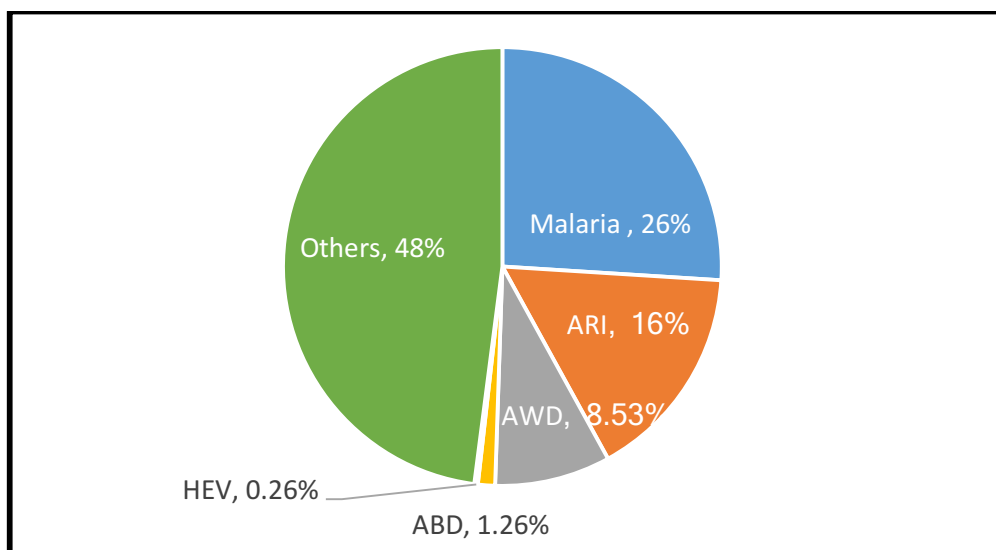


Figure 32 Top Causes of Morbidity in the IDPs Relative to HEV, 2013-2017.

Mortality trends in the IDPs, 2013-2017. Figure 33 presents the top causes of death among the IDPs for the period 2013-2017 for all the age groups. During the period 2013 to 2017, HEV accounted for 6.4% of the total deaths reported among the IDPs. The analyses, therefore, show that HEV the fifth cause of mortality among IDPs for the period 2013-2017 (Figure 33).

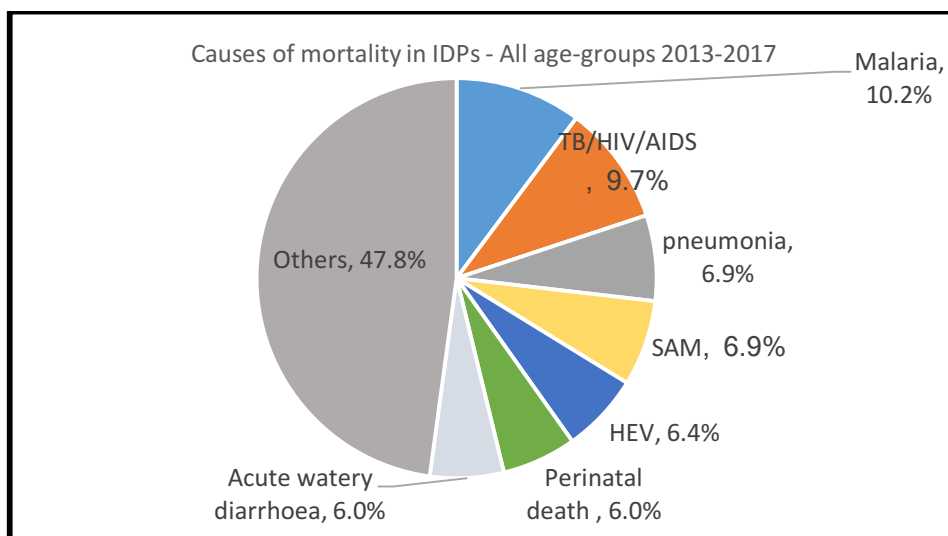


Figure 33 Causes of Mortality in the IDPs (All Age-groups), 2013-2017.

Figure 34 presents top causes of death in IDPs by year from 2013 to 2017 for all the age-groups. The proportionate mortality for HEV was 4% in 2013 but declined to 1% in 2014 before rising to its highest of 13% in 2015, the year when HEV was also the third highest cause of mortality in the IDPs.

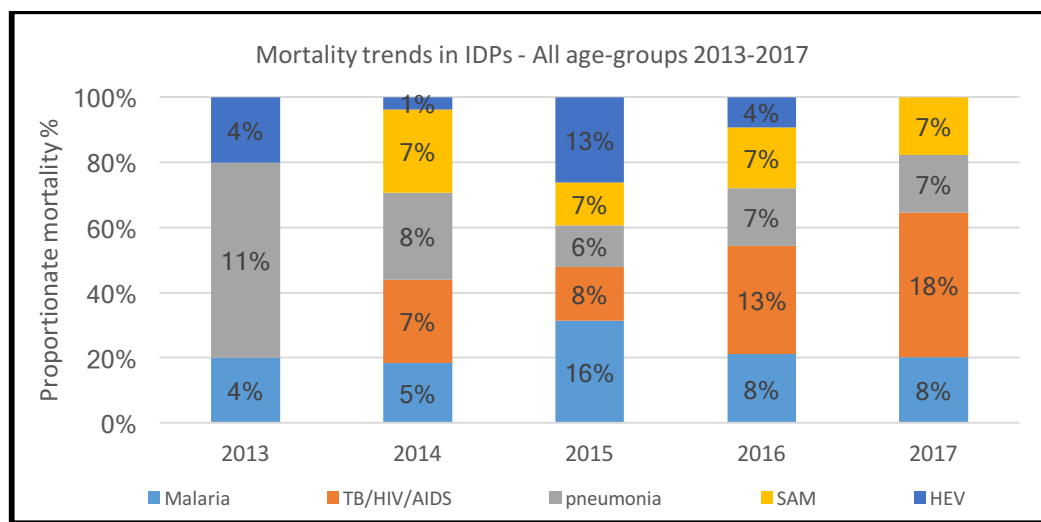


Figure 34 Causes of Mortality in the IDPs by Year (all age-groups), 2013-2017.

The HEV proportionate mortality declined to 4% and 0% in 2016 and 2017 respectively (Figure 34).

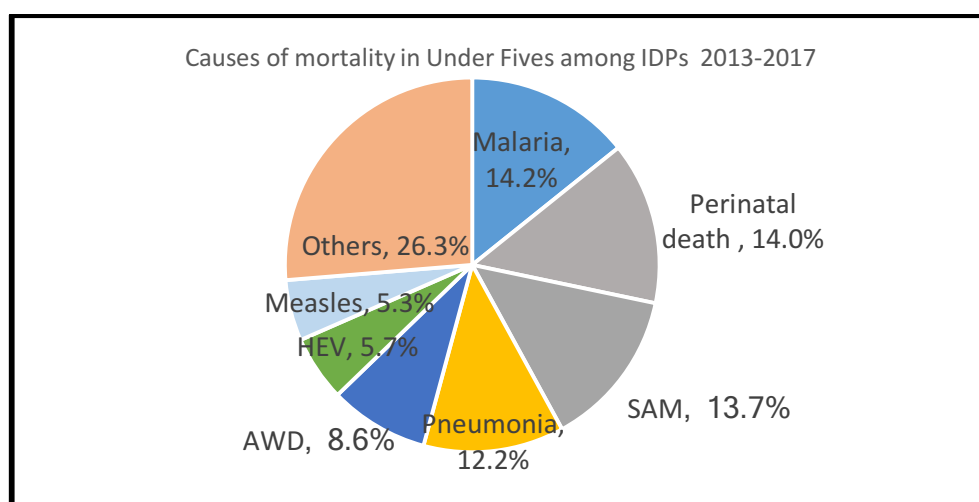


Figure 35 Causes of Mortality in Under-fives Among the IDPs, 2013-2017.

On assessing the IDP mortality trends in the under-fives for the period 2013-2017, HEV had a proportionate mortality of 5.7% thus making it the sixth top cause of death among the under-fives (Figure 35). The highest HEV proportionate mortality of 12% occurred in 2015, the year when HEV was also the sixth highest cause of death in children under five years of age (Figure 36).

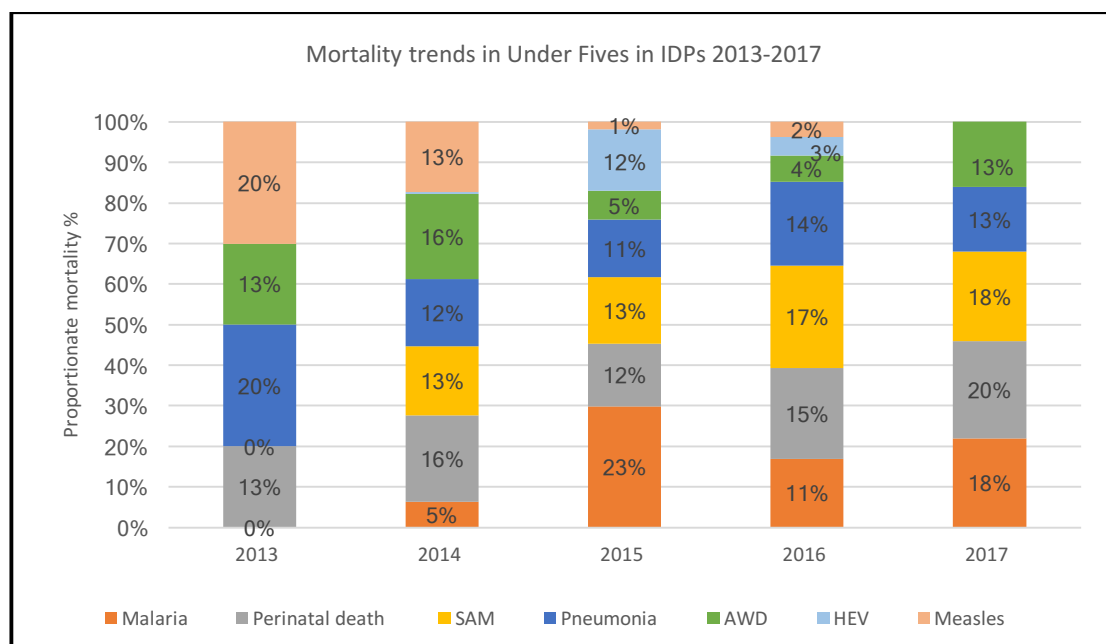


Figure 36 Causes of Mortality in the IDPs by Year (Under-fives), 2013-2017.

Research Question 3

Is there a significant association between demographic, clinical, and epidemiological characteristics and the occurrence of severe symptomatic HEV disease? To answer this research question, I used the HEV dataset from six displaced population sites, namely, Bentiu PoC, Bentiu Town, Doro, Mayom, Lankien, and Mingkaman that had complete data on the main study variables. The demographic variables considered included age, gender, and displacement status. The clinical variables included the

consultation date, the admission date, the discharge date, the date of death, fever, yellow eyes, vomiting, diarrhoea, nausea/loss of appetite, headache, epigastric pain, skin itching, joint pains, the presence of coinfections, mental status, and unexplained bleeding. The epidemiological variables included, travel to affected area, contact with a case, being pregnant or postpartum in females, delay in seeking care, duration of hospital stay, and season of the year. The total number of cases from these six displaced population sites was 4,810 cases of HEV, which falls within the minimum study sample size of 1,372. Of the 4,810 HEV cases, a total of 467 cases had severe symptomatic HEV disease, while 4,343 HEV cases had mild to moderate HEV disease.

The analyses here entailed conducting bivariate analysis using the Pearson chi-square and the unadjusted odds ratio. The researcher used the two tests to identify factors significantly associated with severe symptomatic HEV disease on bivariate analysis. Finally, multivariate analysis was conducted using logistic regression to identify variables significantly associated with severe HEV disease.

Pearson's Chi-square test. A contingency table was constructed to assess factor dependence on severe symptomatic HEV disease. The researcher used Fisher's exact test for cross-tabulations with cell counts of five or less.

The results of the chi-square test are shown in Table 26. The following demographic variables showed a statistically significant association with severe symptomatic HEV disease: age (Pearson χ^2 (5, n=4810) = 74.78, p<0.001), gender (Pearson χ^2 (2, n=4810) = 10.97, p=0.004), and displacement status; (Pearson χ^2 (3, n=4810) = 170.78, p<0.001). A statistically significant association was also established

between severe symptomatic HEV disease and the following clinical variables: mental status (Pearson χ^2 (2, n=4810) = 840.91, p<0.001), nausea/anorexia (Pearson χ^2 (2, n=4810) = 43.82, p<0.001), diarrhoea (Pearson χ^2 (2, n=4810) = 38.22, p<0.001), epigastric pain (Pearson χ^2 (2, n=4810) = 105.01, p<0.001), headache (Pearson χ^2 (2, n=4810) = 64.074, p<0.001), unexplained bleeding (Pearson χ^2 (2, n=4810) = 85.43, p<0.001), skin itching (Pearson χ^2 (2, n=4810) = 50.95, p<0.001), joint pains (Pearson χ^2 (2, n=4810) = 48.21, p<0.001), co-infections (Pearson χ^2 (1, n=4810) = 9.63, p=0.002), females who were pregnant or postpartum (Pearson χ^2 (2, n=4810) = 439.48, p=0.002), outcome (Pearson χ^2 (2, n=4810) = 1625.51, p<0.001), recent travel to an affected area (Pearson χ^2 (2, n=4810) = 74.17, p<0.001), contact with another case (Pearson χ^2 (2, n=4810) = 45.46, p<0.001), and season of the year (Pearson χ^2 (1, n=4810) = 23.70, p<0.001). Similarly, there was a statistically significant association between severe symptomatic HEV disease and the following epidemiological variables: females who were pregnant or postpartum, recent travel to an affected area, contact with another case, and season of the year (Pearson χ^2 (2, n=4810) = 439.48, p<0.001; Pearson χ^2 (2, n=4810) = 1625.50, p<0.001; Pearson χ^2 (2, n=4810) = 74.17, p<0.001; Pearson χ^2 (2, n=4810) = 45.46, p<0.001; and Pearson χ^2 (1, n=4810) = 23.70, p<0.001).

Unadjusted odds ratio. For each of the demographic, clinical, and epidemiological variables significantly associated with severe symptomatic HEV disease based on Pearson's chi-square test, the strength of association was determined using the unadjusted odds ratio (Table 26). Concerning age and with the under 5-years as the reference age-

group, the strength of association between severe symptomatic HEV disease increased with age up to the 18-59-year age-group. The 5-11-year-olds had 0.73 times (95% CI: 0.55 – 0.99) the odds of suffering from severe symptomatic HEV disease than the under 4-year olds. The respective odds of severe symptomatic disease were higher among the 12-17-year-olds (1.50 times (95% CI: 1.07 – 2.10), the 18-59-year-olds (1.99 times (95% CI: 1.55 – 2.57)), and 60-year-olds (1.88 times (95% CI: 1.01 – 3.50) than among the under 5-year-olds. The assessment of the role of gender showed that males had 0.73 times (95% CI: 0.60 – 0.88) the odds of suffering severe symptomatic HEV disease than females. Similarly, the respective odds of severe HEV disease were 0.50 times ((95% CI: 0.41 – 0.62) in the IDPs and 2.59 times (95% CI: 1.86 – 3.61) among cases from the host communities when compared to cases from refugee camps as a reference category. Bivariate analysis for clinical variables showed that severe symptomatic HEV disease was significantly more likely in cases with altered mental status (2.14 times (95% CI: 1.89 – 2.43), nausea/anorexia (1.50 times (95% CI: 1.21 – 1.85), epigastric pain (2.65 times (95% CI: 2.15 – 3.26), headache (0.51 times (95% CI: 0.41 – 0.63), unexplained bleeding (0.45 times (95% CI: 0.37 – 0.56), skin itching (1.45 times (95% CI: 1.13 – 1.87), and coinfections (2.58 times (95% CI: 1.65 – 4.05) than among cases with normal mental status and had no nausea/anorexia, no epigastric pain, no headache, no unexplained bleeding, no skin itching, and no coinfections. Similarly, bivariate analysis for the strength of association of the epidemiological variables showed that severe symptomatic HEV disease was more likely among females who were pregnant or postpartum (12.31 times (95% CI: 8.60 – 17.62), clinical outcome (13.56 times (95% CI: 10.32 – 17.79), contact with another case

(0.64 times (95% CI: 0.50 – 0.83), and season (0.62 times (95% CI: 0.50 – 0.83) than the among cases who were not pregnant, cases who survived the illness, cases that had no contact with another case, and cases that occurred in the dry season.

Table 26

Bivariate Analysis of Characteristics Accounting for Severe HEV Disease in Displaced Populations of South Sudan, 2012-2017.

Variable	Severe HEV disease n=467 (%)	Mild-moderate HEV disease n=4,343 (%)	Chi-square (χ^2); p-value	Unadjusted OR, 95%CI; p-value
Age in years				
<4years*	99 (21.2)	1,147 (26.4)	74.78	
5-11 years	91 (19.5)	1,438 (33.1)	p<0.001	0.73 (0.55-0.99); p=0.04
12-17 years	60 (12.8)	465 (10.7)		1.50 (1.07-2.10); p=0.02
18-59 years	204 (43.7)	1,186 (27.3)		1.99 (1.55-2.57); p<0.01
≥60 years	13 (2.8)	80 (1.8)		1.88 (1.01-3.50); p=0.046
Missing	0 (0)	27 (0.6)		
Gender				
Male	238 (51.0)	1,866 (43.0)	10.97	0.73 (0.60-0.88)
Female*	228 (48.8)	2,467 (56.8)	p=0.004	p=0.001
Missing	1 (0.2)	10 (0.2)		
Displacement status				
Refugee*	151 (32.3)	1,000 (23.0)	170.27	
IDP	240 (51.4)	3,164 (72.9)	p<0.001	0.50 (0.41-0.62); p<0.01
Host community	66 (14.1)	169 (3.9)		2.59 (1.86-3.61); p<0.01
Missing	10 (2.1)	10 (0.2)		
Fever				
Yes	467 (100)	4,339 (99.9)	0.430	
No*	0 (0)	0 (0)	p=0.67	
Missing	0 (0)	4 (0.1)		
Yellow eyes				
Yes	467 (100)	4,343 (100)		
No*	0 (0)	0 (0)		
Missing	0 (0)	0 (0)		
Mental status				
Altered	90 (19.3)	5 (0.1)	840.91	2.14 (1.89-2.43)
Normal*	315 (67.5)	4,053 (93.3)	p<0.001	p<0.01
Missing	62 (13.3)	285 (6.6)		
Vomiting				
Yes	462 (98.9)	4,295 (98.9)	1.41	
No*	0 (0)	11 (0.3)	p=0.49	
Missing	5 (1.1)	37 (0.9)		

Variable	Severe HEV disease n=467 (%)	Mild-moderate HEV disease n=4,343 (%)	Chi-square (χ^2); p-value	Unadjusted OR, 95%CI; p-value
Nausea/anorexia				
Yes	145 (31.0)	1,106 (25.5)	43.82	1.50 (1.21-1.85)
No*	259 (55.5)	2,955 (68.0)	p<0.001	p<0.01
Missing	63 (13.5)	282 (6.5)		
Diarrhoea				
Yes	109 (23.3)	1,066 (24.5)	38.22	1.05 (0.84-1.33)
No*	290 (62.1)	2,987 (68.8)	p<0.001	p=0.66
Missing	68 (14.6)	290 (6.7)		
Epigastric pain				
Yes	258 (55.2)	1,558 (35.9)	105.01	2.65 (2.15-3.26)
No*	159 (34.0)	2,542 (58.5)	p<0.001	p<0.01
Missing	50 (10.7)	243 (5.6)		
Headache				
Yes	264 (56.5)	3,187 (73.4)	64.07	0.51 (0.41-0.63)
No*	145 (31.0)	895 (20.6)	p<0.001	p<0.01
Missing	58 (12.4)	261 (6.0)		
Unexplained bleeding				
Yes	144 (30.8)	2,239 (51.6)	85.43	0.45 (0.37-0.56)
No*	257 (55.0)	1,814 (41.8)	p<0.001	p<0.01
Missing	66 (14.1)	290 (6.7)		
Skin itching				
Yes	86 (18.4)	651 (15.0)	50.95	1.45 (1.13-1.87)
No*	308 (66.0)	3,387 (78.0)	p<0.001	p<0.004
Missing	73 (15.6)	305 (7.0)		
Joint pains				
Yes	160 (34.3)	1,722 (39.7)	48.21	0.93 (0.75-1.14)
No*	234 (50.1)	2,329 (53.6)	p<0.001	p=0.47
Missing	73 (15.6)	292 (6.7)		
Co-infections				
Yes	215 (46.0)	2,327 (53.6)	9.63	2.58 (1.65-4.05)
No*	252 (54.0)	2,016 (46.4)	p=0.002	p<0.01
Missing	0 (0)	0 (0)		
Pregnant and/or postpartum				
Yes	88 (18.8)	62 (1.4)	439.48	12.31 (8.60-17.62)
No*	185 (39.6)	1,604 (36.9)	p=0.002	p<0.01
Missing	194 (41.5)	2,677 (61.6)		
Outcome				
Died	242 (51.8)	116 (2.7)	1625.51	13.56 (10.32-17.79)
Recovered*	176 (37.7)	1,1143 (26.3)	p<0.001	p<0.01
Missing	49 (10.5)	3,084 (71.0)		
Recent travel				
Yes	26 (5.6)	113 (2.6)	74.17	1.26 (0.79-1.98)
No*	174 (37.3)	953 (21.9)	p<0.001	p=0.32
Missing	267 (57.2)	3,277 (75.5)		
Contact with case				
Yes	97 (20.8)	947 (21.8)	45.46	0.64 (0.50-0.83)
No*	210 (45.0)	1,317 (30.3)	p<0.001	p=0.01

Variable	Severe HEV disease n=467 (%)	Mild-moderate HEV disease n=4,343 (%)	Chi-square (χ^2); p-value	Unadjusted OR, 95%CI; p-value
Missing	160 (34.3)	2,079 (47.9)		
Delay in seeking care				
5+days	163 (34.9)	1,629 (37.5)	3.17	0.87 (0.71-1.07)
<5days*	284 (60.8)	2,472 (56.9)	p=0.21	p=0.18
Missing	20 (4.3)	242 (5.6)		
Season				
Wet	272 (58.2)	3,009 (69.3)	23.70	0.62 (0.51-0.75)
Dry*	195 (41.8)	1,334 (30.7)	p<0.001	P<0.01
Missing	0 (0)	0 (0)		

*reference category

Logistic Regression. As part of the multivariate analysis to identify demographic, clinical, and epidemiological variables associated with severe symptomatic HEV disease, logistic regression analysis using the stepwise approach was conducted to build the logistic regression model.

Following bivariate analysis; age, gender, displacement status, mental state, nausea/anorexia, epigastric pain, headache, unexplained bleeding, skin itching, co-infections, pregnant/ postpartum, outcome, contact with a case, and the season had a statistically significant association with severe HEV disease.

Using the stepwise approach to build the logistic regression model; we initially included all the independent variables that had a statistically significant association with severe HEV disease on bivariate analysis ($p < 0.05$). The researcher eliminated all variables with logistic regression-derived p value greater than 0.25 from the model at this stage. Additional variables with a p value of at least 0.15 at bivariate analysis were then introduced one at a time, with only those with a statistically significant association

remaining in the model. The major confounding variables like age and gender stayed in the final model. Table 27 shows the final model.

Table 27

Multivariate Analysis of Characteristics Associated with Severe HEV Disease in Displaced Populations of South Sudan, 2012-2017.

	B	Wald	Sig.	Exp(B)	95% C.I. for EXP(B)	
					Lower	Upper
Age in years						
5-11 years	-.202	.761	.383	.817	.519	1.286
12-17 years	.345	1.502	.220	1.413	.813	2.454
18-59 years	.287	1.482	.223	1.333	.839	2.117
≥60 years	.847	2.533	.111	2.332	.822	6.618
Male gender	.106	.397	.529	1.112	.799	1.548
Abnormal mental state	6.462	128.363	.000	640.236	209.345	1958.021
Nausea/anorexia	.965	20.415	.000	2.626	1.727	3.991
Epigastric pain	.610	8.865	.003	1.840	1.232	2.748
Headache	-.726	13.112	.000	.484	.327	.717
Skin itching	.678	7.264	.007	1.971	1.203	3.228
Co-infections	1.147	7.708	.005	3.149	1.401	7.077
Pregnant or postpartum	2.827	93.914	.000	16.902	9.541	29.942
Outcome as died	3.334	103.733	.000	28.055	14.769	53.292
Wet season	-1.118	41.253	.000	.327	.232	.460
Constant	-2.562	66.993	.000	.077		

Assumptions for conducting logistic regression. Before presenting analyses from logistic regression, the assumptions are reviewed to ensure compliance and reliability of derived estimates. Logistic regression does not assume a linear relationship between the dependent and independent variables. However, the relationship between continuous independent variables and their log odds should be linear. This relationship is usually tested using Box-Tidwell test that entails including in the model, interactions

between the continuous independent variables and their respective natural logs. If such an interaction is significant, then the assumption has been violated. In the current study, there were no continuous independent variables in the model, and therefore the Box-Tidwell test was not undertaken.

The other assumption for logistic regression states that the dependent variable must be a dichotomous variable. In the present study, the dependent variable was severe HEV disease, with two mutually exclusive categories; severe HEV disease and mild to moderate HEV disease. The selection of a categorical variable as the dependent variable, therefore, ensured study conformity to this assumption.

It is also stated that the independent variables need not be interval, nor normally distributed, nor linearly related, nor of equal variance within each group. As part of the study, we used categorical variables for the multivariate analysis. The selected variable type, therefore, complied with the assumption requirements for multivariate analysis.

Multivariate analysis with logistic regression also requires that categories (groups) must be mutually exclusive and exhaustive; that a case belongs only to one group and every case must be a member of one of the categories or groups. To ensure that this assumption was not violated, all variable options were coded into mutually exclusive categories or groups and in addition, all missing values were coded as missing to ensure that every case belonged to at least one group.

Logistic regression also requires larger samples than for linear regression because the maximum likelihood coefficients are large sample estimates. Logistic regression analysis requires a minimum of 50 cases per independent variable. In the present study,

all independent variables included in the final model attained the minimum number of cases needed for logistic regression.

Logistic regression results. The model chi-square was used to assess the overall significance of the final model. The model chi-square in the present study had 23 degrees of freedom, a value of 1598.83 and a probability of $p < 0.001$. The Nagelkerke's R^2 , a proxy indicator of the variation of the dependent variable that is explained by the logistic model was 64.8%, indicating a moderately strong relationship between the independent variables and the occurrence of severe HEV disease. The Hosmer and Lemeshow statistic tests the fit of the model. From the present study, the Hosmer and Lemeshow statistic had 8 degrees of freedom, a χ^2 of 14.45 and was not statistically significant with a p value of 0.071 which means that the model is a good fit. Overall, the model predicted 95% of the variation in the dependent variable with the prediction being 98.1% in the category with mild to moderate HEV disease and 64.3% for the group with severe HEV disease. Of the 4,810 HEV cases with data on the essential demographic, clinical, and epidemiological variables, 4,523 (94%) were included in the logistic regression analysis.

The variables entered into the model in the first step were age, gender, displacement status, mental state, nausea/anorexia, epigastric pain, headache, unexplained bleeding, skin itching, co-infections, pregnant/ postpartum, outcome, contact with a case, and season had a statistically significant association with severe HEV disease. After running the model, two variables, displacement status and unexplained bleeding had p values greater than 0.25 and were therefore eliminated from the model.

There were no additional variables from the bivariate analysis with statistically insignificant p values of up to 0.15.

As seen from Table 27, the results of the logistic regression analysis showed that cases with severe HEV disease were significantly more likely to have an altered mental status than cases with mild to moderate HEV disease (Adjusted odds ratio [aOR], 640.24, 95% CI: 209.35-1958.02). Cases with severe HEV disease were also significantly more likely to present with nausea/anorexia than cases with mild to moderate HEV disease (aOR 2.63, 95% CI: 1.73-3.99). Additionally, cases with severe HEV disease were significantly more likely to have epigastric pain than cases with mild to moderate HEV disease (aOR 1.84, 95% CI: 1.23-2.75). On the other hand, cases with severe HEV disease were significantly less likely to have headache than cases with mild to moderate HEV disease (aOR 0.48, 95% CI: 0.33-0.72). Cases with severe HEV disease were also significantly more likely to report skin itching than cases with mild to moderate HEV disease (aOR 1.97, 95%CI: 1.20-3.23). The analyses also showed that cases with severe HEV disease were significantly more likely to have co-infections than cases with mild to moderate HEV disease (aOR 3.15, 95% CI: 1.40-7.08). In the same way, cases with severe HEV disease were significantly more likely to occur in pregnant or postpartum females than cases with mild to moderate HEV disease (aOR 16.90, 95% CI: 9.54-29.94). In terms of clinical outcomes, cases with severe HEV disease were significantly more likely to die than those with mild to moderate HEV disease (aOR 28.06, 95% CI: 14.77-53.29). With regards to case occurrence by season, cases with severe HEV disease were significantly less likely to occur in the wet season than cases with mild to moderate HEV disease (aOR 0.33, 95%CI:

0.23-0.46). Based on the Wald test in this model, the following variables, in order of importance, were significantly associated with severe HEV disease: (a) altered mental status, (b) death as an outcome, (c) pregnant or postpartum females, (d) wet season, (e) nausea/anorexia, (f) headache, (g) epigastric pain, (h) co-infections, and (i) skin itching (Table 27).

Summary

Cumulative incidence and mortality rates were used in this chapter, to describe HEV seasonality, laboratory characterization, and case distribution by time, place, and person. The spatial-temporal SatScan statistic was used to identify HEV outbreak clusters. Correlation and simple linear regression analyses were undertaken to determine the association between precipitation and HEV cases. The proportionate morbidity and mortality rates were used to assess the overall impact of HEV disease in displaced populations. Finally, bivariate and multivariate logistic regression analyses were performed to identify factors for severe symptomatic HEV disease in displaced populations of South Sudan.

The descriptive analyses showed that from 2012 to early 2017, a total of 14,404 HEV cases including 571 deaths (CFR 3.96%) occurred in the nine displaced population camps of South Sudan. During this period, most cases were reported from Batil and Jamam/Gendrassa refugee camps while the highest case fatality rates of 34% and 8.5% respectively occurred in Mayom and Bentiu PoC respectively. Males constituted 51% of HEV cases, and 18-59-year-olds represented 50% of total HEV cases reported during the period.

The cumulative incidence and mortality rates were computed and used to describe HEV outbreaks, their seasonality, duration, and distribution by time, place, and person. Also, the spatial-temporal SatScan statistic was used to identify HEV outbreak clusters. Transmission of HEV occurred in all the weeks of the period January 2012 to April 2017 with four peaks reported during this period. Two of the peaks occurred in the last two months of the rainy season with the highest peak taking place in the dry season but followed from a shorter peak registered in the rainy season. Correlation and simple linear regression analyses showed that the strength of association between precipitation and HEV cases was low or insignificant for each of the eight displaced population sites. From 2012 to 2017, a total of eight HEV outbreaks occurred in displaced populations of South Sudan with 50% of the outbreaks occurring in refugee camps. The median duration of HEV outbreaks was 96.5 weeks and an interquartile range of 62.25 weeks. The retrospective space-time SatScan statistic identified four HEV outbreak clusters in Bentiu PoC, Yida, Mingkaman, and Lankien but these reduced to two clusters in Maban (Batil, Doro, Jamam/Gendrassa) and Rubkona (Bentiu) after adjusting for the population using the spatial Poisson SatScan statistic. The HEV cumulative incidence and mortality per 10,000 showed that Jamam/Gendrassa, Batil, and Bentiu PoC were the most affected during the period 2012 to 2017. The gender-specific cumulative incidence and mortality showed that the risk of HEV infection and death was higher in males than females. On the other hand, the age-specific cumulative incidence and mortality rates demonstrated that the risk of HEV infection and death was highest in the 18-59-year and 60+year age groups. The age-specific HEV incidence increased with age even after adjusting for sex in all the displaced populations

with 18-59 year and 60+ year age groups being the most affected. The overall HEV cumulative incidence cases per 10,000 increased from 90,917 in 2012 to 173,731 and 202,770 in 2013 and 2014 respectively before reaching the highest peak of 401,212 in 2015. The HEV cumulative declined after that. Laboratory characterization showed that HEV genotype 1 subtype 1e caused the South Sudan outbreaks and these strains were closely related to sequences from Uganda and Chad.

For the second research question, we computed the proportionate morbidity and mortality trends, to assess the overall impact of HEV disease in displaced populations. During the period 2013 to early 2017, HEV accounted for 0.26% of the total consultations and 6.4% of the deaths thus making it the fifth highest cause of morbidity and mortality in the IDPs. During the same period, HEV accounted for 5.7% of the deaths in the under-fives thus making it the sixth highest cause of death among the under-fives living in IDP populations.

Bivariate and multivariate logistic regression analyses were performed to identify the factors for severe HEV disease in displaced populations of South Sudan. Bivariate analyses, showed a statistically significant association between severe HEV disease and the following variables (a) age, (b) gender, (c) displacement status, (d) mental state, (e) nausea/anorexia, (f) epigastric pain, (g) headache, (h) unexplained bleeding, (i) skin itching, (j) co-infections, (k) pregnant/ postpartum, (l) outcome, (m) contact with a case, and (n) season. Logistic regression using the stepwise strategy was then used to determine the independent contributions of each of the factors in the model. On running the model, the logistic regression analysis included 94% of HEV cases and the final model predicted

95% of the variation in the dependent variable, with a Nagelkerke R^2 of 64.8%. The final model showed that the following variables, in order of importance, were significantly associated with severe HEV symptomatic disease (a) altered mental status, (b) death as an outcome, (c) pregnant or postpartum females, (d) wet season, (e) nausea/anorexia, (f) headache, (g) epigastric pain, (h) coinfections, and (i) skin itching.

Chapter 5, presents the interpretation of these findings based on the existing body of literature on HEV distribution and factors for severe symptomatic HEV disease. Furthermore, chapter five presents the limitations of the study. Also presented in the chapter are the recommendations for future studies and the implications for positive social change. The chapter ends with a conclusion.

Chapter 5: Discussion, Conclusions, and Recommendations

Introduction

The present study was a quantitative, nested retrospective cohort design involving 14,404 cases of Hepatitis E Virus in displaced populations of South Sudan from 2012 to early 2017. The study was intended to describe the trends and determine the impact of HEV outbreaks in displaced populations of South Sudan. The study used the host-agent-environment model to identify demographic, clinical, and epidemiological factors associated with severe symptomatic HEV disease in displaced populations of South Sudan. Cumulative incidence and mortality rates were computed to describe the distribution of HEV cases by person, time, and place. The retrospective space-time permutation scan statistic was used to detect HEV outbreak clusters. Proportionate morbidity and mortality rates were computed to determine the impact of HEV in displaced populations. The chi-square test, crude odds ratio, and logistic regression analyses were conducted to identify characteristics associated with severe symptomatic HEV disease.

While the impact of HEV has reduced globally, significant transmission continues in sub-Saharan Africa where displaced populations have emerged as a vulnerable group (Isaacson et al., 2000; E. H. Teshale, Howard, et al., 2010a; Boccia et al., 2006b; Thomson et al., 2013; Ahmed et al., 2013b; Browne et al., 2015; MSF USA, 2017; Azman et al., 2016; Stanaway et al., 2016; MSF, 2017; Anonymous, 2017a; WHO, 2017). These trends highlight the need to optimize HEV control interventions to avert potentially high morbidity and mortality rates in displaced populations. WHO has recommended HEV

vaccines as an additional and complementary tool to existing interventions (WHO, 2015b). However, the paucity of data on detailed characterization of HEV outbreaks in high-risk groups like displaced populations precludes the effective deployment of HEV vaccines. This study, therefore, set out to address this gap in literature to facilitate the effective and complementary use of HEV vaccines for an optimized response for reduced morbidity, mortality, and duration of HEV outbreaks in displaced populations.

A total of 14,404 HEV cases, including 571 deaths (CFR 3.96%), were reported from nine displaced population camps of South Sudan between 2012 and early 2017. Hepatitis E transmission occurred throughout the study period with at least four transmission peaks, two of which took place in the last two months of the rainy season in 2012 and 2015. However, correlation and simple linear regression analyses showed no significant association between precipitation and HEV cases. Based on the standard HEV case definition, eight HEV outbreaks occurred from 2012 to early 2017. The retrospective space-time permutation scan statistic identified four HEV outbreak clusters in Bentiu PoC, Yida, Mingkaman, and Lankien and these reduced to two in Maban (Batil, Doro, and Jamam/Gendrassa) and Rubkona (Bentiu) after adjusting for population using the spatial Poisson SatScan statistic. The median duration of HEV outbreaks was 96.5 weeks with an interquartile range of 62.25 weeks. The most affected camps were Jamam/Gendrassa, Batil, and Bentiu PoC while the risk of HEV infection and death was higher in males than females. On the other hand, the risk of HEV infection and death was highest in the 18-59-year and 60+year age groups. The risk of HEV infection increased with age even after adjusting for sex in all camps with persons aged 18-59 year and 60+years remaining the

most affected. HEV genotype1 subtype 1e caused the South Sudan outbreaks, and these strains were closely related to sequences from Uganda and Chad. On assessing the overall impact of HEV, HEV was the fifth highest cause of morbidity and mortality while among the under-fives, HEV was the sixth highest cause of mortality in IDP populations. Multivariate analysis using logistic regression showed a statistically significant association between severe symptomatic HEV disease and the following characteristics (a) altered mental status, (b) death as an outcome, (c) pregnant or postpartum females, (d) wet season, (e) nausea/anorexia, (f) headache, (g) epigastric pain, (h) coinfections, and (i) skin itching.

Interpretation of the Findings

Displaced populations in sub-Saharan Africa continue to register an increasing and disproportionately high burden of HEV. The affected countries include Namibia, Darfur, Kenya, South Sudan, Ethiopia, Uganda, and more recently in the Lake Chad basin involving Niger, Nigeria and Chad (Isaacson et al., 2000; Guthmann et al., 2006; Ahmed et al., 2013b; Thomson et al., 2013; Browne et al., 2015; Cummings et al., 2014; Patel et al., 2015; MSF USA, 2017; Azman et al., 2017; MSF, 2017; Anonymous, 2017a; WHO, 2017). In the current study, Hepatitis E transmission occurred throughout the study period (2012-2017) with at least four transmission peaks, two of which took place in September and October, the last two months of the rainy season in 2012 and 2015. The 2012 peak coincided with the transmission in two refugee camps (Batil and Jamam/Gendrassa) in Maban county. However, the highest transmission peak occurred in January 2013 during the dry season and was a continuation of the September-October 2012 peak in Batil, Jamam, Gendrassa, and Doro refugee camps. The other two peaks all occurred in 2015,

with the highest occurring during the wet season in August 2015 with most transmission occurring in Bentiu PoC. The initial outbreak investigation conducted by the South Sudan MoH, UNHCR, and the CDC in Batil, Jamam, and Gendrassa refugee camps, showed that the September-October 2012 transmission peak coincided with the rainy season and was associated with floods (Thomson et al., 2013).

HEV outbreaks in developing countries, where the waterborne HEV genotypes 1 and 2 are the predominant strains, usually start during the rainy season. The risk of outbreaks in these settings is accentuated by flooding and increased contamination of unprotected water sources (Hughes, Wilson, Teshale, Hu, & Holmberg, 2010; Yugo & Meng, 2013). Two descriptive HEV case series reports involving displaced populations in Northern Uganda, and Gambella in Ethiopia reported peak transmission during the rainy season (Browne et al., 2015; E. H. Teshale, Howard, et al., 2010a). This trend is consistent with fecal oral transmission of HEV, the predominant mode of HEV transmission in developing countries where the main HEV genotypes are 1 and 2. In the present study, genotyping of samples from Bentiu PoC showed that HEV genotype 1 was responsible for the outbreaks. Since water is the primary reservoir for HEV genotype 1, improving access to safe water remains critical to HEV prevention in displaced populations. In the same way, the seasonal transmission of HEV highlights the need to launch interventions to reduce host susceptibility to HEV infection before the onset of the rain season.

Beyond the qualitative assessments of the association between the occurrence of HEV cases and the rainy season, the present study assessed the relationship between precipitation and HEV cases. While HEV cases were noted to rise and peak during months

with peak precipitation, correlation and simple linear regression analyses showed no significant association between precipitation and HEV cases. These findings therefore suggest that contrary to reports from related studies in developing countries, rainfall was not a significant predictor of HEV case trends for the South Sudan outbreaks. In fact, for sites like Batil, Gendrassa, and Yida, considerable transmission peaks occurred in the dry season. It is therefore highly likely that congestion and suboptimal access to safe water and sanitation contributed significantly as drivers since most transmission occurred during the acute phase of displacement. However, the present study did not assess these variables since they were missing from the secondary datasets.

During the present study, data from eight HEV outbreaks in displaced populations of South Sudan from 2012 to 2017 was analyzed to determine the timing of outbreak onset and duration. This information is critical for gauging the effectiveness of current toolkit of interventions for HEV control. The present study showed that 5 (63%) of the outbreaks started in the dry season with the rest starting in the rainy season. However, following the retrospective space-time permutation SatScan analysis, only four HEV outbreak clusters were detected in Bentiu PoC, Yida refugee camp, Mingkaman IDP settlement, and Lankien IDPs with 3 (75%) starting the rainy season. Further analysis for outbreak clusters after adjusting for the underlying population at risk using the spatial Poisson model revealed only two outbreak clusters in Maban (Batil, Doro, and Jamam/Gendrassa) and Rubkona (Bentiu PoC) where the HEV outbreaks initially peaked in the rainy season. The declining number of detected outbreaks is because the population adjusted SatScan statistics are more precise and specific than the non-population adjusted statistic.

In the present study, the outbreak duration varied from 47-142 weeks with a median duration of 96.5 weeks and an interquartile range of 62.25 weeks. These findings, therefore, highlight the fact that the current toolkit of interventions for HEV control, namely, improving access to safe drinking water, sanitation, and personal and environmental hygiene, are not as effective for securing timely control of HEV outbreaks in displaced populations. Indeed the 2007 HEV outbreak among the IDPs in Northern Uganda persisted for over two years (E. H. Teshale, Howard, et al., 2010a). The effective deployment of HEV interventions should avert the excess HEV cases and deaths reported during the protracted course of the outbreaks.

The risk of HEV infection and death by displaced population and year was analyzed to characterize the outbreaks. The cumulative incidence of HEV increased from 2012 reaching its highest peak in 2013 when most of the transmission was attributed to outbreaks in the three refugee camps in Maban county (Jamam, Gendrassa, Batil, and Doro). This spike in transmission was precipitated by overcrowding and flooding that was most severe in Jamam refugee camp (Thomson et al., 2013). The second but lower peak of HEV transmission occurred during the rainy season of 2015 with most of the cases reported from Bentiu PoC IDP camp. These trends further highlight the link between the onset of rainy season and the increased risk of HEV transmission in displaced populations. These trends are typical of HEV outbreaks in developing countries where HEV genotypes 1 and 2 are predominant and where rains and especially if associated with floods lead to contamination of unprotected open water sources thus increasing the risk of HEV transmission (E. H. Teshale, Howard, et al., 2010b; Kamar et al., 2014). In the current study, Batil, Jamam and

Gendrassa refugee camps that had the highest HEV cumulative attack rates were affected by floods in 2013 (Thomson et al., 2013). During the 2013 HEV outbreak among South Sudan refugees in Gambella, Ethiopia, Bowne et al. (2014) reported peak transmission during the rainy season of June to September 2014. In the same way, the 2007 outbreak among IDPs in Northern Uganda started and reached its highest peak in the wet season (E. H. Teshale, Howard, et al., 2010b). These findings suggest that launching the HEV toolkit before the onset of the rainy season may potentially avert a significant number of cases.

The current study showed that the risk of death was highest in 2012 with most of the deaths occurring in Jamam, Gendrassa, and Batil refugee camps which at the time were going through the acute phase of the refugee crisis after armed conflict broke out in Blue Nile state, Sudan in late 2011 (Thomson et al., 2013). As seen in the present study, displaced populations are very vulnerable to disease and suffering excess mortality (Heudtlass, Speybroeck, & Guha-Sapir, 2016). As a result, the risk of disease outbreaks like HEV is high, and these tend to be associated with high attack rates and an excess risk of death (Boccia et al., 2006b; E. H. Teshale, Howard, et al., 2010b). A serosurvey undertaken in displaced populations in Juba South Sudan in 2015 showed unusually high age-adjusted HEV IgG seroprevalence of 71%, 95%CI 63-78 in a population where symptomatic cases had been reported previously (Azman et al., 2017). These findings suggest that significant HEV transmission goes on undetected in displaced populations. During the present study, HEV mortality peaked during the rainy season of 2015. Most of these HEV deaths occurred in Bentiu PoC where at the time, the under-five mortality rates exceeded the emergency threshold of 2 deaths per 10,000 per day (WHO and MoH, 2015).

These findings show that HEV is a significant cause of mortality in displaced populations thus justifying the need to prioritize and augment current interventions.

The present study assessed the risk of HEV infection and death (as cases and deaths per 10,000) by gender. Overall, the study showed that the risk of HEV infection and death was higher males (590.8 vs 23.1) than the females (487.3 vs 20). These findings are consistent with studies in highly endemic developing countries in Africa and Asia where the gender-specific risk of HEV infection is associated with higher risk of exposure to contaminated water, the common vehicle for HEV transmission in these settings (Li et al., 2013; Kamar et al., 2014). In a study conducted in North Eastern Uganda, Cummings et al. (2014) reported a higher HEV gender-specific attack rate in males than females. Similarly, a meta-analysis into the primary risk factors for HEV infection reported a higher risk of HEV infection in males than females (Li et al., 2013). The observed gender related predilections for HEV most probably relate to gender-specific practices that increase the risk of exposure to contaminated water and/or the risk of developing symptomatic disease. Follow up studies ought to evaluate the gender differences in exposure and risk of symptomatic HEV disease.

The present study computed the age-specific cumulative incidence and mortality rates. The findings from the present study showed that the risk of HEV infection and death were highest among the 18-59-year-olds and in the elderly 60+years. These results are consistent with earlier investigations into the HEV outbreak in the refugee camps of Maban in 2013 during which adults aged 18-59 years registered the highest attack rates (Thomson et al., 2013). Similar findings were reported by Guthmann et al. (2006) during the 2004

HEV outbreak in internally displaced persons in Darfur. During the outbreak, adults aged 15-45 years had 2.13 times (95%CI, 1.02-4.46) the odds of HEV disease than adults older than 45 years (Guthmann et al., 2006). In the same way, a prospective acute jaundice surveillance system implemented in Northern Uganda from 2010 to 2012 showed that serological evidence of HEV infection was highest in adults aged 15-49 years (Gerbi et al., 2015a). Anicteric HEV disease has been reported in children in developing countries with the proportion increasing to over 50% in children 11-15 years (Patel et al., 2015). Clinical diagnosis of cases may therefore grossly underestimate the burden of HEV disease in children and adolescents whose risk of anicteric HEV disease increases with age (Patel et al., 2015). Indeed, the recent findings among IDPs in Juba showed the age-adjusted prevalence of HEV IgG was 71% in a population with no symptomatic cases (Azman et al., 2017). These results suggest that asymptomatic HEV infections may be high even in older age groups.

The current study assessed the overall impact of symptomatic HEV disease. The findings showed that from 2013 to 2017, HEV was the fifth cause of morbidity and mortality in internally displaced populations. However, in children under five years of age, HEV was the sixth cause of death in internally displaced populations. Epidemiological trends in displaced populations show that malaria, acute respiratory infections, and acute watery diarrhoea, measles, and malnutrition are the top causes of morbidity (Gessner, 1994; Owoaje, Uchendu, Ajayi, & Cadmus, 2016; Chelwa, Likwa, & Banda, 2016; Warsame, Chomi, & Ngwatu, 2016). The disease trends in IDPs from the present study were consistent with this pattern with malaria being the top cause of morbidity, followed by

acute respiratory tract infections, and acute watery diarrhoea. The present study thus demonstrated that HEV is a significant cause of morbidity in affected displaced populations since it ranked as the fifth.

Regarding mortality, the present study showed that malaria, Tuberculosis and HIV/AIDS, pneumonia, and medical complications of severe acute malnutrition, and HEV were the top causes of mortality in internally displaced populations. Thus, the findings from the present study show a trend that is consistent with other displaced populations in Afghanistan, Zambia, and other locations where malaria, acute watery diarrhoea, acute respiratory infections, and measles are the top causes of mortality (Chelwa et al., 2016; Gessner, 1994; Owoaje et al., 2016). Among the under-fives, the present study showed that the top causes of mortality were malaria, perinatal complications, medical complications of severe acute malnutrition, pneumonia, acute watery diarrhoea, and HEV. In the under-fives, malaria still comes on top with the other significant causes of mortality being pneumonia, acute watery diarrhoea, and medical complication of acute malnutrition. These are the common illnesses reported in under-fives in vulnerable displaced populations (Gessner, 1994; Chelwa et al., 2016). However, HEV was ranked sixth as a common cause of mortality in the under-fives. It is possible that HEV deaths in this age-group were under reported since HEV cases in this age-group tend to be asymptomatic (Patel et al., 2015).

The present study used the host-agent-environment model to identify demographic, clinical, and epidemiological factors associated with symptomatic HEV disease in displaced populations of South Sudan. The model posits that the risk of an infectious disease is determined by an interplay of a combination of factors in the infectious agent

and appropriate environment that favors survival and transmission of an infectious agent to a susceptible human host through a suitable portal of entry (Gordis, 2008; McLeroy et al., 1988). The current study used the host-agent-environment model to identify demographic, clinical, and epidemiological factors associated with severe symptomatic HEV disease.

Age is one of the demographic host characteristics associated with HEV infection and symptomatic disease. Bivariate analyses conducted in the present study showed that the crude odds ratio for developing symptomatic severe HEV disease increased with age from adolescents aged 12-17 years to adults aged 18-59-years. Bivariate analysis also showed that odds of severe symptomatic HEV disease were higher in the elderly ≥ 60 years than adolescents 12-17 years. As part of the initial outbreak investigations into the HEV outbreak in Maban, Upper Nile, South Sudan, the risk of symptomatic HEV disease was highest in adults 18-59 years of age (Thomson et al., 2013). In an earlier study conducted by Guthmann et al. (2006) in a displaced population of Darfur, the risk of HEV symptomatic disease was highest in adolescents and young adults aged 15-45 years. The findings show that HEV disease risk in developing countries increases with age with adolescents and adults having the highest risk of symptomatic HEV disease (Labrique et al., 2013b; Cummings et al., 2014; Chakrabarti et al., 2016). The risk of HEV infection is high in children, but most of these infections are asymptomatic. For example, during the 2007 HEV outbreak in IDPs in Northern Uganda, Teshale, Howard, et al. (2010) reported a symptomatic attack rate of 6.9% in children under 2 years of age. However, a serosurvey conducted during the same outbreak showed the IgG and IgM seroprevalences were 25.4%

and 31% respectively in children under 15 years of age (Patel et al., 2015). In the present study, the independent effects of age on the risk of severe symptomatic HEV disease were assessed using logistic regression. The findings showed that the risk of severe symptomatic HEV disease increased from adolescents 12-17 years and adults 18-59 years to the elderly ≥ 60 years of age. However, this association did not attain statistical significance. These findings, therefore, suggest that age did not contribute significantly to the risk of severe symptomatic HEV disease during the present study.

Gender is the other demographic characteristic that was assessed in the present study. Bivariate analyses conducted in the present study showed that the risk of severe symptomatic HEV disease was lower in males when compared to females. However, on assessing the independent effects of gender on the risk of severe symptomatic HEV disease, the risk of severe symptomatic HEV disease was higher in the males than females. However, this association did not attain statistical significance. Hepatitis E studies in developing countries have reported a higher risk of HEV infection in the males than females. As part of a case control study conducted to determine the risk factors for HEV disease, Labrique et al. (2013) reported a lower risk of HEV disease in females than males. In Nigeria, a seroprevalence survey reported a higher prevalence of HEV disease in males than females (Junaid et al., 2014). Similar findings were reported by Cummings et al. (2014) as part of a descriptive case-series investigation in Northern Eastern Uganda. In a meta-analysis conducted by Li et al. (2013) into the primary risk factors for symptomatic HEV disease, the adjusted odds ratios showed that males had a 67% higher risk of HEV

disease than females. These findings suggest the possibility of gender-specific risks of exposure to HEV and/or gender-specific predispositions to severe HEV disease.

The current study assessed the association between clinical symptoms and signs and severe symptomatic HEV disease. Bivariate analyses showed that the factors significantly associated with severe symptomatic HEV included age, gender, displacement status, altered mental status, nausea/anorexia, epigastric pain, headache, unexplained bleeding, diarrhoea, skin itching, death as the outcome, contact with a case, and season of onset. Following multivariate analysis using logistic regression, altered mental status, nausea/anorexia, epigastric pain, headache, and skin itching remained significantly associated with severe symptomatic HEV disease. Hepatitis E virus clinical disease spectrum varies from asymptomatic or mild illness to severe life-threatening illness with acute liver failure. In the majority of immunocompetent patients, HEV causes a mildly symptomatic short and self-limiting illness lasting two to six weeks (Kamar et al., 2014; WHO, 2016a). The initial symptoms tend to be mild, nonspecific, and resolve spontaneously without any chronic illness or complications. In one of the original volunteer studies, clinical manifestations of acute hepatitis commenced 36 days after inoculation (Balayan et al., 1983). The initial symptoms were nonspecific and included weakness, abdominal pain, nausea, vomiting and anorexia, followed by more specific symptoms that included fever, dark urine, liver enlargement, and jaundice (Balayan et al., 1983). This presentation has consistently been reported as part of the epidemic and sporadic transmission of HEV in endemic sub-Saharan Africa and Asia (Shujun Zhang et al., 2011; Goumba et al., 2011; Murthy et al., 2014; Chakrabarti et al., 2016). Just like other

hepatotropic viruses, jaundice remains the hallmark of symptomatic HEV disease. Volunteer studies showed that jaundice appears 38-40 days after exposure and persists for 60-120 days (Balayan et al., 1983; Chauhan et al., 1993; CDC, 2015). Though jaundice is the central defining feature of symptomatic HEV disease, it only manifests in a variable proportion of HEV and is estimated at 40% for genotypes 1 and 2 and tends to increase with age with adolescents and young adults reporting higher rates of symptomatic HEV disease (Kamar et al., 2014). To highlight the potential magnitude of anicteric HEV infections, a study conducted in displaced populations of Juba, South Sudan showed a seroprevalence of 71% for HEV IgG in a population with no symptomatic cases (Azman et al., 2017). In the present study, jaundice was not significantly associated with severe symptomatic HEV disease since it was the primary symptom used to identify suspect HEV cases.

Nausea and anorexia usually appear as part of the nonspecific symptoms associated with HEV disease onset (Balayan et al., 1983; Shujun Zhang et al., 2011; Goumba et al., 2011; Murthy et al., 2014; Chakrabarti et al., 2016; Samji, Buggs, & Roy, 2017). The present study showed a statistically significant association between having nausea and anorexia and developing severe symptomatic HEV disease. Though the current study reported a significant association between nausea and anorexia and severe HEV disease, the symptoms are not unique to HEV. These findings suggest that nausea and vomiting, as nonspecific symptoms, are associated with severe HEV illness. In severe symptomatic HEV disease, nausea and anorexia usually occur alongside persistent vomiting (Samji et al., 2017). However, findings from the current study showed that vomiting and severe

symptomatic HEV disease were not significantly associated. The other nonspecific symptoms were headache and joint pains. Bivariate analysis revealed a significant association for both headache and joint pains with severe symptomatic HEV disease. However, only headache remained significant after multivariate analysis. Like nausea and vomiting, headache and joint pains occur as part of the initial non-specific HEV illness during the prodromal phase and usually resolve spontaneously or following symptomatic treatment (Samji et al., 2017). In the present study, however, headache and joint pains were more likely to be reported in patients with severe symptomatic HEV disease as opposed to patients with mild to moderate HEV disease. Headache and joint pains occur as part of the symptoms that characterize the prodromal phase of acute viral hepatitis (Samji et al., 2017).

In the present study, patients with epigastric pain were more likely to develop severe symptomatic HEV disease both on bivariate and multivariate analyses. As part of the acute viral hepatitis symptom complex, epigastric pain occurs along with other gastrointestinal symptoms that predominantly occur during the icteric phase of the disease (Balayan et al., 1983; Chauhan et al., 1993; Remy & Widjaja, 2016; Samji et al., 2017). Epigastric pain continues to be reported during HEV outbreaks in Africa and Asia (Shujun Zhang et al., 2011; Goumba et al., 2011; Murthy et al., 2014; Khaskheli et al., 2015; Chakrabarti et al., 2016). Hepatitis E disease results in liver inflammation and swelling with these manifestations being exaggerated in severe disease due to acute liver failure (Samji et al., 2017). These changes explain the pain in the epigastrium that in the present study was more likely to be reported in HEV cases with severe symptomatic disease than patients with mild to moderate HEV disease. Just like epigastric pain, unexplained bleeding

and skin itching are both related to the inflammation and acute failure of the liver with reduced hepatic synthetic functioning that occurs as part of the HEV disease process (Remy & Widjaja, 2016; Samji et al., 2017).

The current study showed that patients with unexplained bleeding and skin itching were significantly more likely to develop severe symptomatic HEV disease on bivariate and multivariate logistic regression analysis. Severe symptomatic HEV disease entails acute failure of the liver. Acute fulminant liver failure manifests as poor hepatic synthetic functioning with a prothrombin time of 16 seconds or an international normalized ratio (INR) of 1.5 that lead to reduced production of clotting factors and clearance of bilirubin from the body (Samji et al., 2017). This pathophysiology explains in part the unexplained bleeding and skin itching seen in HEV cases, who in the present study were significantly more likely to develop severe symptomatic HEV disease. Low platelet counts and aplastic anemia occur in association with HEV disease with unexplained bleeding being one of the overt characteristic manifestations (Aggarwal, 2011a; Kamar et al., 2014; Khaskheli et al., 2015; Remy & Widjaja, 2016). In the same way, pruritus appears in the prodromal phase and is part of the symptom complex that characterizes the icteric phase especially in cases with acute liver failure (Remy & Widjaja, 2016; Samji et al., 2017). In a study of HEV infection and pregnancy in Hyderabad, pruritus was reported in 90% of women with complicated HEV disease (Khaskheli et al., 2015).

Findings from the present study showed a statistically significant association between altered mental status and having severe symptomatic HEV disease (aOR, 640.24, 95% CI: 209.35-1958.02). Altered mental condition occurs in patients with acute fulminant

viral hepatitis that is complicated by hepatic encephalopathy. The hepatic encephalopathy is attributed to impaired osmoregulation in the brain with increased permeability of the blood-brain barrier, which in turn leads to brain-cell swelling and brain edema that is potentially fatal (Samji et al., 2017). In the current study, both bivariate and multivariate analyses showed that patients with altered mental condition were significantly more likely to have severe symptomatic HEV disease. Severe acute viral hepatitis compromises the ability of the liver to clear toxins from the bloodstream. Consequently, toxins accumulate in the body resulting in hepatic encephalopathy, a condition associated with declining functioning of the brain and other organs in the body. In a study conducted by Khaskheli et al. (2015), altered mental condition was reported in four out of every 10 patients while hepatic coma occurred in one-third of HEV patients and all the patients with hepatic coma died. As part of the initial outbreak investigations into the HEV cases in Maban, South Sudan, critically ill patients with mental confusion, agitation, and coma were reported and had to be admitted since they required intensive care (Thomson et al., 2013). Although fulminant hepatic failure resolves in a few cases, the prognosis is very poor with more than 50% of the cases dying in the absence of timely liver transplant (Samji et al., 2017).

Hepatitis E virus disease due to genotype 1 in developing countries causes fulminant acute hepatic failure in pregnant women. The risk of adverse outcomes following HEV genotype 1 disease in pregnancy increases with increasing gestational age (Kumar et al., 2004; Gad et al., 2012; Thomson et al., 2013; Najma et al., 2014). In the present study, pregnant or postpartum females were significantly more likely to have severe symptomatic HEV disease (aOR 16.90, 95% CI: 9.54-29.94). The risk of adverse outcomes in pregnant

HEV cases increases with gestation age. As such, the 2005 global HEV burden of disease study showed a 10fold higher risk of mortality in pregnant women with HEV than their non-pregnant counterparts (Rein et al., 2012a). In the same way, Thomson et al. (2013) reported a fivefold higher risk of death in pregnant women with HEV than their non-pregnant counterparts aged 18-59 years. During the HEV outbreak in IDPs in Northern Uganda, pregnant women had significantly higher HEV attack rates and mortality rates than their nonpregnant counterparts (E. H. Teshale, Howard, et al., 2010a). In a prospective study that assessed the spectrum and course of HEV in pregnant women, a mortality rate of 27% was reported with 81% of the fulminant hepatic failure cases having HEV (Kumar et al., 2004). The occurrence of acute fulminant hepatitis following HEV disease in pregnancy occurs consistently that the presence of severe disease in jaundiced pregnant women is used to herald the onset of HEV outbreaks in developing countries (Teo, 2012). These findings highlight the need for specialized interventions in the HEV toolkit that are targeted at women of childbearing age since they are prone to adverse outcomes following HEV infection.

Findings from the present study also showed that patients with severe symptomatic HEV disease were more likely to die than their counterparts with mild to moderate HEV disease (aOR 28.06, 95% CI: 14.77-53.29). Studies into HEV continue to show that severe symptomatic HEV disease is more likely to be fatal than mild to moderate HEV disease (Kumar et al., 2004; Rein et al., 2012a; E. H. Teshale, Howard, et al., 2010a; Thomson et al., 2013). The common causes of mortality especially among pregnant women with severe HEV disease in the third trimester are hepatic encephalopathy and disseminated

intravascular coagulation (Kumar et al., 2004; Khaskheli et al., 2015; Samji et al., 2017). Preventive interventions targeted at groups with a high risk of severe symptomatic HEV disease are therefore critical for preventing these deaths.

The present study assessed the role of coinfections as a factor for severe symptomatic HEV disease. It has been established that pre-existing liver disease from other hepatotropic viruses increases the risk of HEV infection and severe disease (Acharya et al., 2007; Gad et al., 2012). The present study showed that HEV patients with co-infections were significantly more likely to develop severe symptomatic HEV disease when compared to HEV patients without co-infections (aOR 3.15, 95% CI: 1.40-7.08). The common coinfections in the present study included Hepatitis B virus, Hepatitis C virus, and malaria. In a study conducted among Egyptian children, several coinfections occurred in HEV cases. The infections implicated aggravated liver injury and included hepatotropic viruses like Hepatitis A virus and Hepatitis B virus (Zaki, Salama, Mansour, & Hossein, 2008). In the same way, a hospital-based study undertaken in Mangalore, India reported high HEV and HAV co-infection rates in patients presenting with acute viral hepatitis (Joon et al., 2015). Overall, the risk of HEV co-infection with other hepatotropic viruses like HBV and HCV is high in developing countries where the three viruses are endemic. Thus, HEV preventive interventions should target populations with high endemicity for HBV and HCV given the risk for severe symptomatic HEV disease.

In the present study, we assessed the effects of several environmental/epidemiological factors including the season of disease onset, type of residence (refugee, IDP, or host community), contact with another case, and travel to an

affected area on the risk of developing severe symptomatic HEV disease. Findings from the present study showed that there were four peaks of HEV transmission, two of which took place in the rainy season (2012 and 2015) while the highest peak occurred in the dry season of 2013 but followed for the peak during the wet season of 2012. It is therefore clear that while the transmission reached the peak at the end of the wet season, the transmission continued in the dry season especially in populations that lacked optimal access to safe water. Multivariate analysis from the present study showed a lower risk of transmission in the wet season (aOR 0.33, 95%CI: 0.23-0.46). The explanation for these findings lies in the fact that in Maban, Upper Nile where the highest transmission peak occurred, the initial transmission started at the end of the wet season but continued into the dry season and even escalated due to suboptimal access to safe water and sanitation during the acute phase of displacement. In developing countries where access to safe drinking water is suboptimal, studies have shown that transmission starts and peaks during the wet season. During the 2009 HEV outbreak among the IDPs in Northern Uganda, transmission reached the peak during the rainy season (E. H. Teshale, Howard, et al., 2010a). Similar findings were reported in the refugees in Maban in 2012 and among South Sudan refugees in Gambella, Ethiopia in 2014 (Thomson et al., 2013; Browne et al., 2015). These findings underscore the need to initiate HEV preventive interventions in high-risk populations before the onset of the rainy season to reduce the risk of symptomatic and severe HEV disease.

In the present study, bivariate analyses showed that in comparison to refugee camp dwellers, the risk of severe symptomatic HEV disease was lower in the IDP sites (crude

OR 0.50, 95% CI: 0.41 – 0.62). These findings suggest that although refugee populations tend to be more stable after the acute phase of displacement, the risk of disease and occurrence of adverse outcomes is high during the acute phase of displacement as was seen in Maban, Upper Nile in 2012 (Thomson et al., 2013). On the other hand, findings from the present study showed that in comparison to refugee camp dwellers, the risk of suffering severe symptomatic HEV disease was higher in the host communities following bivariate analysis (crude OR 2.59, 95% CI: 1.86 – 3.61). These findings underscore the need to extend health services and disease preventive interventions to the host communities since they are vulnerable to new diseases brought in by displaced populations and for which the host community health system is most of the time not prepared to handle.

Two additional factors, namely travel to an affected area and contact with a case were assessed to determine their contribution to the overall risk of symptomatic HEV disease. Bivariate analysis from the present study showed that HEV cases that travelled to an affected area had a 26% higher risk of severe symptomatic HEV disease when compared to individuals who did not travel to an affected area. However, this association did not attain statistical significance and was not even entered into the multivariate logistic regression model since the p value at bivariate analysis was 0.32. The displacements into Maban, Upper Nile state in South Sudan started in late 2011 following armed conflict in Blue Nile state in the Republic of Sudan (Thomson et al., 2013). The data from the present study showed that the initial HEV cases in 2012 reported jaundiced cases in their places of origin in Blue Nile state (Thomson et al., 2013). These movements were therefore responsible for the initial introduction of the virus into the refugee population in Maban,

Upper Nile. Following the introduction of the virus in displaced populations, HEV transmission during the initial phase of displacement was precipitated by sub-optimal access to safe water, the rains and flooding in 2012 (Thomson et al., 2013). In the same way, while the current study showed an association between contact with a jaundiced case and the risk of severe HEV disease on bivariate analysis. However, multivariate analysis did not confirm the apparent relationship. These findings, therefore, suggest that person-to-person transmission may not have played such a significant role. In previous outbreaks, evidence of person-to-person transmission was reported in IDP household contacts in population camps where transmission continued amidst improved access to safe water (E. H. Teshale, Grytdal, et al., 2010). Fecal-oral transmission accounts for most of the transmission in developing countries. Contaminated water is the vehicle of transmission in these settings, and the predominant strain is HEV genotype 1 (Balayan et al., 1983; Naik et al., 1992; Sailaja et al., 2009; E. Teshale & Ward, 2015; Hasan et al., 2016).

As part of the host-agent-environment model, the agent characteristics constitute the other attribute that influences the risk of symptomatic severe HEV disease. Current evidence suggests that severe symptomatic HEV disease is exclusive to outbreaks caused by HEV genotype 1 occurring in developing countries in Africa and Asia. Laboratory test data available from the present study showed that strains collected from Bentiu PoC IDP camp in 2016 belonged to genotype 1 subtype 1e. These South Sudan strains were closely related to sequences from Uganda and Chad. These findings, therefore, suggest that similar strains are responsible for the HEV outbreaks in the region. This assertion is supported by genetic sequencing results for the 2014 HEV outbreak in IDPs of Darfur Sudan which

showed that HEV genotype 1 caused the outbreak and was closely related to the Chad isolates (Nicand et al., 2005). Similarly, the HEV patient samples collected from IDPs in Northern Uganda during the 2007 HEV outbreak belonged to genotype I and were also more closely related to the Chad isolates (E. H. Teshale, Howard, et al., 2010a). In the same way genotype testing of samples collected from refugees in Maban in 2013 showed HEV genotype 1 as the causal strain (E. Teshale & Ward, 2015). The findings suggest that the recent outbreaks of HEV in displaced populations of East Africa are intimately related to the Chadian strain isolated during the 1983-84 HEV outbreak (van Cuyck-Gandré et al., 1997).

Limitations of the Study

The study had several limitations. First, the Ministry of Health acute jaundice syndrome database did not contain all the variables of interest. The database only included variables for routine detection and reporting of HEV cases and therefore lacked the entire list of variables required to assess the factors for symptomatic severe HEV disease. For instance, data reporting individual behavioral factors like use of safe water, handwashing, safe fecal disposal, and knowledge on HEV prevention that are known to influence the risk of infection and possibly severe disease, were not included in the database. The other variables that were missing in the database included environmental variables like access to safe water and improved sanitation facilities, food hygiene, water storage facilities, and presence of animal reservoirs. Thus, the final model did not assess the independent contribution of these variables to the risk of severe symptomatic HEV disease. Consequently, the interpretation of findings from the current study ought to

consider the likelihood of residual confounding. However, this limitation applies to other epidemiological studies that have an inherent risk of residual confounding from known and unknown risk factors.

The second limitation relates to the retrospective cohort study design that does not allow for establishing a temporal relationship between exposure and disease since they were both assessed at the same time (Frankfort-Nachmias & Nachmias, 2008). Establishing a temporal relationship between exposure and disease occurrence is an essential criterion for establishing causality.

The third limitation relates to the healthcare access bias and differential misclassification bias that impact on the internal and external validity of the study findings (Delgado-Rodríguez & Llorca, 2004). Healthcare access bias occurs when there is a mismatch between admitted patients and the community they originated from, thus raising external validity issues. With regards to HEV disease, the mismatch arises from the fact that most of the mildly symptomatic cases usually will not seek care and hence the patients at the treatment center were more representative of cases with moderate to severe disease. Consequently, the findings from the present study can only be generalizable to patients with moderate and/or severe HEV disease that presented to designated treatment centers in displaced populations of South Sudan. The possibility of misclassification in the present study was estimated to be minimal since healthcare workers used a standard case definition to detect and perform laboratory testing for suspect cases.

The fourth limitation is related to the completeness of the datasets regarding the availability of data on the key variables. Since the MoH collected the data for the current study over a duration of five years, the researcher assessed the datasets for completeness and consistency of capturing the key study variables. The variables considered included demographic variables like age, gender, displacement status, and residence. The clinical variables included the consultation date, the admission date, the discharge date, the date of death, fever, yellow eyes, vomiting, diarrhoea, nausea/loss of appetite, headache, epigastric pain, skin itching, the presence of coinfections, mental status, and unexplained bleeding. The epidemiological variables included travel to an affected area and contact with a case. The data on these study variables were consistent and complete for six displaced population sites, namely, Bentiu PoC, Bentiu Town, Doro, Mayom, Lankien, and Mingkaman. Due to the varying versions of the HEV case-based line listing forms, three sites, namely, Yida, Batil, and Jamam/Gendrassa had HEV case-based data sets with incomplete clinical and epidemiological data and were therefore excluded from the assessments to identify factors for severe HEV disease. The total number of HEV cases from the remaining six displaced population sites, which were eventually used to determine the factors for severe symptomatic HEV disease was 4,810 cases, which fell within the minimum study sample size of 1,372.

Recommendations

In May 2015, the World Health Organization released its first position paper on Hepatitis E vaccine (WHO, 2015b). The publication recognized hepatitis E as a major public health problem in developing countries especially in special populations like

pregnant women, displaced populations, and communities affected by outbreaks. The paper also noted that prevention and control of the disease in these settings remains a challenge. While a new highly immunogenic experimental HEV 239 vaccine, Hecolin® is now available, its use to complement existing interventions is precluded by the paucity of epidemiological data for effective use of the vaccine to complement existing interventions (WHO, 2015b). The results of the present study contributed to the limited body of knowledge on epidemiological data needed to facilitate well-timed and targeted toolkit of interventions (including vaccination) for effective prevention and control of HEV.

In developing countries, transmission of waterborne HEV genotypes 1 and 2 is favored by sub-optimal access to safe drinking water with most transmission peaks occurring during the rainy season (Hughes et al., 2010; Yugo & Meng, 2013). In the present study, HEV transmission occurred throughout the study period (2012-2017) with four transmission peaks, three of which either took place in the last two months of the rainy season or happened in the dry season as an extension of transmission that initially peaked in the rainy season. However, a weak and insignificant association between precipitation and HEV cases was observed. Besides, genetic sequencing of samples collected during this period showed that HEV genotype 1 subtype 1e was responsible for the South Sudan outbreaks. As a contribution to the existing body of knowledge on hepatitis E, the present study showed that HEV genotype 1 subtype 1e remains the predominant strain for HEV outbreaks in displaced populations of South Sudan. Though the current study reported a weak and insignificant correlation between precipitation and

HEV cases, it elucidated a seasonal transmission pattern of HEV that usually starts and escalates in the rainy season. Since water is the primary reservoir for HEV genotype 1, improving access to safe water is therefore very critical to HEV prevention in displaced populations. As part of the existing toolkit of interventions for HEV prevention and control, prevention of exposure remains a core strategy that entails controlling the source of infection to reduce the risk of HEV spread. Controlling the source of infections involves several interventions including (a) improving the quality and quantity of drinking water, (b) treating and disposing of human waste correctly, (c) improving personal hygiene, and (d) preparing safe and clean food (WHO, 2014). Findings from the present study show that the strategy is relevant and should, therefore, be uniformly and consistently rolled out to prevent and control HEV outbreaks in displaced populations. The findings from the present study also suggest that the strategy should be rolled out before the onset of the rainy season. Therefore, as part of the prevention of exposure strategy, the following interventions should be rolled out before the beginning of the rainy season to mitigate the risk of HEV outbreaks in displaced populations

- (a) Improve the quality and quantity of drinking water through treatment of water sources at collection points and household level. The process of improving access to safe drinking water entails water safety planning, identification of hazards and risks in the entire water supply chain, followed by prioritization and management of identified risks, and compliance monitoring through periodic confirmation of water quality. During outbreaks, the free chlorine levels should be maintained at 0.5-1mg/Liter at the point of water collection

with the alternative recommendation for communities to boil their water if the free chlorine levels are unsustainable. Also, adequate amounts of water (7.5-15 liters per household per day) should be assured to meet the basic water needs (WHO, 2014).

- (b) Prevention and response efforts should emphasize improving access to, and use of safe excreta disposal since human feces are laden with HEV virus and therefore remain the primary source of the virus. Efforts should, therefore, focus on minimizing open defecation by focusing on immediate interventions like burying of feces especially for rural and peri-urban areas and construction of latrines in displaced populations to attain the recommended one latrine stance for 20 individuals (Damerell, Foubert, Nadig, Furtade, & Beauquis, 2011; WHO, 2014).
- (c) Improving and facilitating handwashing practices through the provision of soap and ensuring that handwashing facilities are available next to the latrine and kitchen. The option of using ash is worth considering in consultation with the communities.
- (d) Improving food safety and hygiene is critical with emphasis on using safe water to prepare foods like vegetables that are eaten raw and ensuring hygienic serving and storage of food. The other interventions entail training of food handlers and conducting regular public health inspections to ensure that public eating places adhere to the minimum sanitation and hygiene standards (WHO, 2014).

In the recent years, large and protracted outbreaks of Hepatitis E virus have occurred in displaced populations (Isaacson et al., 2000; E. H. Teshale, Howard, et al., 2010a; Thomson et al., 2013; WHO, 2015b; Azman et al., 2017; WHO, 2017). During the present study, the interquartile range for the duration of outbreaks was 62 weeks (one year and three months). These findings, therefore, highlight the fact that the current toolkit of interventions for HEV control, namely, improving access to safe drinking water, sanitation, and personal, food, and environmental hygiene, are not as effective for securing appropriate control of HEV outbreaks in displaced populations. The excess HEV cases and deaths reported during the protracted course of the outbreaks are preventable through effective deployment of additional interventions to complement the existing ones.

The current toolkit for HEV prevention and control includes prevention of disease through vaccination as a core intervention (WHO, 2014). However, the use of vaccines to prevent and control HEV outbreaks has not been used widely in the past due to the absence of a safe and effective vaccine. The May 2015 WHO position paper on HEV vaccines refers to the HEV 239 vaccine, Hecolin®, an experimental vaccine at clinical trial stage in humans and is licensed in China for use in high-risk individuals aged 16-65 years (WHO, 2015b). The vaccine is highly immunogenic with nearly 100% seroconversion after three doses on a 0, 1, 6-month schedule. The vaccine has a high efficacy rate in healthy adults aged 16-65 years in China and primarily against genotype 4 (WHO, 2015b). While current data show that the vaccine can protect against all four genotypes there is limited data on safety and effectiveness in pregnant women,

individuals outside the 16-65 year age range, persons with pre-existing liver disease, and protection against genotype 1 (WHO, 2015b). Consequently, WHO does not recommend routine use of the vaccine in endemic or sporadic HEV disease. However, due to the high risk of hepatitis E in outbreak situations, WHO recommends considering the complementary use of HEV 239 vaccine to mitigate the risk of HEV outbreaks for select high-risk groups, namely, pregnant women, travelers, and health and humanitarian relief workers. In these high-risk groups, vaccination is worth considering after evaluating the risks and benefits of vaccination on an individual basis. Based on the current study findings and the recommendations of WHO on the HEV intervention tool kit and position paper on HEV vaccines, the HEV 239 vaccine should be used to complement the conventional HEV control measures. The vaccination campaigns along with the other interventions in the HEV toolkit should be undertaken before the onset of the HEV transmission season to prevent the risk of disease outbreaks.

The present study showed that HEV is a significant cause of morbidity and mortality in displaced populations. Overall, HEV was the fifth cause of morbidity and mortality while in the under-fives, it ranked as the sixth top cause of death. The HEV intervention toolkit includes prevention of death as a core intervention that entails reducing mortality through prompt diagnosis, management, and referral of high-risk and severe cases (WHO, 2014). Since HEV is largely an asymptomatic or mildly symptomatic illness, most of the patients do not require admission and are, therefore, managed as outpatients. Consequently, as part of the response to an ongoing outbreak, accessible, well-staffed and equipped treatment facilities should be made available with

ample outpatient and inpatient/intensive care services. Outpatient care for HEV cases should entail (a) HEV messaging on the need for mild and symptomatic cases to promptly seek care as patients are known to deteriorate quickly, (b) Hygiene messaging to encourage general hygiene and hand washing, improving sanitation, and use of safe drinking water to prevent HEV (c) nutrition messaging to encourage patients to continue eating food and drinking water and the possibility of providing supplemental nutrition if the need arises, (d) symptomatic case management and avoiding hepatotoxic medicines.

In the present study, we used the host-agent-environment model to identify demographic, clinical, and epidemiological factors associated with severe symptomatic HEV disease. Hence as part of the toolkit for HEV prevention and control, the outpatient services should have hospitalization criteria for HEV cases that are likely to develop severe disease. The findings from the present study suggest that such criteria should include but may not be limited to (a) patients with altered mental status, (b) pregnant or postpartum females, (c) patients with any two of the following symptoms – nausea/anorexia, headache, epigastric pain, and skin itching, and (d) patients with co-infections of the liver. The criteria should be used to identify patients for admission, observation, and early initiation of supportive care to prevent complications. Also, within the context of an ongoing outbreak, the maximum benefit from administration of HEV vaccine may be expected by targeting these groups at high-risk for HEV disease. These groups of patients at risk for severe HEV disease should be targeted for vaccination with HEV 239 within the context of an ongoing outbreak.

Since pregnant women are particularly at risk of severe disease with adverse outcomes, the toolkit for HEV prevention and control emphasizes prevention of infection as a core measure. This intervention entails identifying pregnant women at health facilities, antenatal clinics, and outreach clinics and prioritizing them for the provision of safe water and proper sanitation kits and messaging to reduce the risk of infection. Also, all women of childbearing age and pregnant women should be targeted for vaccination with HEV 239 within the context of an ongoing outbreak.

While the present study did not assess social, behavioral, and cultural influences on the risk of disease, it is important that community engagement and communication are undertaken to determine their role on the risk of symptomatic and severe disease. Community engagement facilitates identification of communication needs (WHO, 2014). Community engagement and communication needs assessment are therefore recommended to understand and address the contextual factors to enhance the uptake of technical recommendations on HEV prevention and control. Further research is also required to evaluate the association between HEV and rainfall patterns. In this regard, therefore, correlation analysis of precipitation by location and the occurrence of HEV cases should be undertaken.

Implications

The findings from the present study were useful for individual community members, families, healthcare workers, public health professionals, humanitarian agencies, and public health policy makers for enhanced prevention and control of HEV in displaced populations. The findings from the present study have shown that HEV is one

of the leading causes of morbidity and mortality in displaced populations. In public health practice, assessment is core to characterizing community health problems and informs the initiation of appropriate control measures. As a contribution towards the assessment function, the findings from the present study will inform emergency response planning to ensure that essential health services are optimized to prevent and control HEV in displaced populations.

The present study assessed the effectiveness of the conventional HEV toolkit for preventing and responding to HEV outbreaks. The findings from the present study confirmed that conventional interventions were not as effective for securing timely control of outbreaks. Thus, many of the excess cases and deaths can be prevented through the complementary use of the HEV vaccine. In light of the current constraints in responding to HEV outbreaks in displaced populations, the integrated use of HEV vaccines alongside other conventional interventions to prevent excess morbidity and mortality in IDPs is justified.

While the present study fell short of elucidating a significant correlation between precipitation and the occurrence of HEV cases, it nevertheless showed that HEV cases start rising or peak during the rainy season. Given these findings, the comprehensive HEV tool kit including HEV vaccines should be rolled out before the onset of the wet season with the benefit of improved effectiveness in mitigating the risk of HEV outbreaks. In the same way, the study findings highlighted groups at high risk for severe HEV disease. These findings will be used to update the screening criteria used in

outpatients' departments to identify patients for admission to allow observation and initiation of intensive supportive care to prevent severe disease.

The present study showed that pregnant and postpartum females were at high risk for severe HEV disease. The policy implications for this will entail identifying all women of childbearing age and prioritizing them for primary prevention of HEV using the comprehensive HEV prevention and control toolkit. As part of the assurance function of public health, the current study has highlighted HEV as a significant problem in displaced populations and for which an appropriate package of public health services should be rendered to allow timely access to supportive care and thus mitigate the risk of death. At the individual level, the study findings are expected to mitigate the risk of contracting HEV, ensure timely access to comprehensive care for those with disease symptoms to reduce the risk of severe disease and thus improve the quality of life by preventing adverse outcomes. The implementation of the comprehensive HEV toolkit for HEV prevention and control will reduce HEV attack rates and mortality in families through improved access to safe drinking water, sanitation, and HEV vaccination.

As for the health workers, the present study has identified factors for severe HEV disease that will facilitate screening in outpatients' departments to identify cases for inpatient observation and care. In the same way, health workers and humanitarian workers should receive the HEV vaccine as high-risk groups within the context of ongoing HEV outbreaks. The current study used secondary data and, therefore, could not assess the full range of factors for severe HEV disease. Follow up studies can, therefore, build on findings from the current study to evaluate other factors for severe HEV disease.

Conclusion

A primary objective of responding to humanitarian crises that involve displaced persons entails the rapid deployment of lifesaving public health interventions to prevent excess morbidity, mortality, and disability (WHO, 2009). Detailed epidemiological characterization of disease trends and high-risk groups should inform the effective rollout of public health interventions. Given the current increase in the frequency and duration of HEV outbreaks in displaced populations in Africa, the current study set out conduct a detailed epidemiological characterization of the outbreaks to facilitate effective deployment of the HEV toolkit that includes vaccines for HEV prevention and control.

From 2012 to 2017, HEV cases were reported every month with four transmission peaks, three of which either occurred in the last three months of the rainy season or took place in the dry season as an extension of transmission that initially peaked in the rainy season. However, the study showed a weak and insignificant correlation between precipitation and the occurrence of HEV cases. Genetic sequencing and typing of samples from the 2016 outbreak in Bentiu PoC showed that HEV genotype 1 subtype 1e was responsible for the outbreak.

The risk of HEV symptomatic disease and death was higher in males than females as seen from the cumulative incidence and mortality rates. The age-specific risk of symptomatic HEV disease increased with age even after adjusting for sex. Thus, the risk of infection and death from HEV was highest in adults (18-59) years and the elderly 60 years and above were more affected when compared to children and adolescents. The risk

of symptomatic disease and death was higher in displaced populations in Maban where flooding occurred following heavy rains in 2012.

During the period 2012-2017, a total of eight HEV outbreaks took place in eight displaced population populations of South Sudan. The duration of the outbreaks was protracted as seen from the interquartile range of the outbreak duration of one year and three months.

In relation to other causes of morbidity and mortality in displaced populations, HEV emerged as a significant public health problem. Overall, HEV was the fifth top cause of morbidity and mortality while in the under-fives, HEV ranked as the sixth cause of death.

The current study showed a significant association between the following characteristics and severe symptomatic HEV disease (a) altered mental status, (b) death as an outcome, (c) pregnant or postpartum females, (d) disease onset in the rainy season, (e) nausea/anorexia, (f) headache, (g) epigastric pain, (f) co-infections, and (g) skin itching.

As outbreaks of HEV increase in frequency and duration in displaced populations, the current study provides information that public health experts, humanitarian workers, and policy makers could use to launch timely and well-targeted interventions available from the comprehensive HEV toolkit to reverse the current trends. The data from the present study shows that the outbreaks in South Sudan were protracted and lasted over one year. These findings, therefore, highlight the need to optimize the current response by complementing the conventional HEV control interventions with the HEV vaccines to control disease outbreaks. In addition, the current study suggests that significant benefits of administering the comprehensive HEV toolkit that includes HEV vaccines may be

expected to accrue by targeting women of child bearing age, pregnant and postpartum females, individuals with co-infections of the liver, males, adults (18-59 years), and the elderly (60-65 years).

Finally, in light of the limitations of the present study, further research into the social, behavioral, cultural, and other contextual factors for symptomatic and severe HEV disease in displaced populations should be undertaken. Operational research should be built into every deployment of the comprehensive HEV toolkit that includes the use of HEV vaccines to prevent and control outbreaks. This will provide information on the safety and effectiveness of the HEV toolkit in (a) outbreaks due to genotype 1, (b) pregnant women and, (c) persons outside the age range of 16-65 years.

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[6736\(10\)61030-6](https://doi.org/10.1016/S0140-6736(10)61030-6)

[illegible]

Appendix B: Mortality Line Listing Template

[illegible]

Appendix C: Variables for Symptomatic and Severe HEV Disease

No	Category	Variables	To be assessed in current study (Yes or No)
1	Agent factors	Genotype	No
		Dose of inoculation	No
		Genotypic and sub-genotypic variations	No
2	Host factors	Occurrence of symptomatic severe HEV disease	Yes
		Demographic factors	
		Age	Yes
		Gender	Yes
		Displacement status	Yes
		Residence	Yes
		Clinical factors	
		Date of consultation	Yes
		Presenting clinical symptoms and signs	Yes
		Presence of any co-morbidity	Yes
		Immunosuppression: due to cancer or otherwise	No
		Pregnancy in females	Yes
		Admission status	Yes
		Final clinical outcome	Yes
		Epidemiological factors	
		Date of illness onset	Yes
		History of travel to affected areas	Yes
		Contact with another case	Yes
		Behaviors: hand washing; safe fecal disposal; access to safe drinking water	No
3	Environmental factors	Climatic factors like rainfall and floods	No
		Sanitation and hygiene: access and use of toilets; food hygiene practices	No
		Presence of animal reservoirs like pigs or other wild animals	No

Appendix D: Request for Permission to Use Datasets for Secondary Analysis

WAMALA JOSEPH FRANCIS
College of Health Sciences
Walden University

23 January 2017

The Ethical Committee
Ministry of Health
Republic of South Sudan

Dear Sir or Madam:

Request for Permission to Use Ministry of Health IDSR Datasets for Secondary Analysis

I am pleased to write to your esteemed office in reference to the above subject. My name is Wamala Joseph Francis, a student of the College of Health Sciences, Walden University where I am pursuing a PhD degree in Public Health. My dissertation proposal topic is, **“Hepatitis E: Determinants of Severe Symptomatic Disease in Displaced Populations of South Sudan”**.

I have successfully presented and defended proposal before my dissertation committee. The next stage in the dissertation process entails applying to the University and Local Institutional Review Boards to secure ethical clearance for the data collection to proceed. Since the current study will rely on the Ministry of Health Integrated Disease Surveillance and Response (IDSR) datasets for secondary data analysis, the Walden University Institutional Review Board requires that permission is secured from the institution that owns the data.


The intent of this letter is to request for permission to secure anonymized Ministry of Health IDSR datasets and use them for secondary data analysis as part of the present study. In the same way, I am also submitting the study proposal to facilitate ethical review and approval by the Ministry of Health Ethical Committee.

I thank you.

Wamala Joseph Francis
College of Health Sciences
Walden University
100 Washington Avenue South, Suite 900
Minneapolis, MN 55401

Appendix E: Research Approval Letter from South Sudan Ministry of Health

The Republic of South Sudan



Ministry of Health

21/02/2017

To: Wamala Joseph Francis
University of Walden

RESEARCH APPROVAL LETTER

Dear Wamala,

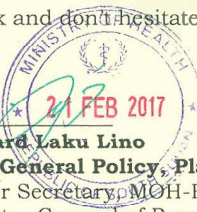
SUBJECT: Hepatitis E. Determinants of Severe Symptomatic Diseases in Displaced Population

I am writing in response to the request for authorization for the study on **"Hepatitis E. Determinants of Severe Symptomatic Diseases in Displaced Population"**. As a part of your secondary data analysis.

After close review on further clarifications and amendments to the proposal made, I am glad to inform you that the ethical committee at the Ministry of Health for the Republic of South Sudan have approved the study. The Ministry acknowledges the importance of the study to provide epidemiological data for decision making on the complementary use of Hepatitis E. Virus (HEV) to control an outbreak of the disease in South Sudan.

Please, keep the Ministry of Health informed in case of any changes regarding the implementation and its progress. I look forward to the report, especially the recommendations that will be generated. Note that any information generated should not be published without the consent of the Ministry.

Good luck and don't hesitate to get in touch should there be any queries.



Dr. Richard Laku Lino
Director General Policy, Planning, Budgeting and Research
 CC: Under Secretary MOH-RSS
 CC: Director General of Preventive Health Services-RSS

Headquarters, Ministerial Complex, Juba, South Sudan - P.O. Box 88, Juba
 Tel: +211 (0) 177 800 281 / +211 (0) 177 800 278

Appendix F: Research Approval Letter from Walden University IRB

3/8/2017

Walden University Mail - IRB Materials Approved



Joseph Wamala <joseph.wamala@waldenu.edu>

IRB Materials Approved

1 message

IRB <irb@mail.waldenu.edu>

Tue, Mar 7, 2017 at 9:39 PM

To: Joseph Wamala <joseph.wamala@waldenu.edu>

Cc: IRB <irb@mail.waldenu.edu>, Daniel Okenu <daniel.okenu@waldenu.edu>, Steven Seifried <steven.seifried@waldenu.edu>

Dear Mr. Wamala,

This email is to notify you that the Institutional Review Board (IRB) confirms that your study entitled, "Hepatitis E: Determinants of Severe Symptomatic Disease in Displaced Populations of South Sudan," meets Walden University's ethical standards. Our records indicate that you will be analyzing data provided to you by [insert CP Name] as collected under its oversight. The IRB approval number for this study is 03-07-17-0297498.

This confirmation is contingent upon your adherence to the exact procedures described in the final version of the documents that have been submitted to IRB@waldenu.edu as of this date. This includes maintaining your current status with the university and the oversight relationship is only valid while you are actively affiliated with Walden University.

If you need to make any changes to your research staff or procedures, you must obtain IRB approval by submitting the IRB Request for Change in Procedures Form. You will receive confirmation with a status update of the request within 10 business days of submitting the change request form and are not permitted to implement changes prior to receiving approval. Please note that Walden University does not accept responsibility or liability for research activities conducted without the IRB's approval, and the University will not accept or grant credit for student work that fails to comply with the policies and procedures related to ethical standards in research.

When you submitted your IRB materials, you made a commitment to communicate both discrete adverse events and general problems to the IRB within 1 week of their occurrence/realization. Failure to do so may result in invalidation of data and/or loss of legal protections otherwise available to the researcher.

Both the Adverse Event Reporting form and Request for Change in Procedures form can be obtained at the IRB section of the Walden web site:

<http://researchcenter.waldenu.edu/Application-and-General-Materials.htm>

Researchers are expected to keep detailed records of their research activities (i.e., participant log sheets, completed consent forms, etc.) for the same period of time they retain the original data. If, in the future, you require copies of the originally submitted IRB materials, you may request them from Institutional Review Board.

Both students and faculty are invited to provide feedback on this IRB experience at the link below:

http://www.surveymonkey.com/s.aspx?sm=qHBjzkJMUx43pZegKlmdiQ_3d_3d

Congratulations!

3/8/2017

Walden University Mail - IRB Materials Approved

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Information about the Walden University Institutional Review Board, including instructions for application, may be found at this link: <http://academicguides.waldenu.edu/researchcenter/orec>