Assessing the Global Burden of Hepatitis E Virus Associated with Genotypes 1, 2, 3 and 4

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Abstract

Globally, policy recommendations on vaccines as provided by the Strategic Group of Experts on Immunization (SAGE) rely on a number of factors including burden of disease. In the scope of this thesis, two studies were conducted in order to inform the World Health Organization HEV working group and ultimately the SAGE: 1) a systematic review and meta-analysis of the global incidence, prevalence and mortality associated with all genotypes of HEV; and 2) a global subgroup analysis of incidence, prevalence and mortality on special populations and high risk populations. Circulation of HEV was documented in all global regions. Severe disease was observed among pregnant women and fulminant hepatic failure patients in both studies conducted. Poor data quality was observed in both studies, this poses a serious challenge for estimating the burden of disease. Countries should consider the implementation of an HEV surveillance system to improve data quality and ultimately to inform decision-making.

Executive Summary

Viral hepatitis is caused by different viruses referred to by different letters A, B, C, D and E. Hepatitis E is transmitted from fecal-oral route. The most common source of Hepatitis E infection occurs through contaminated water. Four types of genotypes are associated with Hepatitis E Virus (HEV). Infections due to genotypes 1 and 2 have often been associated with fecally contaminated water and large outbreaks have been reported predominantly in South East Asia. Genotype 3 is associated with the consumption of uncooked or undercooked game meat. Transmission of HEV through food consumption has also been associated with genotype 4 predominantly in the Western Pacific region. Zoonotic transmission of HEV has also been documented. HEV infection is particularly severe in pregnant women during the third trimester where lethality ranges from 20% to 30%.

In 2011, the first HEV vaccine was licensed in China. In light of this, the World Health Organization (WHO) proceeded to develop global recommendations on the use of this vaccine. A key component to vaccine recommendation is disease burden. In the scope of the WHO work conducted to assess evidence for vaccine recommendations, this thesis assessed the global burden of HEV associated with all genotypes. This was done by completing the following two studies:

The first study consisted of a systematic review and meta-analysis conducted on global incidence, prevalence and mortality associated with all HEV genotypes. The objectives of the systematic review and meta-analysis were to: 1) extract prevalence, incidence and mortality data on HEV at a country level from published articles between December 2009 and January 2014;

2) summarize published evidence of HEV circulation at a country-level, regional level and global level; 3) assess heterogeneity between studies published and when feasible, provide a pooled estimate of the incidence, prevalence and mortality associated with HEV. The results of the systematic review and meta-analysis provided the first ever global analysis of all studies published on HEV genotypes 1, 2, 3 and 4. A high level of heterogeneity was present in studies within a country, region and at the global level.

There are three types of heterogeneity that can result in variation across studies: clinical, methodological and statistical. Clinical heterogeneity refers to differences observed in study populations. Clinical heterogeneity was present in all WHO regions. For example, blood donors consist of a population with specific characteristics that may result in variations when compared to other populations in the same geographical area. Methodological heterogeneity refers to variations in study designs and risk of bias. In the first study, this was assessed by comparing studies that ranked as high quality in the quality assessment to all other studies. Both clinical and methodological heterogeneity contribute to statistical heterogeneity. High levels of statistical heterogeneity were observed in all WHO regions and across countries.

Many factors contributed to the statistical heterogeneity of studies including lack of standardized methods for data collection, differences in laboratory practices including laboratory assays and differences in study populations. We were successful in generating pooled estimates for incidence, prevalence and mortality for a limited number of countries and regions.

The second study consisted of a subgroup analysis on the following three specific populations: blood donors, pregnant women and fulminant hepatic failure (FHF) patients. The objectives of

this second manuscript were to : 1) further explore subgroups of interest; 2) summarize published evidence of HEV circulation at a country-level, regional level and global level for high risk populations; 3) assess heterogeneity between studies published and when feasible, to provide a pooled estimate of the incidence, prevalence and mortality associated with HEV. This study allowed to further exploring heterogeneity due to differences in population observed in the first manuscript. Key findings emerged from the subgroup analysis: a high proportion of sporadic cases of HEV are reported in pregnant women globally. Additionally, fulminant hepatic failure patients accounted for a large proportion of mortality cases reported in the South East Asia region, indicating pronounced impact of disease in that region.

Lack of quality data emerged as a common theme from both manuscripts. The discussion section provided a framework for developing and implementing a surveillance system specific to HEV. Recommendations were developed and intended for use by countries where HEV is endemic. The following are recommendations made based on the work conducted in the scope of the thesis:

1- Modelling of HEV incidence, prevalence and mortality is needed to inform public health actions. Currently, only one modelling exercise was published and was not inclusive of all genotypes and regions of the world resulting in major gaps.
2- More data is needed on high risk groups to inform the use of Hepatitis E vaccines in various populations. Recommendations have not yet been made on high risk populations due to the lack of data available.

3- Studies on the risk of transmission of HEV via transfusion should be specifically assessed in high income countries where high proportions of seroprevalence have been documented in blood donors.

4- Validation studies are needed to compare sensitivity of assays currently used for the detection of HEV. There are currently no global standards to address this issue and inform countries on recommended diagnostic tests.

5- Countries with established viral hepatitis surveillance should consider incorporating HEV as an additional disease. Should an infrastructure for collecting HEV be present, countries should take advantage of this and improve data quality and completeness for HEV infections.

Contribution of the authors

A total of two manuscripts have been prepared in the scope of the thesis. The MSc. Student is the first author on all manuscripts and was responsible for data collection, data quality appraisal, data analysis and dissemination via writing the manuscript. All manuscripts were co-authored by Dr George Wells, Dr Philippe Duclos, David Becking and Joan Peterson. Drs Wells and Duclos provided meaningful feedback on the development and revision of manuscripts; and David Becking and Joan Peterson were the second independent reviewers of the quality assessment and data extraction.

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CHAPTER ONE

INTRODUCTION

1.1 Rationale

The World Health Organization (WHO) has been developing position papers for vaccines since 1998(1). The position papers are intended to provide recommendations on vaccines for national policy makers, international professional associations, non-profit organizations, donor agencies as well as international organizations(1). Within the WHO, the Strategic Group of Experts on Immunization (SAGE) is responsible for developing global policy recommendations on vaccines. At the global level several committees complete work which contributes to the development of vaccine policy recommendations. Among these committees are the Global Advisory Committee on Vaccine Safety (GACVS), the Expert Committee on Biological and Standardization (ECBS), the Immunization Practice Advisory committee (IPAC), and the Immunization and Vaccine related Implementation Research Advisory Committee)(1).

SAGE working groups are established as resources intended to increase the effectiveness of SAGE deliberations by reviewing and providing evidence based information and options for recommendations together with implications of the various options to be discussed by the full SAGE in an open public forum.

The SAGE group is the main body responsible for advising the WHO on vaccines and immunization. A number of expert working groups are developed as key issues on vaccination arise. The working groups report to the SAGE. This group is ultimately responsible for providing

recommendations on the use of vaccines and its conclusions and recommendations are published in the WHO's Weekly Epidemiology Record and used to update the WHO vaccine position papers(2).

SAGE working groups are established for a particular vaccine and are time limited. The working groups are comprised of two SAGE members along with 8-10 external technical experts. Once established, working groups define critical issues and gather evidence to support recommendations for the SAGE members. Systematic reviews are necessary and part of the evidence –based review process to guide the development of recommendations. In order to capture the best available evidence, the expert group defines a research question by developing a PICO (population, intervention, comparator, and outcome) statement. For immunization, the population is defined as the target population for vaccine use, the intervention is the vaccination, the comparator is no vaccine or standard of care and the outcome are the clinical outcomes prevented by or resulting from the vaccination. Once a systematic review has been conducted, the risk of bias is assessed from the evidence retrieved. The last step involved in the evidence gathering process is rating the quality of evidence. This is done by using the Grade of Recommendations, Assessment, Development and Evaluation (GRADE). This tool has been endorsed and adapted by many countries/organizations including WHO. Scores are given to each study based on a number of factors including the type of study. For instance, observational studies are given a substantially lower score than randomized-control trials (i.e. low versus high – see below). A number of factors including limitations in study, publication bias and inconsistency contribute to lowering the confidence while strength of association, dose-response and mitigated biases are factors that contribute in increasing the confidence level. All criteria are

given a score and a summary score is given for each study. Once the quality of evidence has been assessed, a recommendation is made. The following elements are taken in consideration to make a recommendation: quality of evidence, balance benefits/harms, values and preferences, feasibility, and acceptability as well as resource use. There are four levels of grading ranging from level 1 indicating very low level of confidence to level 4 indicating high level of confidence.

Once all the evidence has been assessed by the working group, the following is presented to SAGE: a background document which includes a summary of evidence along with GRADE tables and evidence to recommendations from the table. Additionally, the expert working group provides the complete evidence in the form of systematic reviews or published articles and recommendations for consideration to SAGE are also provided(3).

Along with the information provided by the working group, SAGE takes into account a number of factors including the disease epidemiology, clinical characteristics, vaccine and immunization characteristics, economic considerations along with acceptability of intervention by target population, social and ethical considerations.

As the first HEV vaccine was registered in China in 2011, the World Health Organization proceeded to developing global recommendations on the use of this vaccine. Based in phase II and phase III trials, the vaccine has shown to be highly effective in preventing HEV infections. In the phase III trial, vaccine safety was assessed and similar adverse events following immunization were reported in the vaccine and placebo groups (4). A SAGE working group for Hepatitis E was established in 2013 to review evidence on hepatitis E and to prepare proposed

recommendations on the use of the vaccine for consideration by SAGE(5). SAGE recommendations would then lead to the development of a vaccine position paper. The working group was tasked to review data/evidence including the global prevalence and burden of disease as well as the safety, immunogenicity, effectiveness and cost-effectiveness of the hepatitis E vaccine. The expert working group has successfully reviewed the evidence on HEV and provided recommendations to the SAGE that led to the publication of a position paper in May 2015(6). The work conducted in the scope of this thesis provided information on the disease burden associated with all genotypes of HEV. The goal of providing such evidence was to inform the SAGE Hepatitis E working group on the disease burden associated with HEV. The systematic review conducted and presented in this thesis was used by the WHO working group to quantify the burden of disease associated with HEV.

The present thesis followed a manuscript-based thesis. Two manuscripts have been prepared. In addition, a background chapter and a discussion chapter have been included.

1.2 Objectives

The overall objectives of this thesis were to:

A. Collect evidence on HEV circulation globally via a systematic review concerning all HEV genotypes
B. Develop a quality assessment tool for articles on prevalence, incidence and outbreaks
C. Conduct a meta-analysis and provide global estimates when possible

1.3 Thesis outline

Chapter One

Chapter one provides a rationale and objectives for the thesis along with a description of each chapter.

Chapter Two

Chapter two provides background information on Hepatitis E Virus (HEV), information on the WHO reporting structure and process for establishing a recommendation on vaccines as well as information on the epidemiology of HEV, burden of disease and on the Hepatitis E vaccine.

Chapter Three

Chapter three is a manuscript presenting the result from a systematic review on HEV along with the analysis conducted. The objectives of this chapter were to: 1) conduct an article search on published HEV studies at a country level for years 2009 to 2014; 2) extract data from studies reporting on prevalence, incidence, and mortality by geographical regions worldwide; 3) conduct a quality assessment on the studies included in the systematic review; and 4) analyze data extracted by conducting a meta-analysis on incidence, prevalence and mortality.

Chapter Four

Chapter four is a manuscript presenting an analysis from the systematic review for specific subpopulations. The objectives of the manuscript were to: 1) identify high risk populations; 2) explore inter-regional differences in estimates of prevalence, incidence and mortality; and 3) conduct a meta-analysis on special populations.

Chapter Five

Chapter five provides an overall discussion of the thesis. The discussion chapter also identifies areas for improvement based on findings from the systematic review and from the stratified

analysis conducted in chapters three and four. This chapter also provides concrete recommendations to improve data quality issues related to HEV.

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CHAPTER TWO

BACKGROUND

2.1 Hepatitis E Virus

Viral hepatitis is caused by several different viruses (6). Hepatitis viruses A, B, C and E are responsible for the majority of infections (7). Hepatitis E virus (HEV) is characterized by one serotype and four genotypes (8). HEV is responsible for a considerable amount of non-A and non-B acute viral hepatitis in developing and developed regions (9). Outbreaks related to HEV genotypes 1 and 2 have been documented worldwide and are often linked to poor sanitation and hygiene (7).

In endemic areas, hepatitis E is transmitted via fecal-oral route and can result in mild to severe symptoms including nausea, vomiting, anorexia, jaundice and fever (10) .Severe cases of HEV infections can lead to fulminant hepatitis resulting in liver failure although most cases are self-limiting (4).Vertical transmission of HEV and transmission from blood transfusions have been documented and represent a small proportion of all cases (11). The incubation period of HEV infection ranges from 3 to 8 weeks (4). Young children infected with HEV may present as asymptomatic and infection is often undiagnosed (12). Studies in the literature have identified serological evidence of HEV in individuals with no history of disease. For instance high prevalence of anti-HEV antibodies have been documented in the Egyptian population, however low numbers of documented severe illness have been reported (12). Seroprevalence as high as 80% has been documented in Egypt (13).

In 1978, the first documented outbreak of hepatitis E was reported in India (14). This was the first published evidence of HEV circulation. The outbreak resulted in 52,000 cases and 1700 deaths. Severe complications and higher incidence associated with HEV infection were reported in pregnant women during this outbreak. Since the first reported outbreak, HEV has affected a large number of developing countries with poor water sanitation and disposal systems.

Outbreaks associated with HEV have been documented more frequently in developing countries (15). While the most common source of transmission is the fecal-oral route, zoonotic transmission has been documented. Genotypes 1 and 2 have been associated with waterborne transmission of HEV while genotype 3 and 4 are associated with zoonotic transmission (16). Zoonotic transmission has been documented via consumption of infected swine and wild game. Large epidemics of HEV have often been associated with genotype 1 in Asia, specifically in India and Bangladesh. Apart from vaccination that has been used in China, public health prevention strategies have mostly focused on enhancing hygienic practices, ensuring clean water supplies and disposal systems for human feces.

Severe complications in women can occur as a result of HEV including mortality, intrauterine death, preterm delivery, and stillbirths (17). High mortality rate in pregnant women during the third trimester of pregnancy has also been documented (12). In endemic areas, HEV is the most common cause of hepatitis affecting women during pregnancy (12). Additionally, individuals with chronic liver diseases are at higher risk of acquiring fulminant hepatic failure following infection to HEV (9).The World Health Organization (WHO) estimated that worldwide, 20

million cases of HEV occur annually (4). Global estimates of yearly mortality related to HEV vary in the literature and have been reported to be as high as 300,000 (7). In outbreak settings, higher attack rates are reported in young adults and those 15-40 years (11). However infection in young children is often asymptomatic and less likely to cause illness compared to adults. Refugees, migrants and mobile populations also represent high risk groups for infection due to inadequate access to safe water and sanitation facilities (11).

Acute hepatitis diagnosis is defined per the WHO as an increase in bilirubin and liver enzymes in individuals with a clinical history of hepatitis (11). HEV infections can be confirmed based on direct or indirect tests. Detection of viral nucleic-acid of HEV represents a direct method of identifying HEV. Diagnosis from HEV-RNA can denote a current or past infection as the virus can remain present after infection has occurred (11). Direct tests are more resource intensive and most diagnosis relies on indirect tests such as detection of anti-HEV antibodies including IgM and IgG. Serological evidence of recent exposure to HEV can be obtained from presence of anti-HEV IgM antibodies as they can be detected as early as symptom onset (11). The detection of anti-HEV IgG antibodies indicates recent or past exposure to HEV. Diagnosis of HEV infection can also be done using paired sera, by comparing the acute and convalescent levels of anti-HEV IgG antibody titers. A greater than threefold rise in levels of anti-HEV IgG in paired sera has often been used in the literature to define seroconversion (18-20).

Evidence from the literature indicates that sensitivity from serological assay in immunocompromised individuals may be lower (21). Diagnosis of HEV is conducted based on serologic investigations since symptoms of the infection resemble that of other acute viral

hepatitis infection (22). Several assays have been developed over time to detect the presence of HEV. The sensitivity across different assays varies greatly.

Few countries have surveillance systems specifically designed for HEV surveillance. Objectives of surveillance include monitoring trends over time, timely detection of outbreaks, monitoring circulation, and identifying high risk population. Surveillance data can inform assessment of disease burden. Even though surveillance data likely underrepresents the true number of cases, it can serve as the foundation for modelling outcomes associated with HEV.

2.2 Strategic Advisory Group of Experts on Immunization

The WHO has been developing position papers for vaccines since 1998 (1). The WHO's Strategic Group of Experts on Immunization (SAGE) is the main independent advisory body providing guidance on the development of global policy recommendations for vaccines. At the global level several other more focused committees contribute to the development of vaccine policy recommendations. This includes: the Global Advisory Committee on Vaccine Safety (GACVS) which was established in 1999 and is responsible for risk assessment and responding to vaccine safety issues of potential global importance and perspective (23). The Expert Committee on Biological and Standardization (ECBS) provides guidance and recommendations for a number of products including vaccines. In the scope of this expert working group, international experts come together to develop recommendations on the production and quality control of vaccines (24). The Advisory immunization Practice Advisory committee (IPAC) was established in 2010 to provide advice on immunization practices, operational standards, as well as tools to enhance the delivery of immunization programmes in countries. The Quantitative

Immunization and Vaccine Research Advisory Committee reviews quantitative research methodologies such as modelling and cost-effectiveness and disease burden studies (25).

SAGE working groups are established as resources intended to increase the effectiveness of SAGE deliberations by reviewing and providing evidence based information and options for recommendations together with implications of the various options to be discussed by the full SAGE in an open public forum.

In addition to the epidemiological features of the disease including the disease burden and serotype distribution, other factors taken into consideration for the development of recommendations including vaccine characteristics, as well as considerations of economical, health-care and legal nature (2).

As new findings and developments in disease prevention arise such as the recent development of an HEV vaccine in 2011, the assessment of the burden of disease should also be updated to reflect the current situation. An up-to-date assessment on the evidence of HEV provides a more comprehensive assessment of baseline data in a pre-vaccine era and may provide useful information for future vaccine effectiveness studies. There is a need to assess the burden of disease associated with HEV infections and risk factors associated with HEV to better inform recommendations for use of vaccination. Through this thesis, the burden of all HEV genotypes was assessed. The outcome of this assessment provided evidence that was used for the development of a statement for the recommendation of the newly developed HEV vaccine at the global level.

2.3 Hepatitis E Vaccine

In 2011, Hecolin became the first ever licensed vaccine against HEV. The vaccine has been approved by China's State Food and Drug Administration (SFDA) (4).According to phase III trials, Hecolin was shown to be nearly 100% effective in preventing clinical HEV in individuals who completed all three doses of the vaccine series (26). Produced by Xiamen Innovax Biotech, the HEV vaccine (HEV 239), Hecolin, has been approved for use in individuals 16 years of age or older and is recommended for those at high risk of acquiring an HEV infection (5). The high risk groups listed by the manufacturer include food handlers, members of the armed forces, women of childbearing age, as well as travellers going to endemic areas (5). A phase III study evaluated the immunogenicity of the vaccine on 113, 000 participants. Persons aged 65 years or older and populations at high risk for severe hepatitis E disease were excluded from the study.

2.3.1 Immunogenicity

A phase II study was conducted in 612 healthy seronegative persons 16-55 years of age (14). Study participants either received two doses at 0 and 6 months or 3 doses at 0, 1 and 6 months. The vaccine group received the HEV vaccine while the placebo group received the Hepatitis B vaccine. Seroconversion was observed in 98% of the participants who received two doses and 100% seroconverted in the three dose group of the HEV vaccine.

The phase III trial was conducted on a total of 113 000 participants. Serum samples were taken one month after vaccination. In the vaccine group, 98.7% of subjects seroconverted (4 fold or

greater increase in antibody concentration following vaccination). Immunogenicity has not been evaluated in persons <16 years and >65 years or in population at higher risk of severe HEV disease.

2.3.2 Efficacy

Vaccine efficacy was assessed in both phase II and III trials. The vaccine protected against symptomatic HEV infection, with a very high efficacy rate. Data on efficacy was primarily related to HEV infections caused by genotype 4 as the study was conducted in China where HEV genotype 4 is predominant. In phase II trial, a total of 20 controls had a spontaneous HEV infection compared to 13 in the vaccine group. There were twice as many more subjects in the vaccine group. None of the infections reported were symptomatic. In the phase III trial, a double blind randomized control trial was conducted. Efficacy was assessed by determining the number of symptomatic HEV infections prevented by vaccination. Study participants were followed up for 19 months via active surveillance. In the per protocol analysis a total of 15 of the 48 663 placebo recipients and none of the 48 693 vaccine recipients developed HEV during the 12 months following third dose, reflecting 100% efficacy.

2.3.3 Vaccine safety

In the phase III trial, active surveillance of adverse events was performed. More local reactions in HEV groups were reported compared to the placebo group who received HBV vaccine. Systemic adverse events were similar in the vaccine and placebo group. The Global Advisory Committee on Vaccine Safety concluded that the vaccine has a good vaccine safety profile.

2.3.4 Cost effectiveness

A model was developed to assess the cost-effectiveness of vaccination programs in outbreak settings (26). The model didn't account for increased risk of death for pregnant women and was based on an outbreak that occurred in Uganda. Vaccination at age 1 and vaccination at age 20 were used to assess the cost-effectiveness. Assumptions in the model included a $100 US medical spending on a symptomatic infection and $1000 US associated with a death. The model was also based on a cost of vaccine per unit of $17.18 US. Based on findings from the model, a vaccination rate of 50% would result in a total of $353 904 US of averted health expenditure. Additional a total of $875 US would be needed to avert a DALY. However, this estimate is sensitive to changes in the assumptions used.

2.3.5 WHO's position on the use of the vaccine

Insufficient data on children, adults 65 years or older and immune-supressed was noted in the May 2015 position paper published by the WHO (5). Additionally, no data was available on immunogenicity on pregnant women. Vaccine, efficacy for this vaccine against genotype 1 is limited and unknown for genotypes 2 and 3. In the phase III trial, protection was reported as being high against genotype 4 but lacking for other genotypes. Study participants from phase III trials were followed up 54 months after receiving their first dose and overall protection efficacy was 93%. Overall, the phase III trial demonstrated that the vaccine provides high efficacy in protecting against HEV genotype 4 in adults 16-65 years in China.

In its position paper, the WHO did not recommend the vaccine for routine use due to insufficient data regarding use of vaccine in children and cross protection with other genotypes than type 4.

Additionally the WHO noted insufficient data on safety, immunogenicity and efficacy in children, pregnant women, chronic liver disease patients, patients on organ transplant wait lists, and travellers.

As a result the WHO does not recommend the routine use of the vaccine in children <16 years, pregnant women, liver disease patients and patients on organ transplant lists and travellers. In outbreak settings where the risk of HEV infection and complications or mortality are particularly high, the use of HEV vaccination as a mitigation strategy should be considered.

2.4 Burden studies

Only one study published in the literature has attempted to quantify the global burden associated with HEV infections (27). The first attempt at quantifying the burden of HEV was published in 2010 by David B. Rein. In his paper Rein, assessed the burden of HEV associated with genotypes 1 and 2 for the year 2005 in the continents of Asia and Africa. At the time of study publication Africa and Asia represented approximately 72% of the global population. A clinical model was developed and accounted for pregnancy status.

Age specific incidence was generated by multiplying the incidence rates of HEV generated by DISMOD with the age specific population of each region subcategorized into pregnant and non-pregnant groups.

Calculating the number of pregnant individuals for each age group at a country level was done by multiplying the estimated variant of the United Nations published crude birth rate per 1,000 populations by the total population divided by 1000. The total number of births was then divided

by the number 29 to reflect the number of years of childbearing for women (15-44 years). This number was multiplied by 0.77 to account for the fact that for each birth the mother is pregnant for on average 40 weeks. The number was then distributed to each age category between 15 and 44 years and subtracted the pregnancies from the population in those age groups to avoid double counting.

In order to estimate the probability of symptom a systematic review was conducted. The Monte Carlo Markov Chain simulation method was used as a procedure in SAS to estimate the probability of symptomatic illness based on extracted articles.

The WHO indicates that burden of disease can be assessed using several methodologies including incidence, prevalence, deaths as well as disability-adjusted life years lost (DALYs) (28). Information on incidence, prevalence and mortality is key to provide an insight on trends, epidemiology of the disease as well as determining susceptible populations and to inform public health policies. The quality-adjusted life year (QALY) is a measure of burden that can be used to assess the benefits gained from an intervention. The QALY measure is frequently used to assess cost-effectiveness. DALYs consists of another measure of burden. In order to calculate DALYs, the number of years of life lost (YLL) due to premature mortality are added to the number of years lost due to disability (YLD).

Under a request by the WHO, an initial systematic review was conducted to collect data on HEV for Africa and Asia for the time period of 1990-2010 in order to assess the burden of HEV. The systematic review focused on seroprevalence, incidence and death in both symptomatic and asymptomatic cases of HEV infections. Additionally, estimates were provided for still births.

Extracted data was inputted in a mathematical model developed by the WHO (DISMOD III). The DISMOD model estimated age, sex and region-specific prevalence and incidence based on data extracted from the review (27).

Additionally, the probability of symptomatic infection in individuals infected with HEV, the probability of death in symptomatic and pregnant women versus symptomatic non pregnant women was estimated. The results from the study found that the largest increase in prevalence occurs between the ages of 5 and 20 years of age. The south Asia and East Asia region accounted for the highest seroprevalence (peak of 25%). An estimated seroprevalence ranging from 15% to 25% was reported for all other regions. In Egypt, the model predicted seroprevalence in excess of 50% for individuals 5 years of age or older.

Incidence rates ranged from 0.5% and 1% for children aged 0-15 years based on the model. The incidence rate increased from 1-4% every year for individuals aged 15-20 years. The incidence decreased after 20 years and fell below 0.2% after 30 years of age. However, a high level of uncertainty was noted based on wide 95% confidence intervals obtained from the model. The model estimated the average age of infection to be 17.1 years. The probability of symptomatic illness given infection was estimated to be 0.198. Overall, a total of 20.1 (95% CI 2.8-37.0) million incident cases of HEV were estimated globally. Among the 20.1 million cases, an estimated 3.4 (95% CI 0.5-6.5) million cases displayed symptomatic illness. Additionally, 70,000 (95% CI 12,400-132,732) deaths, and 3,000 (95% CI 1,892-4,424) stillbirths were estimated. The majority of cases estimated were in East and South Asia (61%).

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CHAPTER THREE

A SYSTEMATIC REVIEW AND META-ANALYSIS OF THE GLOBAL INCIDENCE, PREVALENCE AND MORTALITY ASSOCIATED WITH HEPATITIS E VIRUS GENOTYPES 1, 2, 3 AND 4.

The following chapter has been prepared as a manuscript for submission to the Journal of Liver and Clinical Research. This manuscript presents findings from a systematic review conducted on global incidence, prevalence and mortality associated with all Hepatitis E Virus (HEV) genotypes. The objectives of the systematic review and meta-analysis were to: 1) extract prevalence, incidence and mortality data on HEV at a country level from published articles between December 2009 and January 2014; 2) summarize published evidence of HEV circulation at a country-level, regional level and global level; and 3) assess heterogeneity between studies published and when feasible, provide a pooled estimate of the incidence, prevalence and mortality associated with HEV.

Detailed tables of the quality assessment and characteristics of the studies are provided in Appendix 1-30.

The MSc. Student is the first author of this paper and was responsible for data collection, data quality appraisal, data analysis and dissemination via writing the manuscript. This paper was co-authored by Dr George Wells, Dr Philippe Duclos, David Becking and Joan Peterson. Drs Wells and Duclos provided meaningful feedback on the development and revisions of this manuscript; David Becking and Joan Peterson were the second independent reviewers of the quality assessment and data extraction.

Type of Article: Review Article

Title: A systematic review and meta-analysis of the global incidence, prevalence and mortality associated with Hepatitis E virus genotypes 1, 2 3 and 4.

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Title: A systematic review and meta-analysis of the global incidence, prevalence and mortality associated with Hepatitis E virus genotypes 1, 2, 3 and 4.

Abstract:

Hepatitis E virus (HEV) is responsible for a considerable amount of acute viral hepatitis in developing and developed regions. Quantifying the burden associated with HEV is essential for informing public health actions, evaluating interventions, and supporting vaccination programs. The objective of this study was to assess the burden of HEV associated with genotypes 1, 2, 3 and 4 globally. Articles published between December 2009 and January 2014 containing data on HEV seroprevalence, incidence of sporadic cases and mortality associated with HEV were included. An article search was conducted on online databases including PubMed, Scopus and Embase. An analysis for heterogeneity was conducted to determine whether estimates could be pooled at a country, regional and global level. Pooled estimates were included for analysis when heterogeneity scores (I^2) were lower than 75%. Endemic cases of HEV were reported in all regions of the world with genotypes 1, 3, and 4 reported in four of the six global regions (Americas, South-East Asia, Western Pacific and Europe). No evidence of genotype 2 circulation was reported. Eastern Mediterranean reported circulation of genotype 1 and Africa reported circulation of genotype 3. Pooled estimates for seroprevalence, incidence, maternal mortality rate and stillbirth rates were generated. In the region of Africa, incidence of sporadic HEV cases was (65% [95%CI 59%, 72%]). Moratlity estimates were generated for the Eastern Mediterran region (29% [95%CI 18%, 39%]). This systematic review represented the first attempt at quantifying the burden associated with HEV for all genotypes globally. The pooled regional estimates generated are consistent with published findings and highlight the elevated risks associated with HEV infection in pregnant women.

Keywords: Hepatitis E Virus, acute viral hepatitis, epidemiology

Abbreviations: HEV: Hepatitis E Virus

Introduction

Viral hepatitis is caused by several different viruses (6). Hepatitis viruses A, B, C and E are responsible for the majority of infections (7). Hepatitis E virus (HEV) is characterized by one serotype and four genotypes (8). HEV is responsible for a considerable amount of non-A and non-B acute viral hepatitis in developing and developed regions (9). Outbreaks related to HEV genotypes 1 and 2 have been documented worldwide and are often linked to poor sanitation and hygiene (7).

Outbreaks associated with HEV have been documented at a higher frequency in developing countries (28). While the most common source of transmission is fecal-oral route, zoonotic transmission has been documented. Genotypes 1 and 2 have been associated with waterborne transmission of HEV while genotype 3 and 4 are associated with zoonotic transmission (16).

Severe complications in women can occur as a result of HEV including mortality, intrauterine death, preterm delivery, and stillbirths (8). Additionally, individuals with chronic liver diseases are at higher risk of acquiring fulminant hepatic failure following infection to HEV (9). The World Health Organization (WHO) estimates that worldwide, 20 million cases of HEV occur annually (4). Global estimates of yearly mortality related to HEV vary in the literature and have been reported to be as high as 300,000 (7).

Evidence from the literature including systematic reviews is needed to inform the burden associated with HEV. As of June 2016, only one systematic review has been conducted to

summarize published data on HEV at a global level. The systematic review previously conducted was published in 2010 by the World Health Organization (WHO) (29). The review focused on published articles from 1980 to 2008 and summarized data at a country and regional level. Since 2008, mounting evidence on transmission of HEV has been published. The objective of this systematic review was to collect country specific data on the incidence, prevalence and severe outcomes related to Hepatitis E Virus (HEV) infections. The present systematic review complements the previous one. Articles published between December 2009 and January 2014 were been included in the article search and extraction for this systematic review.

Methods

Article Search

The article search was conducted following the exact article search methodology used in the previous WHO systematic review. Articles were searched from the PubMed, Scopus and Embase databases. The selected articles included original articles and reports published between December 2009 and January 2014. In order to address the potential bias in reporting and publication for large outbreaks, case series and case-reports were also included. Articles were extracted by 3 independent reviewers (MS, DB and JP). Articles were assessed against selection criteria by reviewers to ensure all articles that met the inclusion criteria were included. Articles in English, French, Portuguese and Spanish were assessed by reviewers. Google translate was used to assess eligibility of articles in other languages based on the abstract of articles.

Article selection

A total of 1235 articles were identified from PubMed, Scopus and Embase (Figure 1). Articles were first screened at a title and abstract level and a total of 754 articles were excluded. An

additional 106 articles were excluded when reviewed at a full text level. Full text translation was required for a total of 23 articles which were not included in the results of this review. Review articles were not included in this report; this resulted in 86 articles being excluded. Data was extracted from a total of 266 articles and were included for analysis in this systematic review including 82 case series and case-reports.

Eligible articles included original articles, studies reporting on outcomes of interest including HEV prevalence, incidence, mortality, still births or HEV related outbreaks. Case reports, case-series, cohorts, cross-sectional and case-control studies were included in the systematic review. Review articles, animal studies, and environmental studies, studies not reporting any HEV outcomes of interests listed above and articles reporting data solely on travellers were excluded. Additionally, studies not reporting numerators and denominators for HEV outcomes were excluded. Data was extracted from studies that met the inclusion criteria.

Studies were grouped into three categories: seroprevalence, sporadic acute HEV and outbreaks. Studies reporting on anti-HEV IgG antibodies were considered seroprevalence studies unless clinical symptoms consistent with an HEV infection were reported or evidence of seroconversion was documented. Studies reporting on sporadic cases of HEV associated with anti-HEV IgM antibodies or HEV RNA were classified as sporadic acute hepatitis studies. Estimates for prevalence were calculated from seroprevalence studies and incidence estimates were calculated from studies reporting on sporadic HEV cases. For outbreak studies, incidence was reported as a proportion or as an attack rates. Pooled estimates were only provided for incidence in outbreak studies.

The extracted studies from the article search were classified into 6 geographical regions as defined by the World Health Organization: Africa, South-East Asia, Eastern Mediterranean, the Americas, Europe and Western Pacific. For each region, a summary of seroprevalence, sporadic acute HEV and outbreak studies was provided.

Quality assessment

A quality assessment tool was developed for seroprevalence, sporadic acute hepatitis and outbreak studies. No checklists were available at the time of analysis to assess the quality of prevalence and incidence studies. The checklists were developed based on a number of published checklists for observational studies (insert references).

A literature review was conducted to assess the usability of pre-existing quality assessment tools. No existing quality assessment checklist was deemed specific enough for HEV. A quality assessment checklist was therefore developed drawing from five observational quality assessment checklists to assess key items specific to HEV such as diagnostic methods used as well as follow-up criteria for patients.

The checklists were developed to assess representativeness of study population, bias, validity of laboratory test used as well as the interpretation of study results. Three independent reviewers assessed articles extracted based on the developed checklist. Once articles were reviewed, they were re-assessed for consistency. When discrepancies were present between reviewers, consensus was met via discussing articles and assessments. Case reports and case series were not assessed for quality as they were not included in the quantitative analysis. However case reports and case series were included in the results section in the descriptive summary for each region.

Meta-analysis

A meta-analysis was conducted to combine data from studies including in the systematic review. Analysis was done on both a country and regional level. Data from studies were categorized based on the following outcomes/indicators: seroprevalence, sporadic cases, outbreaks, mortality and stillbirths. The initial step in the analysis was to assess whether heterogeneity was present.

A random effect model was used to analyse data from the selected articles. The I^2 value was used to assess for heterogeneity between studies based on the following formula (30):

$$I^2 = \left(\frac{Q-df}{Q}\right) \times 100\%$$

This test assessed whether inconsistencies existed between studies. This indicator was calculated at a country and regional level. Forest plots were generated using the R statistical program (version 3.3.2) to present pooled estimates along with I^2 values for each outcome by country and region designated by the WHO. Confidence intervals for pooled estimates were calculated based on the Clopper-Pearson interval (exact method).

The forest plots were generated at a country, regional and global level. The results were presented according to the following 6 regions defined by the World Health Organization (WHO): Africa, Americas, South East, Eastern Mediterranean, Europe and Western Pacific.

Low heterogeneity was defined as an I^2 value of less than 50%. Medium to high heterogeneity was defined as an I^2 value between 50% and 75% and scores above 75% were considered as high

heterogeneity. A pooled estimate was provided for all countries and regions however, the estimates shouldn't be used when the I^2 value is above 75% due to high heterogeneity.

Results

Global

At the global level, seroprevalence of HEV for all genotypes based on published articles between 2009 and 2014 was 12.0% (95%CI 12.0%, 13.0%). Studies reporting data on seroprevalence were highly heterogenous (I^2=100%). Based on the quality assessment tool, a total of 8 studies were assessed as having the highest level of quality. These studies were highly heterogeneous (I^2>99%). Seroprevalence for high quality studies was comparable to the seroprevalence estimate for all seroprevalence articles: 17% (95%CI 12.0%, 23.0%).

The global incidence of sporadic HEV cases was 5.0% (95%CI 50%, 5.0%). Studies included in the analysis were heterogeneous (I^2=100%). Only one incidence study was assessed as having the highest level of quality. Heterogeneity was not assessed for high quality sporadic and outbreak studies due to insufficient studies available for analysis.

A total of 22 studies reporting incidence from HEV outbreaks were included in the analysis. The global incidence for HEV outbreaks was 42% (95%CI 32.0%, 53.0%). Heterogeneity was high between studies (I^2=100%). Only one study in this sub group was assessed at the highest quality and therefore heterogeneity was not assessed.

A large degree of variation was present in mortality studies across the world. Mortality associated with HEV ranged from 0.2% to 83%. The global mortality associated with HEV was

3.0% (95%CI 2.0%, 4.0%). High levels of heterogeneity was also reported in mortality studies (I^2=90%).

Globally, only three studies reporting data on still births from 2009 to 2014 were included in the analysis. The estimate for still births was 44.0% (95%CI 0.0%, 92.0%). The three studies were heterogeneous (I^2=98%), results should be interpreted with caution due to the small number of studies included in this sub group.

Regions

African region

A total of 4 studies in the African region reported seroprevalence data (Table 1). Seroprevalence ranged from 8.0 % to 29.0% in data retrieved from Burkina Faso, Ghana, Nigeria and Zambia. The seroprevalence for the region was 18.0% (95%CI 11.0%, 25.0%). The lowest prevalence was noted among blood donors. Sporadic cases of HEV infection were reported from Ghana, and Uganda. The highest incidence of HEV was reported among pregnant women in Ghana (64.4%). The overall incidence of sporadic HEV cases for Africa was 65.0% (95%CI 59.0%, 72.0%). A case report study identified circulation of HEV genotype 3 in Mayotte. An additional case report was reported in Angola in a fulminant HEV infection case. A total of 5 outbreak studies reported incidence and attack rates in this region. The incidence of HEV outbreak for the region was 39% (95%CI 22.0%, 57.0%). One outbreak reported an attack rate of 1018.2 per 1000 population (Table 1). An additional outbreak reported in Uganda resulted in an attack rate of 305 per 1000 population. In Ghana, the first death associated with fatal fulminant hepatic failure was reported in 2010. Two studies reported data on mortality and the overall case-fatality rate for the region was 18% (95%CI 0%, 41%)A total of two studies reported data on still births.

The I^2 for seroprevalence of IgG in Africa was 89% based on findings from 4 studies. Based on random effects models the I^2 value for studies reporting on incidence of sporadic HEV cases was 0%. Only two studies were included in the sporadic HEV cases category. Data from studies on incidence of HEV in outbreaks were heterogeneous (I^2=97%). No heterogeneity was present in the mortality studies within the African region (I^2=0%).

Americas region

Seroprevalence data was retrieved from a total of 8 studies conducted in the United States of America and Cuba and ranged from 1% to 32% (Table 2). The overall seroprevalence was 8.0% (95%CI 6.0%, 10.0%). Sex specific seroprevalence was reported in only one study and did not vary greatly between males and females (9% vs. 11% respectively). Sporadic HEV cases have been reported in Brazil, the United States of America, Venezuela and Argentina. Incidence of sporadic HEV cases in this region ranged from 1.6% to 46% and no differences in sex specific incidence were observed. The incidence of sporadic HEV cases in the region of the Americas was 5.0% (95%CI 3.0%, 7.0%). The largest incidence study conducted in the United States reported an incidence of 7 infections per 1000 susceptible persons per year. Four case reports due to HEV were reported in the region of the Americas. Circulation of Genotype 3 was reported in the United States and Uruguay while Genotype 1 was only reported in Uruguay. A total of three American studies reported mortality data. The case fatality rates ranged from 1.0% to 4.0%. Genotypes 1, 3, and 4 have been documented in this region.

Seven studies were included in the analysis for seroprevalence. An I^2 value of 98% was calculated for all seroprevalence studies. Studies reporting data on sporadic HEV cases were 95% heterogeneous. A total of three American studies reporting mortality were analyzed for

heterogeneity. No heterogeneity was detected in the American studies reporting on case fatality rates. Overall, data from this region were highly heterogeneous and no pooled estimates were generated as result.

Eastern Mediterranean region

Seroprevalence studies were retrieved from Egypt, Iran, Iraq, Saudi Arabia and Yemen (Table 3). Seroprevalence in this region ranged from 4% to 59% with Egypt reporting the highest seroprevalence in the Eastern Mediterranean region. The overall seroprevalence for the region was 17.0% (95%CI 13.0%, 21.0%). Seroprevalence was similar between females and males. Age specific seroprevalence increased with age; 1-12% in 1-49 age group versus 14-50% in individuals aged 50 years and above. Data on incidence was reported from Afghanistan, Egypt, Iran, Iraq and Pakistan and the United Arab Emirates. Incidence in this region ranged from 1% to 81% and the overall incidence was 17.0% (95%CI 15.0%, 20.0%). In studies conducted on blood donors, detection of anti-HEV IgM antibodies were reported to be as high 14%. In studies reporting incidence data, a higher incidence was observed in females compared to males. Mortality was reported in pregnant women and still birth was reported as well. Mortality associated with HEV was 29.0% (95%CI 18.0%, 39.0%). One study in the region reported still birth data (36%). Evidence of genotype 1 has been documented in Egypt and Pakistan.

Seroprevalence and sporadic HEV studies from the Eastern Mediterranean regions based on the random effects models was >90% heterogeneous. Mortality data for pregnant women was not heterogeneous (I^2=0%). A total of two studies reporting data on still births was heterogenous (I^2=96%). Studies reporting on mortality in the Eastern Mediterranean were homogeneous and enabled to generate a pooled estimate.

South East Asia Region

Seroprevalence studies were reported from India, Bangladesh, Thailand and Indonesia (Table 4). Seroprevalence ranged from 5% to 34%. Seroprevalence for the South East Asia region was 15% (95%CI 10.0%, 20.0%). Incidence varied from 5% to 79% and the highest incidence rate was reported in adults 16-49 years of age. The overall incidence was 32.0% (95%CI 26.0%, 39.0%). A total of eight outbreaks have been reported between 2009 and January 2014 in this region. Three outbreaks were attributable to genotype 1.

Attack rates for outbreaks ranged from 40 to 16559 per 100 000 population. The incidence of outbreak for the region was 45% (95%CI 23%, 67%). Nine studies reported case fatality rates (0.2%-83%). The pooled cases fatality rate was 2.0% (95%CI 0.0%, 3.0%). Additionally, five case reports were reported in India. Genotypes 1 and 4 were identified in the cases reported from India. Overall, genotypes 1 and 3 have been documented in this region. In South Korea, a case report was the only study to document the presence of HEV in the country (genotype 4). Additionally, a case report in India conducted in 2009 reported the first human case of HEV infection due to genotype 4 acquired in India. A case series in Bangladesh reported an outbreak attributable to HEV genotype 1 that affected 200 people. A total of 11 studies reported data on Hepatitis for this region and have documented the presence of Genotypes 1, 3 and 4. Overall case fatality rates (CFR) were reported from Bangladesh and Thailand. Among pregnant women, CFR's ranged from 20% to 83%. A total of two studies reported on still births and the overall proportion of still births for the region was 48% (95%CI 0-100%).

Seroprevalence data was reported from 3 countries. The I^2 for the region was 97%. Studies from Bangladesh, India, Indonesia and Thailand reported high levels of heterogeneity (I^2>90%). In studies reporting mortality in both the general population and among pregnant women reported

high heterogeneity as well (I^2>90%). Studies reporting on still birth were also highly heterogeneous (I^2=100%).

European region

Seroprevalence in the European region ranged from less than 1% to 52% (Table 5). Among blood donors, seroprevalence ranged from 0.03% to 52.0% . The overall seroprevalence was 10.0% (95%CI 8.0%, 12.0%). Studies conducted on pig farmers and forestry workers in France and Germany reported seroprevalence between 17% and 35%. Only 3 seroprevalence studies reported genotypes. All studies with genotype information identified genotype 3 in patients with evidence of HEV. Data on incidence was retrieved from 13 studies. The incidence of sporadic HEV in Europe was 4.0% (95%CI 3.0%, 5.0%). The highest incidence was reported in a French study conducted on hospitalized patients with acute hepatitis. Sporadic cases of HEV were attributable to genotypes 1, 3 and 4. However genotype 3 was the most common genotype circulating in sporadic cases. Two outbreaks were reported in Europe between 2009 and 2014 in the following countries: France and Uzbekistan. The outbreak in France was attributable to genotype 3. One outbreak was reported as a case series in France and described an HEV outbreak linked to pig liver sausage. Two outbreaks were reported from Uzbekistan (1976 and 1986) with attack rates ranging from 1072 to 1431 per 100 000 population (34). A total of 4 studies from France reported mortality data. The overall case fatality rate ranged from 4.5% to 67% and the most common age group for cases was 50-64 years. Fatal outcomes were reported for 3 cases out of 19 case reports.

Seroprevalence data was retrieved from 7 countries. Data was highly heterogeneous both at the country and regional level (>95%). Blood donors were analyzed as a subgroup and a high level of variability was observed within the studies in this subgroup. Sporadic acute HEV cases were

reported in 10 countries, however heterogeneity was assessed for three countries as data was reported for more than one study. The I^2 for France, Germany and Italy was 97%, 0% and 99%, 98% respectively. When analyzed at a country level, no heterogeneity was found in Germany for sporadic HEV cases. Mortality data from France reported an I^2 of less than 50%. In the European region, high heterogeneity was reported for all outcomes. A pooled estimate should not be used given high variation within countries and the region.

Western Pacific Region

Seroprevalence data was obtained from Mongolia, China, Japan, South Korea and Taiwan (Table 6). Seroprevalence was reported to be as low as 0.01% in Japan and as high as 47% in China. Overall, seroprevalence for this region was 18.0% (95%CI 13.0%, 23.0%). A single seroprevalence study was reported in Mongolia among school children and revealed anti-HEV IgG detection in 0.6% of study participants. Sex specific seroprevalence was greater in females compared to males. A Japanese study focusing on transfusion transmitted HEV cases low detection of anti-HEV IgG in that population (0.01%). Incidence was reported from several studies including Cambodia, China, Hong Kong, Japan, Laos, Singapore and South Korea. Data reported from these studies ranged from 0.01% to 59.3%. The lowest incidence of HEV IgM was reported among blood donors and high incidence was reported among hospitalized acute hepatitis patients. Incidence of sporadic HEV cases for the region was 6.0.0% (95%CI 6.0%, 7.0%). Two outbreaks were reported in this region. All outbreaks occurred in Japan with incidence rates of 4% to 23% and genotype 4 was reported in one study. In studies reporting mortality data, patients diagnosed with fulminant hepatitis displayed the highest case fatality rate (50%). The case fatality rate was 8.0% (95%CI 2.0%, 13.0%). One study reported CFR among

pregnant woman in Japan (11%) (33). In the Western Pacific Region, genotypes 1, 3 and 4 have been documented. All genotypes have been reported in this region.

Data from seroprevalence studies within the Western Pacific ranged from less than 1% to 47% and resulted in a high I_2 value (100%). A total of 25 studies reporting data on sporadic HEV cases were also heterogeneous (>95%). Outbreak data was obtained from Japan where two studies reported incidence rates of 23% and 3.7%. The heterogeneous incidence of HEV outbreak resulted in an I_2 value of 96%. Mortality data for the region was also statistically heterogeneous and resulted in an I^2 value of 85%.

Discussion & conclusion

Overall at the global level, a high degree of variation was observed. High statistical heterogeneity was present in all outcomes assessed. In order to further explore statistical heterogeneity, studies were analyzed at a country level and regional level. In some regions such as the Eastern Mediterranean region, pooled estimates based on an I^2 value of less than 75% were provided. Methodological heterogeneity was assessed by analyzing studies based on the quality of studies as ranked in the quality assessment checklist. A total of eight high quality studies were assessed for the prevalence outcome. There was high statistical heterogeneity amongst the high quality studies. In order to further explore heterogeneity present between studies, characteristics of study populations were also analyzed by countries to determine whether specific study populations could influence the pooled estimates for a given country. In order to fully explore any clinical heterogeneity that could contribute to high statistical heterogeneity, a sub group analysis should be conducted on specific populations such as individuals at high risk of complications due to HEV infections (e.g. pregnant women).

Heterogeneity was 89% for seroprevalence in the African region. All but one study included in the seroprevalence group used ELISA assays (Wantai and Fortress diagnostics). The remaining studies reported having used an EIA assay (Dia Pro). The difference in the type of assay used may have contributed to the increase in heterogeneity. Only two articles were included for acute sporadic cases in Africa and study populations included patients with jaundice in one study while the other study focused on pregnant women. Case-fatality rate was reported for two studies in the African region. Study size was small in both studies (less than 21 participants) and both studies were case-series. Pooled estimates for incidence of sporadic cases and mortality were generated for the African region as I^2 values were less than 75%.

In the region of the Americas, a large study was conducted on blood donors using Wantai assays. The study revealed high seroprevalence among blood donors (18.8%). Another study on blood donors in the United States reported seroprevalence of 3.2% in the same population. The large American study conducted on blood donors reported an increase in seroprevalence with age (3.4% vs. 42.2% in those under the age of 25 years and those over the age of 65 years). A total of 0.4% of the total 1939 donations were positive for IgM. The discrepancy between findings within the same population in seroprevalence studies in the region of the Americas likely played a factor in the high heterogeneity in this subgroup. Large American seroprevalence studies including the NHANES indicate that exposure to HEV occurs frequently in blood donors despite the fact that seroprevalence in this population has been on the decline in the last decades (35).

In the Eastern Mediterranean region, Egyptian studies reported seroprevalence ranging from 39% to 59%. High seroprevalence has been reported in rural areas of Egypt and among pregnant women (36). All articles included in this review in the seroprevalence group for Egypt were in women of childbearing age. This high risk population known to have higher seroprevalence may have contributed to the increase in the regional seroprevalence. A subset analysis should be conducted to determine the effect this population has on the region.

A variety of assays have been developed for laboratory analysis of HEV specimens. Sensitivity and specificity of these assays vary greatly. In seroprevalence studies, a high level of variability has been documented in seroprevalence studies conducted in blood donors and in non-endemic areas (37). Fluorescent antibody blocking assays were considered the first line of serological assays for anti-HEV developed. This type of assay detects HEV via the presence of antigen in tissues and sensitivity ranges from 50% to 70% in patients with acute HEV (37). Western blot assays and enzyme immunoassays (EIAs) were developed to detect the presence of HEV by using recombinant protein-based tests and have been documented to have higher sensitivity than the first line of assays (approximately 90-95% in patients with acute hepatitis). Following EIAs and Western blot assays, a number of immunoassays have been developed with a wide range of sensitivity. Genelabs and Wantai assays are both anti-HEV IgG assays. Wantai assays have been documented to be far more sensitive than the Genelabs assays (98% vs. 56%) (38). Anti-HEV IgM immunoassay include International Immuno-diagnostic, MP biomedicals, Diagnostic systems and Mikrogen. The variation in sensitivity of assays may have resulted in additional heterogeneity in the data analyzed. The positive predictive value (PPV) has also been studied across the world. The PPV is dependent on the prevalence in the population and can vary from

region to region. For example, a study conducted in an endemic area reported a PPV of 95.7% (Florence Legrand-Abravanel). Another study conducted in a non-endemic area reported a PPV of 17.4% (Wen-Chien Wu). The WHO has yet to establish a standard for HEV assays.

In order to further explore heterogeneity a subgroup analysis was conducted on studies that ranked high in the quality assessment checklist. Due to insufficient data, a subgroup analysis was only conducted on the seroprevalence group. A high degree of variation was also observed in the high quality studies. Statistical heterogeneity persisted in this group. Clinical heterogeneity should be assessed further by conducting analysis on specific populations.

A study assessing the burden of HEV associated with genotypes 1 and 2 in 2005 using modelling to estimate prevalence and incidence rates (17). The study estimated that seroprevalence was highest in South Asia and South East Asia. Similar findings were observed in the analysis conducted in the scope of this study. Studies from the South East Asia region reported the highest seroprevalence followed by the Eastern Mediterranean region. In the modelling study, Rein et al. removed Egypt from the North African and Middle East region as this country displayed a high seroprevalence not reflective of the rest of the region. This may potentially explain why the Eastern Mediterranean region included studies with seroprevalence as high as 59%. A subgroup analysis should be conducted on Egypt to further assess differences in the region of the Eastern Mediterranean. The modelling study published in 2010 was the first attempt to estimate the global burden of HEV associated with genotypes 1 and 2 and to summarize articles using techniques from a meta-analysis. Findings from seroprevalence data obtained from the systematic review were comparable to those published in the modelling study. This study represents the first attempt to summarize and assess heterogeneity between studies at

a country and regional level for the years 2009-2014. This study also summarized data for genotypes 1 to 4 for HEV.

A published systematic review conducted in Africa summarized data from several studies that were included in the present systematic review (39). The African systematic review consisted of a comprehensive summary of all articles published on HEV in the African continent. The result from the systematic review from Africa also indicated that Egypt displayed higher prevalence especially in pregnant women when compared to other African countries. Genotype information was not available from studies retrieved for this systematic review however the systematic review published by Kim et al reported that genotypes 1, 2 and 3 were circulating and genotype 1 was the most dominant in the continent.

Seroprevalence for the continent of Africa ranged from 8% to 29%. The findings from the current systematic review are in comparable to the published findings from the previous global systematic review (0% to 24%) (40). High incidence from HEV sporadic cases were noted from both systematic reviews conducted. Additionally, pregnant women displayed high incidence of HEV in this region in both reviews.

In the region of the Americas, low seroprevalence was reported despite one American study reporting seroprevalence of 32%. In the previous WHO systematic review, a seroprevalence study conducted in the United States of America also reported high seroprevalence. In the current review, the United States of America also reported the highest incidence of sporadic HEV cases for the study period of 2009-2014. In the region of the Americas, outbreaks have

been reported in Cuba and Mexico. Two outbreaks were reported in the early 1980s and additional outbreaks were reported in Cuba from the initial systematic review conducted. Since no new outbreaks have been reported in the region of the Americas.

In 2012, Venezuela reported the first identifications of HEV in cases with no history of travel. Two cases were associated with genotype 1 and one case was associated with genotype 3. The genotype 1 strain isolated was similar to isolated strains found in India while genotype 3 was closely related to genotype 3 strain from the United States. In 2009, Brazil reported its first autochthonous case of HEV genotype 3. The isolate was closely related to human strains from Japan and animal strains from Brazil.

Similar to the previous systematic review published and the burden study on HEV, high seroprevalence was reported in Egypt compared to other countries in the region. Outbreaks continued to be reported after 2008 in this region in Egypt, Iraq and Pakistan. Additionally, high seroprevalence of acute HEV was also noted in the updated systematic review.

The South East Asian region continues to be hyper endemic based on updated data retrieved in the current systematic review. Several sporadic HEV studies in this region affected those with fulminant hepatic failure and resulted in high incidence for this population. Pregnant women in this region also continue to display high incidence of HEV. Outbreaks related to genotype 1 were reported in this review. In 2010, the first report of a genotype 4 infection acquired in India was reported. Overall, this region continues to report a large number of cases, outbreaks and case-fatality related studies associated with HEV.

In Europe, one study reported an incidence of 10% in the recent updated systematic review while a previous estimated published in the first systematic review only reported an incidence of 1.9% of anti-HEV IgM antibodies. An outbreak was reported for the first time in this region (42). The outbreak reported from France was linked to the consumption of pig liver sausages and the genotype responsible was 3. Seroprevalence studies reported between 2009 and 2013 in the European region indicated higher detection of anti-HEV IgG was present. Particularly, in France two studies included in the analysis reported seroprevalence of 35% and 52% in swine workers and blood donors respectively. Overall, France reported the highest seroprevalence of anti-HEV IgG, incidence of anti-HEV IgM. Additionally, all mortality and outbreak studies were reported from France. The majority of endemic HEV cases in France are due to HEV genotype 3, and specifically to subtypes 3f, 3c and 3e found in pigs. The findings from the current review indicate that zoonotic transmission of HEV genotype 3 via consumption of raw or undercooked liver-based sausages has been documented and exposure to pigs by swine workers plays an important role in the epidemiology of HEV transmission in France (43).

The systematic review conducted, identified some of the first documented cases of HEV genotype 4 in the European region. In 2009, France reported its first autochthonous human HEV case associated with genotype 4 (insert reference). The case was a 30 year old female with no history of travel abroad. The patients did not consume any uncooked or undercooked pork, wild boar meat or have contact with animals. The case did receive blood transfusions. Person-to-person transmission via blood has been reported in France in 2009. The strain was highly similar to strains isolated from humans in China.

Two years later in 2011, an outbreak of HEV genotype 4 was associated with consumption of liver sausage known to traditionally uncooked. A total of 11 cases were reported during the outbreak period and two were typed as genotype 4. Both had consumed uncooked pig liver sausage. Based on sequencing analysis, the strains from infected cases were similar to strains found China.

In the same year in Italy, an outbreak associated with genotype 4 was reported. A total of five cases were reported. Sequencing analysis revealed that the strains were closely related to strains that originated from China. The strains demonstrated low similarities with the strains from European outbreaks reported earlier such as the one in France. The outbreak was not linked to imported foods or persons travelling from endemic areas. In 2012, Denmark reported three cases of HEV genotype 4. Upon sequence analysis, one strain was similar to the strain from the outbreak reported in Italy and the remaining two strains resembled human and animal strains found in China. The evidence reported in Europe in the last few years suggests that genotype 4 has become endemic to the region.

In the Western Pacific region, China reported seroprevalence cases associated with genotypes 3 and 4. Similar trends in terms of proportion of seroprevalence cases reported in China have been observed in the updated review. In Japan, new data is consistent with previous findings in the literature; no change has been reported in terms of seroprevalence of HEV. Transmission of HEV in individuals working in slaughterhouses has been reported in this region and linked with genotype 4. In this region, recent studies from the current review indicate that blood donors account for a small number of sporadic HEV cases. However, there were reports of detection of

IgM antibodies in blood donors; this was almost inexistent in other regions. In the current review, two outbreaks were reported from Japan (41). In one outbreak study, male gender and pre-existing alcohol liver disease was related to the progression of HEV infection. This observation was also reported in the previous WHO systematic review. Case fatality rates were only reported from China in the current systematic review. High case fatality rates were reported from patients with liver failure and fulminant hepatic failure patients.

Comparing disease estimates between countries is challenging. Firstly, few countries conduct routine surveillance on Hepatitis E. Knowledge dissemination on Hepatitis E cases depends largely on scientific publications. Case definitions for Hepatitis E differ between countries and within countries as well. Case definitions are based on the following three factors: clinical symptoms, virological confirmation and epidemiological links to a laboratory-confirmed case. Additionally, laboratory tests used across the world differ and as mentioned above sensitivity varies greatly. Studies assessing sensitivity of assays have reported sensitivity ranging from 50% to 90%. Several regions had few published articles on HEV cases. This contributed to the high heterogeneity within regions and countries.

Data on HEV continues to be lacking, only a few countries have surveillance systems in place to monitor HEV infections. This limitation poses a serious challenge for estimating the burden associated with HEV at a regional or global level. In certain regions of the world where HEV is not endemic the only documented evidence of HEV circulating is from case reports and case series or abstracts published at a conference. Several regions included in this review only had a few studies available for analysis, this may have resulted in an inaccurate estimation of heterogeneity.

This systematic review represents the first attempt at assessing the global burden associated with HEV genotypes 1, 2, 3 and 4. Pooled estimates were generated for incidence of anti-HEV antibodies for the African region. Mortality estimates for the African, Eastern Mediterranean and European regions were generated. These estimates of incidence, prevalence and mortality may provide a baseline that could possibly be used to estimate the impact of vaccination in the future.

Conflict of Interest

None of the authors reported any conflict of interest.

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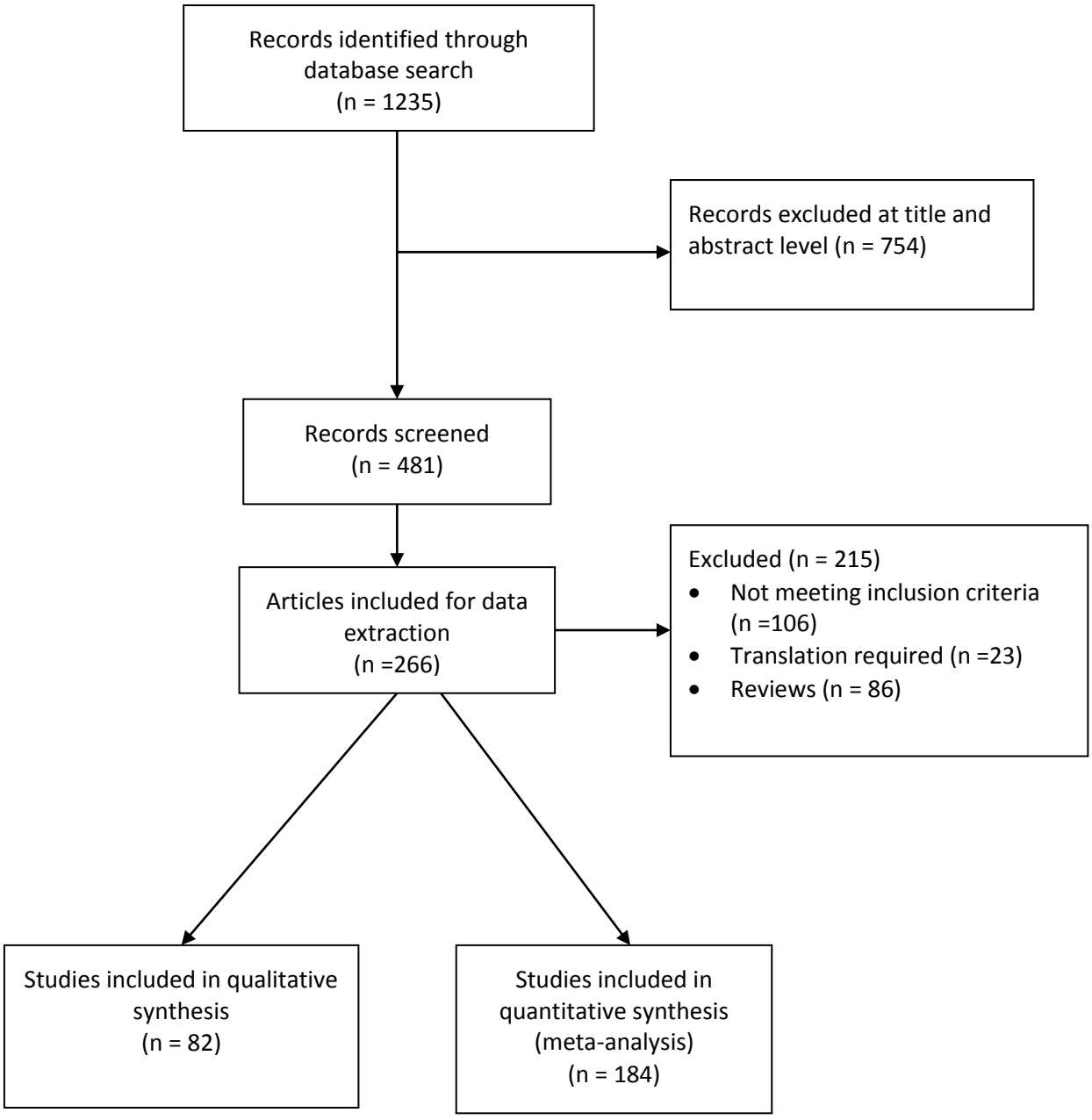
Figures

Figure 1: Flow diagram



Tables

Table 1: Country and regional outcome estimates-Africa

Outcome	Country	Country estimates (%)	Overall regional estimate (%)	I^2 (%)	Comments
Seroprevalence	Ghana	8.0-28.6	18 (95%CI 11.0, 25.0)	89	One study from Zambia reported a prevalence estimate twice as high as the other studies in the region. This factor may explain the heterogeneity present for this outcome. In Ghana, variation between studies was observed. A study conducted in healthy blood donors reported the lowest seroprevalence in the region. The other study from Ghana was conducted on pregnant women.
	Burkina Faso	16.2			
	Zambia	25.0			
	Nigeria	13.4			
Incidence of sporadic HEV	Uganda	65.2	65.0 (95%CI 59.0, 72.0)	0	No statistical heterogeneity was observed in this category.
	Ghana	64.4			
Incidence of HEV outbreaks	Central African Republic	52.0-53.0	39.0 (95%CI 22.0-57.0)	97	Large heterogeneity was present within studies from the Central African Republic and studies from Uganda.
	Uganda	30.5-43.6			
Attack rates*	Uganda	1018.2	……..		No regional estimate was provided as only one country

						provided estimates.
Mortality	Central African Republic	14.2	18.0 (95%CI 0.0-41.0)	0		No statistical heterogeneity was observed in this category.
	Ghana	33.0				
Still births	Central African Republic	14.3	25.0 (95%CI 4.0-46.0)		75	Only two studies were included in this subgroup. Heterogeneity between the two countries resulted in overall heterogeneity for still birth estimate.
	Sudan	36.0				

*Attack rate defined as the number of new laboratory-confirmed HEV cases in the population at risk among the number of persons at risk in the population. Attack rate presented per 100 000 population

Table 2: Country and regional outcome estimates -Americas

Outcome	Country	Country estimates (%)	Overall regional estimate (%)	I^2 (%)	Comments
Seroprevalence	Cuba	10.0	8.0 (95%CI 6.0, 10.0)	98	Two studies in the United States of America reported seroprevalence of 3.1% and 3.2%. As noted by the authors of the American study, low seroprevalence may be attributed to the implementation of universal precautions in the blood donor and animal handler population studied.
	United States of America	3.1-18.8			
Incidence	Argentina	6.1	5.0 (95%CI 3.0,7.0)	95	Both Argentina and Brazil reported lower incidence rates as compared to rates reported by 3 studies in the remainder of the region. Sub group differences were statistically significant as reported by the Chi^2 test of difference reported in R.
	Brazil	1.6			
	United States of America	0.4-46.2			
	Venezuela	29.7			
Case fatality rate	United States of America	1.0-4.0%	……..		No heterogeneity was found in the three studies reported from the United States of America

*Attack rate defined as the number of new laboratory-confirmed HEV cases in the population at risk among the number of persons at risk in the population. Attack rate presented per 100 000 population
**CFR defined as the number of fatal cases among the laboratory-confirmed HEV cases

Table 3: Country and regional outcome estimates-Eastern Mediterranean

Outcome	Country	Country estimates	Overall regional estimate	I_2 (%)	Comments
Seroprevalence	Egypt	39.0-58.6	16 (95%CI 12.0,20.0)	98	Seroprevalence in Egypt was consistently higher than other countries in the region. As documented in the literature, seroprevalence reported from Egyptian studies was high. A total of 5 studies from Iran reported seroprevalence less than 10%. Three other studies from Iran and the studies from Saudi Arabia and Yemen reported seroprevalence ranging from 10-18%. The sources of heterogeneity in this region stemmed from high seroprevlaence in Egypt, and low seroprevalence from 5 Iranian studies.
	Iran	2.3-14.3			
	Iraq	14.2-48.3			
	Saudi Arabia	18.7			
	Yemen	10.7			
Incidence	Afghanistan	28.4	21.0 (95% CI 18.0, 25.0)	98	High heterogeneity was present within studies reported in Egypt. Additionally, one study in Iran and Iraq each reported incidence rates of
	Egypt	0.9-41.2			
	Iran	0.5			
	Iraq	1.6			
	Pakistan	5.4-81.5			
	United Arab Emirates	40.0			

					less than 1%. In Pakistan, high heterogeneity was noted due to one study reporting low incidence (5% prevalence). Four studies reported incidence for outbreaks which ranged from 18% to 81%.
Overall CFR	Sudan	71.9	…….		Estimates were only obtained from one country. No regional estimate was generated.
Maternal CFR	Sudan Pakistan	28.21 29.0	29.0 (95%CI 0.18,0.40)	0	Estimates from both countries were very similar. No heterogeneity was found between studies for this outcome.
Still birth	Pakistan Sudan	50.0 35.9	63.0 (95%CI 9.0,.17)	96	In Pakistan, HEV is endemic and outbreaks. Poor sanitation and access to clean water contribute to the incidence and severe outcomes associated with HEV infections. The study conducted in Pakistan was conducted as a retrospective cohort in a hospital while the study conducted in Sudan was conducted in an outbreak setting.

*Attack rate defined as the number of new laboratory-confirmed HEV cases in the population at risk among the number of persons at risk in the population. Attack rate presented per 100 000 population
**CFR defined as the number of fatal cases among the laboratory-confirmed HEV cases

Table 4: Country and regional outcome estimates -South East Asia

Outcome	Country	Country estimates	Overall regional estimate	I_2 (%)	Comments
Seroprevalence	Bangladesh	3.6-6.0	15.0 (95%CI 10.0,20.0)	97	A study conducted in Java, Indonesia, reported low seroprevalence (5.8%).This estimate contributed to high heterogeneity in this group. High seroprevalence was reported in Bangladesh. The other studies in the region reported seroprevalence that ranged from 11% to 15%. The two studies from Indonesia and Bangladesh contributed to increasing heterogeneity in this regions.
	India	11.3-33.7			
	Indonesia	5.8			
	Thailand	15.8			
Incidence	Bangladesh	6.0-64.2	32.0 (95% CI 26.0-39.0)	98	One study conducted in Bangladesh was one of the first studies to study incidence in a healthy population. This may explain the low incidence reported in this study compared to other estimates generated from studies focusing on acute hepatitis cases as a study population.
	India	4.8-78.6			

					Another study in India reported low incidence (4.6%). This study was conducted among blood donors. This could also explain low incidence reported in that study. A total of 4 studies were conducted on FHF patients. Incidence reported in this group was high (41.4%-64.2%).
Attack rates*	India	40.0-16559	……		Estimates were only obtained from one country. No regional estimate was generated.
Overall CFR**	Bangladesh India Thailand Nepal	65.1 0.2-0.9 9.1 19.6-83.3	2.0 (95%CI 0.0.3.0)	95	One study conducted in Bangladesh reported a CFR of 65%. This study was conducted among Fulminant hepatic failure patients known to be at higher risk for complications associated with HEV infections including death. This contributed to elevated heterogeneity in this group.
Stillbirth	Nepal	5.4	……		Estimates were only obtained from one country. No regional estimate was generated.

*Attack rate defined as the number of new laboratory-confirmed HEV cases in the population at risk among the number of persons at risk in the population. Attack rate presented per 100 000 population
**CFR defined as the number of fatal cases among the laboratory-confirmed HEV cases

Table 5: Country and regional outcome estimates -Europe

Outcome	Country	Country estimates	Overall regional estimate	I^2 (%)	Comments
Seroprevalence	Austria	14.3	10.0 (95%CI 8.0-12.0)	99	High seroprevalence in France was reported in a study focusing on occupational exposure. The other study conducted in France highlighted high endemicity in the south of France that could possibly be linked to dietary practices. A total of 5 studies reporting seroprevalence above 25% contributed to the heterogeneity in this region. The remainder of the studies reported seroprevalence ranging from 0.24% to 17%.
	France	35.2-52.3			
	Germany	0.09-17.3			
	Greece	9.4			
	Italy	1.3			
	Netherlands	1.9-26.7			
	Portugal	33.3			
	Spain	3.7-50			
	Switzerland	4.9			
	Turkey	2.1-12.4			
	United Kingdom	4.7-11.8			
Incidence	Denmark	33.9	4.0 (95%CI 3.0-5.0)	98	A total of 3 studies from France, Spain and Denmark reported incidence tanged from 34% to 88%. All other studies in the region reported incidence from 0.1% to 11%. The three studies reporting high incidence contributed to adding heterogeneity for this outcome.
	France	3.1-51.4			
	Germany	0.03			
	Finland	11.3			
	Hungary	9.6			
	Italy	0.2-48.1			
	Montenegro	6.0			
	Netherlands	11.5			
	Spain	7.0			
	United Kingdom	1.9			

Attack rates	Uzbekistan	1072-1431	……	Estimates were only obtained from one country. No regional estimate was generated.
Mortality	France	4.5-66.7	……..	Estimates were only obtained from one country. No regional estimate was generated.

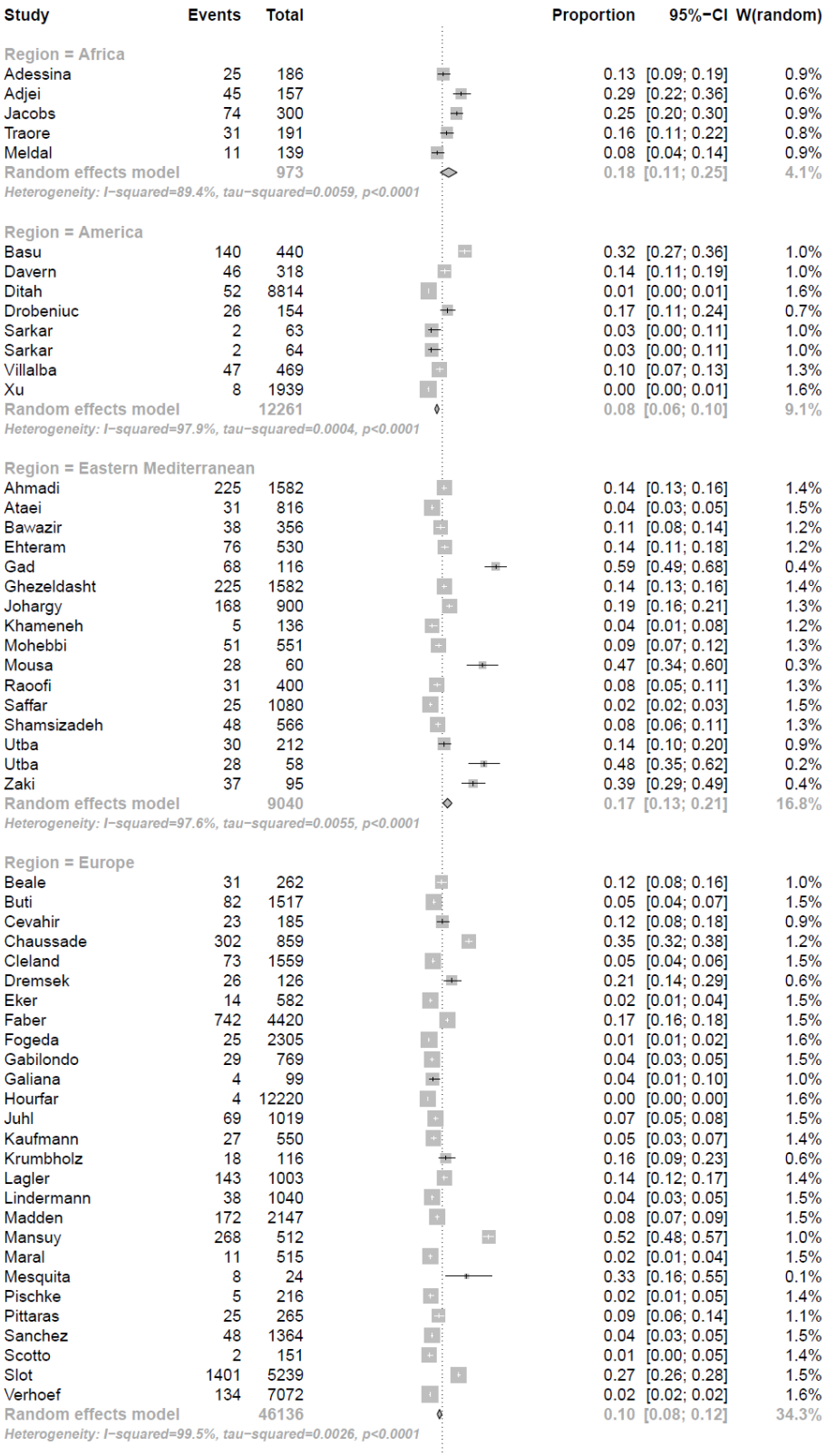
*Attack rate defined as the number of new laboratory-confirmed HEV cases in the population at risk among the number of persons at risk in the population. Attack rate presented per 100 000 population
**CFR defined as the number of fatal cases among the laboratory-confirmed HEV cases

Table 6: Country and regional outcome estimates -Western Pacific

Outcome	Country	Country estimates	Overall regional estimate	I^2 (%)	Comments
Seroprevalence	China	7.9-47.7	18.0 (95%CI 13.0-23.0)	100	Heterogeneity was present within countries and within the region. A study conducted on immigrants residing in various countries reported high seroprevalence.
	Japan	0.01-14.3			
	Mongolia	0.6			
	South Korea	34.0			
	Taiwan	7.7			
Incidence	Cambodia	10.9	7.0 (95%CI 6.0-7.0)	100	Within China, a total of four studies were conducted on large sample sizes (>10 000 study participants). Incidence for these studies were less than 6% compared to other studies reported in China on smaller sample sizes (as high as 92%). Similarly, one study in Japan was conducted on a large sample size and reported low incidence (0.01%) compared to other studies in the country.
	China	0.1-92.2			
	Hong Kong	28.7			
	Japan	0.01-19.9			
	Lao	1.6-18.2			
	Singapore	13.5			
	South Korea	1.4			
Incidence of HEV outbreaks	Japan	3.7-23.3	……..		Estimates were only obtained from one country. No regional estimate was generated.
Overall CFR	China	0.9-16.2	8.0 (95%CI 2.0, 13.0)	87	Studies reporting on acute liver failure patients reported higher CFR and contributed to heterogeneity in this region.
	Japan	1.6-50.0			

*Attack rate defined as the number of new laboratory-confirmed HEV cases in the population at risk among the number of persons at risk in the population. Attack rate presented per 100 000 population
**CFR defined as the number of fatal cases among the laboratory-confirmed HEV cases

Supplement: Forest plots



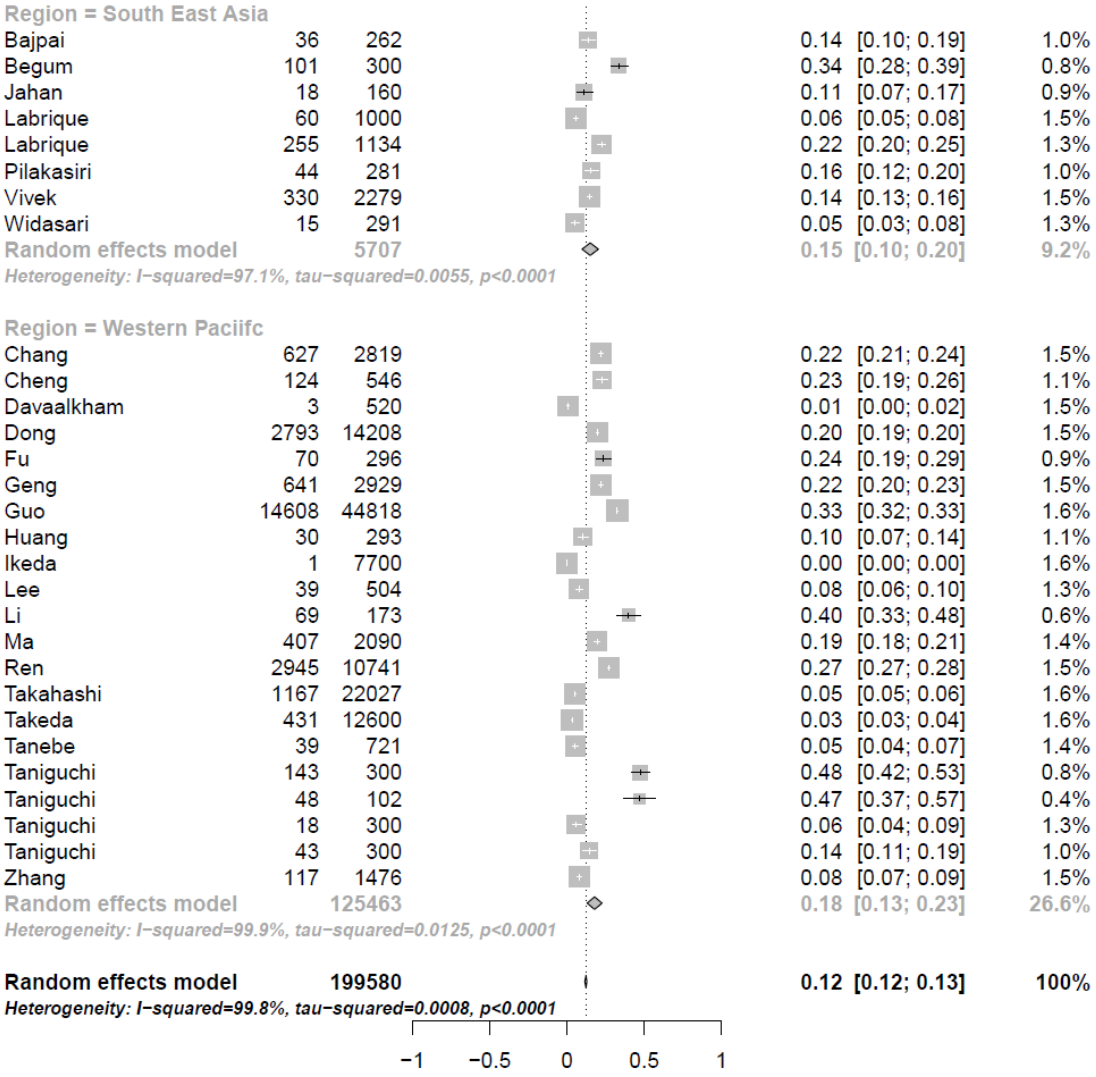
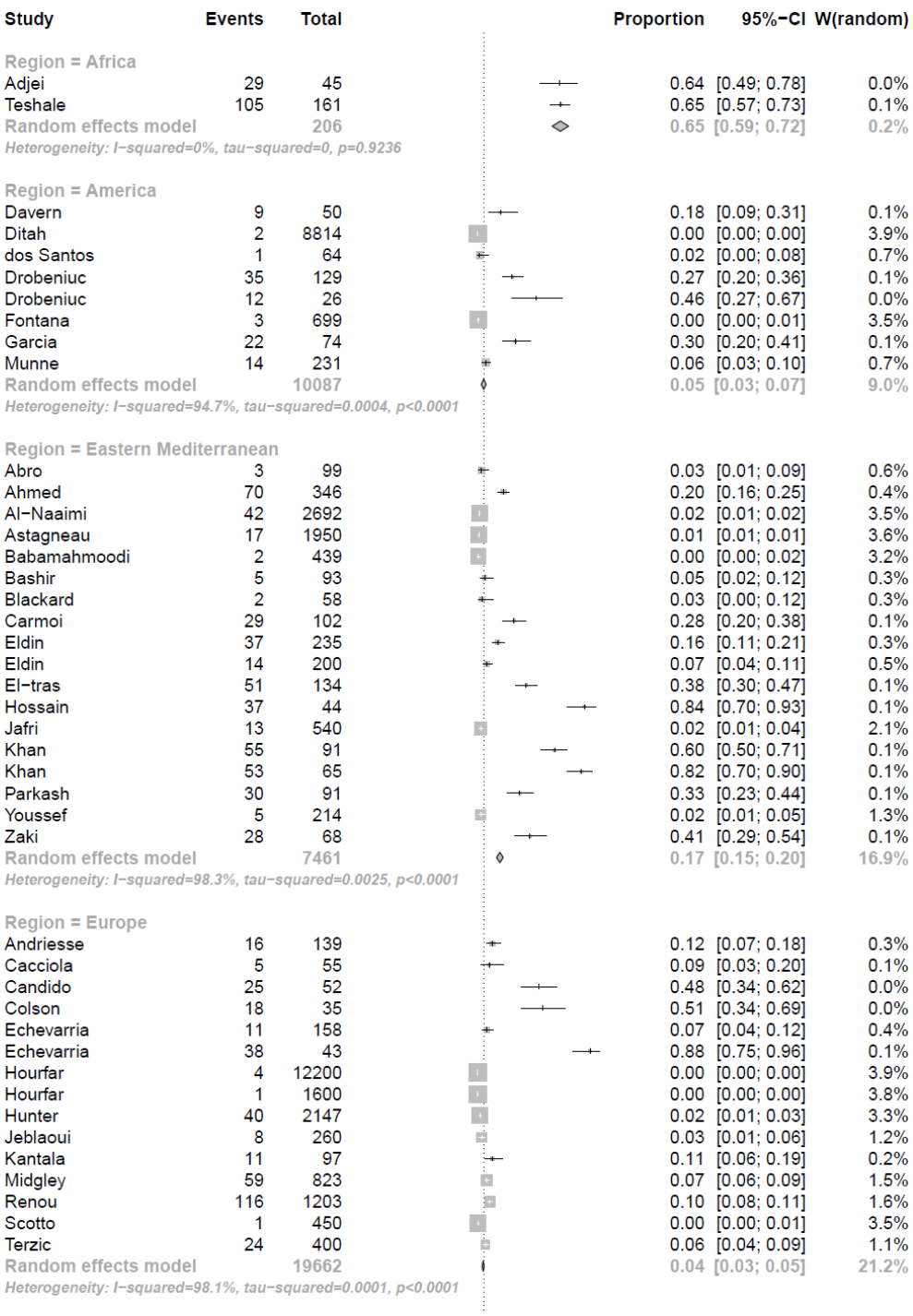


Figure s.1: Forest plot of seroprevalence of anti-HEV antibodies by WHO region


Study Events Total Proportion 95%−CI W(random)
Region = Africa
Adjei 29 45 0.64 [0.49; 0.78] 0.0%
Teshale 105 161 0.65 [0.57; 0.73] 0.1%
Random effects model 206 0.65 [0.59; 0.72] 0.2%
Heterogeneity: I−squared=0%, tau−squared=0, p=0.9236
Region = America
Davern 9 50 0.18 [0.09; 0.31] 0.1%
Ditah 2 8814 0.00 [0.00; 0.00] 3.9%
dos Santos 1 64 0.02 [0.00; 0.08] 0.7%
Drobeniuc 35 129 0.27 [0.20; 0.36] 0.1%
Drobeniuc 12 26 0.46 [0.27; 0.67] 0.0%
Fontana 3 699 0.00 [0.00; 0.01] 3.5%
Garcia 22 74 0.30 [0.20; 0.41] 0.1%
Munne 14 231 0.06 [0.03; 0.10] 0.7%
Random effects model 10087 0.05 [0.03; 0.07] 9.0%
Heterogeneity: I−squared=94.7%, tau−squared=0.0004, p<0.0001
Region = Eastern Mediterranean
Abro 3 99 0.03 [0.01; 0.09] 0.6%
Ahmed 70 346 0.20 [0.16; 0.25] 0.4%
Al−Naaimi 42 2692 0.02 [0.01; 0.02] 3.5%
Astagneau 17 1950 0.01 [0.01; 0.01] 3.6%
Babamahmoodi 2 439 0.00 [0.00; 0.02] 3.2%
Bashir 5 93 0.05 [0.02; 0.12] 0.3%
Blackard 2 58 0.03 [0.00; 0.12] 0.3%
Carmoi 29 102 0.28 [0.20; 0.38] 0.1%
Eldin 37 235 0.16 [0.11; 0.21] 0.3%
Eldin 14 200 0.07 [0.04; 0.11] 0.5%
El−tras 51 134 0.38 [0.30; 0.47] 0.1%
Hossain 37 44 0.84 [0.70; 0.93] 0.1%
Jafri 13 540 0.02 [0.01; 0.04] 2.1%
Khan 55 91 0.60 [0.50; 0.71] 0.1%
Khan 53 65 0.82 [0.70; 0.90] 0.1%
Parkash 30 91 0.33 [0.23; 0.44] 0.1%
Youssef 5 214 0.02 [0.01; 0.05] 1.3%
Zaki 28 68 0.41 [0.29; 0.54] 0.1%
Random effects model 7461 0.17 [0.15; 0.20] 16.9%
Heterogeneity: I−squared=98.3%, tau−squared=0.0025, p<0.0001
Region = Europe
Andriesse 16 139 0.12 [0.07; 0.18] 0.3%
Cacciola 5 55 0.09 [0.03; 0.20] 0.1%
Candido 25 52 0.48 [0.34; 0.62] 0.0%
Colson 18 35 0.51 [0.34; 0.69] 0.0%
Echevarria 11 158 0.07 [0.04; 0.12] 0.4%
Echevarria 38 43 0.88 [0.75; 0.96] 0.1%
Hourfar 4 12200 0.00 [0.00; 0.00] 3.9%
Hourfar 1 1600 0.00 [0.00; 0.00] 3.8%
Hunter 40 2147 0.02 [0.01; 0.03] 3.3%
Jeblaoui 8 260 0.03 [0.01; 0.06] 1.2%
Kantala 11 97 0.11 [0.06; 0.19] 0.2%
Midgley 59 823 0.07 [0.06; 0.09] 1.5%
Renou 116 1203 0.10 [0.08; 0.11] 1.6%
Scotto 1 450 0.00 [0.00; 0.01] 3.5%
Terzic 24 400 0.06 [0.04; 0.09] 1.1%
Random effects model 19662 0.04 [0.03; 0.05] 21.2%
Heterogeneity: I−squared=98.1%, tau−squared=0.0001, p<0.0001

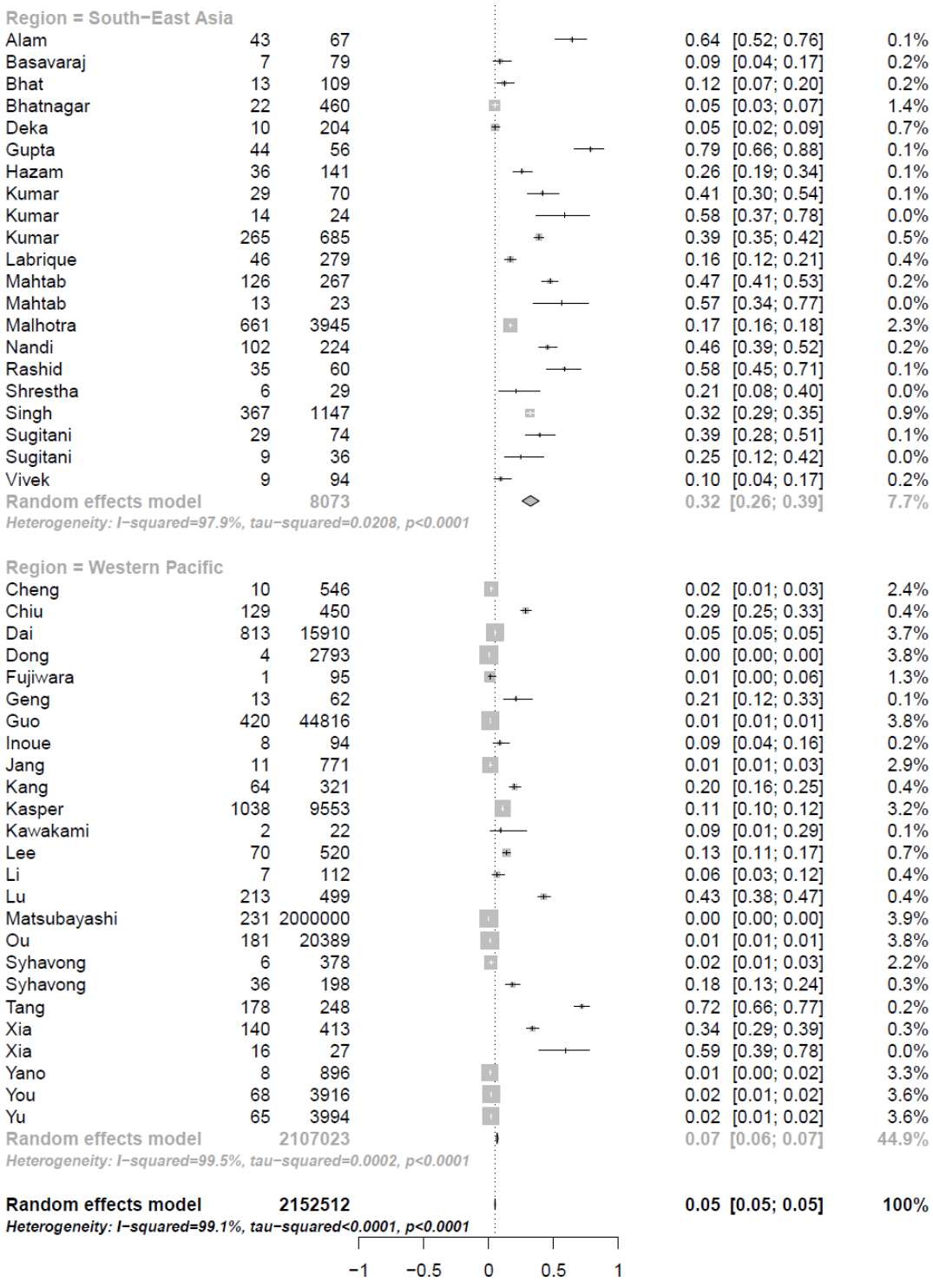


Figure s.2: Forest plot of incidence of sporadic HEV cases by WHO region

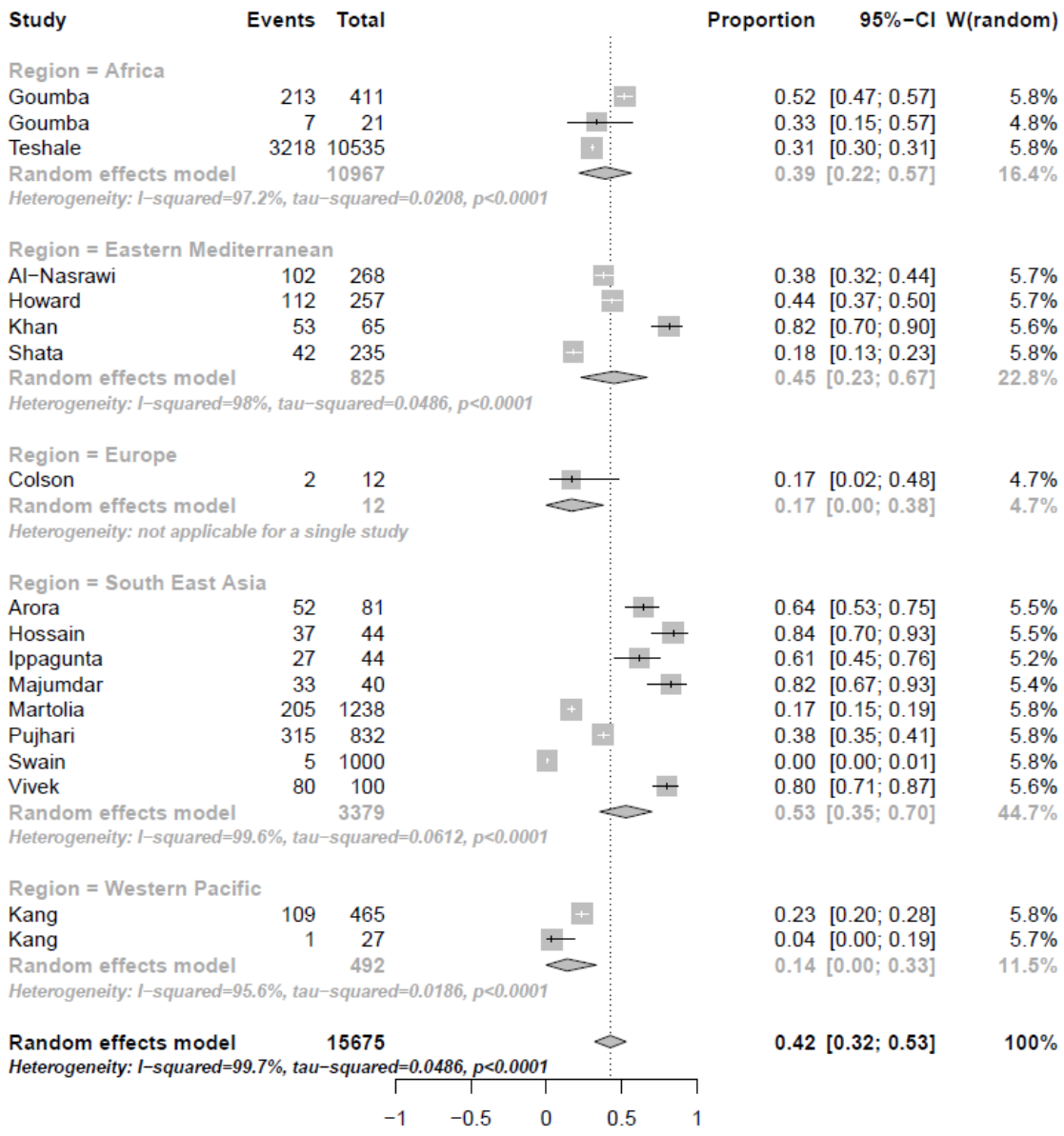


Figure s.3: Forest plot of incidence of HEV outbreaks by WHO region

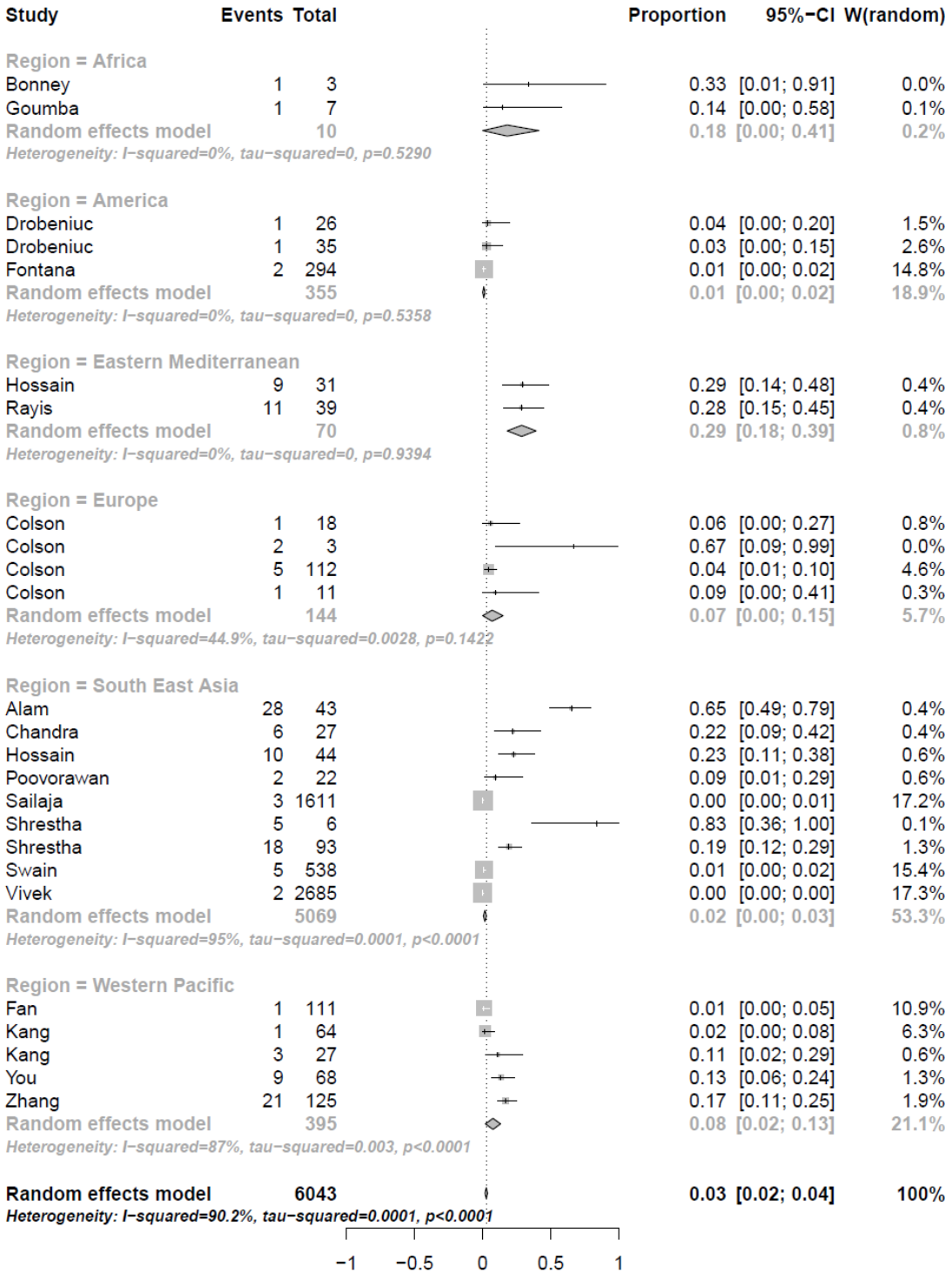


Figure s.4: Forest plot of case-fatality rate (CFR) by WHO region

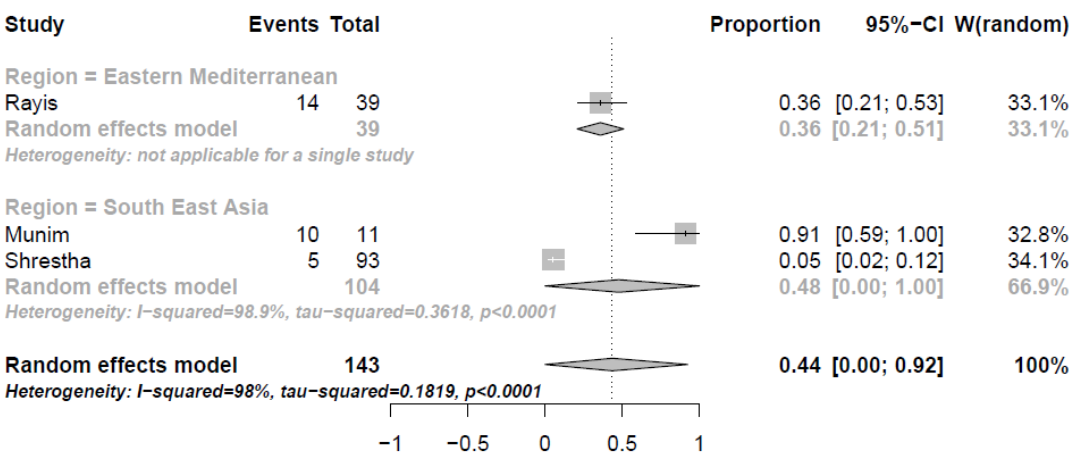
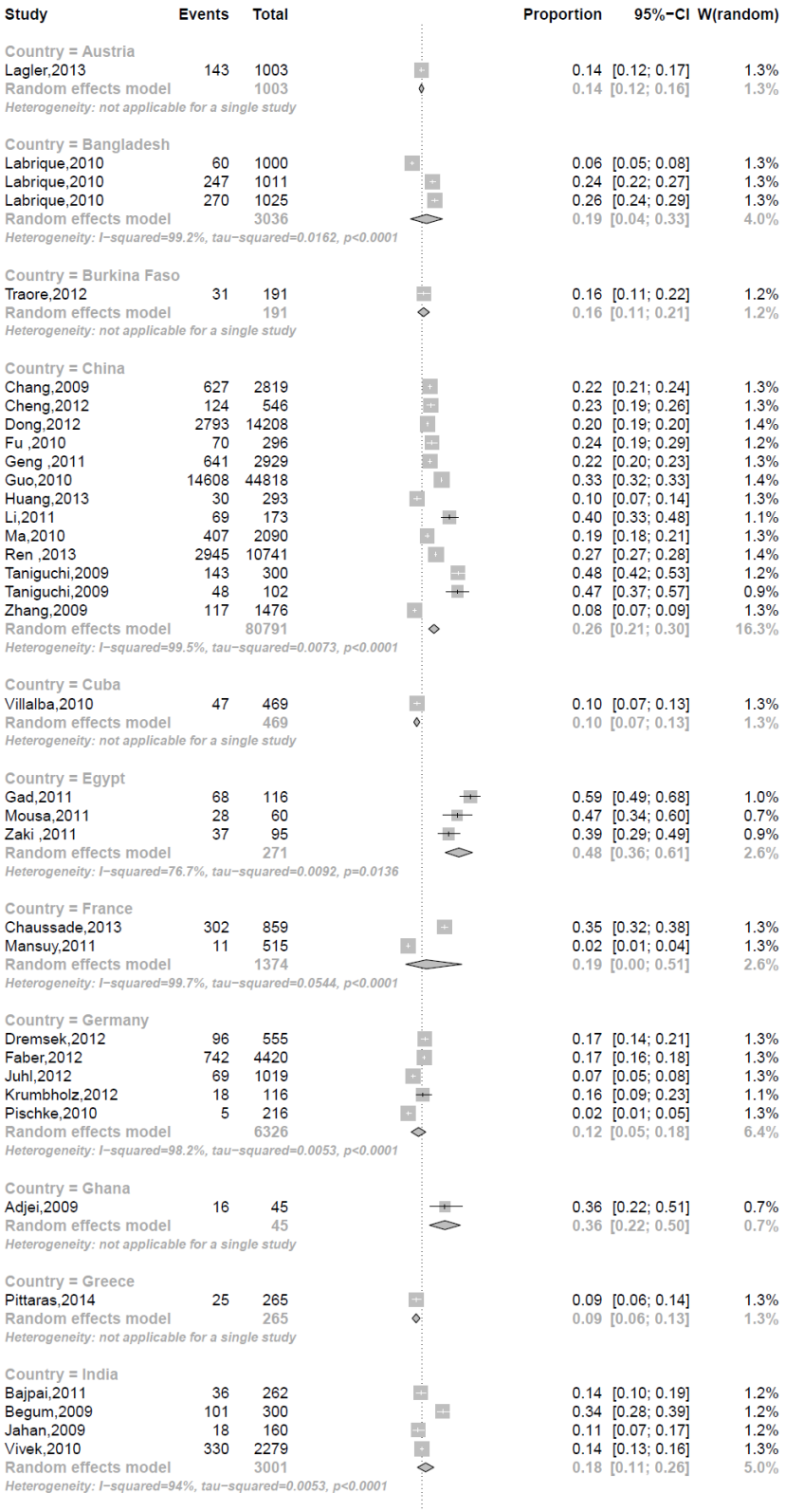


Figure s.5: Forest plot of still births by WHO region


Study Events Total Proportion 95%−CI W(random)
Country = Austria
Lagler,2013 143 1003 0.14 [0.12; 0.17] 1.3%
Random effects model 1003 0.14 [0.12; 0.16] 1.3%
Heterogeneity: not applicable for a single study
Country = Bangladesh
Labrique,2010 60 1000 0.06 [0.05; 0.08] 1.3%
Labrique,2010 247 1011 0.24 [0.22; 0.27] 1.3%
Labrique,2010 270 1025 0.26 [0.24; 0.29] 1.3%
Random effects model 3036 0.19 [0.04; 0.33] 4.0%
Heterogeneity: I−squared=99.2%, tau−squared=0.0162, p<0.0001
Country = Burkina Faso
Traore,2012 31 191 0.16 [0.11; 0.22] 1.2%
Random effects model 191 0.16 [0.11; 0.21] 1.2%
Heterogeneity: not applicable for a single study
Country = China
Chang,2009 627 2819 0.22 [0.21; 0.24] 1.3%
Cheng,2012 124 546 0.23 [0.19; 0.26] 1.3%
Dong,2012 2793 14208 0.20 [0.19; 0.20] 1.4%
Fu ,2010 70 296 0.24 [0.19; 0.29] 1.2%
Geng ,2011 641 2929 0.22 [0.20; 0.23] 1.3%
Guo,2010 14608 44818 0.33 [0.32; 0.33] 1.4%
Huang,2013 30 293 0.10 [0.07; 0.14] 1.3%
Li,2011 69 173 0.40 [0.33; 0.48] 1.1%
Ma,2010 407 2090 0.19 [0.18; 0.21] 1.3%
Ren ,2013 2945 10741 0.27 [0.27; 0.28] 1.4%
Taniguchi,2009 143 300 0.48 [0.42; 0.53] 1.2%
Taniguchi,2009 48 102 0.47 [0.37; 0.57] 0.9%
Zhang,2009 117 1476 0.08 [0.07; 0.09] 1.3%
Random effects model 80791 0.26 [0.21; 0.30] 16.3%
Heterogeneity: I−squared=99.5%, tau−squared=0.0073, p<0.0001
Country = Cuba
Villalba,2010 47 469 0.10 [0.07; 0.13] 1.3%
Random effects model 469 0.10 [0.07; 0.13] 1.3%
Heterogeneity: not applicable for a single study
Country = Egypt
Gad,2011 68 116 0.59 [0.49; 0.68] 1.0%
Mousa,2011 28 60 0.47 [0.34; 0.60] 0.7%
Zaki ,2011 37 95 0.39 [0.29; 0.49] 0.9%
Random effects model 271 0.48 [0.36; 0.61] 2.6%
Heterogeneity: I−squared=76.7%, tau−squared=0.0092, p=0.0136
Country = France
Chaussade,2013 302 859 0.35 [0.32; 0.38] 1.3%
Mansuy,2011 11 515 0.02 [0.01; 0.04] 1.3%
Random effects model 1374 0.19 [0.00; 0.51] 2.6%
Heterogeneity: I−squared=99.7%, tau−squared=0.0544, p<0.0001
Country = Germany
Dremsek,2012 96 555 0.17 [0.14; 0.21] 1.3%
Faber,2012 742 4420 0.17 [0.16; 0.18] 1.3%
Juhl,2012 69 1019 0.07 [0.05; 0.08] 1.3%
Krumbholz,2012 18 116 0.16 [0.09; 0.23] 1.1%
Pischke,2010 5 216 0.02 [0.01; 0.05] 1.3%
Random effects model 6326 0.12 [0.05; 0.18] 6.4%
Heterogeneity: I−squared=98.2%, tau−squared=0.0053, p<0.0001
Country = Ghana
Adjei,2009 16 45 0.36 [0.22; 0.51] 0.7%
Random effects model 45 0.36 [0.22; 0.50] 0.7%
Heterogeneity: not applicable for a single study
Country = Greece
Pittaras,2014 25 265 0.09 [0.06; 0.14] 1.3%
Random effects model 265 0.09 [0.06; 0.13] 1.3%
Heterogeneity: not applicable for a single study
Country = India
Bajpai,2011 36 262 0.14 [0.10; 0.19] 1.2%
Begum,2009 101 300 0.34 [0.28; 0.39] 1.2%
Jahan,2009 18 160 0.11 [0.07; 0.17] 1.2%
Vivek,2010 330 2279 0.14 [0.13; 0.16] 1.3%
Random effects model 3001 0.18 [0.11; 0.26] 5.0%
Heterogeneity: I−squared=94%, tau−squared=0.0053, p<0.0001

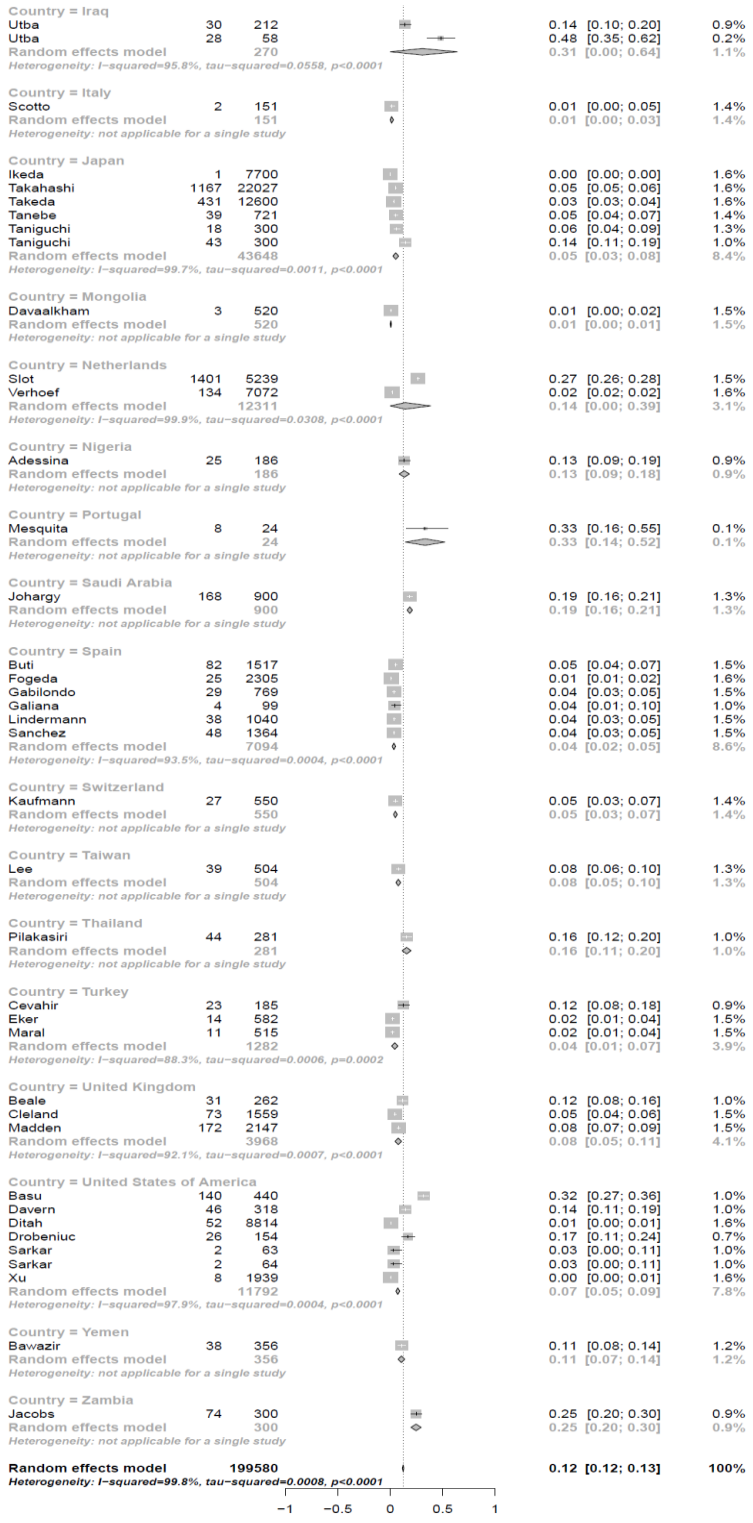
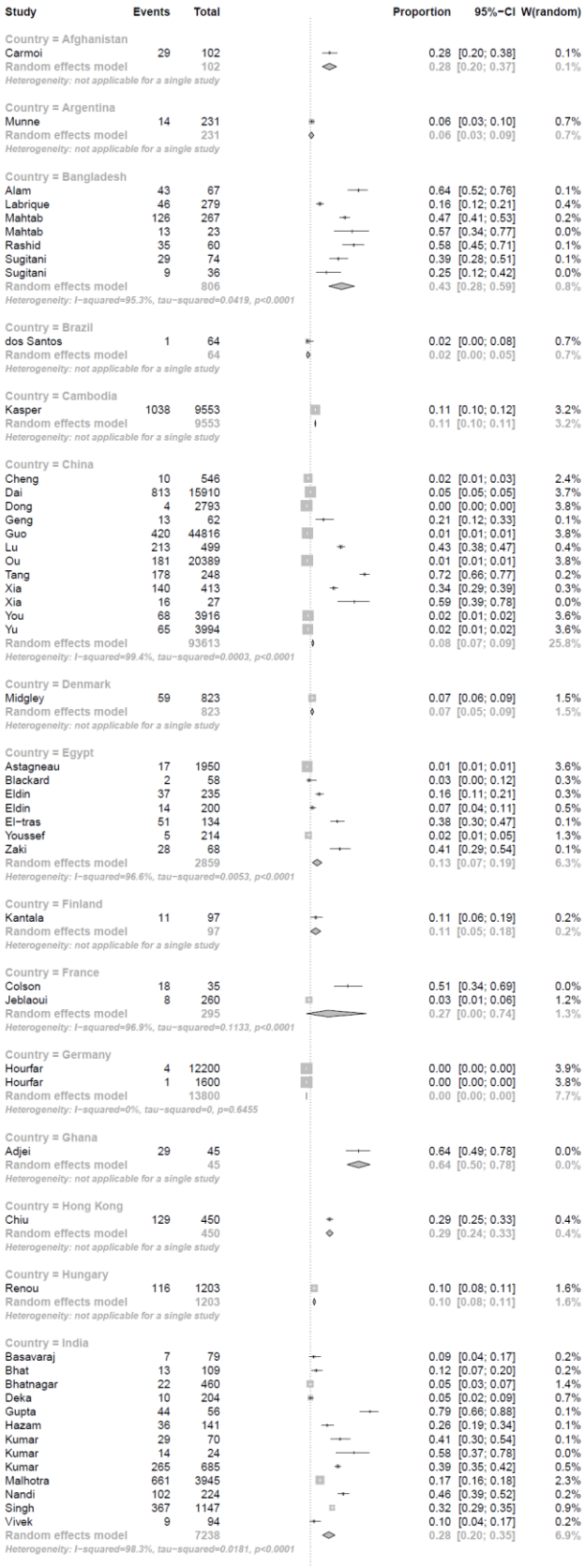


Figure s.6: Forest plot of global seroprevalence of anti-HEV antibodies by country


Study Events Total Proportion 95%−CI W(random)
Country = Afghanistan
Carmoi 29 102 0.28 [0.20; 0.38] 0.1%
Random effects model 102 0.28 [0.20; 0.37] 0.1%
Heterogeneity: not applicable for a single study
Country = Argentina
Munne 14 231 0.06 [0.03; 0.10] 0.7%
Random effects model 231 0.06 [0.03; 0.09] 0.7%
Heterogeneity: not applicable for a single study
Country = Bangladesh
Alam 43 67 0.64 [0.52; 0.76] 0.1%
Labrique 46 279 0.16 [0.12; 0.21] 0.4%
Mahtab 126 267 0.47 [0.41; 0.53] 0.2%
Mahtab 13 23 0.57 [0.34; 0.77] 0.0%
Rashid 35 60 0.58 [0.45; 0.71] 0.1%
Sugitani 29 74 0.39 [0.28; 0.51] 0.1%
Sugitani 9 36 0.25 [0.12; 0.42] 0.0%
Random effects model 806 0.43 [0.28; 0.59] 0.8%
Heterogeneity: I−squared=95.3%, tau−squared=0.0419, p<0.0001
Country = Brazil
dos Santos 1 64 0.02 [0.00; 0.08] 0.7%
Random effects model 64 0.02 [0.00; 0.05] 0.7%
Heterogeneity: not applicable for a single study
Country = Cambodia
Kasper 1038 9553 0.11 [0.10; 0.12] 3.2%
Random effects model 9553 0.11 [0.10; 0.11] 3.2%
Heterogeneity: not applicable for a single study
Country = China
Cheng 10 546 0.02 [0.01; 0.03] 2.4%
Dai 813 15910 0.05 [0.05; 0.05] 3.7%
Dong 4 2793 0.00 [0.00; 0.00] 3.8%
Geng 13 62 0.21 [0.12; 0.33] 0.1%
Guo 420 44816 0.01 [0.01; 0.01] 3.8%
Lu 213 499 0.43 [0.38; 0.47] 0.4%
Ou 181 20389 0.01 [0.01; 0.01] 3.8%
Tang 178 248 0.72 [0.66; 0.77] 0.2%
Xia 140 413 0.34 [0.29; 0.39] 0.3%
Xia 16 27 0.59 [0.39; 0.78] 0.0%
You 68 3916 0.02 [0.01; 0.02] 3.6%
Yu 65 3994 0.02 [0.01; 0.02] 3.6%
Random effects model 93613 0.08 [0.07; 0.09] 25.8%
Heterogeneity: I−squared=99.4%, tau−squared=0.0003, p<0.0001
Country = Denmark
Midgley 59 823 0.07 [0.06; 0.09] 1.5%
Random effects model 823 0.07 [0.05; 0.09] 1.5%
Heterogeneity: not applicable for a single study
Country = Egypt
Astagneau 17 1950 0.01 [0.01; 0.01] 3.6%
Blackard 2 58 0.03 [0.00; 0.12] 0.3%
Eldin 37 235 0.16 [0.11; 0.21] 0.3%
Eldin 14 200 0.07 [0.04; 0.11] 0.5%
El−tras 51 134 0.38 [0.30; 0.47] 0.1%
Youssef 5 214 0.02 [0.01; 0.05] 1.3%
Zaki 28 68 0.41 [0.29; 0.54] 0.1%
Random effects model 2859 0.13 [0.07; 0.19] 6.3%
Heterogeneity: I−squared=96.6%, tau−squared=0.0053, p<0.0001
Country = Finland
Kantala 11 97 0.11 [0.06; 0.19] 0.2%
Random effects model 97 0.11 [0.05; 0.18] 0.2%
Heterogeneity: not applicable for a single study
Country = France
Colson 18 35 0.51 [0.34; 0.69] 0.0%
Jeblaoui 8 260 0.03 [0.01; 0.06] 1.2%
Random effects model 295 0.27 [0.00; 0.74] 1.3%
Heterogeneity: I−squared=96.9%, tau−squared=0.1133, p<0.0001
Country = Germany
Hourfar 4 12200 0.00 [0.00; 0.00] 3.9%
Hourfar 1 1600 0.00 [0.00; 0.00] 3.8%
Random effects model 13800 0.00 [0.00; 0.00] 7.7%
Heterogeneity: I−squared=0%, tau−squared=0, p=0.6455
Country = Ghana
Adjei 29 45 0.64 [0.49; 0.78] 0.0%
Random effects model 45 0.64 [0.50; 0.78] 0.0%
Heterogeneity: not applicable for a single study
Country = Hong Kong
Chiu 129 450 0.29 [0.25; 0.33] 0.4%
Random effects model 450 0.29 [0.24; 0.33] 0.4%
Heterogeneity: not applicable for a single study
Country = Hungary
Renou 116 1203 0.10 [0.08; 0.11] 1.6%
Random effects model 1203 0.10 [0.08; 0.11] 1.6%
Heterogeneity: not applicable for a single study
Country = India
Basavaraj 7 79 0.09 [0.04; 0.17] 0.2%
Bhat 13 109 0.12 [0.07; 0.20] 0.2%
Bhatnagar 22 460 0.05 [0.03; 0.07] 1.4%
Deka 10 204 0.05 [0.02; 0.09] 0.7%
Gupta 44 56 0.79 [0.66; 0.88] 0.1%
Hazam 36 141 0.26 [0.19; 0.34] 0.1%
Kumar 29 70 0.41 [0.30; 0.54] 0.1%
Kumar 14 24 0.58 [0.37; 0.78] 0.0%
Kumar 265 685 0.39 [0.35; 0.42] 0.5%
Malhotra 661 3945 0.17 [0.16; 0.18] 2.3%
Nandi 102 224 0.46 [0.39; 0.52] 0.2%
Singh 367 1147 0.32 [0.29; 0.35] 0.9%
Vivek 9 94 0.10 [0.04; 0.17] 0.2%
Random effects model 7238 0.28 [0.20; 0.35] 6.9%
Heterogeneity: I−squared=98.3%, tau−squared=0.0181, p<0.0001

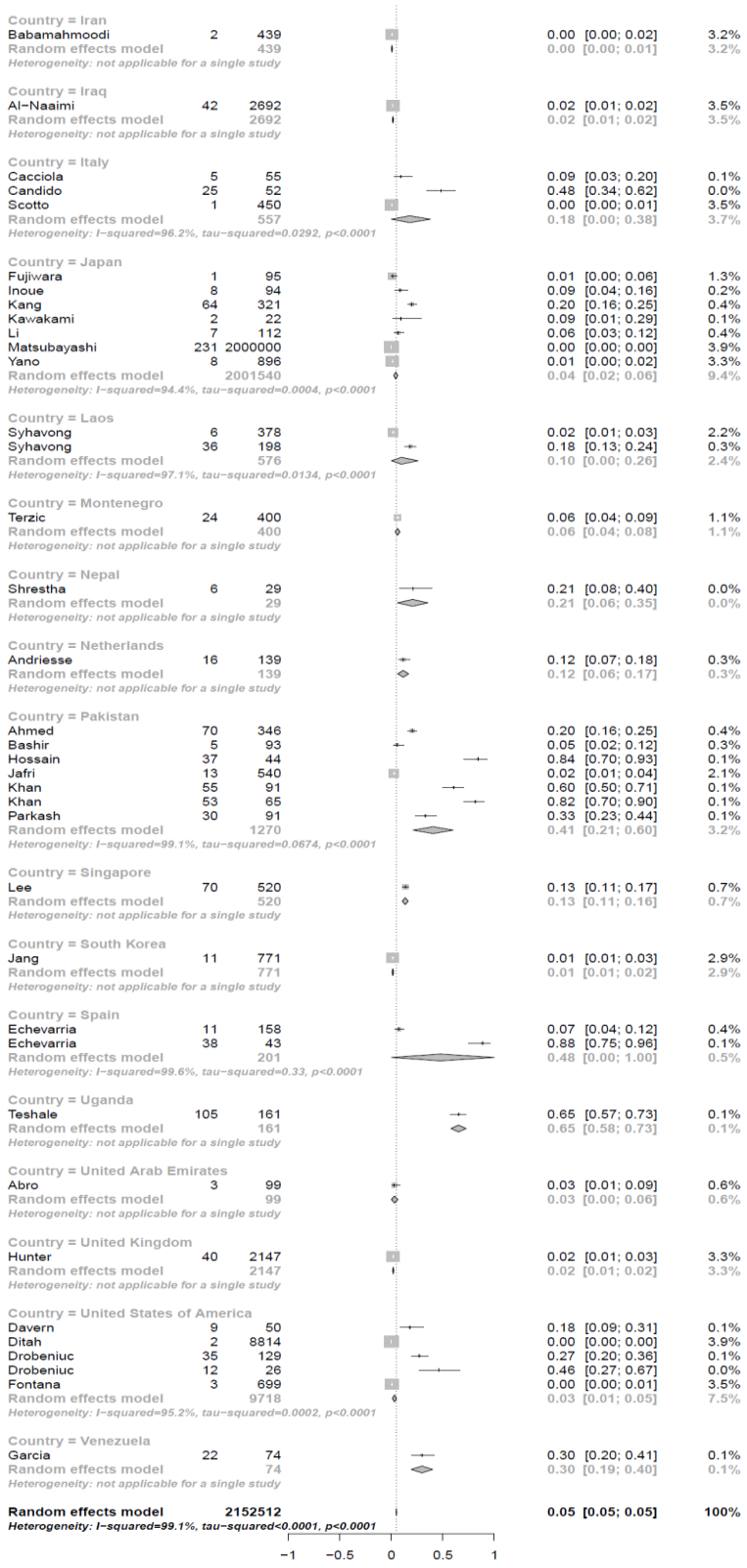


Figure s.7: Forest plot of global incidence of sporadic HEV cases by country

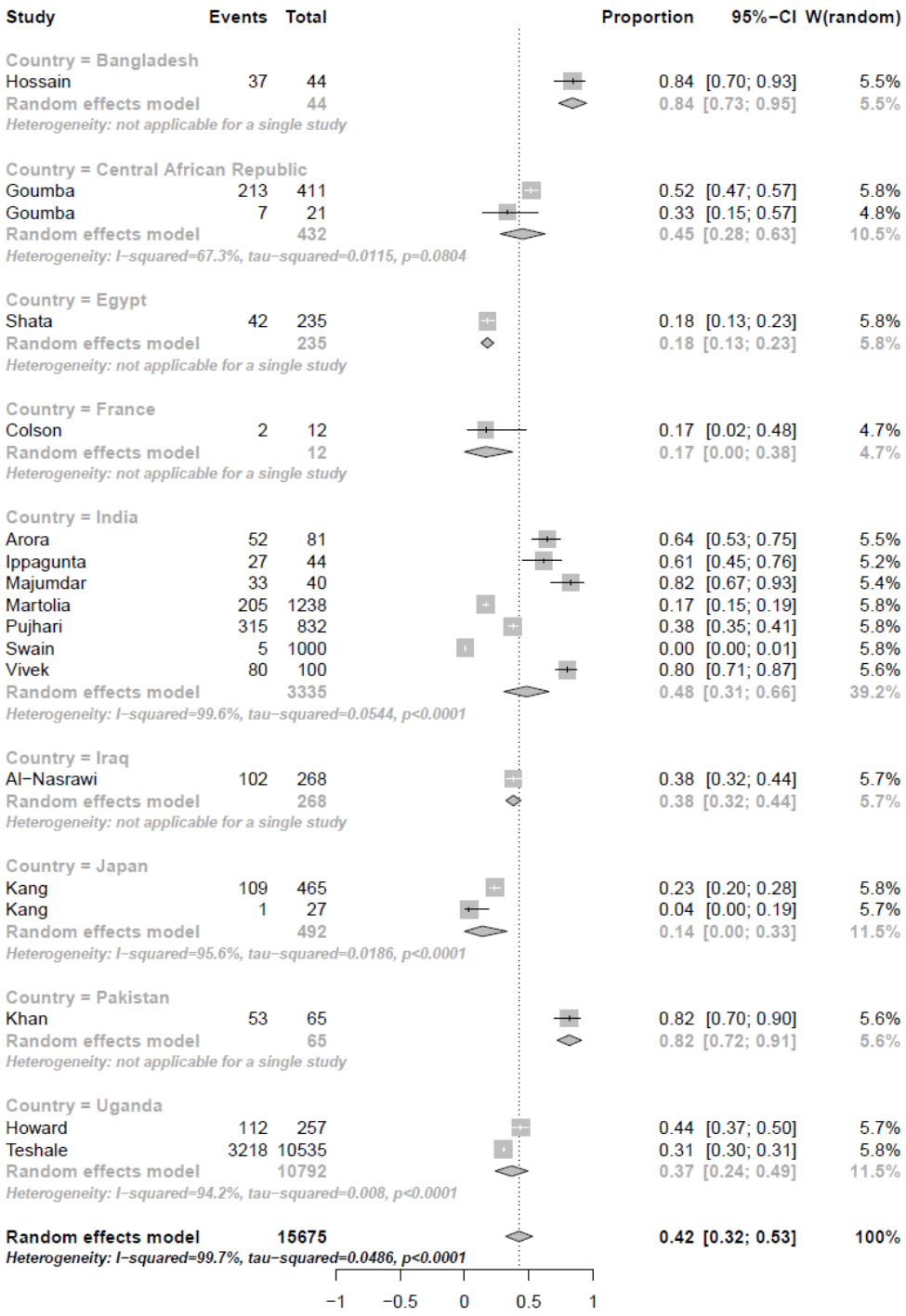


Figure s.8: Forest plot of global incidence of HEV outbreaks by country

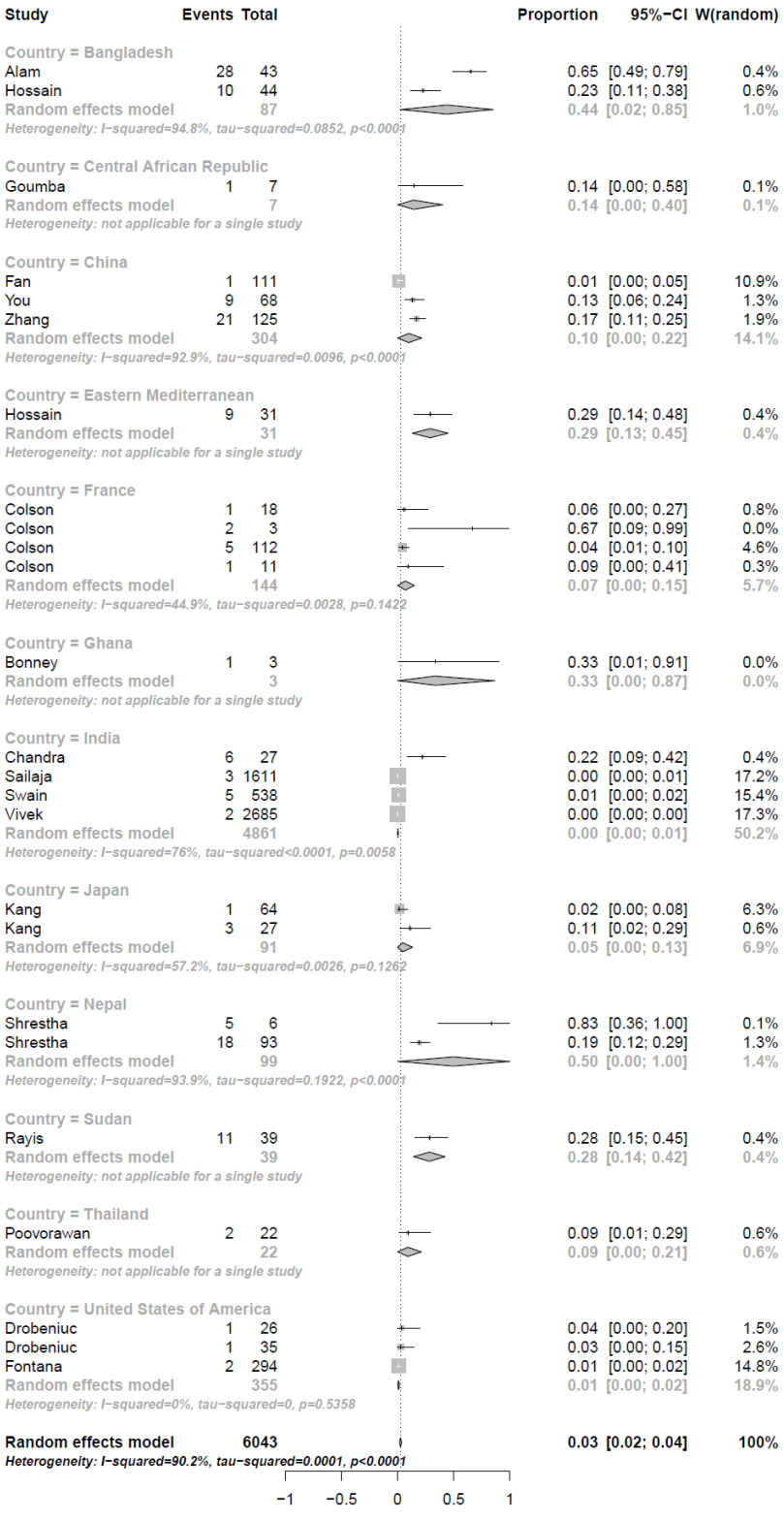


Figure s.9: Forest plot of global Case fatality rate associated with Hepatitis E Virus by country

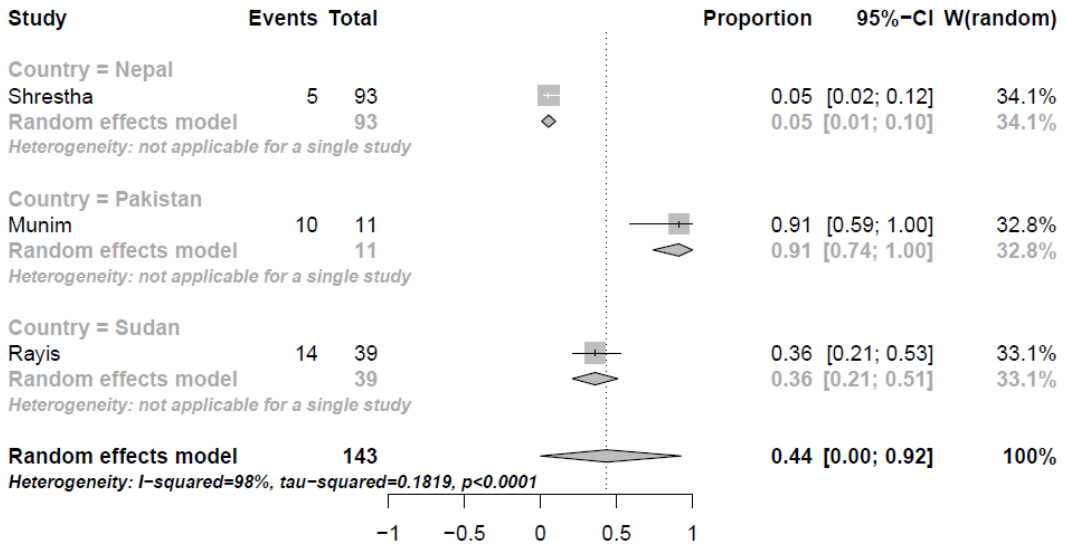


Figure s.10: Forest plot of the global still births by country

CHAPTER FOUR

A SUBGROUP ANALYSIS OF BLOOD DONORS AND HIGH RISK POPULATIONS FOR COMPLICATIONS DUE TO HEPATITIS E VIRUS FROM A GLOBAL SYSTEMATIC REVIEW ON HEPATITIS E VIRUS 1, 2, 3,4

The following chapter has been prepared as a manuscript for submission to the Journal of Liver and Clinical Research. This manuscript builds on the systematic review conducted in the previous chapter. This chapter presents subgroup analysis on the following three specific populations: blood donors, pregnant women and fulminant hepatic failure (FHF) patients. The objectives of this chapter were to: 1) further explore subgroups of interest; 2) summarize published evidence of HEV circulation at a country-level, regional level and global level for high risk populations; 3) assess heterogeneity between studies published and when feasible, provide a pooled estimate of the incidence, prevalence and mortality associated with HEV.

As for Chapter 3, detailed tables of the quality assessment and characteristics of the studies are provided in Appendix 1-27.

The MSc. Student is the first author of this paper and was responsible for data collection, data quality appraisal, data analysis and dissemination via writing the manuscript. This paper was co-authored by Dr George Wells, Dr Philippe Duclos, David Becking and Joan Peterson. Drs Wells and Duclos provided meaningful feedback on the development and revisions of this manuscript; David Becking and Joan Peterson were the second independent reviewers of the quality assessment and data extraction.

Type of Article: Review Article

Title: A subgroup analysis of blood donors and high risk populations for complications due to Hepatitis E virus from a global systematic review on hepatitis E virus genotypes 1, 2, 3, 4

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Disclaimer: One of the authors is a World Health Organization staff member. The opinions expressed in this article are those of the authors and do not necessarily represent the decisions, official policy or opinions of the World Health Organization.

Title: A subgroup analysis of blood donors and high risk populations for complications due to Hepatitis E virus from a global systematic review on Hepatitis E Virus genotypes 1, 2, 3, 4

Abstract: Globally, hepatitis E virus (HEV) is responsible for large outbreaks in endemic countries and sporadic cases throughout the world. Annually, the World Health Organization (WHO) estimates that globally, HEV is associated with 20 million infections and 56 000 deaths. A systematic review was conducted to assess the burden associated with HEV genotypes 1, 2, 3 and 4 in terms of seroprevalence, incidence and mortality. High levels of heterogeneity were reported in the systematic review. We attempted to further analyze the findings from the systematic review and analyzed blood donors, pregnant women and Fulminant Hepatic Failure (FHF) patients as subgroups. Subgroups were analyzed at a global level and by global regions as defined by the WHO. A pooled estimate was generated for pregnant women. Globally, maternal mortality associated with HEV was 20% (95% CI 16%, 25%). In the Eastern Mediterranean regions studies reporting on seroprevalence among blood donors, pooled estimated were generated. Seroprevalence of anti-HEV antibodies in blood donors in the Eastern Mediterranean was 18% (95% CI 14%, 22%) respectively. This study represents the first attempt at quantifying the global burden of HEV genotypes 1, 2, 3 and 4 associated with incidence, prevalence and mortality in pregnant women, FHF patients and blood donors.

Keywords: Hepatitis E virus, acute viral hepatitis, epidemiology

Abbreviations: HEV: Hepatitis E virus

Introduction

Hepatitis E infections are caused by the hepatitis E virus (HEV). Low sanitation has been associated with increased risk of transmission of HEV. Contaminated sources of water are among the most common sources of HEV outbreaks documented worldwide (4). Outbreaks associated with HEV have been documented at a higher frequency in developing countries (28). While the most common source of transmission is fecal-oral route, zoonotic transmission has been documented. Genotypes 1 and 2 have been associated with waterborne transmission of HEV while genotype 3 and 4 are associated with zoonotic transmission (16).

Severe complications in women can occur as a result of HEV including mortality, intrauterine death, preterm delivery, and stillbirths (8). Additionally, individuals with chronic liver diseases are at higher risk of acquiring fulminant hepatic failure following infection to HEV (9). The World Health Organization (WHO) estimates that worldwide, 20 million cases of HEV occur annually (4). Global estimates of yearly mortality related to HEV vary in the literature and have been reported to be as high as 300,000 (7).

HEV infections can be laboratory confirmed by the presence of IgM anti-HEV RNA in feces as well as by detection of the presence of serum anti-HEV IgM and IgG (45). Evidence from the literature indicates that sensitivity from serological assay in immunocompromised individuals may be lower (46). Diagnosis of HEV is conducted based on serology exams since symptoms of the infection reassemble that of other acute viral hepatitis infection (22).

A systematic review was conducted to estimate the seroprevalence, incidence and mortality associated with HEV globally. Heterogeneity between studies, at a country and regional level was assessed using the I^2 statistic. Results from the analysis indicated that with the exception of few countries and region, pooled estimates could not be generated from studies obtained due to high levels of heterogeneity. The aim of

this study was to conduct a subgroup analysis on the following populations: blood donors, pregnant women and fulminant hepatic failure (FHF) patients. The above-mentioned populations were selected to further explore clinical heterogeneity. Results were analyzed at a global level and by global regions as defined by the WHO.

HEV has been identified as transfusion-transmitted pathogen (47). Cases of HEV associated with blood transfusions have been documented in Europe, Asia and the Middle East (48-50). A number of studies reporting data specifically on this population have been documented in the updated global systematic review. A subgroup analysis was conducted in the present study to assess heterogeneity among this subpopulation. Additionally, an analysis was conducted to estimate the risk of infection by estimating the seroprevalence of anti-HEV antibodies, the incidence of sporadic HEV cases and mortality in this subgroup.

Pregnant women are at high risk for complications associated with HEV infections. In endemic areas, HEV is the most common cause of hepatitis in pregnant women (12). This population was assessed as a subgroup in the current study to account for higher outcomes associated with infections such as death.

Fulminant hepatic failure patients also represent a high risk group for complications following HEV infection. A high proportion of cases with hepatic failure patients resulted in fatal outcomes in the systematic review conducted (51). The high proportions of deaths in this subgroup may have resulted in high heterogeneity of overall mortality rates in the systematic review. Articles reporting on fulminant hepatic failure patients were analyzed as a subgroup for seroprevalence of anti-HEV antibodies, incidence of sporadic HEV cases and mortality. Heterogeneity between studies at a country and regional level were also assessed.

Methods

Data were extracted from a previously conducted systematic review (51). The R software version 3.3.2 was used to input seroprevalence, incidence and mortality data from extracted HEV articles included in the systematic review for each subgroup. A detailed methodology of the systematic review is described in the systematic review conducted (51).

Data was retrieved from articles published between December 2009 and January 2014. Forest plots were generated from the R software. The plots were generated at a country and regional level. The results were presented according to the following 6 regions defined by the World Health Organization (WHO): Africa, Americas, South East, Eastern Mediterranean, Europe and Western Pacific.

Low heterogeneity was defined as an I^2 value of less than 50%. Medium to high heterogeneity was defined as an I^2 value between 50% and 75% and scores above 75% were considered as high heterogeneity. A pooled estimate was included in the analysis when the I^2 value was below 75%.

Results

Global level

Blood donors

A total of 17 seroprevalence studies were included in this population. The global seroprevalence in blood donors was 15.0% (95% CI 9.0%, 21.0%). A high level of heterogeneity was reported in this sub group (99%). Seroprevalence reported ranged from 1% to 52% (Table 1). Insufficient data was obtained from sporadic HEV cases, outbreak studies and mortality studies to assess heterogeneity. The largest proportion of studies conducted on blood donors were classified as seroprevalence. Due to the small number of articles retrieved for incidence and mortality studies, stratified analysis on blood donors was not feasible.

Pregnant women

The global seroprevalence in pregnant women was 6.0% (95% CI 4.0%, 9.0%). High heterogeneity was reported among studies reporting seroprevalence in pregnant women (87%). Seroprevalence ranged from 3% to 33% (Table 2). A total of 4 studies reported data on sporadic HEV cases among pregnant women. The I^2 was 98%. The pooled estimated for sporadic HEV cases among pregnant women was 56.0% (95%CI 17.0%, 94.0%). Compared to the other subgroup populations analyzed in this study, pregnant women reported higher seroprevalence. Only one study reported data on pregnant women in outbreak settings, insufficient data was obtained to assess heterogeneity. No heterogeneity was reported when analysis was limited to studies reporting mortality data ($I^2 = 0\%$). The global maternal mortality rate associated with HEV was 20.0% (95% CI 16.0%-25.0%).

Fulminant hepatic failure (FHF) patients

A total of 8 studies reported data on acute liver disease patients. Incidence of HEV anti-IgM antibodies ranged from 1% to 64% (Table 3). Studies in this sub group were highly heterogeneous ($I^2 = 97\%$).The incidence of HEV in this subgroup was 20.0% (95% CI 14.0%, 26.0%). Five studies reported mortality data on FHF patients. Two studies reported case fatality rates over 50%. Two studies reported case fatality rates of less than 15%. The case fatality rate globally for FHF patients was 31.0% (95% CI 5.0, 57.0%).

Regional level or breakdowns

Blood donors

When analyzed as a subgroup, the I^2 for the blood donors was 84% for the African region, indicating that high heterogeneity was present in the data analyzed. The results from the two African seroprevalence studies included in this analysis indicate that this group is highly heterogeneous. The overall regional I^2 value for seroprevalence of anti-HEV antibodies was 84% when the blood donor population was removed

from the analysis compared to 89% when blood donors are included in the regional analysis for Africa. Overall, results from analysis in this region indicated that blood donors are rather a heterogeneous group with comparable results across studies and countries for this population. Only two studies were included in the blood donor group, therefore results from the I^2 test should be assessed with caution and may not be interpreted to all blood donors in the African region.

In the Americas, high heterogeneity was reported in blood donors along with the seroprevalence studies with blood donors and without blood donors in the analysis (I^2=98%). The seroprevalence for the region of the Americas was 11.0% (95% CI 0.0%-26.0%). One study reported 3% seroprevalence of anti-HEV antibodies in blood donors while the other study reported 0.4% seroprevalence in the same population. In the Eastern Mediterranean, Seroprevalence of blood donors as a group were analyzed and an I^2 of 74% was obtained. The pooled estimate for seroprevalence of blood donors in this region was 18.0% (95%CI 14.0%-22.0%). Blood donors were analyzed as a subgroup in Western Pacific. High levels of heterogeneity were reported in this subgroup (I^2=85%). The seroprevalence among blood donors for this region was 25.0% (95%CI 21.0%, 30.0%). In Europe, data for seroprevalence on blood donors was obtained from a total of eight studies. Seroprevalence ranged from 1.3% to 52.3%. The overall estimate for the region was 13.0% (95%CI 7.0%-19.0%). Estimates varied largely between studies and resulted in high heterogeneity in the region (I^2=99%).

Pregnant women

In Europe, a total of 4 studies reporting on incidence in pregnant women were assessed for heterogeneity and the group was highly heterogeneous based on the results from the I^2 score (80%). A pooled estimate was not reported for the region of Europe as all four studies were reported from one country. All other WHO regions had insufficient data to provide a pooled estimate for seroprevalence. There was insufficient data to generate pooled estimates at a regional level for incidence of sporadic HEV cases in pregnant women. A total of two studies were included in the Eastern Mediterranean region for case

fatality rate and a pooled estimate of 29% (95%CI 18%, 39%) was generated. The pooled case fatality rate for South East Asia was 20% (95%CI 14%, 26%). No heterogeneity was observed in the Eastern Mediterranean and South East Region.

Fulminant hepatic failure (FHF) patients

Data from 4 studies reporting on sporadic HEV in FHF patients were pooled to provide an incidence of 41% (95%CI 12%, 69%) for South East Asia. In Western Pacific, the incidence of sporadic HEV infections was 4.0% (95%CI 0.0%,9.0%), studies were highly heterogeneous (I^2=67%).

Discussion

Overall, heterogeneity in studies focusing on maternal mortality was the only subgroup with a heterogeneity score below 75%. A pooled estimate of 20% (95%CI 16%, 25%) was generated for pregnant women. The South-East Asia region is highly endemic to HEV and sporadic HEV incidence was reported to over 60% in the studied subgroup populations.

When analyzed by sub regions, high statistical heterogeneity was observed in all WHO regions. No statistical heterogeneity was found in the Eastern Mediterranean and South East Asian regions for studies reporting on mortality in pregnant women. In the South-East Asia region, fulminant hepatic failure patients reported higher incidence when compared to pregnant women. However pregnant women were the second group with the highest incidence in that region. The same trend was observed when pregnant women were compared to other groups for mortality study in South-East Asia.

Similarly to other studies published, our findings were consists in terms of the Egyptian population (13,52). Compared to the global estimate and the regional estimates, the pooled seroprevalence for Egypt was substantially higher. The incidence in the Egyptian population however was low, even when compared to other countries in the Eastern Mediterranean region.

The results from this meta-analysis by subgroup demonstrate that a high proportion of cases of sporadic HEV are reported in pregnant women globally. Our pooled estimate for global maternal mortality was comparable with previous estimates of maternal mortality. Additionally, fulminant hepatic failure patients account for a large proportion of all incident and mortality cases reported in several regions. Public health actions should target pregnant women and fulminant hepatic failure in endemic areas. Currently, no data on safety, immunogenicity and efficacy is available on these high risk populations for the Hecolin vaccine. There is a need to study the effect of vaccination in pregnant women and fulminant hepatic failure in areas where HEV is endemic and specifically where genotypes causing large outbreaks occur.

Data on HEV continues to be lacking, only a few countries have surveillance systems in place to monitor HEV infections. This limitation poses a serious challenge for estimating the burden associated with HEV at a regional or global level. This is particularly true when analyzing data in different strata of the population.

This study represents the first attempt at quantifying the global burden of illness associated with HEV genotypes 1, 2, 3 and 4 among blood donors, pregnant women and FHF patients. High levels of heterogeneity were reported in studies on blood donors and FHF patients. However, heterogeneity was below 75% and allowed for calculation of a pooled estimate in pregnant women. Globally, the maternal mortality associated with HEV infection was 20.0% (95%CI 16.0%, 25.0%) during the 2009 to 2014 period. When analyzed by subpopulation, few studies were available; this represented a major limitation for analysis. Pooled estimates generated from this meta-analysis should be assessed with cautions due to the small number of studies included in the analysis.

Conflict of Interest

None of the authors reported any conflict of interest.

Acknowledgements

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Tables

Table 1: Country and regional outcome estimates-blood donors

Outcome	Region	Regional estimates (%)	Overall global estimates (%)	I^2 (%)	Comments
Seroprevalence	Africa	5.0.2-14.0	15.0 (95%CI 9.0,21.0)	100	In Africa, only two studies were included. No heterogeneity was identified based on the I^2 value (0%). In the Americas, one study reported 3.1% seroprevalence while the other study reported 18.8% seroprevalence. The study reporting 3.1% was conducted on a large sample (n=1939) while the other study was conducted on a small sample size (n=63). In Europe, a total of 6 studies reported seroprevalence under 10%. A total of 9 studies reported seroprevalence between 12% and 23%. Additionally, one study reported seroprevalence of 52%. The 6 studies under 10% and the study reporting high seroprevalence contributed to the high heterogeneity within this region.
	Americas	3.1-18.8			
	Eastern Mediterranean	14.1-21.0			
	Western Pacific	22.7-32.6			
	Europe	1.3-52.3			

Table 2: Country and regional outcome estimates -pregnant women

Outcome	Region	Region incidence (%)	Overall global estimate (%)	I^2 (%)	Comments
Seroprevalence	Europe	3.5-33.3	6.0 (95%CI 4.0,8.0)	88	All but one study in Europe reported similar seroprevalence (3.5%-5.4%). The other study reported 33.3% seroprevalence. The study was only conducted in 12 pregnant women. Variations in estimates were observed between countries.
	Western Pacific	10.2			
	Eastern Mediterranean	3.7			
	Africa	23.0			
Incidence	South East Asia	17.0-58.0	56.0 (95%CI 17.0,94.0)	98	Two studies reported low incidence (<10%) in South-East Asia. These studies included large sample size (n=110, 473). The other three studies included reported incidence estimates of over 30%.
	Eastern Mediterranean	84..0			
	Africa	64.0			
CFR*	South East Asia	19.3-22.0	20.0 (95%CI 16.0,25.0)	0	Two studies were included for the African region. One study consisted of a case series describing 2 fatal cases based on a study population of 3. Within the regions of South East Asia and Eastern
	Eastern Mediterranean	28.2-29.0			
	Western Pacific	11.1			

	Mediterranean were homogenous. Overall, no statistical heterogeneity was observed across all regions.

*CFR defined as the number of fatal cases among the laboratory-confirmed HEV cases

Table 3: Country and regional outcome estimates-FHF patients

Outcome	Region	Regional estimates (%)	Overall global estimate (%)	I^2 (%)	Comments
Incidence	America	0.43	20.0 (14.0, 26.0)	97	Overall, heterogeneity was 66% for this region and allowed for generating a pooled estimate. The regional estimate was not statistically significant. The two studies included in the Western Pacific resulted in increasing the heterogeneity globally for this subpopulation as the incidence was below 10% vs. over 40% in all other regions.
	South East Asia	8.0-64.0			
	Western Pacific	1.7-8.5			
	Eastern Mediterranean	33.0			
Overall CFR*	America	0.68	31.0 (850, 57.0)	96.0	The estimate from the America was lower than any other region. HEV is not endemic in this region. In Western Pacific, two studies were included. One of the studies consisted of a case series reporting data on only 4 participants. The estimate of 50.0% for that study should be interpreted with caution due to the small denominator.
	South East Asia	64.2			
	Europe	66.7			
	Western Pacific	13.0			

	The CFR estimate globally is not statistically significant.

*CFR defined as the number of fatal cases among the laboratory-confirmed HEV cases

Supplement: Forest plots

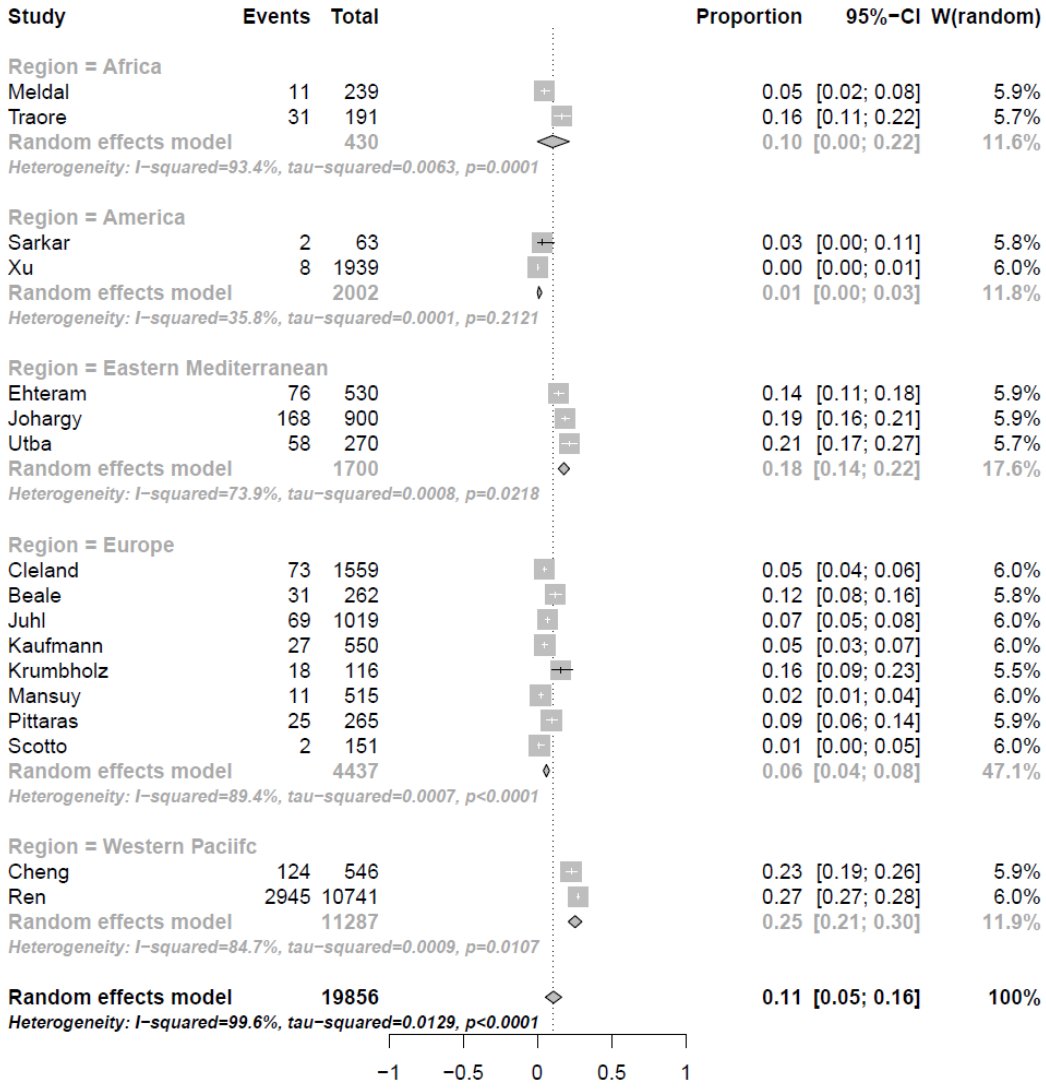

Figure s.1: Forest plot of seroprevalence of anti-HEV antibodies in blood donors by WHO region

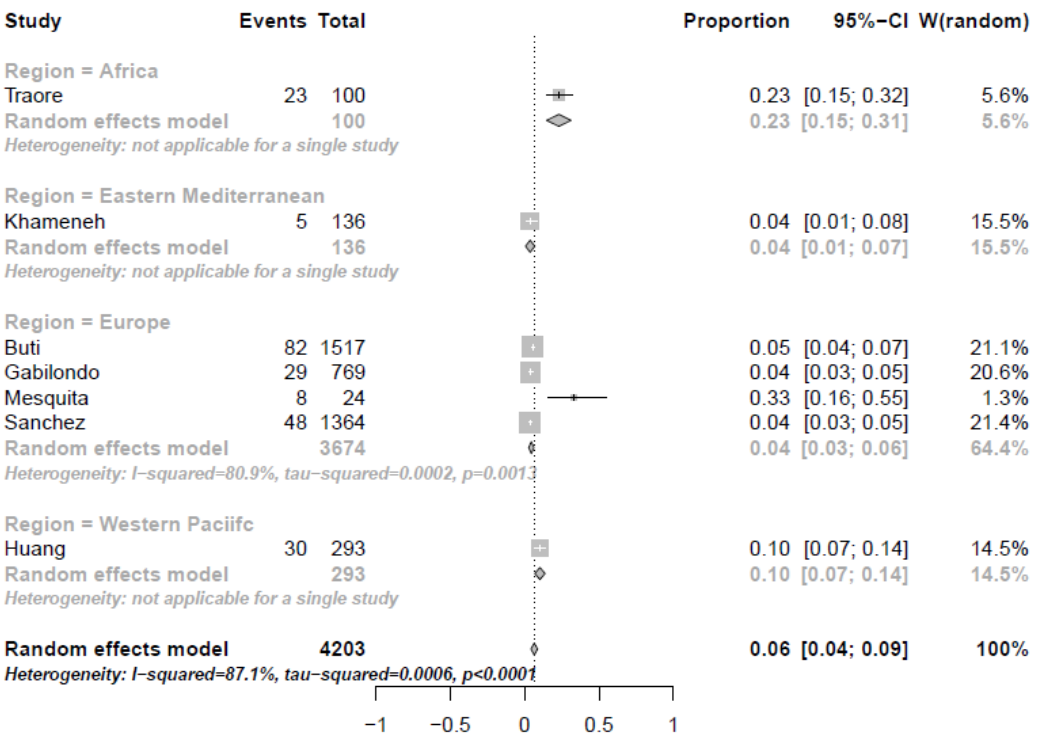

Figure s.2: Forest plot of seroprevalence of anti-HEV antibodies in pregnant women by WHO region

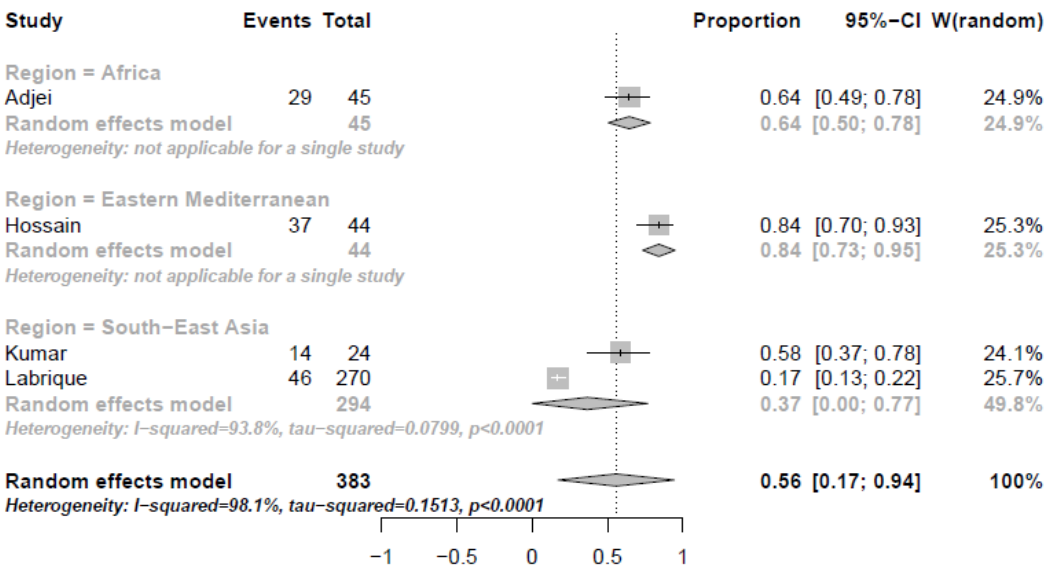

Figure s.3: Forest plot of sporadic HEV cases in pregnant women by WHO region

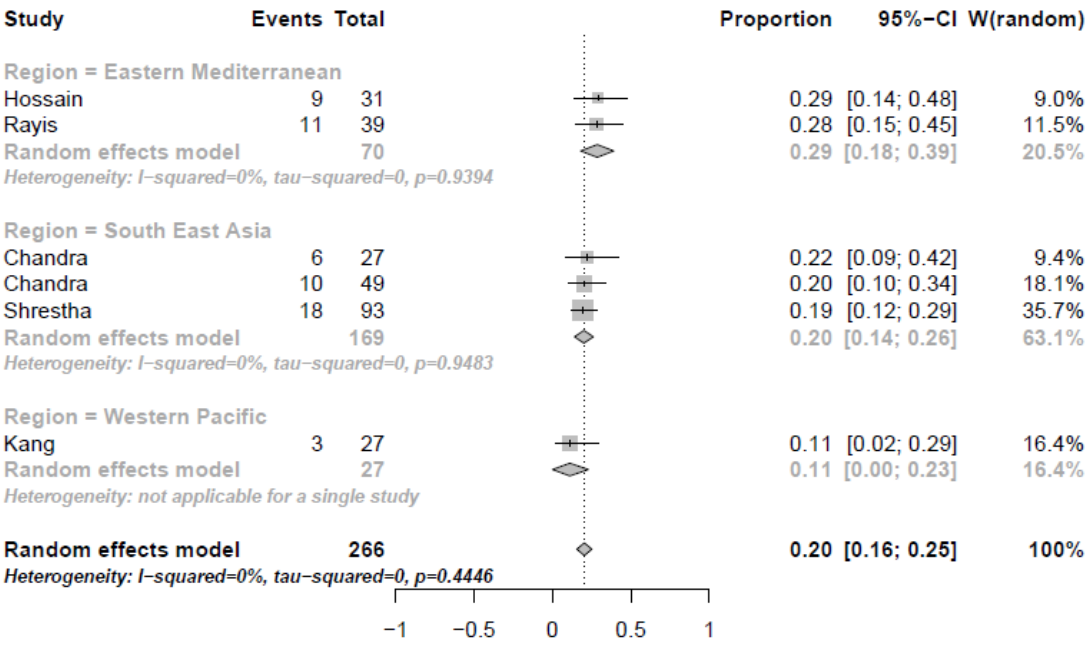

Figure s.4: Forest plot of maternal case-fatality rate in pregnant women by WHO region

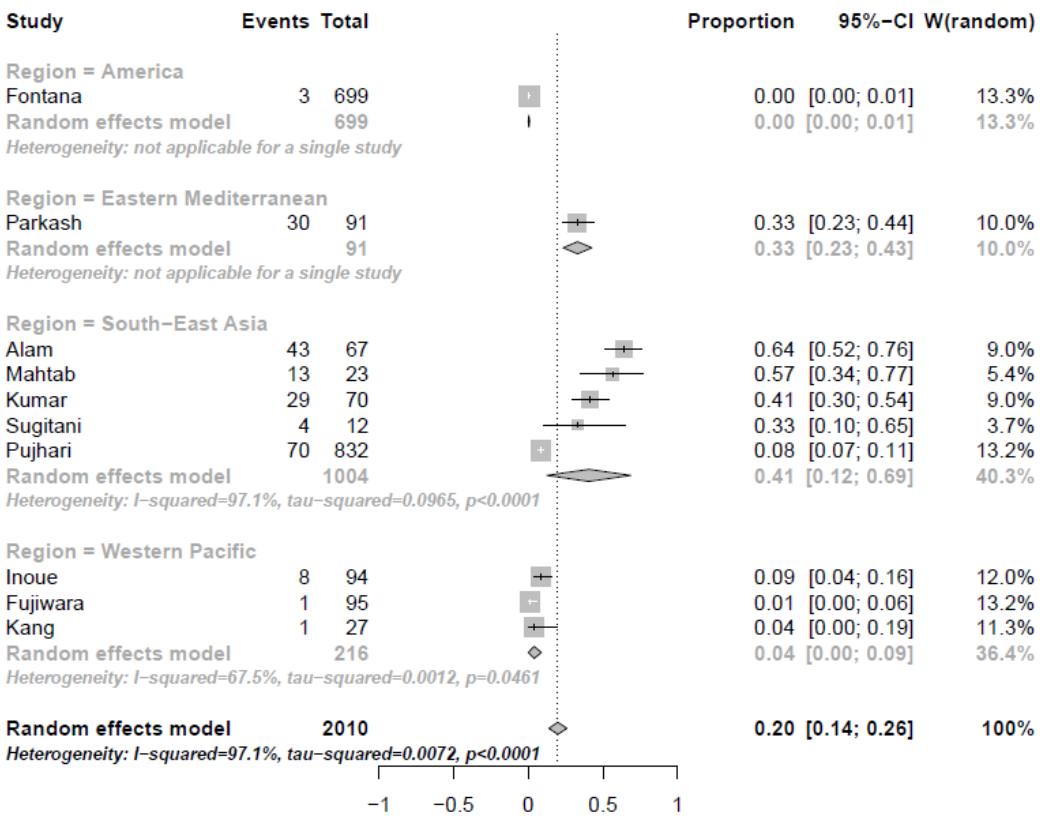

Figure s.5: Forest plot of sporadic cases of HEV in FHF patients by WHO region

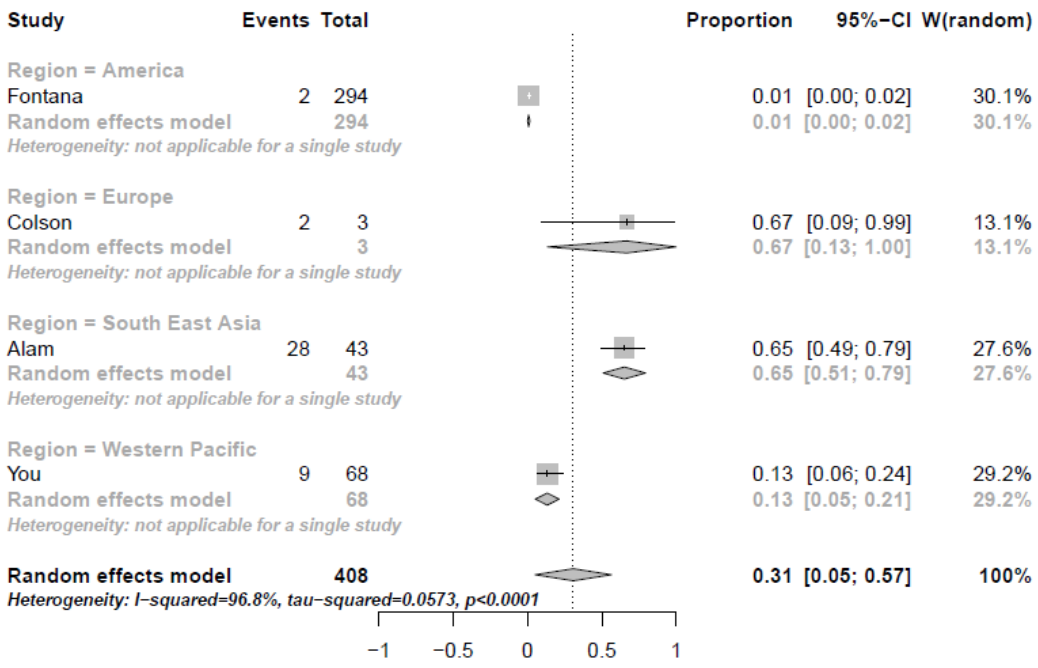

Figure s.6: Forest plot of case fatality rate in FHF patients by WHO region

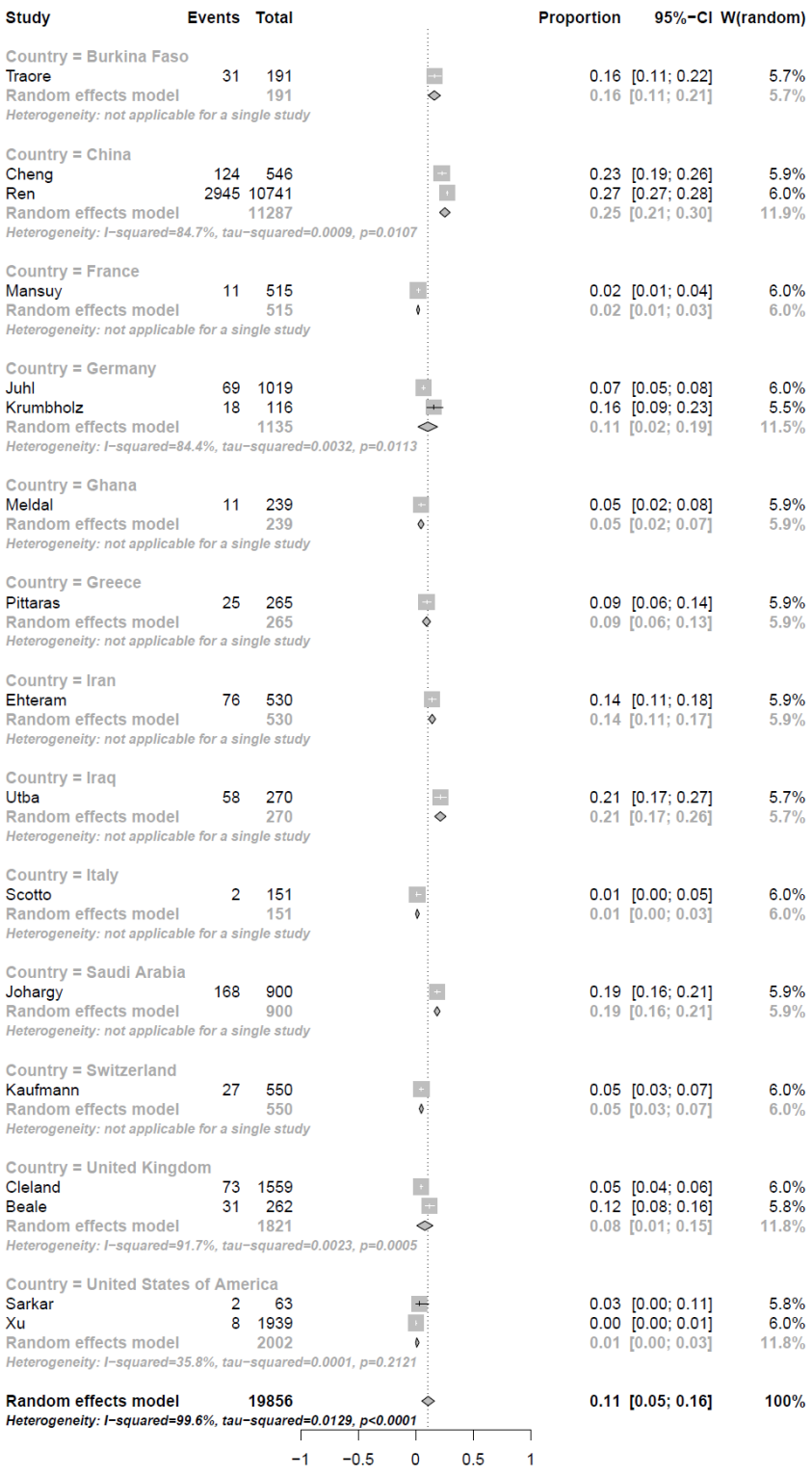

Figure s.7: Forest plot of Seroprevalence anti-HEV antibodies among blood donors by country

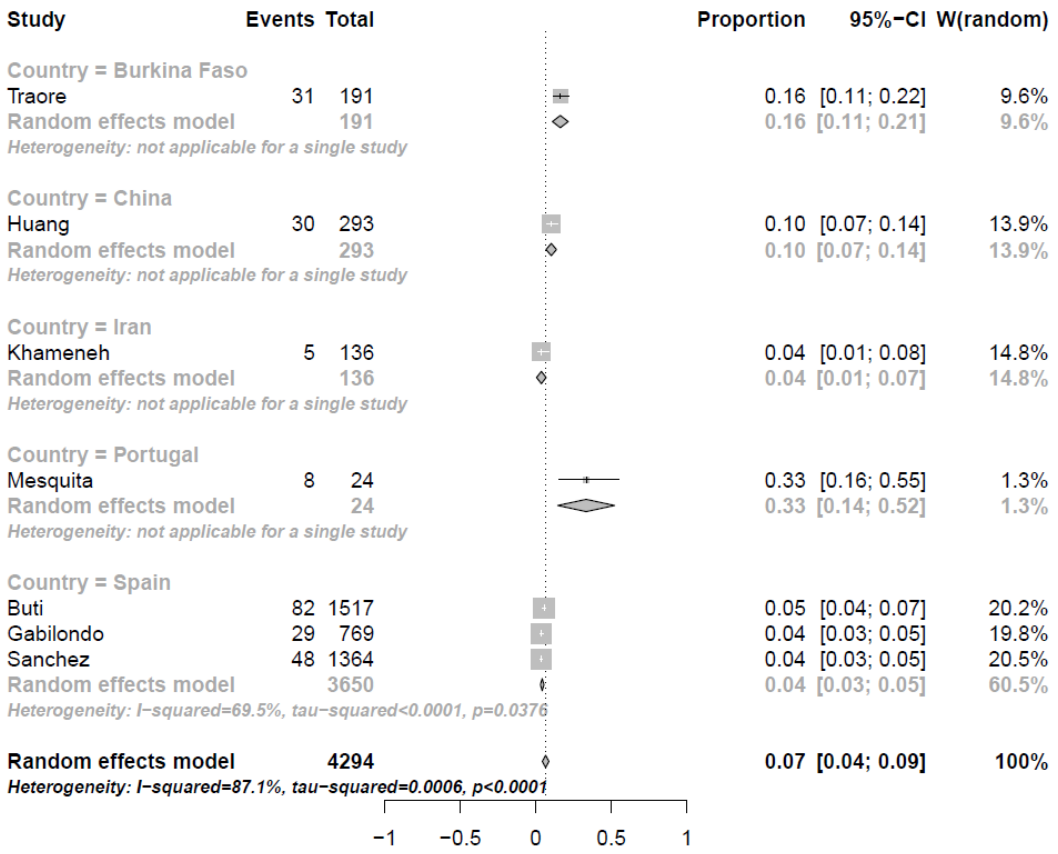

Figure s.8: Forest plot of incidence of sporadic HEV cases among pregnant women by country

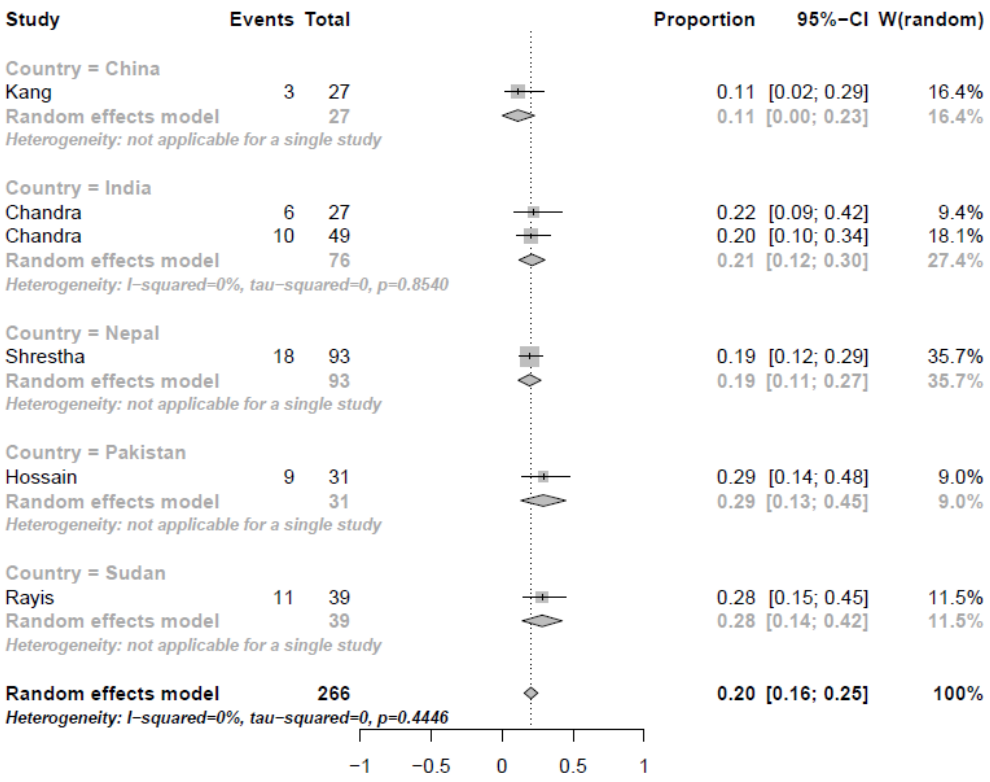

Figure s.9: Forest plot of maternal case-fatality rate in pregnant women by country

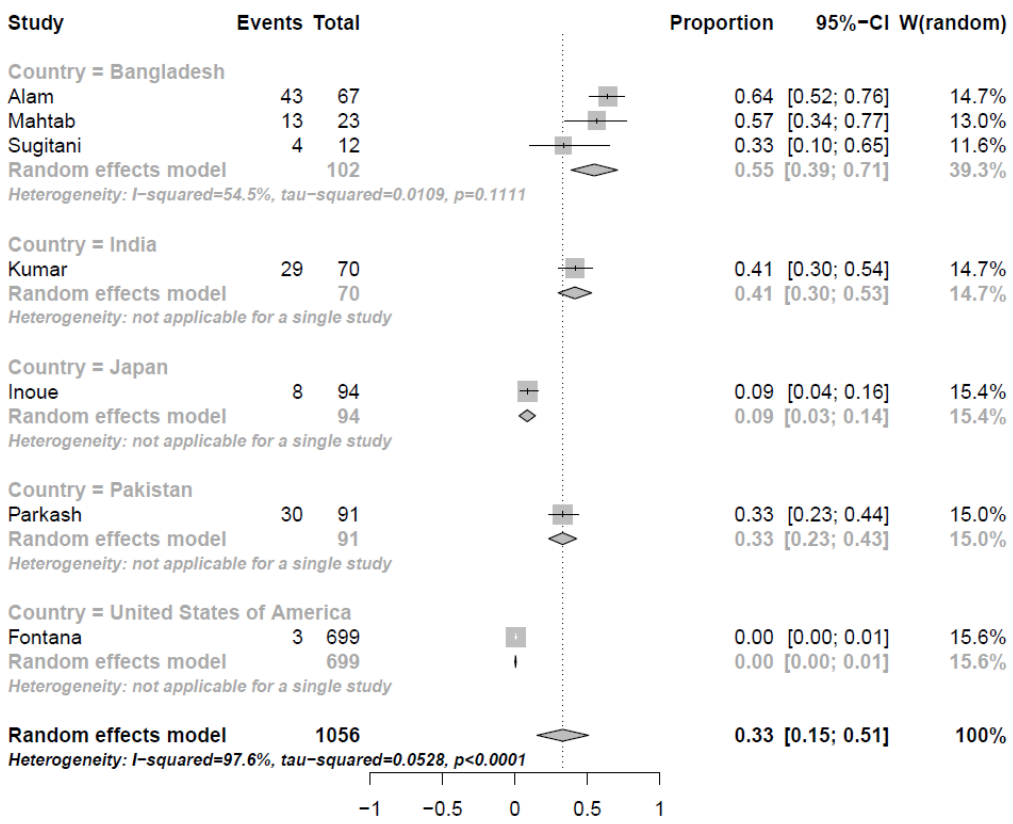

Figure s.10: Forest plot of sporadic cases of HEV in FHF patients by country

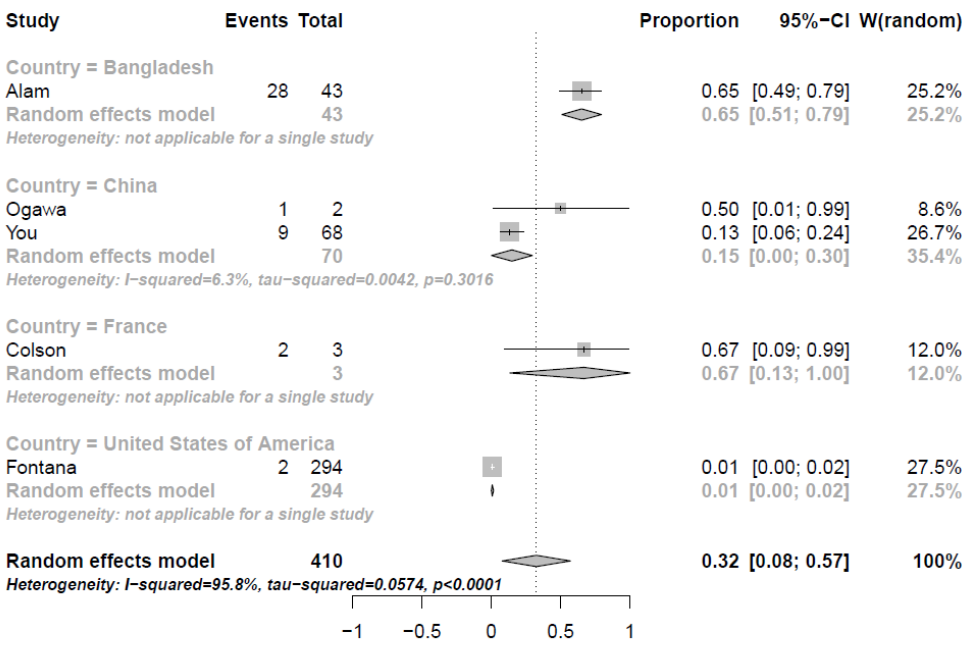

Figure s.11: Forest plot of case fatality rate of HEV in FHF patients by country

CHAPTER FIVE

DISCUSSION

5.1 Overall discussion

The objective of this chapter is to provide recommendations based on findings from the systematic review. The following objectives outlined in the beginning of the thesis were achieved by the following work conducted:

A. Collect evidence on HEV circulation globally via a systematic review concerning all HEV genotypes

The systematic review conducted has demonstrated that HEV is endemic in all WHO regions. The review identified some of the first documented cases of HEV genotype 4 in the European region. Findings presented in Chapter 3 demonstrate that infections of HEV associated with HEV have become endemic to the European region. Additionally, Brazil and Venezuela reported their first autochthonous cases of HEV associated with new genotypes. Data collected in the systematic review was consistent with previous findings in the literature. Large outbreaks continue to be reported in low income countries where access to clean water is a challenge.

B. Develop a quality assessment tool for articles on prevalence, incidence and outbreaks

A quality assessment tool was developed for prevalence, incidence and outbreaks studies. The process of developing the quality assessment tool consisted of initially reviewing the literature. Previously developed quality assessment tools were identified. At the time when the systematic review was conducted, no tool was specific enough to assess quality of articles specific to HEV. Diagnostic methods used to detect HEV can largely influence estimates reported in studies. The

quality assessment tools developed contained specific attributes to capture limitations from identified pre-existing checklists such as diagnostic methods and criteria for follow-up of HEV patients. A tool has since been developed by GRADE (1). The tool was developed to assess quality in prognosis studies and is applies to observational studies. The following criteria are used in the tool to assess quality: risk of bias, sensitivity analysis, inconsistency, imprecision, indirectness and publication bias.

C. Conduct a meta-analysis and provide global estimates when possible

A meta-analysis was conducted in both Chapters 3 and 4. The meta-analysis conducted in Chapter 3 provided pool estimates at a country, regional and global level for the outcomes of interest (prevalence, incidence, mortality and still births). In Chapter 4, three subgroups were selected to further explore clinical and statistical heterogeneity. Pooled estimates were reported at a country, regional and global level for blood donors, pregnant women and fulminant hepatic failure patients.

The results from the systematic review and meta-analysis conducted demonstrate that HEV infections occur globally and in endemic areas result in severe outcomes including liver failure, still births in pregnant women and death. Public health interventions are needed to reduce the burden associated with HEV and its impact on public health infrastructures throughout the world. In order to inform public health actions and the optimal use of the vaccine, evidence on the burden is needed. The only previous review conducted to assess the burden of HEV was conducted in 2010 and only assessed the burden associated with genotypes 1 and 2. The data extracted to produce the systematic review presented in this thesis should be used to reassess the

burden of HEV globally and for all genoytpes. Several mathematical models can be explored for use to model incidence, prevalence and mortality.

In countries where studies on blood donors account for a significant number of studies reporting seroprevalence data, measures to limit transmission of HEV are especially needed. Transfusion-transmitted cases of HEV have been reported in Europe, the Western Pacific region and in the Eastern Mediterranean region (2). According to the US CDC, the ratio of symptomatic to asymptomatic cases may be as high as 1:13 in industrialized countries (2). Clinical illness associated with genotype 3 tends to be milder and thus more likely to be undiagnosed. In high-income countries, genotype 3 has been documented. In the systematic review described in Chapter 3, seroprevalence of over 50% was reported in studies focusing on blood donors in high income countries. Studies focusing on the risk of transmission due to blood transfusion should be conducted in high income countries where high seroprevalence in blood donors has been documented. High quality data remains scarce throughout the world, well designed seroprevalence studies on blood donors are needed to mount good evidence around the risk of transmission. This is of particular importance because the most dominant genotype reported in the systematic review conducted was genotype 3.

Overall, at the global level, high statistical heterogeneity was observed. In order to further explore variations between studies at the global level, a sub group analysis was conducted. The objective of the subgroup analysis was to assess the impact of clinical heterogeneity on statistical heterogeneity. In order to account for methodological heterogeneity, quality assessment checklists were developed and assessed study designs.

Estimates of seroprevalence and incidence vary greatly from one region to another and within countries. The specific laboratory tests used to detect an HEV infection contribute to this issue. This issue was reflected in the findings of the systematic review presented in chapters 3 and 4. Laboratory assays used to detect the presence of HEV vary in sensitivity. Discrepancies in assay sensitivity have been documented predominantly in seroprevalence studies conducted among blood donors in non-endemic areas. High seroprevalence has been documented among blood donors in high income countries. There is a need to conduct further studies and validate assays used to detect the presence of anti-HEV antibodies. A standardized approach for laboratory testing of HEV cases is essential to better understand the true picture of seroprevalence globally.

5.2 Developing an HEV surveillance system

Quality data is lacking for HEV throughout the world. Only a few countries conduct surveillance on HEV. In order to better understand the burden associated with HEV a systematic approach yielding to high data quality is required. The European Center for Disease Prevention and Control (ECDC) along with the U.S. CDC have published guidelines on establishing and evaluating surveillance systems (3,4). The following steps should be taken to establish a public health surveillance system for HEV. These steps have been specifically conducted with respect to HEV surveillance.

5.2.1. Defining surveillance objectives

The first step to establishing a surveillance system consists of defining the surveillance objectives. These could include:

- Monitoring trends of HEV infections over time ;

- Detecting early HEV outbreaks across a country;
- Monitoring circulating genotypes of HEV ,
- Identifying populations at risk of HEV and complications associated with HEV;
- Informing disease burden of HEV;
- Providing information to the World Health Organization (WHO) to inform public health action.

5.2.2 Development of case definitions

Case definitions are based on three factors: clinical, virological and epidemiological link. Cases are typically classified into confirmed, probable or possible. The WHO has developed case definitions for acute hepatitis including Hepatitis E (5). The WHO classifies cases as either presumptive or confirmed cases. Presumptive cases are defined based on clinical criteria and confirmed cases are defined from clinical criteria and biomarkers or epidemiological criteria. The following are the WHO definitions for HEV infections (5):

- Presumptive case: Discrete onset of an acute illness with signs/symptoms of: (i) acute infections illness (e.g. fever, malaise, fatigue); and (ii) liver damage (e.g. anorexia, nausea, jaundice, dark urine, right upper quadrant tenderness, AND/OR raised alanine aminotransferase (ALT) levels more than ten times the upper limit of normal).
- Confirmed cases: Presence of IgM ANTI-HEV OR epidemiological link with a confirmed case.

Chronic HEV cases have not yet been defined by the WHO, case definitions have been developed for Hepatitis B Virus and Hepatitis C.

Case definitions should also be developed for outbreaks. Definitions for outbreaks should take into account time and place. In an outbreak setting, exposure criteria should be established. Exposure criteria include links to a confirmed or probable case during the incubation period. According to the WHO, the incubation period varies from 2 to 10 weeks (5). The exposure criteria for outbreak should be specific to each country/area affected. For example exposure criteria may include travel to affected areas for outbreaks originating from outside the country in non-endemic countries. Additionally, in an outbreak setting, the affected areas should be defined and tied into the case definition. An affected area can be defined as an area human or animal cases of HEV have been detected and where HEV circulation has been reported.

5.2.3 Identification of data sources and flow and types of surveillance

Multiple data sources feed into public health surveillance systems (Figure 1). Virological information on HEV cases are obtained from a laboratory. Laboratories reporting may be part of a network or may be an independent laboratory. Commonly, local public health laboratories send their laboratory confirmed cases to an overall region which will then report to the national level. Additionally, diseases on the national reportable disease list ensure that all cases of a particular disease are reported within a defined timeline. Countries where HEV is endemic should consider including HEV in the national notifiable disease lists. Additionally, initial laboratory testing can be conducted at a local level. Further testing including confirmatory testing and genotype sequencing should be done at a reference laboratory.

Two main types of surveillance systems are used to monitor communicable diseases. Diseases under passive surveillance rely on laboratories, primary and tertiary health care providers to

report a health event to public health authorities. In contrast, public health authorities prompt data providers to report cases on a routine basis under active surveillance systems. Since data quality has been identified as a limitation for HEV, public health authorities should implement active surveillance to enhance the quality and representativeness of data.

Syndromic surveillance may help inform early-on clusters of possible cases. Notification of cases through such a system can be done via primary-health care providers. Population-based surveillance is also another form of syndromic surveillance that can allow understanding the burden of disease associated with HEV in cases that do not seek medical care. Such surveillance system could be implemented in the form of an app where participants would be asked on a weekly basis if they had HEV-like symptoms in the previous week. If participants reported having symptoms in the previous week they would be prompted to answer additional questions including health care behavior seeking questions.

Severe outcome surveillance (hospital admissions and deaths) is conducted through hospitals. Hospitals should provide routinely the number of laboratory confirmed cases of hospitalizations, cases admitted to the Intensive Care Unit (ICU) and fatal cases. Additionally, hospitals should report the number of cases admitted with HEV-like symptoms along with the number of symptomatic cases tested for HEV. This will allow calculating estimates for burden of disease. Data on severe outcomes associated with HEV infections may also be available through different data sources including administrative databases and electronic medical records.

5.2.4 Surveillance system attributes

The CDC identifies nine key surveillance system attributes (3). The attributes should be assessed during the evaluation of surveillance systems. Each attribute should also be addressed during the implementation phase of a new surveillance system.

I. Data quality

High seroprevalence has been reported throughout the world in the systematic review conducted in Chapter 3 (6). Cases of HEV infections identified through population surveys are likely to be missed or underreported in a surveillance system. Failure to capture cases in a surveillance system can be due to a number of reasons including patients not entering the health care system (asymptomatic patients). Cases can also be diagnosed with the wrong administrative code resulting in misclassification and underreporting. The following may help enhance the quality of the data:

- Conducting active surveillance
- Conducting contact tracing during outbreaks to identify contacts at risk
- Screening of high risk groups in the population
- Keeping track of the number of incomplete reports received by data providers. This will help public health authorities to address data quality issues as they arise.
- Setting up data quality validation processes in automated data submission templates. For examples, the system will not accept case report forms with blank or incomplete fields.

II. Representativeness

A representative surveillance system accurately monitors and reports on public health evens over time in the population by place and person. In order to ensure geographical representativeness,

sentinel surveillance systems should target health care providers in a manner that is representative of the population. Additionally, sentinels should be weighted based on the population included in the catchment area.

Data captured should represent the population of interest. Data on both the number of cases (numerator) and the population of reference (denominator) are essential for analysis. Obtaining the denominator can be assessed through a number of data sources including a census of the population and health facility utilization surveys in the population of interest. Studies reporting on HEV outbreaks have reported the number of patients admitted to a hospital during the outbreak period as the denominator. An HEV surveillance system must ensure that denominator data is accessible in order to calculate rates and provide an insight on the burden of illness.

A number of sampling methods can be used to systematically collect samples for virological surveillance. Firstly, interval sampling requires that samples be taken from the N^{th} symptomatic case meeting the case definition (7). Alternate day sampling is another systematic method for selecting patients. The alternate day sampling method requires that all cases meeting the case definition be tested on a particular day of the week (7). In order to eliminate bias from health-seeking behaviors, the selected day of the week should be changed from week-to-week.

III. Timeliness

Timeliness refers to the amount of time required to advance from one step to the next in the surveillance system. The timeliness of a surveillance system can be monitored in several ways. For example, the time between the onset of symptoms and the time of reporting can inform

public health authorities on the timeliness of the system in terms of identifying new cases. Timeliness can also be measured in outbreak settings by calculating the time between the start of an outbreak and the date of reporting to public health authorities. Delays in timeliness of the surveillance system can take place in multiple steps of the data collection and submission process. Time lags in patients seeking health care, physicians ordering laboratory tests, laboratory communicating results to public health authorities will all affect the timeliness of the system.

Timeliness is essential to an HEV surveillance system to ensure early identification of cases and to implement public health actions to respond to outbreaks. In order to ensure timeliness, an online reporting system allows for increased timeliness between steps involved in recording surveillance data. Once a diagnosis is made, an online system would allow to quickly notify local public health officials directly from the physicians' office. Local public health officials would then notify regional and federal public health authority in an automated fashion.

Another feature of a surveillance system that enables early detection of cases is syndromic surveillance. Sentinels could be set up across a jurisdiction and could provide an early signal for HEV cases. Laboratory confirmation of cases is likely to lag in terms of timeliness. For instance acute jaundice could be monitored as a syndrome to provide an indication on acute HEV cases.

IV. Sensitivity

The sensitivity of a surveillance system is measured by the ability to detect cases/outbreaks of HEV. In order to assess sensitivity, data managers can calculate the total number of cases detected by the surveillance system that truly have an HEV infection divided by the total number

of cases detected by the surveillance system. This assessment can be done using external data to validate the number of cases detected by the surveillance system. External data includes administrative data, medical records and registries. The quality of HEV laboratory assays has not been formally assessed by an international body such as the WHO. The laboratory assays used to diagnose an HEV infection should be captured in the surveillance system. In order to account for variability in laboratory testing practices, laboratory samples tested at a local/regional laboratory should be sent to a reference laboratory for confirmatory testing.

V. Predictive Value Positive

Predictive Value Positive (PPV) represents the proportion of cases reported that truly have an HEV infection. PPV is particularly important for outbreak investigations. A high rate of false positives could result in unnecessary investigation and hence inefficient public health resource allocation. The PPV is impacted by the sensitivity of assays used to detect cases of HEV. The limitations in testing assays should be addressed in order to improve PPV for HEV. PPV varies across geographical regions based on the prevalence in the area. A higher PPV is expected in an endemic area compared to a non-endemic area.

VI. Simplicity

The simplicity of a surveillance system refers to the ease of use of a surveillance system. In order to ensure the ease of operation of the system, countries should establish one standard case definition for HEV infections. Additionally, case report forms and guidelines should be developed to guide jurisdictions in identifying and reporting through appropriate channels. Standard Operating Procedures (SOP) should be developed for data managers. Submission of data into the surveillance system should also be adapted to the needs of data providers to

minimize the amount of work required to participate and submit data into the system. A simple surveillance system is more likely to be endorsed by countries if it requires minimal resources to be implemented. Simplicity is a key component for HEV surveillance as the largest proportion of reported cases occurs in low income countries with potentially limited access to public health resources.

VII. Flexibility

A flexible surveillance system has the capacity to accommodate changes. In the scope of an HEV surveillance system, a flexible system is essential to be able to respond to outbreaks. An HEV surveillance system should have the capacity to expand to include additional data elements and additional case definitions or changing reporting forms.

VIII. Acceptability

Acceptability refers to the willingness of individuals/stakeholders and data providers to participate in a surveillance system. This attribute can be assessed by reporting on the number of participants in the system. Participation rate should be monitor throughout time to assess any variation in acceptability. Willingness to participate in a surveillance system is also affected by the amount of resources available. Resource allocations for countries depend on several factors including public health priorities. Countries with endemic circulation of HEV should prioritize the implementation of an HEV surveillance system in order to provide a rationale for public health interventions such as vaccination.

IX. Stability

A stable surveillance system is both reliable and available when needed. Countries implementing sentinel surveillance for HEV should start at a small scale and only expand if attributes of their surveillance systems are not at risk. Despite having less data, the usefulness of the data will be greater if the data quality is high.

Europeans countries have implemented surveillance systems specific to HEV. Germany has published scientific articles on the development and implementation of their HEV surveillance system (8). HEV became nationally notifiable in 2001 in Germany and since being notifiable an increasing proportion of autochthonous cases have been reported. The upfront cost to develop the surveillance system in Germany was estimated to be $170 000 (EUR) and the maintenance cost is approximately $150 000 (EUR) yearly. National surveillance reports on HEV are also published on a yearly basis.

5.2.5 Recommendations

The work conducted in the scope of this thesis has allowed to generate the following recommendations:

1- More data is needed on high risk groups to inform the use of Hepatitis E vaccines in various populations. Recommendations have not yet been made on high risk populations due to the lack of data available.

2- Modelling of HEV incidence, prevalence and mortality is needed to inform public health actions. Currently, only one modelling exercise was published and was not inclusive of all genotypes and regions of the world resulting in major gaps.

3- Studies on the risk of transmission of HEV via transfusion should be specifically assessed in high income countries where high proportions of seroprevalence have been documented in blood donors.

4- Validation studies are needed to compare sensitivity of assays currently used for the detection of HEV. There are currently no global standards to address this issue and inform countries on recommended diagnostic tests.

5- Countries with established viral hepatitis surveillance should consider incorporating HEV as an additional disease. Should an infrastructure for collecting HEV be present, countries should take advantage of this and improve data quality and completeness for HEV infections. The decision to implement a surveillance system depends on a number of factors including public health priority, cost-effectiveness, risks and benefit as well as public perception. Countries could start by implementing a component of a surveillance system such as virological surveillance. A detailed document on evaluating the costs and benefits of developing a national surveillance system has been developed by the WHO and can serve as a useful tool to assess cost-effectiveness of implementing a surveillance system (9).

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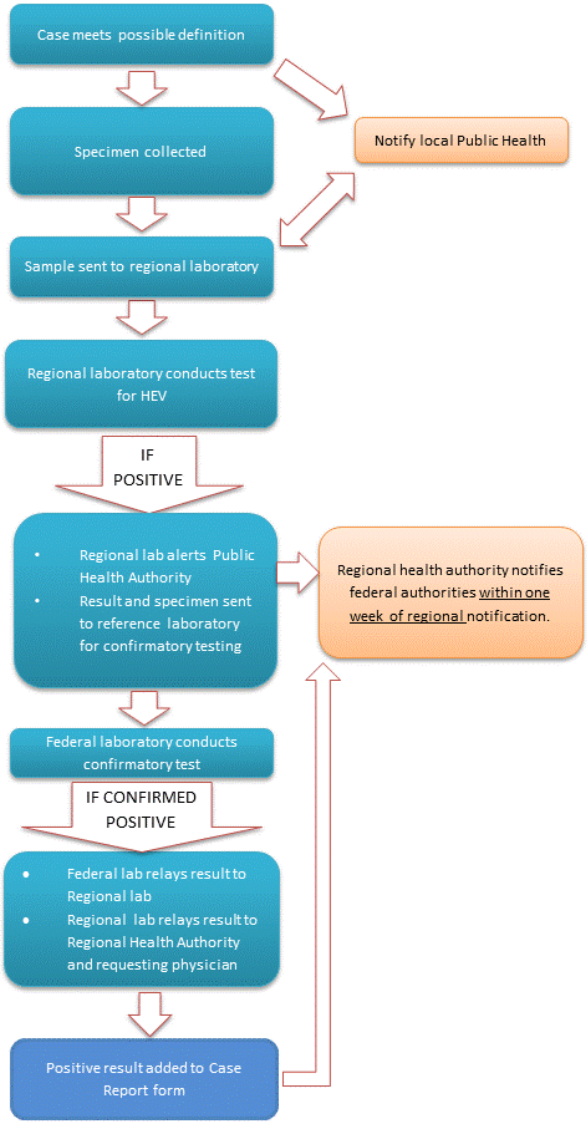
Figures

Figure 1: Data Flow Diagram



Thesis Appendices

Appendix 1: Quality assessment of seroprevalence studies on anti-HEV antibodies

Appendix 1 should be interpreted as follows:

The numbers in the top Row indicate the criteria of the quality assessment tool assessed:

1= Specification of the target population - Are study subjects and the setting described?
2= Representativeness of target population - Was the study population based? Target population: Healthy individuals or subgroups of interest
3= Valid laboratory detection of HEV antibodies - Was HEV seroprevalence detected in a standard and reliable manner?
4= Adequate response rate - Did the study report any completion rate?
5= Are efforts taken to address potential sources of bias?
6= Interpretation of results

The numbers on the second row represent the responses for each criterion. Each response with a designated star counts towards the quality rating of study:
Criterion 1: 1=Yes*, 2= No, 3= Insufficient data for judgment

Criterion 2: 1= Scientific approach to obtain a representative sample (i.e. sufficient size, random sample)* 2= Approach not yielding representative samples (i.e. convenience sample), 3= Not reported in sufficient detail for judgment.

Criterion 3: 1= Seroposivity confirmed according to accepted criteria. (IgG)*, 0= Immunoassay used for detection is identified*, 2= Not reported in sufficient detail for judgment.

Criterion 4: 1= Completion rate reported and > 70% *, 2= Completion rate < 70% and no description of missing subjects, 3= Not reported

Criterion 5: 1= Reported adjusted incidence for potentially non representative samples (eg data broken down by subgroups)*, 2= No adjusted estimates provided, 3=Not reported

Criterion 6: 1= Yes for mortality (1 week)*, 2=No, 3= Not applicable

Criterion 7: 1= Measures of variability are provided to the unadjusted and adjusted point prevalence (e.g. confidence interval, standard error)*, 2= Not reported

Study	Abstract or full text	1			2			3			4			5			6		Rating
		1	0	2	1	0	2	1	0	2	1	0	2	1	0	2	1	0	
Adesina, 2009	Full text	1				1				1	1			1				1	***
Ahmadi, 2013	Full text	1			1					1	1			1				1	****
Ahmed Bawazir, 2011	Full text	1			1			1	1		1			1			1		*******
Ataei, 2009	Full text	1			1					1	1			1				1	****
Bajpai, 2011	Abstract	1					1	1	1		1			1				1	*****
Basu, 2012	Abstract	1					1	1			1			1				1	****
Beale, 2011	Full text	1				1		1	1		1			1				1	*****
Begum, 2010	Full text	1			1			1	1		1			1			1		*******
Bouarma, 2014																			
Buti, 2010	Full text	1			1			1	1		1			1			1		*******
Carmoi, 2009	Abstract	1				1		1	1		1			1				1	*****
Cevahir, 2013	Full text	1				1		1	1		1			1				1	*****
Chang, 2009	Full text	1				1				1	1					1		1	**
Chaussade, 2010	Full text	1				1		1	1		1			1			1		******
Cheng, 2012	Full text	1				1		1	1		1			1			1		******
Chiu, 2013	full text	1			1			1	1		1			1				1	******
Cleland, 2013	full text	1					1	1	1				1	1			1		*****

Dalton, 2010	Abstract	1				1	1	1			1	1				1	****
Davaalkham 2009	fulll text	1			1			1	1			1	1			1	*****
Davern, 2009	Abstract	1				1		1			1				1		1	***
Ditah, 2013	Abstract	1			1			1			1					1	1	*****
Dong, 2012	Full text	1					1	1	1		1		1				1	*****
Dremsek, 2012	Full text	1					1	1	1		1		1				1	*****
Ehteram, 2013	Full text	1				1		1	1		1		1				1	*****
Eker, 2009	Abstract	1					1	1	1			1	1				1	****
Eldin, 2010	full text			1		1		1	1			1	1				1	***
Faber, 2012	Full text	1			1			1	1		1		1			1		*******
Fan, 2011	Abstract	1			1					1	1				1		1	***
Fogeda, 2012	Full text	1			1			1	1		1		1				1	******
Fu, 2010	Full text	1				1		1	1		1				1		1	****
Gabilondo Alvarez, 2010	Abstract	1				1		1	1		1				1		1	****
Gad, 2011	Full text	1					1	1	1		1		1			1		******
Galiana, 2012	Full text	1				1		1	1		1		1				1	*****
Geng, 2011	Abstract	1				1				1	1				1		1	**
Ghezeldasht, 2013	Full text	1			1			1	1		1		1				1	******
Guo, 2010	fulll text	1					1	1	1			1	1				1	****
Harrison, 2013	Full text	1				1		1	1		1		1			1		******
Hourfar, 2012	Abstract			1			1	1	1		1				1		1	***

Huang, 2013	Full text	1			1	1	1	1			1				1	*****
Jacobs, 2013	Full text	1		1		1	1	1			1			1		*****
Jahan, 2009	Abstract	1			1	1	1			1			1		1	***
Juhl, 2009	Abstract	1		1		1	1	1			1				1	******
Juhl, 2012	Abstract	1			1		1	1	1			1				1	*****
Kaufmann, 2011	Full text	1			1		1	1	1			1				1	*****
Khameneh, 2013	Full text	1		1			1	1	1					1	1		******
Krumbholz, 2012	Full text	1			1		1	1	1			1			1		******
Labrique, 2009	Full text	1		1			1	1	1			1			1		*******
Labrique, 2010	Full text	1		1			1	1	1					1	1		******
Labrique, 2013	Full text	1		1			1	1		1		1			1		******
Lagler, 2013	Abstract	1		1			1		1					1		1	****
Lee, 2013	Full text	1				1	1		1			1			1		*****
Li, 2011	Full text	1				1	1	1	1					1	1		*****
Lindemann, 2010	Full text	1		1			1	1	1				1			1	*****
Ma, 2010	Full text	1		1			1	1	1			1				1	******
Madden, 2012	Abstract	1			1		1		1				1		1		****
Mansuy, 2011	Full test	1			1		1	1	1					1	1		*****
Maral, 2009	Full text	1				1	1	1	1					1		1	****
Matsubayashi, 2013	Abstract	1			1		1	1	1			1				1	*****
Meldal, 2013	Full text	1			1		1	1	1					1		1	****
Mesquita, 2013	Full text	1				1	1		1				1			1	***
Mohebbi, 2012	Full text	1			1		1	1		1		1				1	****
Mousa, 2011	Abstract	1			1		1	1	1			1				1	*****
Pilakasiri, 2009	Full text	1				1	1	1	1					1		1	****
Pischke, 2010	Full text	1			1		1	1	1					1		1	****
Pittaras, 2014	Full text	1				1	1	1	1			1				1	*****
Raoofi, 2012	Abstract		1			1	1		1				1			1	**
Ren, 2013	Full text	1			1		1	1	1			1				1	*****
Saffar, 2009	Full text	1		1			1	1	1			1			1		*******
Sanchez-Dias, 2012	Abstract	1			1		1	1	1				1			1	****

Sarkar, 2012	Abstract	1			1		1			1				1		1	***
Scotto, 2012	Full text	1			1		1	1		1				1		1	****
Scotto, 2014	Full text	1				1	1	1		1				1	1		****
Shamsizadeh, 2009	Full text	1		1			1	1		1				1	1		****
Sugitani, 2009	Full text	1				1	1	1		1			1			1	****
Syhavong, 2010	Full text		1			1	1	1		1			1			1	***
Takahashi, 2010	Full text	1				1	1	1		1		1			1		******
Takeda, 2010	Full text	1		1			1	1		1		1				1	******
Tanabe, 2011	Full text	1			1		1	1		1		1				1	*****
Taniguchi, 2009	Full text	1		1			1	1		1		1			1		*******
Traore, 2012	Full text	1				1	1	1			1	1			1		*****
Utba, 2013	Full text	1			1		1	1		1		1				1	*****
Verhoef, 2012	Full text	1		1			1	1		1		1			1		*******
Villalba, 2010	Full text	1		1			1	1		1		1			1		*******
Vivek, 2010	Full text	1		1			1	1		1		1				1	******
Widasari, 2013	Full text	1			1		1	1			1			1		1	***
Xu, 2013	Full text	1			1		1	1		1		1			1		******
Yu, 2009	Full text	1			1		1	1			1			1		1	***
Zaki, 2011	Abstract	1				1			1	1		1				1	***
Zaki, 2013	Full text	1			1		1	1		1		1				1	*****
Zhang, 2009	Full text	1				1	1			1		1			1		*****

Appendix 2: Quality assessment for extracted articles on incidence of sporadic HEV cases

Appendix 2 should be interpreted as follows:

The numbers in the top Row indicate the criteria of the quality assessment tool assessed:

1= Specification of the target population - Are study subjects and the setting described?
2= Valid and repeatable disease definition - Was HEV diagnosed in a standard and reliable manner?
3= Adequate response rate - Did the study report any completion rate?
4= Are efforts taken to address potential sources of bias?
5= Interpretation of results 5. Was follow up long enough for outcomes to occur?
6= Interpretation of results

The numbers on the second row represent the responses for each criterion. Each response with a designated star counts towards the quality rating of study:
Criterion 1: 1=Yes*, 2= No, 3= Insufficient data for judgment

Criterion 2: 1= Incidence confirmed according to accepted criteria. (IgM, HEV RNA via PCR, four fold increase in anti-HEV IgG)*Scientific approach to obtain a representative sample (i.e. sufficient size, random sample)* , 2= Clinical symptoms of HEV including jaundice, anorexia, hepatomegaly, abdominal pain and tenderness, nausea and vomiting, and fever along with laboratory confirmation by*: IgM, HEV RNA, four fold increase in anti-HEV IgG, 3= Immunoassay used is identified*, 4= Not reported in sufficient detail for judgment, not a case

Criterion 3: 1= Completion rate reported and > 70% *, 2= Completion rate < 70% and no description of missing subjects, 3= Not reported

Criterion 4: 1= Reported adjusted incidence for potentially non representative samples (eg data broken down by subgroups)*, 2= No adjusted estimates provided, 3=Not reported

Criterion 5: 1= Yes for mortality (1 week)*, 2=No, 3= Not applicable

Criterion 6: 1= Measures of variability are provided to the unadjusted and adjusted point prevalence (e.g. confidence interval, standard error)*, 2= Not reported

Study	Abstract or Full Text	1	2	3	4	5	6	Rating

		1	0	2	1	0	2	3	1	0	2	1	0	2	1	0	2	1	0	
Abro, 2009	full text	1			1		1		1			1					1		1	*****
Adjei, 2009	Full text	1			1		1		1		1	1			1		1	1		******
Ahmed, 2010	full text	1			1				1					1			1		1	***
Alam, 2009	full text	1				1	1		1			1			1				1	******
Andriesse, 2010	Absract	1			1				1					1			1		1	***
Astagneau, 2011	full text	1			1		1		1			1					1	1		******
Babamahmoodi, 2010	Absrtact	1				1			1			1			1				1	*****
Basavaraj, 2012	full text	1			1		1				1	1					1		1	****
Bashir, 2009	full text	1				1	1				1	1					1		1	****
Begum, 2010	full text	1				1	1		1			1			1				1	******
Bhat, 2011	abstract	1						1	1					1			1		1	**
Bhatnagar, 2013	Abstract	1			1		1		1					1			1		1	****
Blackard, 2009	full text	1				1	1		1					1	1				1	*****
Bonney, 2013	full text	1				1	1				1			1			1		1	***
Bouquet, 2011	Full text	1			1		1		1				1				1		1	****
Caciola, 2011	Full text	1			1		1		1				1				1		1	****
Candido, 2012	Full text	1			1		1		1				1				1		1	****
Chalupa, 2013	Full text	1			1		1		1			1			1				1	******
Chandra, 2012	full text	1				1	1				1			1					1	****

Chang, 2009	Full text	1			1		1		1			1				1		1	*****
Colson, 2012	Abstract	1			1					1			1			1		1	**
Dai, 2013	full text		1		1		1		1				1			1		1	***
Davern, 2009	abstract		1				1			1	1			1				1	**
Davern, 2011	Full text	1			1				1				1			1		1	***
De Silva, 2012	Full text	1			1		1		1			1				1		1	*****
Deka, 2010	full text	1				1	1			1	1					1		1	****
Deshpande, 2013	Full text	1			1				1				1			1		1	***
Despierres, 2011	Full text	1			1		1		1			1		1				1	******
dos Santos, 2010	full text	1			1		1			1			1			1		1	***
Drobeniuc, 2011	Absract	1			1				1				1			1		1	***
Drobeniuc, 2013	Full text	1			1		1		1			1				1		1	*****
Echevarria, 2011	Full text	1			1		1		1				1			1		1	****
Echevarria, 2013	full text	1			1		1			1	1					1		1	****
Eldin, 2010	full text	1			1		1			1	1					1		1	****
El-Tras, 2013	full text	1				1	1			1	1					1	1		*****
Faramawi, 2011	Full text	1					1	1		1	1					1	1		***
Fontana, 2012	Abstract	1			1		1		1				1	1				1	*****
Fujiwara, 2011	Full text	1			1		1		1				1	1				1	*****
Garcia, 2012	Full text	1			1		1		1			1				1		1	*****

Giordani, 2010	Abstract	1		1			1	1					1		1			1	***
Gupta, 2013	Full text	1			1	1	1	1				1				1		1	****
Gurley, 2009	Absrtact	1					1			1	1					1		1	**
Hazam, 2010	Full text	1			1	1		1					1			1		1	****
Hossain, 2012	Abstract	1		1				1					1			1		1	***
Huang, 2014	Full text	1		1				1			1					1	1		*****
Hunter, 2012	Abstract	1					1			1	1					1		1	**
Ijaz, 2012	Abstract full text	1		1						1			1			1		1	**
Chandra, 2012b		1		1	1	1				1	1						1		1
Ijaz, 2013	Full text	1		1		1				1			1			1		1	***
Ikeda, 2009	Abstract	1		1		1		1					1			1		1	****
Inoue, 2009	Full text	1		1		1		1			1			1			1		****** *
Jafri, 2013	Full text	1		1		1		1			1					1		1	*****
Jain, 2013	Full text	1		1		1		1			1					1		1	*****
Jang, 2011	full text	1			1	1		1					1			1		1	****
Jeblaoui, 2013	Abstract	1		1		1		1					1			1		1	****
Johargy, 2013	full text	1		1		1		1					1			1		1	****
Kang, 2011	Abstract	1		1		1				1			1		1			1	***
Kantala, 2009	full text	1			1			1					1			1		1	***
Kasper, 2012	full text	1		1				1					1			1		1	***
Kawakami, 2009	Abstract	1		1				1					1			1		1	***

Khan, 2009	Abstract	1			1			1			1					1				1		1	****
Kumar, 2011	full text	1			1			1					1	1						1		1	****
La Rosa, 2011	Abstract	1			1						1			1						1		1	***
Labrique, 2013	full text	1			1			1			1					1			1			1	****
Lee, 2010	Full text	1								1	1			1						1		1	***
Li, 2012	Full text	1			1			1			1			1						1		1	*****
Li, 2012	Full text	1			1			1			1			1						1		1	*****
Lu, 2013	full text	1			1			1			1					1			1			1	****
Luciano, 2012	full text	1			1			1			1					1				1		1	****
Mahtab, 2009	full text	1			1			1			1					1				1		1	****
Malhotra, 2012	Full text	1			1			1			1			1						1		1	*****
Midgley, 2014		1				1		1			1			1						1		1	
Midgley, 2014	full text	1				1		1			1			1						1		1	*****
Munim, 2011	full text	1				1		1					1	1						1		1	****
Munne, 2011	full text	1			1			1			1					1				1		1	****
Naaimi, 2012	Full text	1			1						1			1						1		1	****
Nandi, 2009	full text	1			1			1			1					1				1		1	****
Nasrawi, 2010	full text	1				1							1	1						1		1	****
Nicand, 2011	Full text	1			1						1			1				1				1	*****
Ou, 2013	Abstract	1			1			1			1			1						1		1	*****
Parkash, 2012	Full text	1								1	1					1		1				1	***
Popov, 2011	Abstract	1			1			1			1				1					1		1	****
Rana, 2011	full text	1			1			1					1			1				1	1		***
Rashid, 2009	Abstract	1			1						1				1					1		1	***
Renou, 2009	full text	1			1			1			1			1						1		1	*****
Romano, 2011	full text	1			1			1			1			1				1				1	******
Shrestha, 2009	full text	1								1	1					1				1		1	**
Shrestha, 2011	Full text	1			1			1			1			1				1				1	******
Singh, 2012	full text			1		1		1					1			1				1		1	**
Slot, 2013	Abstract	1				1		1			1					1				1		1	****
Sugitani, 2009	Full text	1			1			1			1				1					1		1	****

Sugitani, 2009	Full text	1			1			1			1					1				1		****
Syhavong, 2010	Full text	1			1			1			1			1						1		*****
Tan, 2013	Full text	1			1			1			1									1		****
Tang, 2013	Abstract	1			1			1			1					1				1		****
Terzic, 2009	Full text	1			1			1			1			1						1		*****
Terzic, 2010	Full text	1			1			1			1									1		****
Vivek, 2013	Full text	1			1			1					1			1				1		***
Yano, 2010	Full text	1				1		1			1					1				1		****
You, 2013	full text	1						1		1	1					1		1				****
Youssef, 2009	Full text	1				1		1			1					1				1		****
Zaki, 2009	Full text			1		1		1					1			1				1		**
Zaki, 2011	Full text	1				1		1			1					1				1		****
Zhang, 2011	full text	1				1				1	1				1				1			******
Colson, 2011	Abstract	1			1					1				1		1						****
Labrique, 2012	Abstract	1			1					1				1				1				***

Appendix 3: Quality Assessment for extracted articles on HEV outbreaks

Appendix 3 should be interpreted as follows:

The numbers in the top Row indicate the criteria of the quality assessment tool assessed:

1= Specification of the target population - Are study subjects and the setting described?
2= Representativeness of target population - Was the study sample representative of the target population?
3= Valid and repeatable disease definition - Was HEV diagnosed in a standard and reliable manner?
4= Adequate response rate - Did the study report any completion rate?
5= Are efforts taken to address potential sources of bias?
6= Interpretation of results 5. Was follow up long enough for outcomes to occur?
7= Interpretation of results

The numbers on the second row represent the responses for each criterion. Each response with a designated star counts towards the quality rating of study:
Criterion 1: 1=Yes*, 2= No, 3= Insufficient data for judgment

Criterion 2: 1=Study based on census population*, 0= Approach not yielding representative samples (i.e. convenience sample), 2= Study subjects not described in sufficient detail for judgment

Criterion 3: 1= Incidence confirmed according to accepted criteria. (IgM, HEV RNA via PCR, four fold increase in anti-HEV IgG)*Scientific approach to obtain a representative sample (i.e. sufficient size, random sample)* , 2= Clinical symptoms of HEV including jaundice, anorexia, hepatomegaly, abdominal pain and tenderness, nausea and vomiting, and fever along with laboratory confirmation by*: IgM, HEV RNA, fourfold increase in anti-HEV IgG, 3= Immunoassay used is identified*, 4= Not reported in sufficient detail for judgment, not a case

Criterion 4: 1= Completion rate reported and > 70% *, 2= Completion rate < 70% and no description of missing subjects, 3= Not reported

Criterion 5: 1= Reported adjusted attack rates for potentially non representative samples (eg data broken down by subgroups)*, 2= No adjusted estimates provided, 3=Not reported

Criterion 6: 1= Yes for mortality (1 week)*, 2=No, 3= Not applicable

Criterion 7: 1= Measures of variability are provided to the unadjusted and adjusted point prevalence (e.g. confidence interval, standard error)*, 2= Not reported

Study	Abstract or Full Text	1			2			3				4			5			6			7		Rating
		1	0	2	1	0	2	1	0	2	3	1	0	2	1	0	2	1	0	2	1	0	
Arora, 2012	Full text	1				1		1		1		1			1					1		1	*****
Bouscaillou, 2013	Full text	1					1	1		1		1					1	1				1	*****
Chauhan, 2010	Full text	1				1		1						1			1			1	1		****
Colson, 2010	full text	1				1		1	1			1			1					1		1	*****
Garbuglia, 2013	Full text	1				1				1		1			1					1		1	****
Goumba, 2010	full text	1					1		1	1		1			1					1		1	*****
Goumba, 2011	full text	1					1		1	1				1	1					1		1	****
Harun-Or-Rashid, 2013	full text	1					1	1		1		1				1				1		1	****
Howard, 2010	Full text	1				1		1		1		1			1				1		1		******
Ippagunta, 2011	Full text	1					1		1	1		1					1			1		1	****
Jahan, 2009	Abstract	1					1		1	1				1			1			1		1	***
Kang, 2013	Abstract	1				1					1	1				1				1		1	**
Khan, 2011	Full text	1					1	1		1		1					1			1		1	****
Majumdar, 2013	Full text	1				1		1		1		1					1			1		1	****
Martolia, 2009	Full text	1			1			1		1		1				1		1			1		*******
Pujhari, 2010	Full text	1				1		1		1				1	1					1		1	****
Rayis, 2013	Full text	1					1		1	1				1	1					1		1	****
Sailaja, 2009	Full text	1				1		1				1			1			1				1	*****
Sharapov,	Full text	1			1			1		1		1			1			1			1		********

2009											
Shata, 2012	Full text	1	1	1	1	1	1	1	1	1	*****

Appendix 4: Article search methodology

Pub Med

In PubMed, the following search terms were used:

("Hepatitis E" OR "Hepatitis E virus" OR "HEV" OR "Hepatitis E antibody*"OR (enterically transmitted non a non b hepatitis) OR "ET-NANBH")

AND

("2009/01/01"[PDAT]: "2014/01/14"[PDAT])

AND

Country_name

Embase

The following search terms were used in Embase for each country:

Steps	Keyword search
1.	Hepatitis E.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer name]
2.	hepatitis E/ or hepatitis E antibody/ or hepatitis E antigen/ or

hepatitis non A non B/ or

hepatitis E vaccine/ or hepatitis non A non B non C/ or Hepatitis E virus/ or Hepatitis virus

non A non B/

3. #1 OR #2

4. Limit 3 to yr="2009 – 2014"

Scopus

The following search was conducted in Scopus by country:

"HEV" OR "Hepatitis E" OR Hepatitis E virus" OR "Hepatitis E antibodies" OR "et-nanbh" OR "enterically transmitted non a non b hepatitis"
AND
Name of the country

Appendix 5: Quality assessment checklist for prevalence studies

SEROPREVALENCE STUDIES

Specification of the target population

1. **Are study subjects and the setting described?**
   ☐Yes*
   ☐No
   ☐Not reported in sufficient detail for judgment

Representativeness of target population

2. **Was the study population based?**
   Target population: Healthy individuals or subgroups of interest

☐Scientific approach to obtain a representative sample (i.e. sufficient size, random sample)*
☐Approach not yielding representative samples (i.e. convenience sample)
☐Study subjects and setting not described in sufficient detail for judgment.

Valid laboratory detection of HEV antibodies

3. **Was HEV seroprevalence detected in a standard and reliable manner?**

☐Seropositivity confirmed according to accepted criteria. (IgG)*
☐ Immunoassay used for detection is identified*
☐Not reported in sufficient detail for judgment.

Adequate response rate

4. **Did the study report any completion rate?**
   ☐Completion rate reported and > 70% *
   ☐Completion rate < 70% and no description of missing subjects
   ☐Not reported

Bias

5. **Are efforts taken to address potential sources of bias?**
   ☐ Reported adjusted prevalence for potentially non representative samples (eg data broken down by subgroups)*
   ☐ No adjusted estimates provided
   ☐ Not reported

Interpretation of results

6. **Are the estimates of prevalence given with measures of variability and in detail by subgroup, if appropriate?**
   ☐ Measures of variability are provided to the unadjusted and adjusted point prevalence (e.g. confidence interval, standard error)*
   ☐ Not reported

Appendix 6: Quality assessment checklist for incidence studies

SPORADIC ACUTE HEV

Specification of the target population

1. **Are study subjects and the setting described?**
   - ☐Yes*
   - ☐No
   - ☐Not reported in sufficient detail for judgment

Valid and repeatable disease definition

2. **Was HEV diagnosed in a standard and reliable manner?**
   - ☐Incidence confirmed according to accepted criteria. (IgM, HEV RNA via PCR, four fold increase in anti-HEV IgG)*
   - ☐ Clinical symptoms of HEV including jaundice, anorexia, hepatomegaly, abdominal pain and tenderness, nausea and vomiting, and fever along with laboratory confirmation by*:
     - -IgM
     - -HEV RNA
     - -fourfold increase in anti-HEV IgG
   - ☐ Immunoassay used is identified*
   - ☐Not reported in sufficient detail for judgment, not a case

Adequate response rate

3. **Did the study report any completion rate?**
   - ☐Completion rate reported and > 70 % *
   - ☐Completion rate < 70% and no description of missing subjects.
   - ☐Not reported

Bias

4. **Are efforts taken to address potential sources of bias?**
   - ☐ Reported adjusted incidence for potentially non-representative samples*
   - ☐ No adjusted estimates provided
   - ☐ Not reported

Interpretation of results

5. **Was follow up long enough for outcomes to occur?**
   - ☐Yes for mortality (1 week)*
   - ☐Yes for chronic hepatitis (6 months between 1st and second evaluation). *
   - ☐No
   - ☐Not applicable
6. **Are the estimates of incidence given with measures of variability and in detail by subgroup, if appropriate?**
   - ☐ Measures of variability are provided to the unadjusted and adjusted incidence (e.g. confidence interval, standard error)*
   - ☐Not reported

Appendix 7: Quality assessment checklist for outbreak studies

OUTBREAK STUDIES

Specification of the target population

1. **Are study subjects and the setting described?**
   ☐Yes*
   ☐No
   ☐Not reported in sufficient detail for judgment

Representativeness of target population

2. **Was the study sample representative of the target population?**
   ☐ Study based on census population*
   ☐Approach not yielding representative samples (i.e. convenience sample)
   ☐ Study subjects not described in sufficient detail for judgment

Valid and repeatable disease definition

3. **Was HEV diagnosed in a standard and reliable manner?**
   ☐Incidence confirmed according to accepted criteria. (IgM, HEV RNA via PCR, four fold increase in anti-HEV IgG)*
   ☐ Clinical symptoms of HEV including jaundice, anorexia, hepatomegaly, abdominal pain and tenderness, nausea and vomiting, and fever along with laboratory confirmation by*:
   -IgM
   -HEV RNA
   -fourfold increase in anti-HEV IgG
   ☐ Immunoassay used is identified*
   ☐Not reported in sufficient detail for judgment, not a case

Adequate response rate

4. **Did the study report any completion rate?**
   ☐Completion rate reported and > 70 % *
   ☐Completion rate < 70% and no description of missing subjects.
   ☐Not reported

Bias

5. **Are efforts taken to address potential sources of bias?**
   ☐ Reported adjusted incidence for potentially non-representative samples*
   ☐ No adjusted estimates provided
   ☐ Not reported

Interpretation of results

6. **Was follow up long enough for outcomes to occur?**
   ☐Yes for mortality (1 week)*
   ☐Yes for chronic hepatitis (6 months between 1st and second evaluation)*
   ☐No
   ☐Not applicable

7. **Are the estimates of incidence given with measures of variability and in detail by subgroup, if appropriate?**
   ☐ Measures of variability are provided to the unadjusted and adjusted incidence (e.g. confidence interval, standard error)*
   ☐Not reported

Appendix 8: Table of characteristics on seroprevalence of anti-Hepatitis E Virus antibodies in in Africa

Country	Study period	Seroprevalence (%)	Sample demographics	Sample size	Diagnostic methods	Genotype	Quality Assesssment score	Reference
Ghana	NS	4.6	Blood donors	239	IgG	NS	****	Meldal, 2013 (1)
Ghana	2008	28.7	Pregnant women	157	IgG	NS	*****	Adjei, 2009 (2)
Burkina Faso	2010-2012	16.2	Blood donors and pregnant women	191	IgG	NS	*****	Traoré, 2012 (3)
Zambia	1999-2011	24.7	Individuals living in urban settings	300	IgG	NS	*****	Jacobs, 2013 (4)
Nigeria	2007	13.4	Healthy and sick individuals	186	NS	NS	***	Adesina, 2009 (5)

Abbreviations: NS: Not specified

Appendix 9 Table of characteristics on Incidence of sporadic Hepatitis E Virus cases in Africa

Country	Study period	Incidence (%)	Sample demographics	Sample size	Diagnostic methods	Genotype	Quality Assessment score	Reference
Ghana	2008	64.4	Pregnant women	157	IgM	NS	******	Adjei, 2009 (2)
Uganda	2007-2008	65.2	Displaced persons	161	IgM	NS	******	(Teshale, 2010) (6)

Abbreviations: NS: Not specified

Appendix 10: Table of characteristics on Hepatitis E Virus outbreaks in Africa

Country	Sample demographics	Incidence (%)	Attack rate (per 1000 population)	Diagnostic methods	Attack rate by age	Attack rate by gender	Genotype	Quality Assessment score	Source
Central African Republic	Pregnant women receiving prenatal care at a maternity centre	33.3	NS	IgM	NS	NS	NS	*****	Goumba, 2010 (7)
Central African Republic	Male workers	72.7	NS	IgM	NS	NS	NS	*****	Bouscaillou, 2013 (8)
Central African Republic	Patients with jaundice Patients with GI complaints and fever leading to a clinical diagnosis of malaria with ineffective malaria treatment	51.8	NS	IgM	NS	NS	NS	****	Goumba, 2011 (9)
Uganda	Acute hepatitis E cases	43.6	1018.2	NS	1-15 years: 44.7%; 16-49 years: 47.5%	NS	NS	******	Howard, 2010 (10)
Uganda	Residents of Madi Opei	30.5	305.5	Self-reported	NS	NS	1	*******	(Teshale, 2010) (11)

Abbreviations: NS: Not specified

Appendix 11: Table of characteristics on case fatality rate associated with Hepatitis E Virus in Africa

Country	Study period	Sample size	Sample demographics	CFR in pregnant women (%)	Genotype	Stillbirth (%)	Overall CFR (%)	Reference
Central African Republic	2002	21	Pregnant women	14.3	NS	14.3	NS	Goumba, 2010 (7)
Ghana	2010	3	Pregnant women	33.3	NS	66.7	NS	Bonney, 2012 (12)
Sudan	NS	39	Pregnant women	28.2	NS	36.0	NS	Rayis, 2013 (13)

Abbreviations: NS: Not specified

Appendix 12: Table of characteristics on seroprevalence of anti-Hepatitis E Virus antibodies in the Americas

Country	Study period	Seroprevalence (%)	Sample demographics	Sample size	Diagnostic methods	Genotype	Quality Assessment score	Reference
Cuba	2003	10.0	Patients without a history of jaundice	469	IgG	NS	*******	Villalba, 2010 (14)
United States of America	NS	12.9	Healthy individuals	140	IgG	NS	****	Basu, 2012 (15)
United States of America	1988-1994	6.0	U.S. general population from the National Health and Nutrition Evaluation Survey (NHANES)	8814	IgG	NS	*******	Ditah, 2014 (16)
United States of America	2006-2012	18.8	Blood donors	1939	IgG	NS	******	Xu, 2013 (17)
United States of America	NS	3.1	Personnel with frequent workplace contact with pigs	64	IgG	NS	***	Sarkar, 2012 (18)
United States of America	NS	3.2	Blood donors	63	IgG	NS	***	Sarkar, 2012 (18)
United States of America	NS	14.5	Patients in clinical sites Patients with suspected drug induced liver injury from 5 clinical	318	IgG	NS	***	Davern, 2009 (19)

sites

Abbreviations: NS: Not specified

Appendix 13: Table of characteristics on incidence of sporadic Hepatitis E Virus cases in the Americas

Country	Study period	Incidence (%)	Sample demographics	Sample size	Diagnostic methods	Genotype	Quality Assessment score	Reference
Argentina	2005-2010	6.1	Acute hepatitis patients	231	NS	3	****	Munne, 2011 (20)
Brazil	2004-2008	1.56	Patients with acute hepatitis	64	IgM	3	***	dos Santos, 2010 (21)
United States of America	NS	18.0	Drug-induced liver injury patients	318	IgM	3	***	Davern, 2011 (22)
United States of America	2005-2012	27.1	Acute non-A, non-B, non-C hepatitis patients	129	IgM	1, 3, 4	***	Drobeniuc, 2011 (23)
United States of America	2009-2010	0.59	Individuals who participated in the National Health and Nutrition Examination Survey	8814	IgM	NS	*****	Ditah, 2013 (16)
United States of America	1998-2011	0.43	Patients with acute liver failure	699	IgM	NS	*****	Fontana, 2012 (24)
United States of America	2005-2012	46.2	Persons seronegative for HAV or HBV	154	IgM	NS	*****	Drobeniuc, 2013 (25)
Venezuela	2009	29.8	Hepatitis A patients	74	IgM	1, 3	*****	Garcia, 2012 (26)

Abbreviations: NS: Not specified

Appendix 14: Table of characteristics on case fatality rate associated with Hepatitis E Virus in the Americas

Country	Sample demographics	CFR in pregnant women (%)	Stillbirth (%)	Overall CFR (%)	Genotype	Reference
United States of America	Individuals seronegative for HAV and HBV	NS	NS	3.9	NS	Drobeniuc, 2013 (23)
United States of America	Acute non-A, non-B, non-C hepatitis patients	NS	NS	2.7	1, 3, 4	Drobeniuc, 2011 (27)
United States of America	Patients with acute liver failure	NS	NS	0.7	NS	Fontana, 2012 (24)

Abbreviations: NS: Not specified

Appendix 15: Table of characteristics on seroprevalence of anti-Hepatitis E Virus antibodies in the Eastern Mediterranean

Country	Study period	Seroprevalence (%)	Sample demographics	Sample size	Diagnostic methods	Genotype	Quality Assessment score	Reference
Egypt	2009	58.6	HCV and non-HCV positive pregnant women	116	IgG	NS	******	Gad, 2011 (28)
Egypt	NS	46.7	Asymptomatic pregnant women	60	IgG	NS	*****	Mousa, 2011 (29)
Egypt	NS	39.0	Healthy adolescent females	95	NS	NS	***	Zaki, 2011 (30)
Iraq	NS	14.2	Blood donors	212	NS	NS	*****	Utba, 2013 (31)
Iraq	NS	48.3	Cleaning service workers in health care centers and hospitals	58	NS	NS	*****	Utba, 2013 (31)
Iran	2009	14.2	Residents of the great Mashhad capital	1582	NS	NS	****	Ahmadi, 2013 (32)
Iran	2005	3.8	Residents of Isfahan province	816	NS	NS	****	Ataei, 2009 (33)
Iran	NS	3.7	Pregnant women	136	NS	NS	******	Khameneh, 2013 (34)
Iran	NS	14.2	Local population during pilgrimage	1582	NS	NS	******	Ghezeldasht, 2013 (32)
Iran	2006-2007	9.3	Randomly selected individuals by postal code	551	NS	NS	****	Mohebbi, 2012 (35)

Iran	2009	7.8	NS	400	NS	NS	**	Raoofi, 2012 (36)
Iran	2006-2007	8.5	School children	566	IgG	NS	****	Shamsizadeh, 2009 (37)
Iran	2003	2.3	Healthy children and young adults	1080	IgG	NS	*******	Saffar, 2009 (38)
Iran	2012	14.3	Blood donors	530	IgG	NS	*****	Ehteram, 2013 (39)
Saudi Arabia	2009	18.7	Blood donors	900	IgG	NS	****	Johargy, 2013 (40)
Yemen	2005	10.7	Patients attending primary health care facilities	538	IgG	NS	*******	Bawazir, 2010 (41)

Abbreviations: NS: Not specified

Appendix 16: Table of characteristics on incidence of sporadic Hepatitis E Virus cases in the Eastern Mediterranean

Country	Study period	Incidence (%)	Sample demographics	Sample size	Diagnostic methods	Genotype	Quality Assessment score	Reference
Afghanistan	2008	28.4	Residents of Kabul who visited a field hospital	102	IgM	NS	*****	Carmoi, 2009 (42)
Egypt	2006-2008	20.2	Patients with acute hepatitis	287	NS	1	*****	Blackard, 2009 (43)
Egypt	2010-2011	38.1	Patients with acute hepatitis	134	IgM	NS	*****	El-Tras, 2013 (44)
Egypt	2002-2007	0.9	Acute hepatitis patients	1950	IgM	1	******	Astagneau, 2012 (45)
Egypt	NS	41.2	Pediatric patients with acute hepatitis	68	IgM	NS	**	Zaki, 2009 (46)
Egypt	NS	2.3	Patients with high ALT and AST	214	IgM	NS	****	Youssef, 2009 (47)
Egypt	2007-2008	15.8	Patients with acute hepatitis	235	IgM	NS	****	Eldin, 2010 (48)
Egypt	2007-2008	7.0	Asymptomatic healthy individuals	200	IgM	NS	****	Eldin, 2010 (48)
Iraq	NS	1.6	Patients with suspected acute viral hepatitis	2692	IgM	NS	****	Al-Naaimi, 2012 (49)

Iran	2003-2008	0.5	Residents of northern Iran	439	IgM	NS	*****	Babamahmoodi, 2010 (50)
Pakistan	2007	5.4	Suspected hepatitis patients	93	IgM	NS	****	Bashir, 2009 (51)
Pakistan	2008-2009	2.4	Children residing in an urban slum area of Karachi	540	IgM	NS	*****	Jafri, 2013 (52)
Pakistan	NS	60.4	Acute viral hepatitis patients	91	IgM	NS	****	Khan, 2009 (53)
Pakistan	2005-2007	33.0	Patients with acute liver failure with jaundice	91	NS	NS	***	Parkash, 2012 (54)
Pakistan	2009-2011	66.0	Pregnant women with jaundice	47	NS	NS	***	Hossain, 2012 (55)
Pakistan	1987-2007	20.2	Patients with viral liver disease	346	NS	NS	***	Ahmed, 2010 (56)

Abbreviations: NS: Not specified

Appendix 17: Table of characteristics on Hepatitis E Virus outbreaks in the Eastern Mediterranean

Country	Study period	Sample demographics	Incidence (%)	Attack rate (per 1000 population)	Diagnostic methods	Attack rate by age	Attack rate by gender	Genotype	Quality Assessment score	Source
Egypt	2007-2008	Symptomatic HEV cases	17.9	NS	IgM	NS	Male: 84.0; Female: 67.8	NS	*****	Shata, 2012 (57)
Iraq	2005	Patients with jaundice	38.1	NS	IgM	1-15 years: 12.9; 16-49 years: 39.5;	Male: 35.6; Female: 39.5	NS	****	Al-Nasrawi, 2010 (58)
Pakistan	NS	symptomatic acute liver disease cases	81.5	NS	IgM	NS	NS	1	****	Khan, 2011 (59)
United Arab Emirates	2006-2007	Acute hepatitis patients	40	NS	NS	NS	NS	NS	*****	Abro, 2009 (60)

Abbreviations: NS: Not specified

Appendix 18: Table of characteristics on case fatality rate associated with Hepatitis E Virus in the Eastern Mediterranean

Country	Sample demographics	CFR in pregnant women (%)	Stillbirth (%)	Overall CFR (%)	Genotype	Reference
Sudan	Pregnant women in outbreak setting	28.21	35.9	71.9 in non-pregnant women	NS	Rayis, 2013 (13)
Pakistan	Pregnant women with fulminant hepatic failure	NS	90.01	NS	NS	Munim, 2011 (61)
Pakistan	Pregnant women with jaundice	29.0	NS	NS	NS	(Hossain, 2012) (55)

Abbreviations: NS: Not specified

Appendix 19: Table of characteristics on seroprevalence of anti-Hepatitis E Virus antibodies in South East Asia

Country	Study period	Seroprevalence (%)	Sample demographics	Sample size	Diagnostic methods	Genotype	Quality Assessment score	Reference
Bangladesh	2003-2004	6.0-24.4	General population from a random representative sample	1134	NS	NS	******	Labrique, 2010 (62)
Bangladesh	2001-2007	5.6	Pregnant women	1127	IgG	NS	***	Labrique, 2012 (63)
Bangladesh	2007-2010	3.6	Pregnant women	1100	IgG	NS	***	Labrique, 2012 (64)
India	NS	13.7	Blood donors	262	IgG	NS	*****	Bajpai, 2011 (65)
India	2006-2007	33.7	Pregnant women	300	IgG	NS	*******	Begum, 2009 (66)
India	2008	14.5	Healthy individuals	2279	IgG	NS	******	Vivek, 2010 (67)
India	2008-2009	11.3	Medical students	160	IgG	NS	***	Jahan, 2009 (68)
Indonesia	2011	5.8	Healthy residents of Bali and Java	277	NS	3, 4	***	Widasari, 2013 (69)
Thailand	NS	15.7	Nursing students	281	NS	NS	****	Pilakasiri, 2009 (70)

Abbreviations: NS: Not specified

Appendix 20: Table of characteristics on incidence of sporadic Hepatitis E Virus cases in South East Asia

Country	Study period	Incidence (%)	Sample demographics	Sample size	Diagnostic methods	Genotype	Quality Assessment score	Reference
Bangladesh	2003-2008	64.2	Patients with Fulminant Hepatic Failure (FHF)	67	IgM	NS	******	Alam, 2009 (71)
Bangladesh	2004-2006	17.0	Symptomatic hepatitis cases	279	IgM	NS	****	Labrique, 2013 (72)
Bangladesh	2004-2006	47.2	Patients with acute hepatitis	267	IgM	NS	****	Mahtab, 2009 (73)
Bangladesh	2004-2006	56.5	Patients with FHF	23	IgM	NS	****	Mahtab, 2009 (74)
Bangladesh	1995-1996	25.0	Patients with acute sporadic hepatitis	36	IgM	1	****	Sugitani, 2009 (75)
Bangladesh	1995-1996	33.3	Patients with fulminant hepatitis	12	IgM	1	****	Sugitani, 2009 (75)
Bangladesh	NS	58.3	Viral hepatitis patients	60	NS	NS	***	Rashid, 2009 (76)
India	NS	41.4	Patients with FHF	70	IgM	1	****	Kumar, 2011 (77)
India	NS	58.3	Pregnant women	24	IgM	1	****	Kumar, 2011 (77)
India	NS	38.7	Viral hepatitis patients	685	IgM	1	****	Kumar, 2011 (77)
India	NS	9.6	Clinical acute hepatitis patients with jaundice and liver disease	104	IgM	1	****	Deka, 2010 (78)
India	2010-2012	78.6	Outpatient clinic	156	IgM	NS	****	Gupta, 2013

			patients					(79)
India	2000-2013	25.5	Sporadic cases of viral hepatitis	141	IgM	NS	****	Hazam, 2010 (80)
India	2003-2004	45.5	Acute viral hepatitis cases	224	IgM	NS	****	Nandi, 2009 (81)
India	2012	4.8	Male blood donors	460	IgM	NS	****	Bhatnagar, 2013 (82)
India	2003-2004	8.9	Suspected cases of hepatitis	79	IgM	NS	****	Basavaraj, 2012 (83)
India	2009-2011	16.8	Acute hepatitis patients	3945	IgM	NS	*****	Malhotra, 2012 (84)
India	2005-2007	11.9	Children	109	NS	NS	**	Bhat, 2011 (85)
India	NS	32.0	Acute viral hepatitis cases	1147	IgM	NS	**	Singh, 2012 (86)
India	2006-2010	9.6	Acute sporadic HEV cases	94	RNA	1	***	Vivek, 2013 (87)
Nepal	2001-2007	20.7	Pregnant women with acute hepatitis	29	NS	NS	**	Shrestha, 2009 (88)

Abbreviations: NS: Not specified

Appendix 21: Table of characteristics on Hepatitis E Virus outbreaks in South East Asia

Country	Study period	Sample demographics	Sample size	Incidence (%)	Attack rate (per 1000 population)	Diagnostic methods	Attack rate by age	Attack rate by gender	Genotype	Quality Assessment score	Source
Bangladesh	2008-2009	Women of reproductive age		84.1	74	IgM	NS	NS	NS	****	Hossain, 2009 (89)
India	2011-2012	Patients with jaundice and acute hepatitis		64.2	NS	IgM	NS	NS	NS	*****	Arora, 2013 (90)
India	NS	Acute hepatitis cases		61.4	NS	IgM	NS	NS	NS	****	Ippagunta, 2011 (91)
India	2010	Patients with suspected acute viral hepatitis		NS	4080	IgM	NS	NS	1	****	Majumdar, 2013 (92)
India	2005	Jaundice patients		NS	16 559	NS	<9years: 5263 10-14 years: 17164 15-44 years: 19067 >44 years: 14176	Male: 17509 Female: 15884	NS	*******	Martolia, 2009 (93)
India	2002-2006	Patients with acute viral hepatitis		37.9	NS	IgM	NS	NS	1	****	Pujhari, 2010 (94)

India	2004	Cluster of acute hepatitis	NS	509	IgM	NS	NS	NS	*******	Swain, 2010 (95)
India	2008	Icteric hepatitis cases	NS	5731	IgM	NS	Male: 7887 Female : 3430	1	*******	Vivek, 2010 (96)
India	2005	Acute hepatitis cases	NS	40	IgM	0-4 years: 3.3 5-9 years: 22.8 10-19 years: 46.8 20-44 years: 53.0 >45 years: 27.3	Male: 50.1; Female : 29.2	NS	*****	Sailaja, 2009 (97)
India	NS	Jaundice patients	NS	75	IgM	NS	NS	NS	****	Chauhan, 2010 (98)

Abbreviations: NS: Not specified

Appendix 22: Table of characteristics on case fatality rate associated with Hepatitis E Virus in South East Asia

Country	Sample demographics	CFR in pregnant women (%)	Stillbirth (%)	Overall CFR (%)	Genotype	Reference
Bangladesh	Patients with FHF	NS	NS	65.1	NS	Alam, 2009 (71)
Bangladesh	Women of reproductive age	NS	NS	22.7	NS	Hossain, 2009 (89)
India	Cluster of acute hepatitis	NS	NS	0.9	NS	Swain, 2010 (95)
India	Acute hepatitis cases	NS	NS	0.2	NS	Sailaja, 2009 (97)
India	Acute hepatitis cases	22.2	NS	NS	NS	Chandra, 2012 (99)
Nepal	Pregnant women	19.6	5.4	NS	NS	Shrestha, 2011 (100)
Nepal	Pregnant women with acute hepatitis	83.3	NS	NS	NS	Shrestha, 2009 (88)
Thailand	Acute HEV cases	NS	NS	9.1	3	Poovorawan, 2012 (101)
India	Icteric hepatitis cases	NS	NS	1.3	1	Vivek, 2010 (96)

Abbreviations: NS: Not specified

Appendix 23: Table of characteristics on seroprevalence of anti-Hepatitis E Virus antibodies in Western Pacific

Country	Study period	Seroprevalence (%)	Sample demographics	Sample size	Diagnostic methods	Genotype	Quality Assessment score	Reference
China	NS	22.2	Pig farmers, general population and slaughterhouse workers	2819	NS	4	**	Chang, 2009 (102)
China	2007-2008	23.7	Healthy individuals	296	NS	4	****	Fu, 2010 (103)
China	NS	10.2	Pregnant asymptomatic women	293	IgG	NS	*****	Huang, 2013 (104)
China	NS	39.9	Residents of Kunming	173	IgG	NS	*****	Li, 2011 (105)
China	NA	19.5	Healthy individuals	2090	IgG	4	******	Ma, 2010 (106)
China	2005-2008	47.7	Indigenous Chinese age 40 and over	300	NS	NS	*******	Taniguchi, 2009 (107)
China	2005	27.4	Blood donors	10741	IgG	3	*****	Ren, 2013 (108)
China	2002-2008	32.6	Eligible blood donors from 6 urban centres	44816	IgG	1, 4	****	Guo, 2010 (109)
China	2006-2008	19.7	Chinese and ethnic minority populations	14208	IgG	NS	*****	Dong, 2012 (110)
China	NS	21.9	Pig farm workers, general population and slaughterhouse workers	2929	NS	4	**	Geng, 2011 (111)
China		47.1	Koreans living in China age 40 and over	300	NS	NS	*******	Taniguchi, 2009 (107)

China	NS	7.9	NS	1776	IgG	4	*****	Zhang, 2009 (112)
China	2005`	22.7	Illegal blood donors	546	IgG	4	******	Cheng, 2012 (113)
Japan	2002-2007	5.3	Healthy individuals who underwent health check ups	22027	IgG	3	******	Takahashi, 2010 (114)
Japan	2005	3.4	Blood donors	12600	IgG	NS	*****	Takeda, 2010 (115)
Japan	NS	2.0-5.4	Healthy individuals	NS	IgG	NS	*****	Tanabe, 2011 (116)
Japan	2004-2008	0.01	Transfusion-transmitted cases of HEV	7700	IgG	3	****	Ikeda, 2009
Japan	2005-2008	6.0	Indigenous Japanese age 40 and over	300	NS	NS	*******	Taniguchi, 2009 (107)
Japan	2005-2008	14.3	Koreans living Japan age 40 and over	300	NS	NS	*******	Taniguchi, 2009 (107)
Mongolia	2004	0.6	School children	1145	IgG	NS	*****	Davaalkham, 2009 (117)
South Korea	2005-2008	34.0	Indigenous Koreans age 40 and over	300	NS	NS	*******	Taniguchi, 2009 (107)
Taiwan	2012-2013	7.7	Swine farmers, students and pregnant women	504	IgG	NS	*****	Lee, 2013 (118)

Abbreviations: NS: Not specified

Appendix 24: Table of characteristics on incidence of sporadic Hepatitis E Virus cases in Western Pacific

Country	Study period	Incidence (%)	Sample demographics	Sample size	Diagnostic methods	Genotype	Quality Assessment score	Reference
Cambodia	2006-2009	10.9	Patients with febrile illness	9997	IgM	NS	***	Kasper, 2012 (119)
China	NS	2.21	Blood donors	20389	RNA	NS	*****	Ou, 2013 (120)
China	2006-2008	0.1	Chinese and ethnic minority populations	14208	IgM	NS	*****	Dong, 2012 (110)
China	2001-2013	5.1	Suspected acute viral hepatitis	15910	IgM	4	***	Dai, 2013 (121)
China	2002-2008	0.9	Blood donors	44816	IgM	NS	****	Guo, 2010 (109)
China	NS	21.0	Acute hepatitis E patients	62	RNA	4	****	Geng, 2011 (122)
China	2010-2012	92.2	Hospitalized patients	116	RNA	1, 3, 4	****	Geng, 2013 (123)
China	2011-2012	71.8	Acute hepatitis E patients	248	IgM	4	****	Tang, 2013 (124)
China	2002-2011	1.7	Liver failure patients	3916	NS	NS	****	You, 2013 (125)
China	NS	1.6	Individuals with rare contact with swine	3994	IgM	4	***	Yu, 2009 (126)
China	2005-2011	42.7	Acute sporadic hepatitis E cases	499	RNA	4	****	Lu, 2013 (127)
China	2009	59.3	Sporadic HEV cases	27	RNA	4	****	Xia, 2010 (128)
China	2005-2008	33.9	Sporadic HEV cases	413	RNA	4	****	Xia, 2009 (129)
Hong Kong	2008-2009	28.7	Hospitalized patients	450	IgG	NS	******	Chiu, 2013 (130)
Japan	2007-2010	19.9	Patients with acute hepatitis	321	IgM	3, 4	***	Kang, 2011 (131)

Japan	1999-2008	8.5	Acute fulminant hepatitis cases	94	IgM	3	*******	Inoue, 2009 (132)
Japan	2005-2011	0.01	Blood donors	2000000	RNA	NS	*****	Matsubayashi, 2013 (133)
Japan	1990-2009	1.1	Autoimmune fulminant liver failure	95	IgG	NS	*****	Fujiwara, 2011 (134)
Japan	1989-2004	6.3	Acute hepatitis patients	112	IgM	4	*****	Li, 2012 (135)
Japan	2004-2007	9.1	Patients diagnosed as etiology-obscure acute liver injury	22	IgM	3	***	Kawakami, 2009 (136)
Japan	1980-2008	0.9	Cases of acute viral hepatitis	4302	IgM	4	****	Yano, 2010 (137)
Lao	2001-2004	1.6	Patients admitted to hospital with acute jaundice or elevated AST or ALT	392	IgM	NS	*****	Syhavong, 2010
Lao	2001-2004	18.2	Blood bank control	198	RNA	NS	*****	Syhavong, 2010 (138)
Singapore	1998-2007	13.5	Viral hepatitis cases	520	NS	NS	***	Lee, 2010 (139)
South Korea	2006-2008	1.4	Symptomatic viral hepatitis	771	IgM	NS	****	Jang, 2011 (140)

Abbreviations: NS: Not specified

Appendix 25: Table of characteristics on Hepatitis E Virus outbreaks in Western Pacific

Country	Study period	Sample demographics	Incidence (%)	Attack rate (per 1000 population)	Diagnostic methods	Attack rate by age	Attack rate by gender	Genotype	Quality Assessment score	Source
Japan	2007-2010	Patients with acute liver injury		23.4	NS	NS	NS	4	**	Kang, 2013 (141)
Japan	2007-2010	Fulminant Hepatitis E patients		3.7	NS	NS	NS	NS	**	Kang, 2013 (142)

Abbreviations: NS: Not specified

Appendix 26: Table of characteristics on case fatality rate associated with Hepatitis E Virus in Western Pacific

Country	Sample demographics	CFR in pregnant women (%)	Stillbirth (%)	Overall CFR (%)	Genotype	Reference
China	Patients admitted to hospital	NS	NS	0.9	NS	Fan, 2011 (143)
China	Liver failure patients	NS	NS	13.2	NS	You, 2013 (125)
China	NS	NS	NS	16.8	4	Zhang, 2011 (144)
Japan	Cases of fulminant Hepatitis E	NS	NS	50.0	4	Ogawa, 2012 (145)
Japan	Pregnant women	11.1	NS	NS	NS	Kang, 2011 (131)
Japan	Patients with acute hepatitis	NS	NS	1.6	3, 4	Kang, 2011 (131)

Abbreviations: NS: Not specified

Appendix 27: Table of characteristics on seroprevalence of anti-Hepatitis E Virus antibodies in Europe

Country	Study period	Seroprevalence (%)	Sample demographics	Sample size	Diagnostic methods	Genotype	Quality Assessment	Reference
Austria	2009	14.3	Military personnel	1003	IgG	NS	****	Lagler, 2013 (146)
France	2011-2012	35.2	Pig farmers, Swine vets and forestry workers	859	IgG	NS	******	Chaussade, 2013 (147)
France	2003-2004	52.3	Blood donors	512	IgG	NS	*****	Mansuy, 2011 (148)
Germany	2007-2012	15.5	Blood donors	116	IgG	3	******	Krumbholz, 2012 (149)
Germany	2006-2008	2.3	Healthy individuals and chronic liver disease patients	216	IgG	NS	****	Pischke, 2010 (150)
Germany	NS	17.3	Forestry workers	864	IgG	NS	*****	Dremsek, 2012 (151)
Germany	2011	16.8	Individuals from subset of Germany Health Examination Survey for adults	4420	IgG	NS	*******	Faber, 2012 (152)
Germany	NS	6.8	Blood donors	1019	IgG	NS	******	Juhl, 2012 (153)
Germany		0.03	Blood donors	12200	NS	NS	***	Hourfar, 2012 (154)
Greece	NS	9.43	Blood donors	265	IgG	NS	*****	Pittaras, 2014 (155)
Italy	NS	1.3	Blood donors	151	IgG	NS	****	Scotto, 2012 (156)
Netherlands	2006-2007	1.9	Healthy	7073	IgG	NS	*******	Verhoef, 2012

			individuals					(157)
Netherlands	2011-2012	26.7	Blood donors	45415	IgG	3	****	Slot, 2013 (158)
Portugal	1993	33.3	Pregnant women and newborns	24	IgG	NS	***	Mesquita, 2013 (159)
Spain	2004	5.4	Pregnant women	1517	IgG	NS	*******	Buti, 2010 (160)
Spain	2007-2009	3.8	Pregnant women	769	IgG	NS	****	Gabilondo Alvarez, 2010 (161)
Spain	2008	2.2	Residents of Madrid	2305	NS	NS	******	Fogeda, 2012 (162)
Spain	2007-2010	3.7	Pregnant women	1040	IgG	NS	*****	Lindemann, 2010 (163)
Spain	NS	4.0	Blood donors	99	IgG	NS	*****	Galiana, 2010 (164)
Spain	2007-2011	3.5	Asymptomatic pregnant women	1364	IgG	NS	****	Sanchez Diaz, 2012 (165)
Switzerland	2009	4.9	Blood donors	550	IgG	NS	*****	Kaufmann, 2011 (166)
Turkey	NS	12.4	School children	185	NS	NS	*****	Cevahir, 2013 (167)
Turkey	2003-2005	2.1	School children	515	NS	NS	****	Maral, 2010 (168)
Turkey	2005	2.4	Individuals living in Edirne city centre	582	IgG	NS	****	Eker, 2009 (169)
United Kingdom	NS	8.1	Blood donors with a history of jaundice	333	IgG	NS	*****	Beale, 2011 (170)
United	2004-2008	4.7	Blood donors	1559	IgG	3	*****	Cleland, 2013

Kingdom								(171)
United Kingdom	1999-2011	8.0	Healthy individuals	2147	NS	NS	****	Madden, 2012 (172)

Abbreviations: NS: Not specified

Appendix 28: Table of characteristics on incidence of sporadic Hepatitis E Virus cases in Europe

Country	Study period	Incidence (%)	Sample demographics	Sample size	Diagnostic methods	Genotype	Quality Assessment score	Reference
Denmark	2010-2012	33.9	Patients with clinically suspected hepatitis	823	RNA	1, 3, 4	*****	Midgley, 2014 (173)
France	2011	3.1	Viremic infections	280	RNA	4	****	Jeblaoui, 2013 (174)
France	2008	51.4	Adult hospital patients	35	NS	3	****	Colson, 2011 (175)
Germany	NS	0.03	Blood donors	94	NS	NS	***	Hourfar, 2013 (176)
Finland	2000-2008	11.3	Patients with acute hepatitis	105	RNA	1	***	Kantala, 2009 (177)
Hungary	2001-2006	9.6	Acute hepatitis patients	1203	IgM	3	*****	Renou, 2009 (178)
Italy	2009	9.1	Acute hepatitis cases	430	IgM	1	****	Cacciola, 2011 (179)
Italy	2004-2010	48.1	Hospitalized patients	52	IgM	1, 3	****	Candido, 2012 (180)
Italy	2010-2011	0.2	Asymptomatic individuals	450	IgM	1	****	Scotto, 2014 (181)
Montenegro	2000-2008	6.0	Patients with acute viral hepatitis	400	IgM	NS	*****	Terzic, 2009 (182)
Netherlands	2007-2008	11.5	Patients with infectious hepatitis	139	NS	NS	***	Andriesse, 2010 (183)
Spain	2004-2011	7.0	Patients with acute hepatitis	277	RNA	3	****	Echevarria, 2013 (184)
United	1999-2011	1.9	Jaundice patients	2147	NS	NS	**	Hunter, 2012

Kingdom	(185)

Abbreviations: NS: Not specified

Appendix 29: Table of characteristics on Hepatitis E Virus outbreaks in Europe

Country	Study period	Sample demographics	Incidence	Attack rate	Diagnostic methods	Incidence by age	Attack rate by gender	Genotype	Quality Assessment score	Source
France	NS	Control of HEV cases in HEV outbreak	16.7	NS	IgM	NS	NS	3	*****	Colson, 2010 (186)
Uzbekistan	1976	Hospitalized patients with acute hepatitis	NS	1072	IgM	NS	NS	NS	********	Sharapov, 2009 (187)
Uzbekistan	1987	Hospitalized patients with acute hepatitis	NS	1431	IgM	<3 years: 68.7% 3-6 years: 56.7% 7-10 years: 57.1% 20-29 years: 85.2% 30-39 years: 82.5%			********	Sharapov, 2009 (187)

Abbreviations: NS: Not specified

Appendix 30: Table of characteristics on case fatality rate associated with Hepatitis E Virus in Europe

Country	Sample demographics	CFR in pregnant women (%)	Stillbirth (%)	Overall CFR (%)	Genotype	Reference
France	HEV cases	NS	NS	9.1	3, 4	Colson, 2012 (188)
France	Adult hospital patients	NS	NS	5.6	1	Colson, 2011 (175)
France	Hospital patients	NS	NS	4.5	3	Colson, 2012 (189)
France	Fulminant hepatitis E patients	NS	NS	66.7	3	Colson, 2011 (190)

Abbreviations: NS: Not specified

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