EPIDEMIOLOGY, IMMUNOLOGY AND GENETICS OF VIRAL HEPATITIS IN ADEN CITY, YEMEN

By

AMIN AHMED BAWAZIR

Thesis submitted in accordance with the requirement of the University of Liverpool for the degree of Doctor in Philosophy

Liverpool School of Tropical Medicine

July 2009

DEDICATION

To my parents who are always supporting me...

My wife

My sons Ala, Ahmed, Mohammed

My daughter Abeer

And to my brothers and sisters.

ACKNOWLEDGEMENT

I am very grateful to many people for assisting with the preparation, field work, writing and completion of this work.

I would like to acknowledge with deep gratitude, the excellent guidance and meticulous supervision rendered by Dr. Luis Cuevas who devoted many hours from his valuable academic time to help me study, analyse and organise every detail of this study. I am very grateful to the late Professor Charles Anthony Hart, for his professional support and valuable assistance. I am also very grateful to Dr. Christopher Parry whose generosity and timely advice motivated me at the different stages of this work to its completion. Dr. Cuevas, Prof Hart and Dr. Parry generously invested their talent and high standard of excellence in their supervision, which hopefully is reflected in this work.

Many thanks to Mr. Harper who supervised my laboratory work and Ms. Broughton and Mark for the PCR analysis.

My sincere and special gratitude to Drs. John and Muriel Berkeley for their generous support and help in revising my editing of the text and I appreciate their valuable suggestions and comments.

My heartiest thanks to my postgraduate colleagues at the LSTM, PhD room, especially Iman, A.Wahab, Gertrude, Nabil, Faiza, Samira, Asma, Gibby, Victoria, Rukhsana, Daniel and Ahmed Makki who extended all co-operation, valuable advice, friendship and guidance during the conduct of this study. I am also thankful to my friend Adel Aulaqi for his constant support and encouragement to accomplish this work.

In Yemen, I am grateful to Professor Abdulla Hattab for his constant encouragement and support and Dr. Talal Sallam for his helpful advices on laboratory requirements.

I would like to express my sincere gratitude to Ramiz Madhi, Firas Abubaker and Lola Murshed who helped me in the difficult time of field work and enrolment of the participants and preparation of the samples. I should also remember the group of third year students at the Aden Medical College who assisted with the household survey.

My heartfelt thanks and gratitude goes to all members of my family, who have been with me for every step of my life and (have always been) beside me with their unlimited care, prayers, support and love, which has brought me this far.

ABSTRACT

Background: Viral hepatitis is a significant public health problem with millions of humans infected worldwide. There are very few studies of hepatitis in Aden, Yemen.

Aims: The study aims to determine the prevalence of viral hepatitis (A, B, C and E), their co-infection with Epstein - Barr virus (EBV), cytomegalovirus (CMV) and human herpes virus (HHV6) and the risk factors for HBV infection among individuals attending primary health care facilities. Risk factors for HBV and HCV infection were also identified in patients with chronic liver disease (CLD), multi-transfusions and those undergoing haemodialysis (HD). The genotypes of HBV are studied. The HBV vaccination coverage in children < 5 years in Aden is also described.

Methodology: A cross-sectional study of individuals attending primary health care facilities in Aden was conducted to identify the prevalence of the viruses. Participants were recruited stratified by age. A case-control study of hospital patients with CLD, polytransfusion and HD was used to identify risk factors for HBV and HCV. Both cases and healthy participants were interviewed and blood samples were analysed using ELISA assays. A community-based survey of children < 5 years was used to identify vaccination coverage and to interview parents. PCR sequencing method was used for HBV genotyping.

Results: The overall seroprevalence of exposure to HAV (anti-HAV antibodies), HBV (anti-HBc antibodies), HEV (anti-HEV antibodies) and HCV (anti-HCV antibodies) were 86.6%, 16.2%, 10.7% and 0.4%, respectively. HBV and HCV had low prevalence in children and no HBV carriage. Perinatal transmission does not seem to be a major route of transmission for HBV. Acupuncture and cupping are risk factors for chronic liver diseases in this setting. The duration of the haemodialysis and a history of malaria were associated with increased rates of HBV and HCV infections among polytransfused/HD patients. This is the first report of the prevalence of EBV, CMV and HHV6 in Yemen. The three viruses had high seroprevalences and co-infections with another herpes virus or hepatitis viruses were common. The Expanded Programme of Immunisations in Aden has achieved HBV vaccination coverage of 63% in children < 5 years old which was lower than its target (85%), but the highest reported in the country. Lack of parental education and access to health care facilities were associated with lack of vaccination. The predominant genotype of hepatitis B was genotype D.

Conclusions: Viral hepatitis is a major public health problem in this community. Viruses causing hepatitis varied from hyperendemic (HAV) to low prevalence (HCV); and prevalence varies with age. None of the children < 15 years were HBV carriers or had HCV infection. The detection rates in the study would classify Aden as a low HBV endemic zone. This is the first description of HEV in Yemen revealing it to be a significant problem. Polytransfusion and HD are important risk factors for contracting HBV and HCV. The information yielded in this study on the prevalence and risk factors for HBV and HCV infection on patients with CLD would improve our understanding on the role of these viruses and the application of preventive and control measures in this population.

TABLE OF CONTENTS

LIST OF TABLES

No table of figures entries found.

LIST OF FIGURES

List of abbreviations

ACC	Aden Cancer Centre
AHSR	Annual Health Statistical Report
AIDS	Acquired immune deficiency syndrome
ALT	Alanine transaminase
Anti-HAV	Antibody to hepatitis A virus
Anti-HBc	Antibody to hepatitis B core antigen
Anti-HBe	Antibody to hepatitis B e antigen
Anti-HBs	Antibody to hepatitis B surface antigen
Anti-HCV	Antibody to hepatitis C virus
Anti-HEV	Antibody to hepatitis E virus
AOR	Adjusted odds ratio
AST	Aspartate transaminase
Au	Australia antigen
CDC	Centers for Disease Control and Prevention
cDNA	Complementary DNA
CI	Confidence interval
CLD	Chronic liver disease
CMV	Cytomegalovirus
CNS	Central nervous system
CP	Core promoter
DNA	Deoxyribonucleic acid
EBNA	Epstein-Barr virus nuclear antigen
EBV	Epstein-Barr virus
EIA	Enzyme immunoassay
ELISA	Enzyme-linked immunosorbent assay
EPI	Expanded programme for immunisation
GAVI	Global Alliance for Vaccines and Immunisation
HAV	Hepatitis A virus
HBeAg	Hepatitis B epsilon antigen
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HD	Haemodialysis
HDV	Hepatitis D virus
HEV	Hepatitis E virus
HGV	Hepatitis G virus (GBV-C virus)
HHV6	Human herpes virus 6
HIV	Human immunodeficiency virus
IB	Immune blot
ICD-10	International Classification of disease –edition 10
IDU	Intravenous drug use
IFA	Immunofluorescence assay
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IM	Infectious mononucleosis
IQR	Inter quartile range
IV	Intravenous

LATH	Liverpool Associates in Tropical Health
MOPHP	Ministry of Public Health and Population
MOPIC	Ministry of Planning and International Co-operation
NAAT	Nucleic acid amplification tests
NANB	Non-A non-B hepatitis
NCR	Non-coding region
NOS	Not otherwise specified
NS	Non significant
OD	Optical Density
OHB	Occult hepatitis B
OR	Odds ratio
ORFs	Open reading frames
PC	Pre core
PCR	Polymerase chain reaction
PHC	Primary health care
RNA	Ribonucleic acid
rpm	Revolutions per minute
RT-PCR	Reverse transcription-polymerase chain reaction
SD	Standard deviation
SPSS	Statistical Package for the Social Sciences
TAT	Transfusion-associated transmission
UAE	United Arab Emirates
UK	United Kingdom
UNICEF	United Nations Children's Fund
USA	United States of America
VCA	Virus capsid antigen
WHO	World Health Organization
YR	Yemeni Riyal

CHAPTER 1: INTRODUCTION

Hepatitis, or the inflammation of the liver, can be caused by biological and non biological agents including viruses, bacteria, drugs, toxins, excessive alcohol intake and autoimmunity (WHO 2002b; Horn & Learned 2006). Despite ample achievements to control viral hepatitis and a considerable pool of information and prevention tools, hepatitis is still considered as a significant problem all over the world (Lavanchy 2002), with millions of humans infected worldwide (Zuckerman 1999; Simmonds 2001; Lavanchy 2002; Alter 2006; van der Sande et al., 2006).

Several viruses have been shown to cause hepatitis, which vary in their mode of acquisition, clinical course and outcome, ranging from asymptomatic infection to fulminant hepatitis, hepatic cirrhosis, hepatocellular carcinoma and premature death (Beutels et al., 1997; Lau 2009). Although, most of these infections have common features, the diagnosis can only be made reliably by laboratory methods identifying the presence of specific viral antigens or antibodies (Mahoney 1999; WHO 2004d).

Five hepatotropic human hepatitis viruses are recognized to date. These include hepatitis A (HAV), hepatitis B (HBV), hepatitis C (HCV), hepatitis D (HDV) and hepatitis E viruses (HEV) (Tameda et al., 1996; Beutels et al., 1997; Lemon 1997; Aguilera Guirao et al., 2006a; CDC 2006). Hepatitis G virus (HGV), was previously designated hepatitis G virus but is now called GBV-C. Although GBV-C was initially thought to be associated with chronic hepatitis, extensive investigation failed to identify any association between this virus and any clinical illness (Alter et al., 1997) and it does not cause hepatitis and was not considered further to be pathogenic in humans (Laskus et al., 1997; Moenkemeyer et al., 2008). However, GBV-C infection is common in humans and may persist for decades, although most infected persons clear the virus and subsequently develop antibodies to the envelope glycoprotein (George et al., 2006). In contrast to HCV GBV-C does not replicate in hepatocytes but is a lymphotropic virus

(Xiang et al., 2000). Because GBV-C is blood borne and sexually transmitted, prevalence of GBV-C infection is high in individuals infected with HIV-1 (Zuckerman 1996; Lefrere et al., 1999; Wiwanitkit 2005). The epidemiology and route of transmission of these viruses will be reviewed later in chapter three, as they are relevant to tailor control measures.

The prevalence of viral hepatitis is different across countries worldwide depending on factors such as the viral characteristics; the prevalence of high risk groups; the route of transmission; the suitability of the environment and the efficacy of preventive and control measures (Arora et al., 1996; Lin & Kirchner 2004; van der Sande et al., 2006; Zuckerman 2006). For HBV, the World Health Organization (WHO) classifies its prevalence into three categories as countries of high, intermediate and low prevalence, defined as prevalences of $\geq$ 8%, 2%-7% and $\leq$2%, respectively (Mahoney 1999; WHO 2002b). Most industrialized countries have low prevalences (WHO 2002b; Raptopoulou et al., 2009; Romano et al., 2009). Countries of the Middle East fall into all the three groups of low, intermediate and high prevalence, varying from 2% to 20% (Mahoney 1999; Qirbi & Hall 2001; WHO 2002b). Most studies conducted in Yemen to establish the prevalence of HBV were undertaken in tertiary health care settings in Sana'a and included patients with acute or chronic hepatitis admitted to hospital (Abdel Raheem et al., 1991; Gunaid et al., 1997; Al-Moslih & Al-Huraibi 2001; Al-Nassiri & Raja'a 2001; Haidar 2002) or blood donors (Sallam et al., 2003a; Sallam et al., 2003b). As these populations are known to be highly selected in other settings, such studies may not reflect the real prevalence in the community and patients in these settings might have different risk factors for infection. Community surveys however have rarely been conducted in Yemen and are on a small scale (Scott et al., 1992; Al-Jarba & Al-Sayyari 2003; Sallam et al., 2003a; Yousef Khalidah 2003). Data on the prevalence of these infections in the community are described in chapter three, based on a primary health setting attendants of children and adults.

Numerous studies have described the importance of hepatitis genotypes on the clinical and therapeutic outcome of disease, and these genotypes vary across geographical regions (Blumberg 1977; Nousbaum 1998; WHO 2002b; Sugauchi et al., 2003; WHO 2003a; Lin & Kirchner 2004; Yuen et al., 2004; Hipgrave et al., 2006). Up to eight genomic groups are defined for HBV (designated with A-H; may be more) (Magnius & Norder 1995; Nousbaum 1998; Lunel et al., 2000; Arauz-Ruiz et al., 2002; Sugauchi et al., 2002; Fung & Lok 2004; Hagiwara et al., 2006). Genotype A and D are most common in Europe, while genotype B and C are most common in Asia (Lunel et al., 2000; Sugauchi et al., 2002; Fung et al., 2006). The last part of chapter 3 describes the genotypes of HBV in Aden.

Risk factors for hepatitis infection are well established worldwide. There is however a paucity of information regarding the prevalence of these factors in Yemen. There are no community-based studies in Aden to determine the risk factors for hepatitis A or E. A small scale study in pregnant women, health workers and blood donors for detecting HBsAg showed a low prevalence of carriers in Aden (Sallam et al., 2003b; Yousef Khalidah 2003). Factors associated with HBV or HCV infection vary by risk groups and include exposure to unscreened blood, blood products and organs, chronic haemodialysis, parenteral exposure to blood through contaminated or inadequately sterilized instruments and needles, traditional medicine, the use of unsterilized objects for rituals, intravenous drug use, health care workers and high-risk sexual practices (Mahoney 1999; WHO 1999a; WHO 2002b). In Yemen, the relative contribution of these factors has not been defined with population-based epidemiological studies. Chapter four therefore describes the risk factors for HBV infection in the community to inform health decision maker in the selection of preventive and control measures.

Globally, an estimated 2 billion people have been infected with HBV and more than 350 million have chronic HBV infection (WHO 2002b). Among persons with chronic infection, 15% will die prematurely of related chronic liver diseases, including cirrhosis

and hepatocellular carcinoma (HCC) (WHO 1999a; WHO 2003a; Zarski 2006). Epidemiological studies have described that chronic HBV infection is a common cause of cirrhosis and HCC, particularly in endemic countries (Yim & Lok 2006). Factors associated with increased risk of cirrhosis include older age, male sex, superinfection with HDV and specific genotypes (Fung & Lok 2004; Yim & Lok 2006). Chapter five in this thesis describes the role of HBV and HCV as risk factors among patients with liver cancer, chronic hepatitis and cirrhosis in Aden.

The risk of transfusion-associated transmission (TAT) of blood-borne infections including HBV and HCV has been greatly minimized as a consequence of the strict application of blood screening methods before transfusion. Industrialized countries achieved a dramatic progress in this concern particularly with the use of sensitive tests such as Nucleic Acid Amplification Testing (NAAT) that enhance the early detection of infection (Busch et al., 2003; Ameen et al., 2005; Busch et al., 2005; Montgemry S et al., 2006). However many blood transfusion services in resource limited countries still process only a limited numbers of specimens. The WHO encourages countries to use blood screening tests before blood donation. As a result of this screening, the risk of transfusion-transmitted viral infections has decreased dramatically, with a reduction in the prevalence of chronic hepatitis, cirrhosis and HCC (WHO 2003a). Chapter six describes the prevalence of HBV and HCV infection among polytransfused and haemodialysis patients in Aden and discusses the current screening practices in these high risk groups.

To understand the epidemiological features of viral hepatitis in the community, it is necessary to assess the coverage of the HBV vaccine (WHO 1999a; WHO 2001b). This vaccine has between 85% and 100% efficacy to prevent chronic hepatitis infection (Andre & Zuckerman 1994; Mahoney 1999; WHO 2001b; Fitzsimons et al., 2005; van der Sande et al., 2006) and is currently included in Yemen's Expanded Programme of Immunization (EPI). Chapter seven describes a community-based study among children < 5 years old in Aden Governorate to assess current HBV vaccination coverage.

Many studies describe Epstein-Barr Virus (EBV), Cytomegalovirus (CMV) and Human herpes virus 6 (HHV6) as having a high prevalence all over the world (Macsween & Crawford 2003; De Bolle et al., 2005a; Zerr et al., 2005; Takeuchi et al., 2006). However, there is no information in regard to the association between the three herpes viruses' infection and liver problems such as chronic hepatitis, cirrhosis and liver cancer. Moreover, few studies have shown that there is a relation of these viruses with the manifestation of viral hepatitis infection, as well as their way of transmission could be further than the usually known route of transmission to include blood and blood products (Okuno et al., 1989; Liu et al., 1990; Braun et al., 1997; Godshall & Kirchner 2000; Michalek & Horvath 2002).

EBV was reported as has been associated with the infectious mononucleosis (IM), nasopharyngeal carcinoma in China and proliferative diseases such as Hodgkin's diseases and Burkett's lymphoma in Africa (Spano et al., 2003; Pai et al., 2007). However, CMV was reported to be linked with occurrence of congenital infection and a major cause of hearing loss and mental retardation. Also re-activation is a major problem in immunocompromized patients like those with AIDS and post transplant cases (Staras et al., 2006). HHV6 infection is often asymptomatic and sometimes is associated by roseola infantum accompanied by febrile convulsions (Zerr et al., 2005). Mostly its seroconversion occurs by the age of 2 years and it's seroprevalence in the adult population is high (> 95%) (Hall et al., 1994). There is no information regarding the prevalence of EBV, CMV or HHV6 in Yemen, therefore, chapter eight will described the seroprevalence of these viruses in Aden and their past exposure with other hepatic viruses.

Finally chapter nine provides a general discussion of the findings reported on chapters' three to eight and discusses their implication for the health system in Yemen.

1. Objectives

This study therefore aims to:

1.a. determine the prevalence of exposure to viral hepatitis (A, B, C and E) by age among a population attending primary health care facilities in Aden Governorate.

1.b. describe the predominant HBV and HCV genotypes in the study populations.

2. identify the risk factors for HBV infection among this population.

3. to identify the seroprevalence of HBV and HCV markers among chronic liver disease patients.

4. estimate the prevalence of HBV and HCV exposure and infection among multi-transfused and haemodialysis patients in Aden.

5. determine the vaccination coverage rate of the hepatitis B vaccine among children under five years in Aden Governorate.

6. describe past exposures between viral hepatitis viruses and Epstein-Barr, cytomegalovirus and human herpes virus 6.

CHAPTER 2: METHODOLOGY

This chapter describes the methods used to investigate each study objective. Technical aspects that apply only to a specific objective are further expanded in subsequent chapters. Methods are described separately for each objective.

2.1 Objective 1.a: Prevalence of hepatitis viruses

To determine the prevalence of hepatitis A, B, C and E by age among a population attending primary health care facilities in Aden Governorate.

2.1.1 Study design and setting

This study was conducted in Aden city, the second largest city of Yemen. Aden is one of the main coastal cities of the country, located at the south-west of the Arabic Peninsula and the capital of the previous Democratic Republic of Yemen until unification in 1990 (fig. 2.1). The 2004 population census reported a population of 590,413 (MOPIC 2005). Administratively, the city is divided into eight districts. Each district has one to two public clinics called polyclinics which contain basic health care services and mother and child health care facilities for a total of 10 polyclinics. Of these one is currently being repaired and one is under construction.

A cross-sectional survey was conducted among the attendants to the eight functioning polyclinics within Aden's Governorate (Al-Tawahi, Al-Maala, Al-Maidan, Khormaksar, Dar-Saad, Al-Mansoora, Al-Shiek-Othman, and Little Aden polyclinic). Polyclinic users usually reside within the district. These facilities are used for medical consultation and for mother and child health care (primary health care facilities). Individuals attending for preventive other than hepatitis and curative purposes were considered eligible for recruitment, independently of age and sex. Recruitment took place through April to July 2005. A letter of introduction from the Ministry of Health Aden Office

was sent to all executive directors of the polyclinics with instruction to facilitate the conduct of this study.

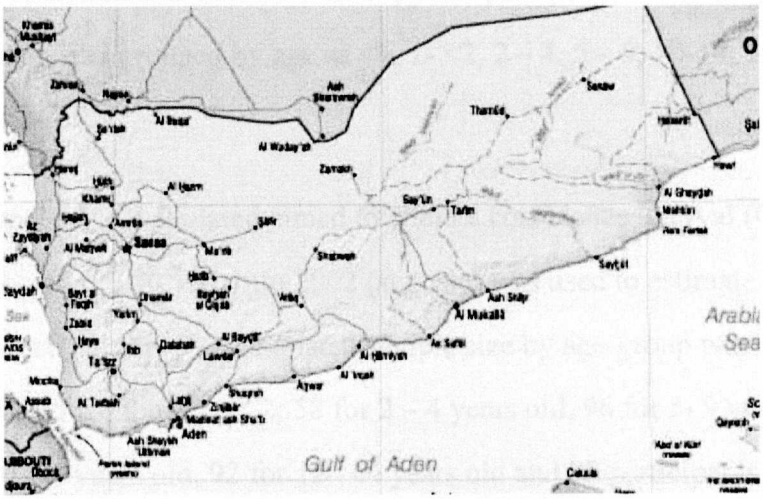


Figure 2.1 Map of the study site: Aden City, Yemen

2.1.2 Data collection (Interview)

Outpatient clinics (private rooms) were used to undertake the interview with the participants to ensure confidentiality and secure a convenient environment. Face-to-face interviews were used. The participants were briefed about the objectives of the study, their right to stop/withdraw from the interview and to refuse to answer question. Assurance was given that the data would be used only for the purpose of this study. Formal written consent was requested from adult participants or the parents/adult guardian of children. The questionnaires were completed by the investigator and his assistant. Close-structured questionnaire (Appendix A) was used to collect information on the general characteristics of the participants, past medical history, risk factors for hepatitis transmission, household characteristics and general health indicators. The average duration of the interview was 10 minutes.

A pre-test was applied on 5% of the sample size with a population and circumstances similar to the site of the study to ensure that the questionnaire was easy to use and acceptable to the interviewees. Each questionnaire was reviewed by the investigator.

2.1.3 Sample size

The sample size calculated to establish the seroprevalence of viral hepatitis among the study population was based on the expected prevalence for each age group. The study population was grouped by age as <1, 1- <2, 2 – 4, 5 – 9, 10-14, 15 – 44 and ≥ 45 years old.

The sample size calculated aimed to attain a confidence interval (CI) of 95% and a precision of ± 10%. Epi-Info 2002 (statcalc) was used to estimate the required sample size for each group. The estimated sample size by age-group was: 55 of <1 year old children, 55 for those 1- < 2; 58 for 2 – 4 years old, 96 for 5- 9 years old, 90 for those aged 10 -14 years old, 92 for 15– 44 years old and 92 participants that were ≥ 45 years and above (Table 2.1).

Table 2.1 Projected prevalence and sample size calculation by age groups for hepatitis viruses.

Age-group / years	HAV		HBV		HCV		HEV		Total
	P %	SS	P%	SS	P %	SS	P %	SS	
< 1	[a]12.2	41	[a] 17.3	55	[d]5.0	18	[a]4.8	18	55
1 - < 2	[a]12.2	41	[a] 17.3	55	[d]5.0	18	[a]4.8	18	55
2-4	[a] 18.6	49	[a] 21.1	58	[d]5.0	18	[a]3.7	14	58
5-9	[b] 43.9	96	[d]20.0	61	[d]4.0	15	[b] 1.6	8	96
10-14	[b] 68.0	84	[d]37.0	90	[e] 5.0	35	[g] 10.3	1	90
15-44	[c] 72.7	75	[d]40.0	92	[f] 14.4	61	[g] 18.9	92	92
≥ 45	[c] 72.7	72	[d]40.0	92	[f] 20.0	61	[g] 18.9	92	92
Total	-	458	-	503	-	226	-	243	538

a is Sidal et al., 2001; b is Colak et al.; c is Cesur et al., 2002; d is Mayama et al., 1998; e is Sallam et al 2003[a]; f is Nafeh et al., 2000; g is Mathur et al., 2001.

2.1.4 Sample strategy

The sample was proportionally distributed to the eight polyclinics according to the number of attendants reported in the annual statistical health report available at the local health office (AHSR/Aden 2003) as shown in appendix B. It was also distributed internally in each health facility proportionally to the age group strata. Participants were selected systematically with a sampling interval varying from 5-8 based on the daily

registered attendants in the previous year (47-82 daily) in each polyclinic. The average number of participants enrolled differed from 6-18 per day. Participants were selected systematically at the beginning of the day and enrolment continued on a daily basis until completing the required sample size.

2.1.5 Identification of the participants and confidentiality

Before the time of data collection and blood sampling the investigator allocated a unique code for the identification of each participant. This code was entered into a unique register (identification register), and consist of two letters for the type and site and four digits to number participants sequentially (0001 to 9999). For confidentiality this register was under the responsibility of the investigator (Appendix C).

2.1.6 Preparation of specimens

Laboratory technicians were recruited for blood collection. Talks were given to explain the importance of the study, motivation and instruction for blood collection. Laboratory technicians were responsible for the identification of specimens and transport to the laboratory.

Blood specimens (7ml for children and 10 for adults) were obtained by venipuncture, allowed to clot and centrifuged at 1500 rpm for 15 minutes in vacutainer tubes. Serum was divided into 3 to 4 aliquots of 300 µl, labelled, arranged in cardboxes and stored at -70 °C until analyzed.

2.1.7 Statistical analysis

Data were entered into computer database SPSS Version 14 for Windows (2006 SPSS Inc. Chicago. USA). Statistical analysis included quantitative descriptive analysis and summary statistics (means, median, percentages, standard deviations, inter quartile rates, etc) for describing the prevalence of HAV, HBV infection [HBV core antibodies (Anti-HBc)], HBV carriage [(HBV surface antigen (HBsAg)], HCV and HEV in the studied population.

Quantitative analysis of all the studied variables included 95% CI, Chi squares for trends and rates stratified by gender, location and other variables.

2.1.8 General Ethical considerations

Recruitment of the participants was preceded by an informed consent that included an essential explanation of the study objectives and a clear declaration on the confidentiality of the obtained data and that will not be used out of the research dimension (Appendix G). In the community –based survey for assessing the rate of vaccination coverage by HBV vaccine, a verbal consent was given. The researcher ascertained that the enrolled person/parent of the enrolled child understood the information and answered any questions or enquiry before starting the questionnaire and taking the blood sample.

2.1.9 Ethical consideration

Study protocol was attested from the higher ethical committee at the Liverpool School of Tropical Medicine, Liverpool University, UK and from the Local Ethical Committee in Yemen at the Ministry of Public Health and Population (MOPHP).

2.2 Objective 2: Risk factors for HBV infection

To identify the risk factors for HBV infection among this population.

2.2.1 Study design and setting

A case-control study was conducted to investigate the risk factors for infection with hepatitis viruses among the participants recruited for objective one. The prevalence of HBV reported in Aden before this study was 17.4% (Sallam et al., 2003b). This prevalence was estimated based on healthy blood donors and it was considered that this prevalence would be higher in the current study at around 18-25% as it would include some older participants and individuals with other illnesses (Al-Nassiri & Raja'a 2001; Sallam et al., 2003b). The cross sectional study included 538 subjects investigated for HBV. It was thus expected to identify 90 cases with evidence of HBV infection and the remaining 356 were categorized as controls, giving a case: control ratio of 1:4. This

sample size would allow investigating factors with Odds ratio (OR) above 2.2. Factors with ORs below 2.2 were deemed not to attain sufficient power and were considered a limitation of the study. A list of the risk factors investigated and their expected prevalence in cases and controls is shown in table 2.2.

2.2.2 Statistical analysis

The data was statistically analyzed using univariate analysis to describe associations between the characteristics of the participants and the presence of hepatitis viruses. Variables with p value < 0.2 were entered into multivariate regression analysis. Odds ratios and adjusted odd ratios (AOR) with 95 % CI were calculated. Variables with significant AOR were used to evaluate the risk factors of HBV on this study.

2.3 Objective 3: Risk factors for chronic liver disease

To estimate the prevalence of HBV and HCV among patients with chronic hepatitis, cirrhosis and liver cancer and to identify if infection with these viruses is a significant risk factor.

2.3.1 Study design and setting

A case-control study was conducted to identify the relationship between HBV and HCV and liver disease, including chronic hepatitis; liver cirrhosis; and hepatocellular carcinoma (HCC) among patients admitted to three hospitals of Aden Governorate (Al-Gamhouria teaching Hospital, Al-Wehdah Teaching Hospital and Aden General Hospital) or registered at Aden's Cancer Registry as having hepatocellular carcinoma.

2.3.2 Sample size calculation and sampling strategy

The expected prevalence of HBV/HCV among cases having HCC, cirrhosis or chronic viral hepatitis is likely to be within the range of 25%-65% (El Guneid et al., 1993; Okada et al., 1998). Thus the sample size was calculated with an expected prevalence of 24% HBV in cases and 7% for controls.

The sample was divided into the three main hospitals according to their admission rates. Enrolment of the case was based on confirmed diagnosis of these diseases by clinical, histopathology, ultrasound and /or biochemical and serological markers. The recruitment of the control group was community-based enrolment, and from the same catchment area of the cases and comprised adults with the same sex and age as cases. This required a minimum sample size of 49 cases and 147 controls with a proportion of 1:3 (Table 2.3).

2.3.3 Data collection

For each recruited individual, information concerning socio-demographical and risk factors were obtained with a structured questionnaire (Appendix D).

A method for blood sample, specimen preparation and storage was the same for objective one.

2.3.4 Data Analysis

Data were entered into computer database SPSS Version 14 for Windows (2006 SPSS Inc. Chicago. USA). Univariate analyses based on the "odds-ratio" (prevalence ratio was denominated OR) and 95% CI and P values <0.05. Analyses was based on chi-square tests, exact Fisher tests, followed by logistic regression analysis for categorical variables and evaluation of the relationship between different risk factors and prevalence of hepatitis B and/or C viruses in the study population.

Table 2.2 Risk factors for HBV infection and sample calculation

Risk Factor	Cases %	Controls %	OR	Sample size required 1:4	References	Country
Chronic liver disease	24.1	18.5	1.3	886:3544	(El Guneid et al., 1993)	Yemen
Dental therapy	17.8	11.7	1.6	369:1476	(Sagliocca et al., 1997)	Italia
Blood transfusion	15	8.1	2.0	214:856	(Al-Nassiri & Raja'a 2001)	Yemen
Hospitalization	17.5	10.4	1.9	207:828	(Sagliocca et al., 1997)	Italia
Surgical Procedure	12	3.1	4.4	81:324	(Sagliocca et al., 1997)	Italia
Liver diseases	26.9	12.7	2.48	83:332	(Scott et al., 1990; Gunaid et al., 1997)	Yemen
Health care workers	18	4.2	5.2	48:192	(Beltrami et al., 2000)	USA
Acute hepatitis	33.6	13.3	3.45	41:164	(Al-Moslih & Al-Huraibi 2001)	Yemen
Haemodialysis patient	38.9	1.5	32.7	13:52	(Cendoroglo Neto et al., 1995)	Brazil

2.4 Objective 4: Risk factors with polytransfusion/HD

To estimate the prevalence of HBV and HCV among polytransfused patients in Aden and to identify if infection with these viruses is a significant risk factor.

2.4.1 Study design and setting

A case-control study was conducted targeting patients admitted to Al-Gamhouria teaching Hospital, Al-Wehdah Teaching Hospital and Aden General Hospital or to the haemodialysis centre who presented with at least one of the following conditions: bleeding disorder such as haemophilia A; haemophilia B; Von Willebrand's disease; homozygous for thalassemia; homozygous for sickle cell disease; congenital methemoglobinemia; other bleeding disorder or had undergone haemodialysis for more than 3 time or had been transfused at least once with a total of at least ten units of allogeneic blood or other blood components (i.e. whole blood, plasma, red blood cells or platelets), before the date of inclusion in the study. The diagnosis was checked through hospital records for the recruited individuals before enrolment.

2.4.2 Sample size calculation and sampling strategy

A sample size was calculated, taking in consideration the highest expected proportion HBV/HCV infection among the patients, and based on previous reports of HBV/HBC infection among multi transfused and haemodialysis patients (table 2.4). The required sample size was 39 cases and 174 controls with in a proportion of 1:4. The recruitment of the control group was community-based, and from the same catchments area as the cases. The sample was divided proportionally into the three main hospitals according to their admission rates.

2.4.3 Data collection

For each recruited individual, information concerning sociodemographic and risk factors were obtained with a structured questionnaire (Appendix E). A method for blood sample, specimen preparation and storage was the same for objective one.

Table 2.3 Risk factors for hepatocellular cancer, chronic hepatitis and cirrhosis and sample calculation

Risk Factor	parameter	Cases %	Controls %	OR	Sample size1:1	Sample size1:2	Sample size1:3	References	Country
Hepatocellular cancer	Anti-HBc	65	43	2.46	88:88	66:132	59:177	(Okada et al., 1998)	Japan
Chronic hepatitis	HBV	24.1	7.4	4.2	81:81	57:114	49:147	(El Guneid et al., 1993; Al-Nassiri & Raja'a 2001)	Yemen
Chronic liver disease	HCV	30	9.0	4.33	64:64	45:90	39:117	(Chu et al., 2002b)	China (H.K)
Chronic liver disease	HCV	21.5	3.8	6.77	67:67	46:92	39:117	(El Guneid et al., 1993)	Yemen
Acute hepatitis	HCV	24.2	2.9	10.21	51:51	35:70	29:87	(Yang et al., 2002)	Taiwan
Hepatocellular cancer	HCV	75.8	42.9	4.17	40:40	30:60	27:81	(Hassan et al., 2001)	Egypt
Hepatocellular cancer	HCV	29.5	2.5	13.86	41:41	28:56	23:69	(Donato et al., 1999)	Italy
Hepatocellular cancer	HBsAg	63.2	5.2	32.34	12:12	9:18	7:21	(Zhang et al., 1998)	China
Hepatocellular cancer	HBsAg	63.2	4.4	40.86	11:11	8:16	7:21	(Ruiz et al., 1998)	Peru
Chronic liver disease	HCV	61.3	4.2	35.68	13:13	9:18	7:21	(Al-Moslih & Al-Huraibi 2001)	Yemen

2.4.4 Data Analysis

Data were entered into computer database SPSS Version 14 for Windows (2006 SPSS Inc. Chicago. USA). Univariate analyses based on the "Odds ratio" and 95% CI and P values <0.05. Analyses was based on chi-square tests, exact Fisher tests, followed by logistic regression analysis for categorical variables and evaluation of the relationship between different risk factors and transmission rate of hepatitis B and/or C viruses.

2.5 Objective 5: vaccination coverage of HBV vaccine in children <5

To determine the vaccination coverage rate of the hepatitis B vaccine among children under five in Aden Governorate.

2.5.1 Study design and setting

A household-based cross-sectional survey was conducted with the aim of assessing the coverage rate of the HB vaccine among children 6 months to five years old residing in Aden Governorate.

2.5.2 Sample size calculation and sampling strategy

Children < 5 years old were included in the survey. For the purpose of calculating the sample size, it was assumed that the vaccination coverage rate was a conservative 50%. A sample size of 96 children per age group (< 1, 1-2 and 2-5 years old) would allow establishing the vaccination prevalence with an error margin of +/-10% for each age strata. An overall sample size of 500 children < 5 years old would allow calculating the coverage rate with +/-5 % margin of error. In general, 150 (31%) children < 1 year old, 137 (28.5%) 1-2 years old and 193 (40%) 2-5 years old were enrolled, for a total of 480 participants.

Children were selected using stratified sampling for defines blocks of between 140-170 households. A map has 385 blocks and is used by the MOPHP for vaccination campaigns showed 385 blocks. From these, a total of sixty blocks were randomly selected, representing 16 % of the all.

Table 2.4 Risk factors on polytransfused and haemodialysis patients and sample size calculation

Risk Factor	parameter	Cases %	Controls %	OR	Sample size1:1	Sample size1:2	Sample size1:3	References	Country
Haemophiliacs	HCV	82.5	50.66	4.69	38:38	29: 58	26: 78	(Garson et al., 1990)	England
History blood transfusion	HCV	9.7	1.1	9.93	219:219	157: 314	135:405	(Chang et al., 2001)	Taiwan
HCV seropositive	HCV	75	3.2	97.0	8 : 8	12 : 6	5 : 15	(Abdulkarim et al., 1998)	Yemen
Haemodialysis	HCV	43.4	2.3	32.6	20 : 20	14:28	11:33	(Ayele et al., 2002)	Ethiopia
Blood donors	HCV	68	3.2	0.02	10 : 10	8:16	7:21	(Abdelaal et al., 1994; Huraib et al., 1995)	Yemen
Haemodialysis	HCV	40	2	10	25:25	17:34	15:45	(Haidar 2002)	Yemen
Haemodialysis	HCV	30.4	1.5	7	39:39	27:54	23:69	(Hayashi et al., 1991)	Japan
Blood donors	Anti-HBc	29.9	8.8	4.3	64:64	45:90	39:117	(Bernier et al., 1982)	USA
Renal dialysis	HCV	63.4	9	13	15 :15	11: 22	9: 27	(Chanpong et al., 2002)	Indonesia

A systematic sampling method was used to identify the houses. The first household was randomly chosen in each selected block and houses were identified with a sampling interval of 17-21 to obtain at least 7 respondents. To validate the response of the participants we used the vaccination card of each child to confirm if the vaccine had been given and the number of doses received. Verbal consent was obtained from one of the parents or the child guardian

2.5.3 Data collection

The questionnaire was tested during a pilot study to include 5% of the sample size and was modified accordingly. A team of 6 female medical students (3rd year and above) and nurses were trained to help in gathering the data. Only female interviewers were used as mothers have limitations to face male persons in the study setting. Interviews were undertaken in the subject's home to ensure confidentiality and ensure a convenient environment. Face-to-face interviews were used with one of the parents or guardians. After explaining the objectives of the study and informed consent, the questionnaires were completed by the investigator and/or his team members.

Close-structured questionnaires were used to collect information on the general characteristics of the child, past medical history, vaccination against HBV, risk factors for hepatitis transmission, household characteristics and general health indicators. The average duration of the interview was 15 minutes. Data entry and archiving of the questionnaires were the same as explained in objective one and two.

2.5.4 Data analysis

Data were entered into computer using SPSS Version 14 for Windows (2006 SPSS Inc. Chicago. USA). Statistical analysis including means, median, percentages, standard deviations, inter quartile rates were used to describe the characteristics of the population under study and the coverage rate of HB vaccine. Chi square for trends and rates stratified by gender, location and other variables were used with 95% CI.

2.6 Objective 6: Past exposures between hepatitis viruses and herpes viruses

To describe past exposures between hepatitis viruses and Epstein-Barr virus, Cytomegalovirus and Human herpes virus 6.

2.6.1 Study design and setting

This was a cross sectional study to describe past exposures between hepatitis A, B, C, E viruses and other blood-borne viruses such as Epstein-Barr virus (EBV), Cytomegalovirus (CMV) and Human herpes virus 6 (HHV6). All participants enrolled from the polyclinics and hospitals were tested for EBV and CMV. Only children <5 years were tested for HHV6.

2.6.2 Sample size calculation and sampling strategy

Sera of the 538 individuals enrolled in the polyclinics of Aden City as described in previous objective were included. All 182 children < 5 years old were tested for HHV6.

2.6.3 Data collection

Information concerning sociodemographic characteristics of the participants was available from questionnaires I (appendix A). Stored blood samples collected for objective one were used for the test of EBV and CMV, while 182 children < 5 years old were tested for HHV6. The laboratory methods are described in a separate section below.

2.6.4 Data analysis

Descriptive statistics were used to describe the characteristics of these three infections in the study population. A correlation analysis was used to estimate the proportion of co-infections with EBV, CMV, HHV6 and hepatitis viruses. P values of < 0.05 were considered statistically significant.

2.7 General laboratory methods

2.7.1 Management of blood samples and laboratory processing

Each blood sample was identified by a form designed by the investigator (Appendix H and I), which included the participant's and technical information, steps for collection, transport and storage of specimens.

Blood specimens were obtained by venipuncture and vacutainers were used for blood collection from participants by a trained laboratory technician. Gloves and aseptic techniques were used throughout. Needle-disposal boxes were used and disposed in the hospital incinerator.

Blood specimens were allowed to coagulate and then centrifuged at 1500 rpm for 15 minutes. Serum was divided into 3 to 4 aliquots of 300 µl, labelled, arranged in cardboxes and stored at - 70 °C until analyzed.

Sera were tested for HAV, HBcore, HCV and HEV antibodies and HBsAg, following the manufacturers' instructions. Reactive sera for HBcore, HCV antibodies and HBsAg were retested in duplicate. Five hundred and thirty eight samples were tested for HAV and HBcore; 259 for HCV and 356 for HEV. Random selection of the samples was used from the total 538 to eliminate bias in age or sex. All 87 samples positive for HBcore antibodies were tested for HBsAg.

Serum samples were analyzed by using ELISA. ETI-AB-HAVK PLUS (N0136) Anti-HAV Enzyme Immunoassay Kit to measure antibodies for HAV (DiaSorin S.p.A. 13040 Saluggia, Vercelli, Italy). For Anti-HBc, a Bio-Rad Kit was used (Monalisa anti-HBC PLUS, 3, Boulevard Raymond Poincare 93430 Marnes- La coquette- France). The HBsAg was detected with a Monalisa Ag HBS PLUS ELISA kit (Bio-Rad 3, Boulevard Raymond Poincare 93430 Marnes- La coquette- France). Bio-Rad ELISA kit was used (Monalisa Anti-HCV Plus Version 2. 3, Boulevard Raymond Poincare 93430 Marnes-

La coquette- France) to detect antibodies against HCV. An MP Diagnostic HEV ELISA kit (MP Biomedicals Asia Pacific Pte. Ltd. 85 Science Park Drive #04-01, The Cavendish Singapore) was used to detect IgG antibodies to HEV.

EBV antibodies were detected by an ELISA Anti-EBV VCA IgG (Biotest, Landsteinertr.5, D-63303 Dreieich, Germany). An ELISA kit coated with CMV antigen (Biotest, Landsteinertr.5, D-63303 Dreieich, Germany) was used for the qualitative identification of CMV-IgG. Samples were also tested for HHV-6 using the PANBIO Herpesvirus-6 IgG ELISA (Pan bio-diagnostics, 532 Seventeen Mile Rocks Rd, Sinnaman Park QLD 4073 Australia).

Optical Density (O.D.) measurements of the plates were made using a Bio-Rad Microplate reader (Model 550, USA) with the wavelength of light specified in the manufacturer's instructions for a qualitative measuring of samples and controls. O.D. data were transferred to a spreadsheet (Microsoft Excel) via a hyperlink terminal and calculation of the data was followed according to the manufacturer's instructions. Internal positive and negative controls were used to determine the presence or absence of significant levels of the marker under investigation. Instructions were followed for interpreting the results as specified on each kit's instruction manual.

Typically, in the assessment of Anti-HBc in the sample, this was determined by comparing the recorded absorbance with that of the calculated cut-off value for each sample. The cut-off value was measured by taking the average of three O.D. readings of the positive control serum divided by five; as per the manufacturer's instructions. Validation criteria were taken into consideration for negative (it should be < 0.100) and positive controls (≥ 1.000 and ≤ 2.400).

2.8 Genotyping

All ELISA positive sera for HBsAg from the different studies (15 samples) were tested for HBV genotyping in the Department of Medical Microbiology, University of Liverpool. Two methods were used as described below.

2.8.1 Multiplex PCR assay

This method uses a specific genotyping system that involves a multiplex PCR with type-specific primers (Naito et al., 2001). Positive HBV DNA samples were initially amplified by a first-round PCR based on the amplification of the nucleotides of the envelope ORFs (PreS1 to S). The product was then subjected to two second round of PCR reactions using combination of mix A primers to specify genotypes A, B and C while mix B primers for genotypes D, E and F were used to differentiate the HBV into the six genotypes (A-F) (Naito et al., 2001), as shown in table 2.5. These primer combinations for the second-round PCRs were designed on the basis of the differences in the sizes of the genotype-specific bands. The type specific primers were designed on the basis of the conserved nature of those sequences within a genotype and on the basis of their poor homology with the sequences driven form other HBV genotypes" (Naito et al., 2001). The strategy for HBV genotyping is illustrated in fig. 2.2.

The nucleic acid was extracted from 100 µl serum samples using the QIAamp MinElute Virus Spin Kit (QIAGEN Ltd. QIAGEN House, Fleming Way, Crawley, West Sussex, UK) for simultaneous purification of viral RNA and DNA. Nucleic acid was eluted into 60 µl AVE buffer (from the kit) and stored at -20°C overnight. HBV genomic DNA was amplified by nested PCR using the universal primers (P1 and S1-2) for the outer primers, followed by two different mixtures containing type-specific inner primers as described above. The first PCR was carried out in a tube containing a 20 µl reaction made up of Bioline BioMix Red (16 The Edge Business Centre Humber Road London, U.K) containing 50 ng of each outer primer and a 200 µM concentration of each of the four deoxynucleotides. The DyNAmo™ cDNA Synthesis Kit (F-470S) is intended for

cDNA synthesis for two-step quantitative reverse transcription-PCR (qRT-PCR) applications, where amplicons are usually around 100 bp in length.

Table 2.5 Primer sequences used for HBV genotyping by nested PCR (Naito et al., 2001)

Primer	Sequences [a] (position, specificity, and polarity)
First PCR	
P1[b] ...	5'-TCA CCA TAT TCT TGG GAA GA-3' (nt 2823-2845, universal, sense)
S1-2	5'-CGA ACC ACT GAA CAA ATG GC-3' (nt 685-704, universal, antisense)
Second PCR	
Mix A	
B2	5'-GGC TCM AGT TCM GGA ACA GT-3' (nt 67-86, types A to E specific, sense)
BA1R	5'-CTC GCG GAG ATT GAC ATG-3' (nt 113-134, type A specific, antisense)
BB1R	5'-CAG GTT GGT GAG TGA CTG GAG A-3' (nt 324-345, type B specific, antisense)
BC1R	5'-GGT CCT AGG AAT CCT GAT GTT G-3' (nt 165-186, type C specific, antisense)
Mix B	
BD1	5'-GCC AAC AAG GTA GGA GCT-3' (nt 2979-2996, type D specific, sense)
BE1 ...	5'-CAC CAG AAA TCC AGA TTG GGA CCA-3' (nt 2955-2978, type E specific, sense)
BF1 ...	5'-GYT ACG GTC CAG GGT TAC CA-3' (nt 3032-3051, type F specific, sense)
B2R ...	5'-GGA GGC GGA TYT GCT GGC AA-3' (nt 3078-3097, type D to F specific, antisense)

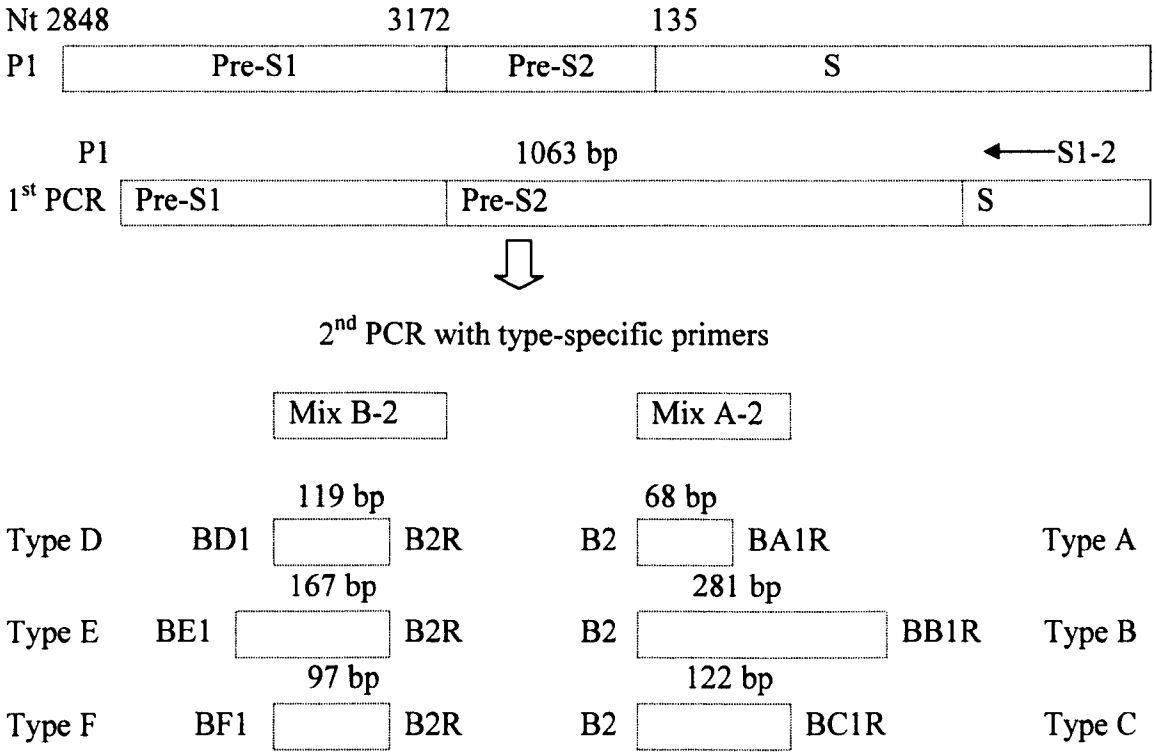
[a] An "M" represents a nucleotide that could be either an A or a C; a "Y" represents a nucleotide that could be either a C or a T. nt. Nucleotide.

[b] The sequence for primer P1 was determined by Lindh et al. (Lindh et al., 1999)

The thermocycler equipment used in this method, (GenAmp® PCR system 9700, Base Module, Applied Biosystems. U.S.A) was programmed to first incubate the samples for 10 min at 95 °C, followed by 40 cycles consisting of 94 °C for 20 s, 55°C for 20s, and 72 °C for 1 min. Two-second round PCRs were performed for each sample, with the common universal sense primer (B2) and mix A for type A through C and the common universal antisense primer (B2R) and mix B for type D through F. A 1-µl aliquot of the first PCR product was added to two tubes containing the second sets of each of the inner primer pairs, each of the deoxynucleotides, PCR buffer, as in the first reaction. These were amplified for 40 cycles with the following parameters: preheating at 95 °C for 10

min, 20 cycles of amplification at 94 °C for 20 s, 58°C for 20 s, and 72°C for 30 s and an additional 20 cycles of 94°C for 20s, 60°C for 20 s, and 72°C for 30 s. Genotypes of HBV for each sample were determined by identifying the genotype-specific DNA bands. The two different second-round PCR products from one sample were separately electrophoresed on 2% agarose gel, stained with ethidium bromide, and evaluated under ultraviolet light. The sizes of PCR products were estimated according to the migration pattern of a 100-bp DNA ladder (Pharmacia Biotech, Uppsala, Sweden). Mix A allows for the specific detection of PCR products for types A, B, and C and mix B allows for detection of types D, E, and F. The type-specific PCR products were recognized by their distinct sizes in gel electrophoresis.

Figure 2.2 Strategy for HBV genotyping using type-specific primers.
(Naito et al., 2001).



2.8.2 Sequencing method

Method two was used as the results of the first method were unexpected. This method was based on amplifying the entire HB surface antigen gene and then sequencing the product. Primers Z0404, HBV3, PolF, and M5877 were used with the Qiagen Hotstar Taq PCR kit to amplify a 900bp fragment covering the entire gene. Nucleotide sequences of both strands were determined using the BigDye® Terminator v1.1 seqencikit (Applied Biosystems, USA) on an ABI Prism® 3100-Avant Genetic Analyser (Applied Biosystems, USA). The webtool (www.hiv-grade.de/hbv_grade) was used to infer the HBV genotype from the resultant consensus sequences. The different steps of the applied method were conducted following the manufactures' instructions.

CHAPTER 3: PREVALENCE OF VIRAL HEPATITIS (A, B, C AND E)

3.1 Introduction

This chapter describes the epidemiological characteristics of hepatitis viruses (A, B, C and E) among a population seeking health care for them or their children and attending a primary health care setting in Aden Governorate.

The literature review focuses on the epidemiological dimensions and characteristics of each virus. The epidemiology of hepatitis viruses is not uniform, and reflects the route of transmission of the virus and the susceptibility of the population. For example HBV is highly prevalent among older generations and individuals with blood exposure, while HAV has a high prevalence from early age and infection is usually acquired through faeco-oral transmission.

The chapter also describes the predominant genotypes of patients with positive HBsAg and compares them to the genotypes reported from the region.

3.2 Objective

To determine the prevalence of viral hepatitis (A, B, C and E) by age among a population attending primary health care facilities in Aden Governorate.

To describe the predominant genotypes of patients with positive hepatitis B surface antigen.

3.3 Literature review

Since ancient times, there was awareness about hepatitis viruses (poison) among the ancient Chinese, Greeks and Romans. Hippocrates called it "Epidemic Jaundice" five centuries Before Christ (B.C.) (Mahoney 1999). According to Cockayne (1912), the first report on catarrhal jaundice as an epidemic form is that of Cleghorn, who described the prevalence of the disease occurs in Minorca from 1744 to 1749 (Cockayne 1912). It was McDonald who implicated a virus as an etiological agent of what we now call hepatitis A in 1908 and again in 1918 (Zuckerman 1969; Cuthbert 2001). Between 1930 and 1970 studies on human volunteers confirmed the transmission route, incubation period and clinical manifestations of HAV and HBV (Zuckerman 1969; Mahoney 1999). These viruses were named in the order of their discovery and differ widely in their mode of transmission, clinical features and epidemiological trends. All of these viruses can cause acute hepatitis while HBV, HDV and HCV can also cause chronic hepatitis. (Aguilera Guirao et al., 2006b).

Hepatitis viruses are not host-specific (Robertson 2001) as non-human primates are equally susceptible to produce antibody markers and cultured mammalian cells have been shown to be permissive for HAV replication (Robertson 2001).

3.3.1 Hepatitis A virus

HAV is considered one of the most common causes of viral hepatitis in the world. It varies clinically from asymptomatic to fulminant fatal disease and its presentation is influenced by age (Koff 1998). Symptoms are reported in only 4%–16% of children compared to 75%–95% of the adults reported by the routine surveillance systems (WHO 2000a; Ansaldi et al., 2008). Therefore, a large proportion of infections are undetected by clinical studies and seroprevalence surveys provide a more accurate description of the prevalence of HAV infection in a given population.

The availability of the electron microscope in the second half of the twentieth century allowed the visualization of HAV in stools (Martin & Lemon 2006) and several

diagnostic methods are nowadays available for the detection of infection in the research laboratory or clinical setting. These methods vary from simple humoral and cellular immune responses and serological kits to liver biopsy (Cuthbert 2001).

3.3.1.1 Epidemiology of HAV

Hepatitis A, one of the oldest diseases known to humankind, is mostly a self-limited disease which may results in fulminant hepatitis and death in a small proportion of patients. It is a significant cause of morbidity and socio-economic losses in many parts of the world (WHO 2000a). HAV outbreaks were frequently reported during the wars of the 19th and 20th centuries (Mahoney 1999) and was recognized as a separate entity from other types of hepatitis during World War II, with later studies providing convincing evidence of the prevalence and transmission of HAV (Aguilera Guirao et al., 2006a).

The transmission of the virus was recognized by the early 1900s and throughout the first quarter of the last century (Cuthbert 2001). The oro-faecal route is the most common frequent route of transmission via unsafe water, food or person to person contact (Cuthbert 2001; Martin & Lemon 2006). Between 80% and 95% of children <5 years old infected with the virus do not develop acute manifestations, while most adults (75%-90%) develop symptoms (Craig & Schaffner 2004). HAV infection does not result in chronic disease (WHO 2000a).

HAV is a stable virus at low PH and can resist heat up to 56 °C for 30 minutes. It is frequently found in urban sewage and transmission of the virus by blood products is rare (Cuthbert 2001; Glas et al., 2001; Martin & Lemon 2006). Infection by the virus is not very different from other water borne diseases and it thrives in environments where sanitation is poor and living conditions crowded, where infection occurs early in life (Fujiwara et al., 2002; Martin & Lemon 2006).

The endemicity of HAV in developing countries is mostly influenced by its transmission either by person-to-person contact or by faecal contamination of food and

water, resulting in common-source outbreaks. The prevalence of HAV is thus highest in areas with inadequate sanitation, where the seroprevalence can reach 100% before adolescence (Keystone & Hershey 2008). In contrast, in areas with good sewage and water supplies and sanitation, seroprevalence rates have fallen to ≤10% in adolescence although it can still be up to 70% in adults over the last 50 years (Zuckerman 2006).

The annual estimated incidence of HAV is 1–4 million new cases worldwide, although the true incidence, including asymptomatic and anicteric infections, is at least 10 times higher (Zuckerman 2006; Keystone & Hershey 2008). The epidemiology of the infection is best defined by measurement of anti-HAV antibodies. Based on seroprevalence data, hepatitis A is estimated to cause ten million infections occurs each year and 1.5 million clinically confirmed cases worldwide (Lavanchy 2002; Wasley et al., 2006). This prevalence varies over time and geography, with wide differences across countries and cities (Lavanchy 2002).

There is, nowadays, substantial evidence of the linkage between the reduction in HAV prevalence, mainly among children and young adults, and the improvement of living standards and sanitation. For example, in Japan the prevalence among adults over 50 years old shifted from 97% to 50% in 1973 and 2003, respectively (Kiyohara et al., 2007). Similarly, in Catalonia (Spain), a marked reduction was observed in the prevalence among children <15 years age from 13% in 1986 to 6% in 2001 (Dominguez et al., 2004). In Korea, there was an overall decline in the seroprevalence of HAV in individuals <20 years of age from 64% in 1979 to around 5% in 1996 (Sohn et al., 2000). In some Middle east countries such as Saudi Arabia, the HAV rates of infection by age ranged from 7% in children <8 years to 52% of those >16 in 2005, compared to 68% and 93% in 1989 for the same age groups (Al Rashed 1997; Fathalla et al., 2000; Almuneef et al., 2006). In Lebanon, HAV rates decreased from 40% in children aged 1-5 years and 98% in adults in 1982 to 11% and 78% in 2000 (Sacy et al., 2005). This shift in rates of HAV was attributed to countries with significant improvement of

socioeconomic status; increased urbanization with improvement in water resources, sewage disposal, decreasing illiteracy rates and the application of the HAV vaccine in some developed countries (Jacobsen & Koopman 2004). The improvement in sanitation prevented most children from infection and therefore, large sectors of the population have become susceptible to the infection. The implementation of the vaccine has since become more important to promote their health in certain areas.

The WHO International Classification of Diseases classifies hepatitis A into B15 (Acute hepatitis A), B15.0 (Hepatitis A with hepatic coma), B15.9 (Hepatitis A without hepatic coma) and Hepatitis A (acute, viral) Not Otherwise Specified (NOS) (WHO 2005).

Despite the availability of diagnostic tests, it is still difficult to conduct epidemiological studies. HAV is eliminated in the stools since the first week of infection. However it is difficult to diagnose the disease using stool cultures because of its poor growth and complexity of the techniques required (Jaffray & Flint 2003). During the acute phase of infection, immunoglobulin M (IgM) production starts 21-42 days after the ingestion of the inoculum (WHO 2000a; Cuthbert 2001). The production of Immunoglobulin G (IgG) antibodies develops very rapidly afterwards and these antibodies persist throughout life, conferring complete protection against infection (WHO 2000a; Martin & Lemon 2006). Virus and antibody can be detected by commercially available RIA, EIA or ELISA kitswith high sensitivity (100%) and specificity (99.5%) (WHO 2000a) which are also could detect either the IgM (acute) or IgM (past infection) or total (both in the same time). Viraemia, which can be transient but persist for an average of 2 to a maximum 4 months after clinical onset has limited use for clinical management and the complex technique for detection limits its use for surveys (Costa-Mattioli et al., 2002; Rezende et al., 2003). Polymerase chain reaction (PCR) methods have been used by different laboratories for the detection of HAV RNA but the variation in the sensitivity and specificity of the assays is well documented (Saldanha 1999). Recently, Real-Time PCR methods have been used with high sensitivity and specificity, providing

quantitative data (Hutin et al., 1999). However these are infrequently used and are only available in a limited number of research laboratories (Goswami et al., 1997).

In industrialised countries, injection drug users, institutionalized persons and their caretakers, and those who travel from low-to high-prevalence countries are considered among the high-risk groups (Cuthbert 2001; Webster et al., 2001; Craig & Schaffner 2004). Improvements in sanitation and hygiene have delayed infection, resulting in a shift of HAV prevalence from childhood to adulthood (Bonanni et al., 1998; Glas et al., 2001), thus adults being increasingly susceptible to HAV (Fujiwara et al., 2002).Additionally, the incidence of hepatitis A in industrialised countries has fallen dramatically since the introduction of the vaccine (Martin & Lemon 2006). The lowest seroprevalence is found in the Nordic countries (about 15%). In other parts of Europe and Australia, Japan and the United States, 40%-70% of the adult populations have demonstrable antibodies (WHO 2000b). Studies from Africa have shown that almost all children have antibodies to HAV by the age of 12, indicating high endemicity (Tufenkeji 2000).

The prevalence of infection in most Middle East countries ranges from intermediate such as in Qatar, the United Arab Emirates and Saudi Arabia (64%, 60% and 45% respectively) to high endemicity in Oman, Egypt and Sudan (Tufenkeji 2000).
No data are available from community-based studies in Yemen. A hospital-based study in 1988 showed a high rate of HAV antibodies (99.7%) in four northern governorates (Scott et al., 1990). Al Gunaid et al conducted a study in Sana'a hospitals among patient with acute and chronic liver diseases reporting that only 5 % had HAV IgM antibodies (Gunaid et al., 1997).

3.3.1.2 Genomic structure

The HAV is a small non-enveloped virus with a single stranded RNA (7.5 kb length) which contains a single open reading frame (ORF) encoding a polyprotein (Cuthbert

2001). The viral proteins are translated directly from the messenger-sense genomic RNA, which is delivered to the cytoplasm after uncoating of the viral particle (Martin & Lemon 2006). Recently a number of features that are unique to the hepatoviruses have been described by molecular cloning of the viral genome and have led to the recognition that HAV is a member of the Picornaviridae family (Nainan et al., 2006; Yun et al., 2008). The RNA genome of the virus was reverse-transcribed, a complementary DNA (cDNA) copy was molecularly cloned and, in 1987, RNA transcripts derived from a genome-length cDNA clone were shown to be infectious when transfected into cultured cells (Martin & Lemon 2006).

Genotypic differences are useful for the study of global hepatitis epidemiology. Their importance is emphasized by the association of specific genotypes with severity of the disease and poor responses to therapy (Nousbaum 1998). Sequencing of viral genomes has now become a major goal of descriptive virology and sequence data is now used to trace the routes of infection, to reconstruct the phylogenetic history of the viruses and to delimit genetic subtypes (Magnius & Norder 1995).

The HAV-RNA genome contains a 5' non-coding region (NCR) of 734 to 740 nucleotides (Martin & Lemon 2006), a coding region of 2,225 to 2,227 nucleotides, and a 3' non-coding region of 40 to 80 nucleotides (WHO 2000a; Cuthbert 2001). Three major proteins of the viral capsid (VP1, VP2, and VP3) on the capsid surface define the conformational immunodominant antigenic site of HAV (Probst et al., 1999; WHO 2000a). HAV is neutralized by both anti-HAV IgG and anti-HAV IgM which are used in laboratory settings for the detection of acute or past infections (WHO 2000a). Genotypes sequencing were based on VP1-2A region. Seven distinct genotypes have been identified to date (WHO 2000a; Nainan et al., 2006), four genotypes (I, II, III, and VII) are of human origin, and three (IV, V, VI) are of simian origin (Nainan et al., 2006)

3.3.2 Hepatitis B virus

Of the many viral causes of human hepatitis, few are of greater global importance than hepatitis B virus. Hepatitis B is a serious and common infection of the liver, affecting millions of people throughout the world (Michel et al., 2001; WHO 2002b; Sprengers et al., 2003; Lin & Kirchner 2004; Lok & McMahon 2004). It has been previously called type B hepatitis, serum hepatitis and homologous serum jaundice (WHO 2002b). Through a series of studies between the 1960s and 1970s, Krugman, Blumberg and others described the HBV, which was then called the Australia antigen (Au) with a long incubation period (Zuckerman 1969; Blumberg 1977; Mahoney 1999). The established association between the Au and the appearance of symptoms of hepatitis, its percutaneous transmission and the existence of homologous immunity after hepatitis A or hepatitis B infection were also described (Blumberg 1977; Mahoney 1999).

Subsequent studies revealed that the antigen had a variable distribution across populations and occurred more commonly among patients who received multiple transfusions and blood products (Prince 1968; Mahoney 1999). The distribution of the Au antigen in various population groups and patients whose diseases did not appear to be related to hepatitis were also described. The association of the Au antigen with acute hepatitis B was subsequently demonstrated and led to the development of specific tests for the identification of HBV infection (Blumberg 1977; Prince et al., 1982; Mahoney 1999).

Later, the use of the electronic microscope facilitated the demonstration of the Dane particle of the HBV, with its surface component designated hepatitis B surface antigen (HBsAg) and its core component containing endogenous DNA, hepatitis core antigen (HBcAg) (Mahoney 1999). Since then, the discovered of antibodies of both HBsAg and HBcAg have become diagnostic indicators to differentiate the chronic from the acute phase, stage of the disease and therefore developing strategies to prevent transmission (Mahoney 1999; Lin & Kirchner 2004). Other antigens/antibodies were also discovered

later such as HBe antigen (HBeAg), hepatitis B core antigen (HBcAg) which contributed in identifying the prognosis of the disease and responses to antivirus therapies (table 3.1).

Table 3.1 Markers of viral hepatitis B infection and abbreviations used.

Name	Abbreviation
Hepatitis B core antigen	HBcAg
Anti-HBcore antibody	Anti-HBc
Hepatitis Be antigen	HBeAg
Antibody to HBe antigen	Anti-HBe
Hepatitis B surface antigen	HBsAg
Antibody to HBs antigen	Anti-HBs

Carriage of HBV is defined by the presence of detectable HBsAg, whereas the presence of anti-HBc without HBsAg is taken to indicate past HBV infection. Overall, the prevalence of HBV infection is determined by the presence of HBsAg and/or anti-HBc in the population (WHO 2004a).

3.3.2.1 Epidemiology of HBV

The importance of hepatitis B infection is demonstrated by the severe pathological consequences of infection, such as chronic hepatic insufficiency, cirrhosis, hepatocellular carcinoma (HCC) and long term carriage (West & Calandra 1996; WHO 2002b; Lin & Kirchner 2004; Vogt et al., 2006). Approximately one third of the world's populations (nearly 2 billion persons) have serologic evidence of HBV infection (WHO 1999a; WHO 2003a; Zarski 2006). Of these, an estimated 350 million have chronic HBV infection and at least 500 000 chronically infected persons die each year from liver cancer and cirrhosis (Shiratori et al., 1995; Rivkina & Rybalov 2002; WHO 2002b). HBV ranks among the highest causes of mortality from infectious diseases worldwide despite the presence of an effective vaccine since 1982 (Hall 1993; Mast et al., 1999; Sprengers et al., 2003).

The strong association between HBV infection and HCC has been known for a long time in a distinct study conducted in male Taiwanese health workers where it illustrated that the rates of HCC approximately 100 times higher among positive HBsAg than

negative group (Beasley et al., 1981; Beasley 1988; Kane 2003). Other studies thereafter also have documented the close relation between the incidences of HCC and the role of age factor among old populations who are HBsAg carriers than among children who have more recent infections (Beasley 1988; Alter et al., 1989; el-Refaie et al., 1996; Branda & Wands 2006; Knisely et al., 2006; Thorgeirsson et al., 2006). Numerous studies have described the natural mode of transmission of HBV, its activity, relation to the body immune system, mutation, genotypes and subtypes, transmission routes as well as the role of vaccines in the control of the diseases and its consequences (Blumberg 1977; Mast et al., 1999; WHO 2002b; WHO 2003a; Lin & Kirchner 2004; Fitzsimons et al., 2005; Hipgrave et al., 2006; van der Sande et al., 2006; Zuckerman 2006). Transmission of the virus is mainly parenteral (percutaneous) or by contact with infected blood, body fluids, sexual intercourse, intravenous drug use and acupuncture (Gitlin 1997; Lai et al., 2003). As the virus does not cross the placenta, infected pregnant women with HBV can transmit the virus to their babies at birth (Mahoney 1999; WHO 2002b). Infection of children during the perinatal period from mothers who are HBsAg and HBeAg positive tend to give symptoms in only 1%. However, the likelihood of progression to chronic infection, cirrhosis and/or HCC in later age is as high as 90%. This risk is very high when compared to 5%–10% of immunocompetent adults infected during adulthood that progress to these chronic stages (Goldstein et al., 2005; Zuckerman 2006).

The prevalence of HBV varies worldwide depending on factors such as the prevalence of high risk groups; the route of transmission; the suitability of the environment and the efficacy of preventive and control measures (Arora et al., 1996; Lin & Kirchner 2004; van der Sande et al., 2006; Zuckerman 2006). Based on the existence of HBsAg in the population, WHO classifies the prevalence of HBV into three categories as countries of high, intermediate and low prevalence, defined as prevalence of $\geq$ 8%, 2%-7% and <2%, respectively (Mahoney 1999; Andre 2000; Qirbi & Hall 2001; WHO 2002b; Sallam et al., 2003a). Most industrialized countries have a low prevalence (Mahoney

1999; WHO 2002b) while around 90% of HBV carriers reside in the developing world, where access to health care services is limited (West & Calandra 1996; Reda et al., 2003).

In the Middle East region, the disease exists with different degrees of prevalence, from low to hyperendemicity varying from 2% to 20% (Mahoney 1999; Qirbi & Hall 2001; WHO 2002b). For example, countries of low endemic HBV include Bahrain, Iran, , and Kuwait (0.9%-1.25%, 1%, 1.5% respectively); countries with intermediate prevalence such as Cyprus, Iraq, Libya and United Arab Emirates (2%-2.5%, 4%-5%, 2%-6% and %2-6% respectively); countries with high endemicity include Egypt, Jordan, Oman, Palestine, Sudan, Saudi Arabia, Tunisia and the Republic of Yemen (3%-11%, 3%-10%, 2%-10%, 5%-6%, 16%-20%, 7%-17%, 6.5% and 12%-18.5% respectively) (Andre 2000; Qirbi & Hall 2001). These prevalence rates are also not constant in the same country. A recent study conducted in 14 sites of the Saudi show that the prevalence ranged from 2.8% in Hail City to 12.6% in Tabouk City (Al-Faleh 2003).

Most studies conducted in Yemen to establish the prevalence of HBV were undertaken in tertiary health care settings in Sana'a and included patients with acute or chronic hepatitis admitted to hospital (Abdel Raheem et al., 1991; Gunaid et al., 1997; Al-Moslih & Al-Huraibi 2001; Al-Nassiri & Raja'a 2001; Haidar 2002) or blood donors (Sallam & Tong 2002; Sallam et al., 2003b) as shown in table 3.2. As these populations are known to be highly selected in other settings, such studies may not reflect the real prevalence in the community and patients in these settings might have different risk factors for infection than the general population. Community surveys however have rarely been conducted and are on a small scale (Scott et al., 1992; Al-Jarba & Al-Sayyari 2003; Sallam et al., 2003b; Yousef Khalidah 2003). Sallam studied the prevalence of HBV in a group of residents from Socotra Island (450 Km south-East to Aden) and show around 60% were infected (Sallam et al., 2003b). The prevalence of infection among blood donors in Aden city was 17.4% (Sallam et al., 2003b). These

findings indicate that there are geographical and population differences in the prevalence of HBV

The prevention of chronic infection has become a high priority in the global community with an increased importance is given from international agencies to countries with high HBV prevalence including Yemen, encouraging them to apply preventive and control measures. These include among others, safe blood transfusions, appropriate use of injections, sexual health promotion, and immunization (WHO 2001b).

3.3.2.2 Genomic structure

Hepatitis B virus is a hepatotropic enveloped virus belonging to the family hepadnaviridae containing a double stranded, circular DNA genome with four open reading frames (Naito et al., 2001; Kar 2005). This DNA is enclosed in a nucleocapsid (core antigen) surrounded by a spherical envelope (surface antigen) (Lin & Kirchner 2004). The Dane particle referred to the entire virion. The genome encodes a DNA polymerase that acts as a reverse transcriptase (WHO 2002b; Lin & Kirchner 2004). The HBcAg and HBeAg components are translated from a common gene. HBcAg is targeted to the endoplasmic reticulum and is considered to be essential for viral packaging and is an integral part of the nucleocapsid as well as an indicator of past infection (Mahoney 1999). HBeAg (the precore fragment) is a soluble protein which is not essential for viral replication but can be detected in the serum of patients with high virus titers (WHO 2002b).

Table 3.2 Prevalence of hepatitis B virus in Yemen

Year	Region	Study population	Sample size	HBsAg alone %	Total/anti-HBc antibody %	Reference
1988	Sana'a, Hajja, Taiz and Hudeidah	Hospital, clinic and school volunteers	868	12.7	45.5	(Scott et al., 1990)
1997	Sana'a	Patients with acute liver disease, blood donors and pregnant women attending hospitals and clinics	126	26.9	--	(Gunaid et al., 1997)
1999	Sana'a	Children attending family planning centres	272	4.0	--	(Al-Shamahy 2000)
1999	Sana'a	Mothers attending family planning centres	272	13.2	--	(Al-Shamahy 2000)
1999	Sana' a	Blood donors	721	7.1	--	(Al-Nassiri & Raja'a 2001)
1999	Sana' a	Patients at Sana'a Central Health Laboratory	600	5.5	--	(Al-Nassiri & Raja'a 2001)
1999	Sana' a	Pregnant women from family planning centres	182	6.6	16.5	(Al-Shamahy et al., 2003)
2001	Sana'a	Cases from hospitals and clinics	143	33.6	--	(Al-Moslih & Al-Huraibi 2001)
2001	Sana'a	Controls from hospitals and clinics	20	13.3	--	(Al-Moslih & Al-Huraibi 2001)
2003	Sana'a	Community	99	19.6	60 .8	(Sallam et al., 2003b)
2003	Socotra	Community	97	20.2	85.9	(Sallam et al., 2003b)
2003	Sana' a	Blood donors at central and hospital laboratories	494	15	33.5	(Sallam et al., 2003a)
2003	Aden	Blood donors at central and hospital laboratories	493	6.7	17.4	(Sallam et al., 2003a)

Genotyping of HBV has been accomplished based on a partial sequence of the HBV genome such as the pre-S or S gene. Up to eight genomic groups are defined for HBV (designated A-H) (Magnius & Norder 1995; Nousbaum 1998; Lunel et al., 2000; Arauz-Ruiz et al., 2002; Sugauchi et al., 2002; Abe et al., 2004; Bartholomeusz & Schaefer 2004; Fung & Lok 2004; Kar 2005; Hagiwara et al., 2006; De Mitri & Bernardi 2008). Genotype A and D are most common in Europe, while genotype B and C are most common in Asia (Lunel et al., 2000; Sugauchi et al., 2002; Kar 2005; Fung et al., 2006).

The genotypes are classified to subtypes (Lai et al., 2003). Studies on Asian groups revealed that genotype B is divided into two subtypes: Ba (found among Asian countries except Japan) show recombination in genotype C in the pre core (PC)/core promoter (CP) region, while Bj (found almost exclusively in Japan) does not have recombination with other genotypes (Magnius & Norder 1995; Lindh et al., 1997; Sugauchi et al., 2002; Lai et al., 2003; Fung et al., 2006; Yim & Lok 2006). Recent studies indicate a clear association between the HBV genotype B and HBeAg seroconversion at an earlier age, less active liver disease, slower progression to cirrhosis and a higher rate of HBeAg response to interferon therapy compared with genotype C (Chan et al., 2003; Yuen et al., 2004; Janssen et al., 2005).

3.3.3 Hepatitis C virus

The Non-A non-B hepatitis (NANB), as it was named since 1970 (Choo et al., 1989; Hayashi 1992), was designated as HCV in 1988 and the first antibody tests to identify individuals infected with the virus become available in 1990 (Choo et al., 1989).

According to the WHO data (1999), hepatitis C is a major health problem and the global prevalence of chronic hepatitis C is estimated to average 3% ranging from 0.1–5% in different countries with approximately 170 million people worldwide are infected with HCV (WHO 1999a; Lauer & Walker 2001; Osoba 2002). However, based on the few studies performed in representative cohort samples, it is evident that the prevalence varies substantially among different countries (WHO 1999a; Bellentani et al., 2000).

Progression to chronic disease occurs in the majority of HCV-infected persons (Lauer & Walker 2001; Soza et al., 2004). Cirrhosis and HCC are considered the most frequent complications resulting in considerable morbidity and mortality and substantial social and economic burden (Lee et al., 2006). HCV is becoming the main indication for liver transplantation (Seaberg et al., 1998) as well as the main aetiological agent for post-transfusion non-A, non-B hepatitis mainly in industrialised countries (Laurent et al., 2001).

Diagnosis of HCV is based on immunoassays (EIA) which include immunoblot techniques and enzyme linked absorbent assays (ELISA) for the detection of antibodies or PCR techniques for the molecular detection of HCV RNA (Lauer & Walker 2001).

To date, numerous data have accumulated on the epidemiology and natural history of HCV infection, its genetic components and classification, mode of transmission and therapeutic regimens (Higuchi et al., 2002; Ramia & Eid-Fares 2006). However, many aspects still need to be further explored, particularly in tropical countries, where most of the data available is based on studies of blood donors, which are likely to bias the estimates of prevalence and mode of transmission (Tibbs 1997). Well-designed prevalence studies of the general population are needed in many regions of the world to obtain a more accurate estimate of infection and disease burden (WHO 1999a).

Transmission of HCV is mainly through blood transfusion and its products, organ transplantation and haemodialysis (HD) and it is considered as endemic in many of these units (Abdulkarim et al., 1998; WHO 1999a; Osoba 2002; WHO 2003b; Loftis et al., 2006). Other routes of transmission have also emerged as the primary mode of transmission in many countries, such as intravenous drug users (60%-90%) in various countries and inadequately sterilized medical and dental equipment (London & Evans 1996; Grebely et al., 2006).

3.3.3.1 Epidemiology of HCV

Worldwide statistics demonstrate significant geographical variations in the characteristics of the disease, showing different transmission patterns, prevalence and incidence (Tibbs 1997; WHO 2003b; Ramia & Eid-Fares 2006). Although, European countries have low prevalence of HCV infection in the general population, high prevalence was encountered among risky group such as IDUs. For example, the prevalence is 2.7% vs. 80% in Italy (Bellentani et al., 1999; Trepo & Pradat 1999; Ansaldi et al., 2005), 0.22% vs. 75% in Germany (Grumbach et al., 1998; Reimer et al., 2007) and 0.7 vs. 43.7- 67% in the UK (Lamden et al., 1998; Mohsen 2001; Judd et al., 2005). In the USA the reported community prevalence was 1.8% in 1999 and up to 66% in IDU (Garfein et al., 1996; Alter et al., 1999; Thorpe et al., 2002). In Puerto Rico, a report indicated that the prevalence of HCV reached 6.3% (Perez et al., 2005). In Africa, the prevalence of infection varies widely (Wansbrough-Jones et al., 1998). The highest prevalence was found among Egyptian blood donors, ranging from 6% to 38% (Nafeh et al., 2000; Stoszek et al., 2006a). In some African communities in Niger, Ethiopia and Gabon it reaches 0.5%, 1.4% and 6.6% of the population, respectively (Tsega et al., 1995; Laurent et al., 2001).

One-fifth of the estimated 170 million chronic carriers of HCV worldwide live in the Eastern Mediterranean region where the prevalence range between 1% and 12% of the population (WHO 1999a; Shobokshi 2003). In Saudi Arabia, a community-based and a blood donor study revealed a rate of 1.2%-1.7% and 0.4% respectively (El-Hazmi 2004). Egypt has a higher prevalence of antibodies to HCV when compared to other countries in the region that may exceed 20% in some population (Arthur et al., 1997; Tibbs 1997; Shepard et al., 2005). It is recorded as the largest known global iatrogenic epidemic after an extensive use of parenteral antischistosomia therapy in a mass treatment setting (Frank et al., 2000; Strickland et al., 2002). The strong homogeneity of HCV isolate subtypes among the population suggests this hypothesis of an epidemic

spread of HCV throughout the infected individuals (El Gohary et al., 1995; Mellor et al., 1995).

This condition probably was similar to some areas in Yemen with known schistosomal infestations and people were treated with parenteral antischistosomia therapy. On the contrary, Scott et al. (1992), did not reveal any relation between treatment with antischistosomia medication and infection with HCV in the Yemeni population from Sana'a, Taiz, Hajja and Hudeidah (Scott et al., 1992).

Simmonds (1993), on his analysis of the pattern of nucleotide sequence for HCV has suggested a geographical dispersion of hepatitis C virus variants (Simmonds et al., 1993a). However he and others have approved the existence of genotype 4 in most of the Middle East HCV infected individuals (Simmonds et al., 1993b; Dusheiko et al., 1994). Many studies from Egypt have described that genotype 4 is accounting for almost all HCV infections in the population (Dusheiko et al., 1994). Other studies have also indicated the predominance of genotype 4 in the Middle East countries as it was reported among infected individuals from Saudi Arabia, Kuwait, Iraq and Yemen (Bukh et al., 1993; Simmonds et al., 1993a; Al-Faleh et al., 1995; Mellor et al., 1995; Al-Ahdal & Kessie 1997; Shobokshi et al., 1999). Although, no study conducted in Yemen concerning the identification of HCV genotypes, however the study in the Western Province of Saudi Arabia which is close adjacent to the northern governorate of Yemen, have shown that genotype 4 was the dominant among the study population from this area (Al-Faleh et al., 1995). These findings probably support that also this genotype could be dominant in Yemen, in addition to the reports from neighbouring Gulf countries (Simmonds et al., 1993a; Shobokshi 2003).

Some studies in Yemen have also reported that the prevalence of HCV among hospital acute hepatitis patients is 7.7% (Al-Moslih & Al-Huraibi 2001) and blood donors have a prevalence ranging from 0.2% to 6.4% (Gunaid et al., 1997; Haidar 2002; Sallam et al., 2003b). There are no data on the prevalence of HCV in the community of Aden

Governorate except that in blood donors (Sallam et al., 2003b) and in hospital employees (Al-Jarba & Al-Sayyari 2003) (0.6% and 1.3%) respectively.

3.3.3.2 Genomic structure

HCV is a small enveloped RNA virus of negative stranded ribonucleic acid with 9401 base pairs (WHO 1999a). Taxonomically the virus is classified as a separate genus in the Flaviviridae family (between the Flaviviridae and Pestiviridae) (Choo et al., 1989). Genotyping of HCV is based on sequences of forward and reverses at the outer region 5' in both directions. Though, six major genotypes (1-6), numerous subtypes and around 100 different strains are described for HCV and their distribution varies across the world (WHO 1999a; Bortolotti et al., 2005; Franco et al., 2006; Germer et al., 2006; Moghaddam et al., 2006). Genotype 1b and 2 are prevalent in Southern Europe; 1a in the USA and Northern Europe; genotype 3 occurs most often in the Indian subcontinent and drug abusers in Europe and genotype 4 is mostly reported from Africa and the Middle East (WHO 1999a; Corbet et al., 2003; Sy & Jamal 2006). In Saudi Arabia genotype 4 is dominant with a minority of infections being due to genotypes 1 (Shobokshi et al., 2003b). In contrast to the Mediterranean and Middle East regions, Tunisia has reported subtypes 1a and 1b as dominant HCV genotype among the infected population (Djebbi et al., 2003).

To date, numerous data have been accumulated on the role of genotype as one of the important parameters used to determine the duration of antiviral therapy with the greatest chance of success (Ramia & Eid-Fares 2006). For example, some studies have shown that hepatitis C virus genotype 4 is frequently associated with cirrhosis and a poor response to interferon (Remy et al., 1998; Shobokshi 2003). Others also have considered that the genotyping of HCV isolates is a key factor in molecular studies aimed to establish the source of outbreaks in blood banks, haemodialysis units and other nosocomial settings (Hinrichsen et al., 2002; Othman et al., 2004). Although, some studies in the past shows no association between the HCV genotypes in chronic hepatitis C with mode of virus acquisition (Zein 2000), recently, other studies have

found a correlation between the rout of infection mainly among risky groups such as IDU and the genotypes (Remy et al., 1998; Dal Molin et al., 2002; Bortolotti et al., 2005). Even so, HCV genotypes in Yemen have not been studied yet.

3.3.4 Hepatitis E Virus

In 1980, HEV was recognized as an enterically transmitted virus (Balayan 1993; Harrison 1999; Aggarwal & Krawczynski 2000; WHO 2001a). It is now well recognised to be an enterically transmitted infection with a distinct clinical illness compared to other hepatitis viruses (Aggarwal & Krawczynski 2000; Lu et al., 2006). The first proof of the virus was obtained in 1990 from faecal material of infected patients by the use of an immune electronic microscope (Balayan 1993; Tsarev et al., 1994; Irshad 1999).

Since 1990, serological and nucleic acid tests have been developed for both epidemiologic and diagnostic purposes. The serological assays were designed for the detection of serum antibodies to HEV (IgA, IgM, and IgG) and the nucleic acid tests were used to detect serum, bile and/or faecal HEV RNA (Tokita et al., 2003; Takahashi et al., 2005). These assays made it easier to differentiate recent and/or ongoing from remote infections and allowed the clinician to make fewer diagnostic errors based on incomplete serological characterization (Mushahwar 2008).

The development of effective diagnostic tests, however, has been hampered due to the lack of a cell culture system capable of growing HEV (Li et al., 2000). Several recombinant antigen-based assays have been developed, and it is clear that, in HEV-endemic areas, 90–95% of the patients with acute hepatitis during outbreaks of hepatitis E develop anti-HEV antibodies (Favorov et al., 1996).

Earliest serologic tests utilized a variety of HEV antigen sources from different strains of HEV. These included synthetic peptides (Dawson et al., 1992; Favorov et al., 1996); or expressed proteins from both the non-structural and structural regions encoded by

ORF 2 and 3 of HEV (Dawson et al., 1992; Anderson et al., 1999); putative structural proteins expressed in insect cells (He et al., 1993; Emerson et al., 2006); and use of empty virus-like particles (Li et al., 2000). These earlier assays were used widely for studying the epidemiology of HEV infections in both endemic and non-endemic areas. The assays, however, varied in sensitivity despite an excellent specificity (Mast et al., 1998). Up to recently, little was known then of the autochthonous HEV infections in many of these countries (Schlauder et al., 1998) and of the existence of a zoonotic reservoir for the virus in both developed and developing countries (Meng et al., 1997). At present, many authors have been agreed that HEV antibody tests based on ORF2 of HEV have broad activity and yield data that are reproducible in many laboratories and definitely are superior to tests based on combinations of ORF2 and ORF3 antigens of HEV (Ghabrah et al., 1998; Engle et al., 2002; Meng et al., 2002). In 2004, Zhou et al., found had described the ELISA for putative neutralizing antibodies to HEV genotypes 1–4 as a very practical and useful assay with high specificity for neutralizing antibodies against HEV. It is also used for quantifying the humoral immune response for hepatitis E vaccine and to measure the durability of the immune response (Zhou et al., 2004). Although today, there are many types of EIA assays have been developed, variation in their sensitivity and specificity to detect past infections is still considered (Mushahwar 2008). These assays used a recombinant HEV antigen to capture antibodies, with no consideration to geographical differences in strain distribution (Ayoola et al., 2002). Thus, antigens were replaced with peptide-based (solid phase) antigens and the addition of a buffer system in ELISA assays increased their sensitivity (96%) and specificity (98%) (Mast et al., 1998). Currently, the diagnosis of HEV infection is obtained using ELISA by identifying antibody markers in serum, such as anti-HEV IgM, IgA and IgG by ELISA (Mast et al., 1998; Irshad 1999). HEV IgM develops 3 weeks after infection and is followed by IgG. The former declines rapidly during early convalescence while IgG persists for long periods of time (>14 years) and provides protection against subsequent infections (WHO 2001a; Zhang et al., 2002). In addition to the clinical and

biochemical parameters used in the diagnosis of HEV, confirmative tests can be undertaken after the incubation period (4-6 weeks), when the virus is present in blood, stool or bile or its antigen can be shown in the liver by using PCR or histopathology (not common) (Irshad 1999; WHO 2001a). In most instances, there is a very good co-relation between the positivity in RT-PCR and EIA. However, there are several limitations of EIA and the sensitivity of such assays in an endemic area for disease diagnosis remains undetermined, particularly during epidemics (Panda et al., 2007).

3.3.4.1 Epidemiology of HEV

Reports on the high morbidity and mortality associated with outbreaks of HEV infection, involving many people, mainly in areas of low socioeconomic status are becoming more frequent since the discovery of the virus (Harrison 1999; Zhang et al., 2002). Mostly the virus transmitted through faecal contamination of water or food supplies and poor sewage disposal and frequently implicated in major outbreaks (Tanaka et al., 2005; Bendall et al., 2008). Over 50 massive outbreaks of HEV have been reported and occurred in many parts of the world, mainly in developing countries such as Central and South-East Asia, North and West Africa, and in Mexico, where faecal contamination of drinking water is common (Skidmore et al., 1990; Balayan 1993; Castera & Pawlotsky 2001; Lewis et al., 2006; Myint & Gibbons 2008). Preventive measures similar to those described for HAV could be applied for HEV, including good personal hygiene, improved water supplies and disposal of sanitary waste.

The prevalence in developing countries ranges from 7.2% to 24.5% (Balayan et al., 1994; Arankalle et al., 2001a; Krawczynski et al., 2001; WHO 2004d; Chau et al., 2006). In some areas in developing countries, HEV causes more than half of the cases of acute viral hepatitis with a high mortality among infected pregnant women (Engle et al., 2002; WHO 2004d). It has been reported that the infection become severe among pregnant women particularly in the third trimester, leading to fulminant hepatic failure with a high mortality rate and foetal malformations, death of the foetus in uterus and

stillbirth (Irshad 1999; WHO 2001a; Takahashi et al., 2002b; WHO 2004d; Bendall et al., 2008; Mansuy et al., 2008). Although, HEV infection is a self-limiting disease with short lasting viremia, however, chronic HEV viremia and chronic hepatitis has been recently reported in organ transplant recipients or immunesuppressed patients (Haagsma et al., 2008; Kamar et al., 2008; Schildgen et al., 2008).

There is increase anti-HEV IgG titre through the acute phase and decrease during the convalescent phase and supposed to persist for a longer time. Some reports have shown that past infection with development of anti-HEV IgG is protective in humans and primates (Bryan et al., 1994; Tsarev et al., 1994), but the correlation between the durability of the antibodies and developed immunity is not yet fully elucidated (Myint et al., 2006b). Seroepidemiological studies of HEV suggest that previously infected individuals are protected during epidemics (WHO 2005), although, it is not clear, whether or not the young adults with symptomatic disease in hepatitis E epidemics have been exposed in childhood (Tsarev et al., 1994; WHO 2004d). Generally, limited data are available concerning the level of protection afforded by IgG anti-HEV which is shown in the differences of serological readings between children and adults (Harrison 1999). Some authors have documented that two-thirds of paediatric cases became seronegative by 9 months after the onset of disease, whereas 100% of young adults were still positive 20 months after onset (Goldsmith et al., 1992; Bryan et al., 1994). Previous study has found the persistent of positive HEV antibodies in adults after 14 years of being with history of infection (Khuroo et al., 1993); however, the possibility of repeated infection being the cause of persistent IgG cannot be excluded (Wu et al., 2008). In addition, there is some evidence that antibody to hepatitis E virus is not as long lasting as is antibody to hepatitis A virus (Emerson & Purcell 2003).
On the other hand, HEV infection in endemic areas is seen in adults in comparison to other water borne infections like polio and HAV infections, which are seen mostly in childhood. By 10 years of age more than 90% of the population in India has antibodies against HAV (Acharya et al., 2003) and poliovirus, whereas, only about 26% of

children (Balayan 1997) have antibody against HEV IgG. Therefore, the immune response for HEV appears to be different from that of HAV and poliovirus. The occurrence of disease in adults associated with low-level circulation of the virus in asymptomatic individuals indicates lack of long-term immunity. Therefore, the possibility of developing a long-term protective vaccine appears to be an ambitious challenge.

Although few studies in the past have evaluated the performance of existing assays for anti-HEV antibodies, a wide range of sensitivity and specificity has been reported for these assays (Favorov et al., 1996; Mast et al., 1998). It is probably that the concordance between these assays was not sufficient and that the anti-HEV antibody profiles reported may be variable, so it should be interpreted with caution (Mast et al., 1998; Labrique et al., 1999; Aggarwal & Krawczynski 2000). The recently used EIA methods have been developed to detect anti-HEV antibodies using recombinant proteins and only those generated to ORF2 with a longer half-life are considered reliable for studying seroepidemiology (Ghabrah et al., 1998; Waar et al., 2005). Also RT-PCR assays was shown to be sensitive and specific for the detection of HEV genotypes 1–4 in acute-phase sera, stool samples, as well as in contaminated water and sewage (Jothikumar et al., 2006).

Hepatitis E virus is also reported in non-endemic regions, in patients with no history of travel to endemic areas (Thomas et al., 1993; Krawczynski et al., 2000; McCrudden et al., 2000; Emerson & Purcell 2004; Sookoian 2006). Numerous genetically distinct strains of HEV were recently identified (Erker et al., 1999; Wang et al., 1999; Zanetti et al., 1999; Schlauder et al., 2000) has led to a hypothesis that an animal reservoir for HEV exists (Balayan 1993; Li et al., 2005; He 2006; Sookoian 2006; Dalton et al., 2007). Accordingly, reports on evidence of HEV infection in domestic and farm animals have been well documented and anti-HEV was detected in more than 10 species of non-human primates and animals are known to be susceptible to HEV infection

(Balayan 1997; Arankalle et al., 2001b). These includes pigs from different countries, such as Nepal, China, Thailand, Vietnam, the US, Canada, Japan, Korea, Spain, Taiwan and Australia (Clayson et al., 1995; Chandler et al., 1999; Hsieh et al., 1999; Kabrane-Lazizi et al., 1999; Meng et al., 1999; Pina et al., 2000; Meng et al., 2002; Clemente-Casares et al., 2003). In addition, anti-HEV has also been detected from rodents caught in the wild in the US (Kabrane-Lazizi et al., 1999; Aggarwal & Krawczynski 2000; Favorov et al., 2000; Schlauder & Mushahwar 2001; Chau et al., 2006; De Deus et al., 2006). In Vietnam, anti-HEV was detected in chickens, dogs and in rats (Tei et al., 2003). Positive anti-HEV was found in cows from Somali, Tajikistan and Turkmenistan and sheep and goats from Turkmenistan. Transmission via ingestion of undercooked sika deer or wild boar meat was also reported (Arankalle et al., 2001b; Tei et al., 2003; Takahashi et al., 2004).Naturally acquired anti-HEV have also been detected in rhesus monkeys (Palmer et al., 2005; van Cuyck et al., 2005). Reports of a HEV sequences in chickens were also identified from Spain, Canada and Australia (Li et al., 2006).

In western countries, Clemente-Casares (2003), had analyzed the excretion of HEV strains by the populations of Spain, France, Greece, Sweden, and the United States and has concluded that HEV strains are more widespread in the human population than previously thought in these countries (Clemente-Casares et al., 2003). Moreover, endemic HEV infections are likely present in Europe and the United States (Suzuki et al., 2002). In relation to genotypes identification, it was appear that genotypes 3 and 4 are relate more to zoonotic origin from a different animals species in different parts of the world, whereas the relative conservation of genotypes 1 and 2 is consistent with their primary circulation in humans and less frequent isolation from animals (Lu et al., 2006). However, recently Caron et al. (2006) were identified genotype 1 in a pig in Cambodia, representing the first and only animal isolate of this genotype (Caron et al., 2006).

In the Middle East and North Africa for example, two major endemic regions for hepatitis E whereas swine are not very common, epidemiological features of the

infection are compatible with an anthropogenic infection (Balayan 1997). Stoszek et al (2006), has found high seroprevalence of anti-HEV among healthy adults and pregnant women in rural areas of Egypt (Stoszek et al., 2006b). The study' authors hypothesized that both zoonotic and anthroponotic transmission of a virulent HEV is occurring extensively in these rural villages. Although, no previous study of HEV infection has been conducted in animals at Aden City, the habit of growing animals in the city (including domestic dogs or cats) is not common; however, this did not have to exclude those people moving frequently to the neighbour villages where domestic animals such as caws and sheeps were frequently raised.

Symptoms of HEV infection are mostly similar to those seen with HAV. However, there was some ambiguity in the aetiological confirmation of outbreaks reported before the advent of specific tests (Tsarev et al., 1999). The global distribution of HEV strains is of great importance, as these have been associated with infections varying in clinical picture from asymptomatic to severe disease (Arankalle et al., 2001a). The infection is commonly reported among young adults (15-40 years); although, children are infected, the frequency of these infections is lower in this age group (Kurbanov et al., 2003; Chau et al., 2006). In endemic countries, HEV seroprevalence studies have reported that only 5% of children < 10 years are infected, compared to 10%–40% of adults (Balayan 1997). In the Middle East, Jordan, Oman, Egypt and Saudi Arabia reported a high prevalence of infection, although waterborne outbreaks have not been reported, moreover, population-based studies suggest that the virus is highly endemic (WHO 2001a).

In Yemen, the only study of Gunaid et al., (1997) showed that 14% of the cases of acute viral hepatitis were due to HEV in a hospital-based study in Sana'a, suggesting a wide spread distribution of the agent over the country (Gunaid et al., 1997). No data are available on community-based studies in Yemen.

Despite the growing number of hepatitis E vaccine candidates have been considered recently; the only one advanced to evaluate efficacy is a vaccine using a recombinant

HEV (rHEV) capsid antigen (Wang & Zhuang 2004; Myint & Gibbons 2008). This vaccine (rHEV 56-KDa) has passed preclinical and clinical trials in primates and humans (Trautwein et al., 2009). Shrestha et al. (2007) has reported a phase 2 trial of rHEV vaccine administered to young male soldiers in Nepal, who had not been exposed to HEV before but were at risk of infection (Shrestha et al., 2007). The study found no difference in adverse events between vaccine and placebo groups, but demonstrated a vaccine efficacy of 95.5% after three vaccine doses (Shrestha et al., 2007). Recently other randomized-controlled phase II clinical trial of a recombinant hepatitis E vaccine was conducted in a rural area of southern China to evaluate the safety and immunogenicity of the HEV 239 vaccine. The study concluded that the course of three doses of the vaccine induced 100% seroconversion and it was safe and immunogenic for humans (Zhang et al., 2009). However, the duration of protection by the rHEV vaccine is also unclear; further studies are needed to validate appropriate indications for these vaccines and to determine its long-term effects (Tacke & Trautwein 2007).

Despite that the vast majority of HEV-related diseases immunocompetent people are benign; it is generally encouraging that rHE vaccine was effective in preventing hepatitis E disease in a high-risk population mainly those in endemic areas and those under compromised living conditions, for example refugee camps or serving the army (Bryan et al., 2002; Tacke & Trautwein 2007) in addition to traveller from developed countries to endemic regions.

3.3.4.2 Genomic structure

HEV is a small non-enveloped RNA virion, of positive –sense single –stranded polyadenylated molecule. It is of 7.5 kb length and 32-34nm diameter with 7500 Nucleotides. Its short 5' and 3' terminals at the non-coding regions are of 27 and 68 nucleotides respectively (Reyes 1993; Emerson & Purcell 2003; Kar et al., 2008) Despite the similarity in the transmission routes of HEV and HAV, there are some key differences. The HEV virion is bigger than HAV; the viral proteins of HAV consist of 4

capsid proteins while HEV contains a mix of structural (ORF1) and non-structural proteins (ORF2), and other proteins of unknown functions (Aggarwal & Krawczynski 2000; WHO 2001a; Naoumov 2007).

Currently, HEV has four major with a clear geographical distribution (Naoumov 2007). Genotype 1 has been isolated from tropical countries in Asia and Africa, genotype 2 was found in Mexico, Nigeria and Chad, whereas genotype 3 has a worldwide distribution, including Europe, Asia, North and South America. Genotype 4 is found exclusively in Asia. Some reports indicate that multiple genotypes of HEV can co-circulate in the same area (Enouf et al., 2006; Naoumov 2007); and that even distinct genotypes of HEV can exist in the same patient (Takahashi et al., 2002a)

Most strains of the identified four genotypes of HEV are belonging to a single serotype (Meng et al., 1997; Schlauder and Mushahwar, 2001; Wang et al., 2000). Many studies have reported a relative stability of HEV genome sequences; although the genome of strains isolated from geographically distinct locations are generally more diverse (Payne et al., 1999; Agunos et al., 2006; Peralta et al., 2009). The phylogenic analysis divided HEV genotype 1 into five subtypes, genotype 2 into 2 subtypes, whereas genotypes 3 and 4 were divided into 10 and 7 subtypes, respectively. It is proposed that 5, 2, 10 and 7 subtypes of HEV genotypes 1, 2, 3 and 4 be designated alphabetised subtypes. A total of 24 subtypes (1a, 1b, 1c, 1d, 1e, 2a, 2b, 3a, 3b, 3c, 3d, 3e, 3f, 3g, 3h, 3i, 3j, 4a, 4b, 4c, 4d, 4e, 4f and 4g) were given (Lu et al., 2006; Panda et al., 2007). Although single serotype is recognised, extensive genomic diversity has been observed among HEV isolates (Lu et al., 2006).

The zoonotic nature of HEV was further substantiated with the recent isolation of HEV from many swine samples and from chickens, plus the identification of patients who were likely to have contracted HEV due to consumption of wild boar or deer meat (He et al., 2006). This zoonotic property has provided HEV some unique features that differ

from other enterically transmitted diseases (Haqshenas et al., 2001; Tei et al., 2003; Tamada et al., 2004). Most swine isolates have been genotype 3 strains with the exception of the Taiwanese strain which belongs to a recently discovered genotype 4 (Hsieh et al., 1999). Intriguingly, the virus isolated from swine in nonendemic countries is usually closely related to the strain responsible for rare cases of HEV within that country (Emerson & Purcell 2003). In general, these observations are consistent with the hypothesis that swine serve as a reservoir for human disease, but direct transmission of virus from swine to human has not been documented yet. Serosurveys have suggested people who have contact with swine may have a somewhat higher seroprevalence of anti-HEV than the general population, but variables other than contact with swine have not been eliminated as the cause (Drobeniuc et al., 2001; Meng et al., 2002).

3.4 Results

3.4.1. Characteristics of the participants

3.4.1.1. Sociodemographic characteristics

A total of 538 participants attending primary health care facilities in Aden City, were recruited. The general characteristics of the participants are described in Table 3.3

The age of the participants ranged from one month to 79 years with a mean of 18.2 years. Two thirds of the population (364) were children <18 years old (67.7%) and of these, 234 (43.5%) were under 7 years old. Both genders were represented, with 52% being male. Of 130 children over 7 and under 18 years old, all were attending school, 114 were attending primary (88%) and 16 (12%) secondary school. Adult education ranged from illiterate (14%) to university education with 55% of participants having achieved at least primary education. Around 59% of the adult participants were employed.

The number of participants enrolled in each clinic reflected the proportion of the population residing in areas served by the clinics, as explained in the methods section, and ranged from 9.5 % to 20.1% per clinic.

3.4.1.2. Household characteristics of the participants

The household characteristics of the participants are shown in table 3.4. There was a median of 7 members per household with an IQR ranged of 5 to 10 individuals. The median number of children <18 was 4 per household, while the median number of adults was 3 (IQR of 2-5 for both). Households had a median of 2 bedrooms. The crowding ratio (number of residents/number of bedrooms) was 3.4.

Electricity, piped water and toilet facilities were available in 98.1%, 93.8% and 86.7% of the households, respectively. One hundred and one participants had not visited a public clinic during the previous year (18.8%), 236 (44%) had visited 1-2 times, 135 (25%) had visited 3-4 times and 66 (12%) had paid 5 or more visits.

Despite the widespread availability of private clinics in Aden, 341 (63%) participants reported that they had not visited one in the previous year. The average time reported to reach a public clinic was 16 minutes.

Table 3.3 Socio-demographic characteristics of the participants

		N= **538** (%)
Age / years	mean (S.D) [IQR]	18.2 (19.4) [3, 32.3]
	adults: children <18 (adults %)	174: 364 (32.3)
Gender	male : female (% male)	280: 258 (52%)
Education	< 7 years old	234 (100.0)
	minor	198 (84.6)
	primary	36 (15.5)
	7-<18 years	130 (100.0)
	Primary	114 (87.7)
	Secondary	16 (12.3)
	Adults	174 (100.0)
	illiterate	25 (14.1)
	read and write only	53 (30.6)
	basic education	38 (21.9)
	secondary school	30 (17.3)
	higher	28 (16.1)
Employment (adults only)	employed	102 (58.6)
	unemployed	72 (42.4)
Clinic of enrolment	Sheikh Othman	108 (20.1)
	Khormaksar	71 (13.2)
	Buraika	69 (12.8)
	Dar Saad	69 (12.8)
	Crater	64 (11.9)
	Muala'a	58 (10.8)
	Mansura	51 (9.5)
	Tawahi	48 (8.9)

±SD= standard deviation, IQR = inter quartile range

Table 3.4 Household characteristics of the participants

Variable		N= 538 (%)
Number of household members	Median [IQR]	7 [5-10]
	1-4	154 (28.4)
	5-9	244 (45.6)
	10-14	110 (20.4)
	≥ 15	30 (5.6)
Number of household members <18 years old	Median [IQR]	4 [2-5]
	1-2	151 (28.1)
	3-4	230 (42.8)
	≥ 5	157 (29.2)
Number of adult household members ≥18 years old	Median [IQR]	3 [2-5]
	1-2	221 (41.1)
	3-4	140 (26.0)
	≥ 5	177 (32.9)
Number of bedrooms	Median [IQR]	2 [2-3]
	1-2	326 (60.6)
	3-4	188 (34.9)
	≥ 5	24 (4.5)
Median crowding ratio*	Median [IQR]	3.4 [2-4]
Availability of	electricity	528 (98.1)
	piped water	502 (93.8)
	toilet	463 (86.7)
Frequency of public clinic attendance /year	none	101 (18.8)
	1-2 times	236 (43.9)
	3-4 times	135 (25.1)
	≥ 5	66 (12.3)
Frequency of private clinic attendance /year	none	341 (63.4)
	1-2 times	129 (23.9)
	3-4 times	50 (9.3)
	≥ 5	18 (3.3)
Average time taken to reach the clinic (in minutes)	Mean (SD)	16 (11.1)

±SD= standard deviation. IQR = inter quartile range. * number of resident/number of bed rooms

3.4.2. The prevalence of HAV, Anti-HBc, HBsAg, HCV and HEV

The prevalence of HAV, anti-HBc, HBsAg, HCV and HEV are shown in table 3.5. Out of 538 samples tested for HAV, 466 (87%, 95% CI 83.7-89.5) were HAV IgG positive. Past infection by HBV (anti-HBc) were detected in 87 (16%, 95% CI 13.1-19.3) participants. All of the anti-HBc positive subjects were further tested for the presence of HBsAg and 8 (9%, 95% CI 3.1-15.3) were positive. The overall carriage rate was therefore 1.5% (95% CI 0.7-2.8). One adult (0.4%, 95% CI -0.4-1.2) of 259 samples

tested was positive for HCV and 38 (11%, 95% CI 7.5-13.9) of 356 samples tested were positive for HEV.

Table 3.5 Prevalence of HAV, anti-HBc, HBsAg, HCV and HEV.

	positive	negative	(95% CI)	all
	N (%)	N (%)		N (%)
HAV	466 (86.6)	72 (13.4)	(83.7-89.5)	538 (100)
Anti-HBc	87 (16.2)	451 (83.8)	(13.1-19.3)	538 (100)
HBsAg	8 (1.5)	530 (98.5)	(0.5-2.5)	538 (100)
HCV	1 (0.4)	258 (99.6)	(-0.4-1.2)	259 (100)
HEV	38 (10.7)	318 (89.3)	(7.5-13.9)	356 (100)

3.4.3 Prevalence of HAV, HBV and HEV by characteristics of the participants

3.4.3.1 Sociodemographic characteristics

The association between the characteristics of the study participants and the prevalence of HAV, anti-HBc and HEV are shown in table 3.6. Children < 18 years old had a lower frequency of HAV, Anti-HBc and HEV seropositive markers than adults ($p < 0.001$, $p < 0.001$ and $p < 0.05$, respectively). Among children, the prevalence of the infection by these viruses was 80%, 7% and 8% respectively, while in adults it was 99%, 35% and 15.4%, respectively.

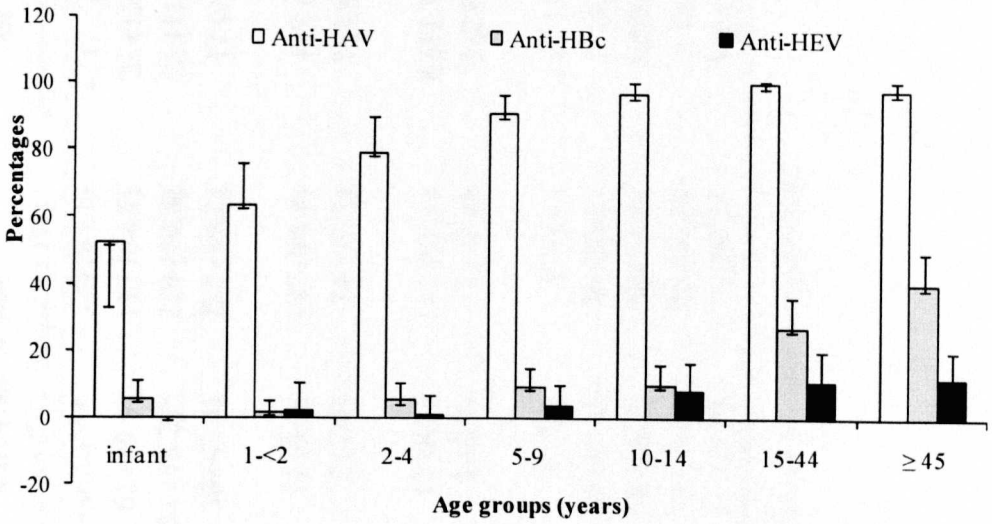
Infants were less frequently infected with HAV than young adults (53% vs. 100% and $p < 0.001$). Similar trends were found for anti-HBc with infants (5.5%) having the lowest rate and up to 40% of adults being anti-HBc positive ($p<0.001$). No antibodies for HEV were found among infants, and these increased with age reaching 16% in adults, as shown in fig 3.1.

The seroprevalence of HAV, anti-HBc and HEV in males (87%, 18% and 12%, respectively) was similar to the seroprevalences among females (86%, 14% and 10%, respectively).

There was no significant difference in the prevalence of HAV or anti-HBc by educational level, as HAV infection was nearly universal in adults, but statistical significant differences was seen in HEV ($p< 0.05$). It seems children not yet enrolled in

school had a lower frequency of infection than those of the same age already in primary or secondary education (69% vs. 94% and 100%, respectively, $p < 0.05$).

Figure 3.1 Seroprevalence of anti-HAV, anti-HBc and anti-HEV markers by age (percentages and 95% confidence interval)



Adult participants either employed (100%, 33% and 17%, respectively) or not (97%, 40% and 13%, respectively) had similar rates of infection by HAV, Anti-HBc and HEV.

Ninety eight percent of participants at Mansura clinic were infected with HAV, which was higher than for other clinics ($p < 0.05$), with the lowest frequency (76%) being observed in Mualla clinic. For HBV and HEV, the highest rates of infection were seen in Dar Saad and Crater (25% and 20%, respectively) and the lowest in Muala'a and Khormaksar clinics (7% and 3%, respectively), but this was not statistically significant.

A higher prevalence of HAV (98%, $p < 0.001$) and HEV (92%) was observed in participants with a history of hepatitis/jaundice but not for positive Anti-HBc (22%). Ninety one percent of participants with a history of hepatitis or jaundice in their family members were HAV positive, which was higher than those without family history (85%, $p<0.05$). However, Anti-HBc and HEV showed a reversed trend with 14% vs. 17% and 13% vs. 10%, for those with and without a family history, respectively. However these differences were not statistically significant.

Table 3.6 Prevalence of HAV, Anti-HBc and HEV according to the characteristics of the participants.

		HAV N=538		Anti-HBc N=538		HEV N=356	
		Positive (%)	Negative (%)	Positive (%)	Negative (%)	Positive (%)	Negative (%)
Age	children <18	294 (80.8)***	70 (19.2)	25 (6.9)***	339 (93.1)	17 (7.7)**	203 (92.3)
	adults	172 (98.9)	2 (1.1)	62 (35.6)	112 (64.4)	21 (15.4)	115 (84.6)
Sex	male	243 (86.8)	37 (13.2)	51 (58.2)*	229 (50.8)	21 (11.7)	158 (88.3)
	female	223 (86.4)	35 (13.6)	36 (41.4)	222 (49.2)	17 (9.6)	160 (90.4)
Education	< 7 years old						
	not in school	137 (69.2)**	61 (30.8)	11 (5.6)	187 (94.4)	4 (3.4)*	112 (96.6)
	primary	24 (92.3)	2 (7.7)	2 (7.7)	24 (92.3)	2 (13.3)	13 (86.7)
	7-<18 years						
	primary	117 (94.4)	7 (5.6)	11 (8.9)	113 (91.1)	8 (11.1)*	70 (89.7)
	secondary	16 (100)	0 (0)	1 (6.3)	15 (93.8)	3 (27.3)	8 (72.7)
	adults						
	illiterate	25 (100)	0 (0)	8 (32)	17 (68)	2 (8.3)**	22 (91.7)
	read and write only	51 (96.2)	2 (3.8)	23 (43.4)	30 (56.6)	2 (6.5)	29 (93.5)
	basic education	38 (100)	0 (0)	10 (26.3)	28 (73.7)	7 (21.9)	25 (78.1)
	secondary school	30 (100)	0 (0)	10 (33.3)	20 (66.7)	1 (4.5)	21 (95.5)
	higher	28 (100)	0 (0)	11 (39.3)	17 (60.7)	9 (33.3)	18 (66.7)

* $p<0.2$ ** $p<0.05$ *** $p<0.01$ Anti-HBc= hepatitis B virus core antibodies

Continued Table 3.6.

		HAV N=538		Anti-HBc N=538		HEV N=356	
		Positive (%)	Negative (%)	Positive (%)	Negative (%)	Positive (%)	Negative (%)
Employment (adults)	employed	102 (100)*	0 (0)	34 (33.3)	68 (66.7)	15 (17.2)	72 (82.8)
	unemployed	66 (97.2)	2 (2.8)	27 (38.9)	41 (61.1)	6 (12.2)	39 (87.8)
Clinic of enrolment	Tawahi	43 (89.6)**	5 (10.4)	6 (12.5)*	42 (87.5)	1 (5.9)*	16 (94.1)
	Muala'a	44 (75.9)	14 (24.1)	4 (6.9)	54 (93.1)	1 (2.9)	33 (97.1)
	Crater	54 (84.4)	10 (15.6)	9 (14.1)	55 (85.9)	10 (19.6)	41 (80.4)
	Khormaksar	55 (77.5)	16 (22.5)	15(21.1)	56 (78.9)	1 (2.6)	37 (97.4)
	Mansura	50 (98.0)	1 (2.0)	11 (21.6)	40 (78.4)	5 (17.2)	24 (82.8)
	Sheikh Othman	96 (88.9)	12 (11.1)	14 (13)	94 (87)	11 (14.9)	63 (85.1)
	Buraika	59 (85.5)	10 (14.5)	11 (15.9)	58 (84.1)	4 (7.7)	48 (92.3)
	Dar Saad	65 (94.2)	4 (5.8)	17 (24.6)	52 (75.4)	5 (8.2)	56 (91.8)
History of hepatitis	(individual) yes	79 (97.5)***	2 (2.5)	18 (22.2)*	63 (77.8)	4 (7.8)	47 (92.2)
	(individual) no	386 (84.6)	70 (15.4)	69 (15.1)	387 (84.9)	34 (11.2)	270 (88.8)
	(family) yes	157 (90.8)*	16 (9.2)	24 (13.9)	149 (86.1)	15 (13.3)	98 (86.7)
	(family) no	308 (84.6)	56 (15.4)	63 (17.3)	301 (82.7)	23 (9.5)	219 (90.5)

* $p<0.2$ ** $p<0.05$ *** $p<0.01$ Anti-HBc= hepatitis B virus core antibodies

3.4.3.2 Household characteristics

A large family size seemed to be associated with higher exposure to infection. The number of household members was higher among individuals with HAV and HBV infection but not for HEV ($p < 0.001$ and <0.05, respectively) as shown in table 3.7. A similar pattern was seen for HBV in families with high number of members <18 years ($p <0.001$). No marked difference was seen if household members were mostly adults, except for HAV, where a statistical difference was observed ($p <0.001$).

The number of bedrooms per household was associated with the prevalence of HAV ($p<0.05$) but not with Anti-HBc or HEV. The crowding ratio was not associated with the proportion of participants infected with HAV, Anti-HBc or HEV.

The availability of electricity and other domestic services was high among participants. The former was associated with a higher rate of HAV infections ($p<0.05$). However there was no association with the availability of piped water or toilet facilities.

Attending public or private clinics in the past was not associated with a higher prevalence of HAV, HBV or HEV. The mean time to reach the clinic was short for both HAV and Anti-HBc positive and negative participants. Despite the closeness of the clinics, HAV- positive cases took slightly longer to reach the clinic than HAV-negative cases ($p<0.05$).

Table 3.7 Household characteristics of participants according to their HAV, Anti-HBc and HEV status.

		HAV N=538		Anti-HBc N=538		HEV N=356	
		Positive (%)	**Negative (%)**	**Positive (%)**	**Negative (%)**	**Positive (%)**	**Negative (%)**
Number of household members	1-4	43 (69.4)***	19 (30.6)	5 (8.1)**	57 (91.9)	2 (6.3)	30 (93.8)
	5-9	301 (89.6)	35 (10.4)	50 (14.9)	286 (85.1)	24 (10.7)	200 (89.3)
	10-14	98 (89.1)	12 (10.9)	26 (23.6)	84 (76.4)	6 (8)	69 (92)
	≥15	23 (79.3)	6 (20.7)	6 (20.7)	23 (79.3)	5 (20.8)	19 (79.2)
Number of members <18 years old	<3	126 (83.4)*	25 (16.6)	13 (8.6)***	138 (91.4)	8 (8.6)	85 (91.4)
	3-4	197 (85.7)	33 (14.3)	36 (15.7)	194 (84.3)	19 (12.8)	129 (87.2)
	≥ 5	143 (91.1)	14 (8.9)	38 (24.2)	119 (75.8)	11 (9.6)	104 (90.4)
Number of adults	1-2	179 (81)***	42 (19)	30 (13.6)	191 (86.4)	13 (9.3)	127 (90.7)
	3-4	129 (92.1)	11 (7.9)	26 (18.6)	114 (81.4)	7 (8.1)	79 (91.9)
	≥ 5	157 (89.2)	19 (10.8)	31 (17.6)	145 (82.4)	17 (13.2)	112 (86.8)
Number of bedrooms	1-2	281 (86.2)**	45 (13.8)	43 (13.2)*	283 (86.8)	21 (10.3)	183 (89.7)
	3-4	168 (89.4)	20 (10.6)	40 (21.3)	148 (78.7)	16 (11.9)	118 (88.1)
	≥ 5	17 (70.8)	7 (29.2)	4 (16.7)	20 (83.3)	1 (5.6)	17 (94.4)

* $p<0.2$ ** $p<0.05$ *** $p<0.01$ Anti-HBc= hepatitis B virus core antibodies

Continued Table 3.7

		HAV		Anti-HBc		HEV	
		Positive (%)	**Negative (%)**	**Positive (%)**	**Negative (%)**	**Positive (%)**	**Negative (%)**
Median crowding ratio*		3.6*	3.3	3.7	3.6	3.7	3.6
Availability of	electricity	460 (87.1)**	68 (12.9)	87 (16.5)	441 (83.5)	38 (10.9)	312 (89.1)
	pipe water	437 (87.1)*	65 (12.9)	84 (16.7)	418 (83.3)	37 (11.1)	295 (88.9)
	toilet	406 (87.7)*	57 (12.3)	81.(17.5)*	382 (82.5)	33 (10.9)	271 (89.1)
Frequency of public clinic attendance /year	none	82 (80.4)	20 (19.6)	15 (14.7)	87 (85.3)	11 (16.4)	56 (83.6)
	1-2 times	209 (88.6)	27 (11.4)	35 (14.8)	201 (85.2)	15 (9.4)	145 (90.6)
	3-4 times	119 (88.1)	16 (11.9)	23 (17)	112 (83)	8 (9.3)	78 (90.7)
	≥ 5	54 (86.6)	9 (13.4)	14 (21.5)	51 (78.5)	4 (9.3)	39 (91.7)
Frequency of private clinic attendance /year	none	309 (90.6)***	32 (9.4)	59 (17.3)	282 (82.7)	24 (10.7)*	201 (89.3)
	1-2 times	101 (78.3)	28 (21.7)	16 (12.4)	113 (87.6)	13 (15.3)	72 (84.7)
	3-4 times	41 (82)	9 (18)	9 (18)	41 (82)	1 (2.9)	34 (97.1)
	≥ 5	15 (83.3)	3 (16.7)	3 (16.7)	15 (83.3)	0 (0)	11 (100)
Average time to reach the clinic (in minutes) [±S.D.]		15.3 [9.98]**	17.6 [9.3]	15.1[10.67]	15.75 [9.77]	15.8 [10.39]*	12.38 [8.06]

* $p<0.2$ ** $p<0.05$ *** $p<0.01$ Anti-HBc= hepatitis B virus core antibodies

3.4.4 Prevalence of HBsAg according to the characteristics of the participants

3.4.4.1 Socio-demographic characteristics

The prevalence of HBsAg according to the characteristics of the participants is shown in table 3.8. All 8 carriers were adults (12.9% of the 62 Anti-HBc positive adults) resulting in a carriage rate of 4.6% (95% CI 2 - 8.9) among adults. Three of the eight HBsAg positive adults were male (6%) and five (14%) female, however it is not statistically significant.

HBsAg positive participants have varying education, ranging from illiterate (3) to higher education (3). However numbers were too low for statistical analysis. Employed participants were more likely to be HBsAg positive than unemployed participants but again, this was not significant. Three cases were identified in Dar Saad clinic (18%) and one case for each of the remaining clinics except Muala'a and Mansura clinic. Three individuals (18%) with a history of hepatitis were HBsAg positive and one person (4%) with a family history of hepatitis was positive.

Overall, only one (0.6%) of the 259 participants tested was infected by HCV. This adult was an unemployed, illiterate female attending Dar Saad clinic. She had a history of hepatitis but did not report a family history of hepatitis.

3.4.4.2 Prevalence of HBsAg according to characteristics of the household.

The prevalence of HBsAg carriage and HCV infection did not show significant differences by the numbers of household members, been those below 18 years old or adults. Data for HBsAg are shown in table 3.9. Data for HCV was omitted as there was only one positive case. There was no association between HBsAg and the crowding ratio, nor the availability of electricity, pipe water or toilet facilities or clinic attendance per year. The mean time required to reach the nearest health clinic was similar for HBsAg positive and negative cases

Table 3. 8 Sociodemographic characteristics of the HBsAg positive subjects among the Anti-HBc positive participants.

			† HBsAg (N=87)	
			Positive (%)	Negative (%)
Age		children <18	0 (0)*	25 (100)
		adults	8 (12.9)	54 (87.1)
Sex		male	3 (5.9)	48 (94.1)
		female	5 (13.9)	31 (86.1)
Education	< 7 years old	minor	0 (0)	11 (100)
		primary	0 (0)	2 (100)
	7 <18 years	primary	0 (0)	11 (100)
		secondary	0 (0)	1 (0)
	adults	illiterate	3 (37.5)*	5 (62.5)
		read and write only	1 (4.3)	22 (95.7)
		basic education	0 (0)	10 (100)
		secondary school	1 (10)	9 (90)
		higher	3 (27.3)	8 (72.7)
Employment (adults)		employed	5 (14.7)	29 (85.3)
		unemployed	3 (10.7)	25 (89.3)
Clinic of enrolment		Dar Saad	3 (17.6)	14 (82.4)
		Tawahi	1 (16.7)	5 (83.3)
		Crater	1 (11.1)	8 (88.9)
		Buraika	1 (9.1)	10 (90.9)
		Sheikh Othman	1 (7.1)	13 (92.9)
		Khormaksar	1 (6.7)	14 (93.3)
		Mansura	0 (0)	11 (100)
		Muala'a	0 (0)	4 (100)
History of hepatitis	(individual)	yes	3 (17.6)	15 (83.3)
	(individual)	no	5 (7.2)	64 (93.8)
	(family)	yes	1 (4.2)	23(95.8)
	(family)	no	7 (11.1)	56 (88.9)

* p<0.2 ** p<0.05 *** p<0.01 †HBsAg= hepatitis B virus surface antigen

Table 3.9 households characteristics in HBsAg positive subjects among the Anti-HBc positive participants.

		† HBsAg (N=87)	
		Positive (%)	Negative (%)
Number of household members	1-4	0 (0)	5 (100)
	5-9	5 (10)	45 (90)
	10-14	3 (11.5)	23 (88.5)
	≥15	0 (0)	6 (100)
Number of members <18 years old	<3	1 (7.7)	10 (92.3)
	3-4	2 (5.6)	34 (94.4)
	≥ 5	5 (13.2)	33 (86.8)
Number of adult	1-2	1 (3.3)*	29 (96.7)
	3-4	5 (19.2)	21 (80.8)
	≥ 5	2 (6.5)	29 (93.5)
Number of bedrooms	1-2	2 (4.7)	41 (95.3)
	3-4	5 (12.5)	35 (87.5)
	≥ 5	1 (25)	3 (75)
Median crowding ratio		3.2	3.8
Availability of	electricity	8 (9.2)	79 (90.8)
	pipe water	7 (8.3)	77 (91.7)
	toilet	7 (8.6)	74 (91.4)
Frequency of public clinic attendance /year	none	0 (0)	15 (100)
	1-2 times	4 (11.4)	31 (88.6)
	3-4 times	2 (8.7)	21 (91.3)
	≥ 5	2 (14.3)	12 (85.7)
Frequency of private clinic attendance /year	none	4 (6.8)*	55 (93.2)
	1-2 times	1 (6.3)	15 (93.8)
	3-4 times	3 (33.3)	6 (66.7)
	≥ 5	0 (0)	3 (100)
Average time to reach the clinic (in minutes) [S.D.]		11.25 [5.18]	15.49 [11.01]

* $p<0.2$ ** $p<0.05$ *** $p<0.01$ HBsAg= hepatitis B virus surface antigen

3.4.5 Past exposure between HAV, HBV, HCV and HEV

From the total of 466 participants with positive HAV, 354 (76%) were not co-infected with other hepatitis viruses and 112 (21%) were co-infected with one or more viruses in this study, as shown in table 3.10 and 3.11. Seventy four (15.9%) HAV positive cases were co-infected with HBV and 30 (6.4%) with HEV. In addition, 7 cases were co-infected with HBV and HEV and one (0.2%) with HBV and HCV.

Five cases with HBV infection (5.8%) were not co-infected with other viruses and 82 were co-infected with one or more viruses. The majority 74 (85%) were co-infected with HAV, 7 (8%) were co-infected with HAV and HEV and one (1.2%) with HAV and HCV. Of the 38 cases of HEV, one did not have past exposure with other viruses, while 30 (79%) were co-infected with HAV and 7 (18%) with both HAV and HBV. The single case of HCV was co-infected with HAV and HBV but not with HEV.

Analysis of association between HAV with HEV and HAV with HBV was made. Findings show that there was an association between HAV and HEV with p- value <0.05. Both viruses were having similar route of transmission (Faeco-oral). It is surprising that an association was found between HAV and HBV ($P= 0.02$) where their route of transmission is different, albeit that refereeing to the universal infection of HAV and probably to other factors such as rate of crowdedness in the house as well as the habit of sharing inter familial properties.

Table 3.10 Multiple past exposure between HAV and the other viruses (HBV, HCV and HEV)

		+ one virus				+ two viruses		Total
	Single	HAV	HBV	HEV	HCV	HBV/HEV	HBV/HCV	
HAV (%)	354 (76)	NA	74 (15.9)	30 (6.4)	0 (0)	7 (1.5)	1 (0.2)	466
						HAV/HEV	HAV/HCV	
HBV (%)	5 (5.8)	74 (85.0)**	NA (-)	0 (0)	0 (0)	7 (8.0)	1 (1.2)	87
						HAV/HBV		
HEV (%)	1 (2.6)	30 (79)**	0 (0)	NA	0 (0)	7 (18.4)		38
						HAV/HBV		
HCV (%)	0 (0)	0 (0)	0 (0)	0 (0)	NA	1 (100.0)		1

** p value < 0.05

Table 3.11 Association of past exposure between HAV, HBV and HEV

		HEV		HBV				
		positive N (%)	negative N (%)	Positive N (%)	negative N (%)		HEV positive N (%)	HEV negative N (%)
HAV	positive N (%)	37 (11.7)	278 (88.3)	***83 (17.8)	383 (82.2)	HBV positive N (%)	7 (11.1)	56 (88.9)
	negative N (%)	1 (2.4)	4 0 (97.6)	4 (5.6)	68 (94.4)	negative N (%)	31 (10.6)	262 (89.4)

*** p value < 0.01

3.5 Discussion

For the purpose of planning an adequate health system and implementing sound preventive control measures, it is important to obtain baseline information on the prevalence of infection and its markers. This study shows a varied prevalence in HAV, HEV, HBV and HCV in Aden, and that this prevalence varies with age. A significant difference was observed in the prevalence between adults and children <18 years for HAV and HBV. This is the first description of the epidemiological characteristics of HAV and HEV in this community and for the south-eastern governorates of Yemen.

3.5.1 Enterically transmitted viruses (HAV and HEV)

In this study, the prevalence of HAV increased steadily from 53% among infants, 82% in pre-school children to 99% amongst adults. Although the overall prevalence was very high (87%), it was lower than what was found by Scott et al., (99.7%) in 1988, in the northern governorates of Yemen (Takahashi et al., 2002b). The mean age of Scott et al population (24.2 ±14.6 years) was higher than the age of the participants in this study (18.3 ± 19.4 years). The resulting difference of 13% in the prevalence between the studies could be an artefact of different population structures. However, data for Scott's study is not reported by age and is not possible to investigate whether the effect is due to this confounder.

A high prevalence of HAV infection in pre-school children was observed (82%), which was similar to other studies on pre-school children in developing countries with a high HAV prevalence. For example, the prevalence of this age group was 86% in India (Scott et al., 1990) and 100% in South Africa (Abdool Karim & Coutsoudis 1993). However, in Oman the rate of infection in this age group was 53.2% (Acharya et al., 2003), in Saudi Arabia 39% (MOH-SO 1999) and in Lebanon 10.5% (Arif M 1995). Other countries with very low prevalence among pre-school age are Italy (13%) (Sacy et al., 2005), Korea (5%) (Ansaldi et al., 2008) and Luxembourg (<5%) (Sohn et al., 2000), Japan (1.3%) (Mossong et al., 2006).

Most reports from the Middle East indicate that there is high endemicity of HAV in the adult population, which varies with age. The majority of countries in the region have prevalence > 95%, including Egypt, Qatar, some areas of Saudi Arabia, Oman, Syria, Iran, Morocco and Algeria (Kiyohara et al., 2007), which resemble our results in the adult population. Nearly, the same rate of infection was seen in Japan 30 years earlier (1973), while in 2003 only 50% were positive showing a remarkable shift (MOH-SO 1999; Fathalla et al., 2000; Tufenkeji 2000; Jacobsen & Koopman 2004).

Yemen, still suffers from a low availability of potable water and sanitation (Kiyohara et al., 2007). Based on the 2005 report from the Ministry of Planning and International Cooperation (MOPIC) access to water and sanitation services was available for only 65% and 35% of the population, respectively (WorldBank 2006). Similarly, there is a poor control of food hygiene, low level of health awareness, high rates of illiteracy and poverty and high rates of infection among young children. All these factors contribute to an increasing spread of hepatitis A in the community and the country, not surprisingly, has a high endemicity.

In general, no significant differences in the HAV prevalence were observed by gender, education and occupational status, although, participants enrolled in Mualla and Mansura had the lowest and highest prevalence. Mualla area was constructed at the time of the British Colony in Aden (1839 - 1967) and was used for settling the British Army in the mid of the last century. The area has adequate sanitation services and drinking water that was better than the Mansura where some of its areas have a mixture of new and old constructions and with limited sewerage system. In addition, proportions of children <18 years old, was higher in Mualla than in Mansura (42 and 22, respectively). In comparing Aden with other cities in Yemen, piped water and sanitary toilet services are available for 94% and 87%, respectively, of the population in Aden which could not found in other city in the country whereas they confronting the rapidly depleting water sources particularly in main cities such as Sana'a and Taiz. To confront the water crisis in these cities residents have been forced to buy untreated

water from private sources. A microbiological examinations study was conducted in Sana'a and Taiz (main cities in northern governorates) to assessed the quality of partially treated drinking water in private establishments in the City have shown that 83%-90% of the samples were contaminated; 50% with faecal coliforms and 33% with total coliforms (Raja'a et al., 2001; Metwali 2003). This indicated a probably a gross contamination with other viruses or parasites. Therefore, a substantial changes would be needed to achieve the control of HAV and HEV infection in Yemen, as 42% of the population live below the poverty line and the Gross National Income (GNI) per capita is $760/ year (MOPIC 2006). The priority for the control of HAV at this stage therefore should focus on improving the water quality, sanitation coverage, public food hygiene and awareness.

In this study, an HEV-ELISA was used to detect IgG. This assay utilises three absorbent recombinant HEV antigens which correspond to the structural region of the viral genome and is reported to have high sensitivity and specificity to detect the past infections (WorldBank 2006; Worldbank 2007).
Reverse-transcription PCR can be used to confirm infection and to identify the strains. However, because of its technical complexity and cost, it is only used in reference laboratories (Myint et al., 2006a; MPDiagnostics 2007). Western blot assays to detect anti-HEV IgM and IgG, are costly and not frequently used for prevalence surveys (Irshad 1999; WHO 2001a).

The prevalence of HEV has not been studied in Yemen. The only study available was based on patients with acute hepatitis and reported a rate of 14% (WHO 2001a). This is higher than the reported in this study and is likely to reflect differences in the study populations. However, such rates suggest a widespread prevalence of the agent.

Population-based studies in the Middle East show a high prevalence of HEV, in Egypt (30%-50%), (Kumar et al., 2001)(20%), Somalia (71%) and Saudi Arabia (Jeddah) (14.9%) Somalia

(Abdelaal et al., 1998; Irshad 1999; Fix et al., 2000; Kumar et al., 2001; Meky et al., 2006; Stoszek et al., 2006c).

It was thought that HEV had a restricted distribution to developing countries. However, most recent studied have revealed that anti-HEV antibodies can be observed in all countries including developed countries (Abdelaal et al., 1998; Irshad 1999; Fix et al., 2000; Kumar et al., 2001; Meky et al., 2006; Stoszek et al., 2006c), although the prevalence can range between 3% - 20% and 0.4% - <3% in endemic and non-endemic countries, respectively (WHO 2001a). An epidemiological survey in 11 countries showed that the rate of infection varied across countries from 18% in Bolivia to 80% in Egypt (Balayan 1997). Sporadic infections also seem to be common in both developing and developed countries (Abe et al., 2006).
Countries have been categorized into low, moderate and high HEV prevalence zones (0-2%, >2-5% and >5%, respectively) (Irshad 1999). Based on these categories, Yemen would be placed on the high prevalent zone.

No significant differences were seen by gender, occupational status, clinic of enrolment, availability of electricity, piped water or toilets. The education level was associated with the prevalence of infection with higher rates of infection among those with higher education (33%) than among those having secondary school (5%), however no explanation was found.

At present, no vaccine is available against HEV, although several studies are in progress (Irshad 1999). Studies in monkeys showed that protective immunity is induced by vaccination with recombinant proteins and suggested that a vaccine for hepatitis E might be feasible (Tsarev et al., 1997). However recently, the study by Shrestha et al. (2007) looks encouraging; in a group of almost exclusively male subjects (898 in the vaccine group and 896 in the placebo group) received three vaccine doses; that recombinant HEV vaccine effectively prevents clinical hepatitis (Shrestha et al., 2007). Findings from other trial from China of a recombinant hepatitis E vaccine have concluded that the course of three doses of the vaccine

induced 100% seroconversion and it was safe and immunogenic for humans (Zhang et al., 2009). However, the duration of protection by the rHEV vaccine is also unclear; further studies are needed to validate appropriate indications for these vaccines and to determine its long-term effects (Tsarev et al., 1994; Zhang et al., 2002; Tacke & Trautwein 2007). Despite that the vast majority of HEV-related diseases immunocompetent people are benign; it is generally encouraging that rHEV vaccine was effective in preventing hepatitis E disease in a high-risk population mainly those in endemic areas and those under compromised living conditions, for example refugee camps or serving the army (Bryan et al., 2002; Tacke & Trautwein 2007) in addition to traveller from developed countries to endemic regions.

3.5.2. Blood-borne viruses (HBV and HCV)

Hepatitis B and C continue to be global public health problems despite the efforts to control these infections through education, screening, the application of preventive safety measures in medical care and vaccination programmes for HBV (Krawczynski 2007; Shrestha et al., 2007).

Studies across the world show differences in the prevalence of HBV when stratified by age or exposure factors (Alexander & Kowdley 2006). In our study, of the 538 participants enrolled, 7% of <18 years olds were Anti-HBc positive versus 36% of the adults. The overall carrier rate (HBsAg positive) was low (1.5%) and none of the children <18 years old were carriers compared to 4.6% of the adults. The latter is nearer to the 5.5% rate found among employees of three major hospitals in Aden reported by Al-Jarba (El-Sayed et al., 1997; WHO 2002b; Lobato et al., 2006; Michos et al., 2008). Similarly, 6.7% of blood donors in Aden City were reported to be carriers. Similar rates were reported from Sana'a, where 4% of healthy mothers (Al-Jarba & Al-Sayyari 2003; Sallam et al., 2003a) and 7.4% of individuals presenting to the central laboratory were identified to be carriers (Al-Shamahy 2000). Despite the differences in the source of the study populations in these studies findings among adult seen to be remarkably similar.

Adults in Aden were more likely to have a higher prevalence of HBV infection than children reflecting their longer exposure to hepatitis. This is also a possible a reflection of lack of education, inadequate blood transfusion safety measures, unsafe use of needles and syringes and the lack of vaccination against HBV in the past. The prevalence found therefore is a mixture of the cohort effect of the markers of infection used, with a likely decreasing rate of infection in younger cohorts which might be protected by the vaccine. To our knowledge there is no previous study of the prevalence of HBV among children in Aden to enable a valid comparison with the current situation. However, studies in other locations have shown that the prevalence of HBV is lower among children than adults. In Egypt the prevalence of HBV was reported to be 10% among Sinai children (Al-Nassiri & Raja'a 2001); similarly in Western Brazilians (Amazon region) around 7% of children under 16 years of age (El-Sayed et al., 1997); in a Romany population of children, under 16 years old in Athens the prevalence was 22% (Lobato et al., 2006) and 6% of children under 12 years old in Colombia were HBV core positive (Michos et al., 2008).

HBV transmission can occur via multiple routes namely, perinatally, horizontally and percutaneous or per-mucosal exposure to infectious body fluids including sexual or parenteral contact (de la Hoz et al., 2008). The major mode of transmission in Yemen is not well established. Sallam and Al-Nassiri findings support the notion that HBV in Yemen is most likely acquired during adulthood reflecting the increasing HBsAg prevalence with age and the longer exposure to high transmission rates in the past (Mahoney 1999; Hens et al., 2007). Our findings were similar, with lower prevalence of infection among children and an increase in early adolescence supporting the hypothesis that HBV infection is more likely to be acquired horizontally in early adulthood. The absence of control measures in blood transfusion, the unsafe use of needles and the lack of vaccination in the past would also have contributed to the longstanding exposure of the adult cohorts in these studies. Furthermore recent studies, on pregnant women in the perinatal period attending primary health care facilities in Aden City, show a low rate of carriers (2.8%) (Al-Nassiri & Raja'a 2001; Sallam et al., 2003a). The low

prevalence of HBV infection in children supports the hypothesis that vertical transmission is not a likely common cause in Aden.

The low prevalence of HBV infection among young participants in Aden may be due to several factors. Firstly, prior to 1992, there was no clear policy for screening blood donors in the main hospitals. From 1992 to 1998 the Abbot Latex test was used to detect HBV and since 2003 the ELISA method was introduced. More recently, a fourth generation ELISA analysis system has been in use in the central referral laboratory (Yousef Khalidah 2003). In addition, immunisation against HBV was introduced in late 1999 in most public health facilities. The coverage rate for the target group (under one year old) achieved 93% for the first dose and 87% for the third dose with a default rate of 6% by 2006 (Omer 2007).

The effectiveness of vaccination programmes against HBV has been clearly demonstrated. Study from Taiwan had shown that the rate of HBV infection decreased from 34% to 10% and the carriage rate fell from 11% to 4%, before and after the implementation of vaccination (EPI/Aden 2007). In Italy, the prevalence in HBV has progressively decreased over the last 20 years, as a result of general improvements in the standard of living, the use of preventive measures, implementation of vaccination programmes and the implementation of blood screening procedures (Chen et al., 2007a). It is likely that these measures would lead to a reduction in the prevalence of HBV infection in the recipient population, although data on the prevalence of HBV before 2000 are not available.

There were some limitations in the present study. Although HBV vaccine was a protective factor against infection, the participants' detailed vaccination history was not recorded, which would unavoidably miss some important information on vaccination failure. Secondly, only two HBV markers (Anti-HBc and HBsAg) were investigated. Therefore, no clear idea was found in estimating the proportion of the population who were previously infected or those who may have received the vaccine. With these limitations in mind a further study could be helpful to be conducted to address the issue of susceptibility to HBV infection in Yemen.

Our study also found past exposure of HBV with other hepatitis viruses varying between one virus and two viruses. The majority of HBV cases were co-infected with HAV (74/87). Past exposure with two hepatitis viruses was also seen among 7 participants with HAV and HEV and only one case with HAV and HCV simultaneously. In addition, an association was found between HAV and HBV however, their routes of transmission are different. HAV transmitted via oro-faecal route while HBV is parenteral. Nevertheless, the rate of HAV infection was seen in the majority of the population under study which explain this type of association.

The overall findings in this study indicate that HBV is still an important public health problem in adults, despite improvements in blood transmitted infections and immunisation in the last decades. Similar studies could be undertaken in other areas of Yemen.

The prevalence of HCV was low with only 0.4% of the population infected and only one adult case infected. Al-Jarba and Al Sayyari study of HCV prevalence among hospital employees in Aden reported an overall rate of 1.3% (576 participants) (Zanetti 2001). The rate of 0.4% is lower than and at variance from those found in other studies conducted in Yemen, namely in a Soqotrian population (5.1%), blood donors in Sana'a (5.2%) (Al-Jarba & Al-Sayyari 2003) and Mukalla City (1.6%) (Sallam et al., 2003b). Globally, it is reported that the transmission of HCV is less common than that of HBV in the general population (Bahaj 2003). Some risk factors such as multiple partners, intravenous drug use and commercial sex work are not common in Yemen. However, further study would be needed to develop a comprehensive picture of the HCV infection in the country and looking in-depth on the underling risk factors in different communities.

3.6 Genotyping for Hepatitis B virus

3.6.1 Objective 1.b

To describe the predominant HBV genotypes in the study populations.

3.6.2 Literature review

Numerous studies on have described the importance of viral hepatitis genotypes on the clinical and therapeutic outcome of liver disease and genotypes vary across geographical regions (WHO 2003b).

In Yemen, HBV genotypes were previously reported from sera of male blood donors (Blumberg 1977; Nousbaum 1998; WHO 2002b; Sugauchi et al., 2003; WHO 2003a; Lin & Kirchner 2004; Yuen et al., 2004; Hipgrave et al., 2006) and genotypes A and D were reported with a predominance of the latter. This current however, describes the genotype distribution among asymptomatic carriers and patients with liver diseases.

3.6.2.1 Genetic characteristics of HBV DNA

Historically, HBV variation was defined by means of antigenic analysis of the surface antigen of the virus. On this basis 9 distinct subtypes have been described (Sallam & Tong 2004). These serotypes (adw, ayw, adr and ayr) are defined by two mutually exclusive determinant pairs, d/y and w/r, and a common determinant a (Bowyer et al., 1997). Sequence analysis of viral DNA has led to the definition of 8 genotypes from A through H, based on the relatedness of whole the genome (Ding et al., 2001; Sakugawa et al., 2002; Westland et al., 2003; Kao et al., 2004; Chu & Liaw 2005). Each genotype differs from the other by nucleotide distances of approximately 10%–13% (Norder et al., 1994; Hardie & Williamson 1997; Lindh et al., 1997; Stuyver et al., 2000; Arauz-Ruiz et al., 2002; Ding et al., 2002; Mahtab et al., 2008). The genotypic variation of HBV is reflected in a partial sequence of the HBV genome, for example in the pre-S or S gene (Magnius & Norder 1995; Nousbaum 1998; Lunel et al., 2000; Simmonds 2001; Arauz-Ruiz et al., 2002; Sugauchi et al., 2002; Fung & Lok 2004; Hagiwara et al., 2006). As the sequence of the S gene is more conserved than the pre-S region, analysis

of the S gene is much more suitable for genotyping (Norder et al., 2004; Kramvis et al., 2005).

3.6.2.2 Determination of HBV genotype

Reliable and easy methods to differentiate HBV genotypes and subgenotypes are a pre-requisite for molecular epidemiological tests and clinical studies. Several methods have been employed for HBV genotyping, such as direct sequencing, where samples are amplified by PCR and the pre-S or S region is used as primer for the reaction (Lindh et al., 1999; Kao et al., 2000; Ding et al., 2001; Sakugawa et al., 2002; Westland et al., 2003; Kao et al., 2004; Chu & Liaw 2005). The products of amplification are directly sequenced and the sequences are then compared against published sequences to determine its homology with known genotypes (Chan et al., 2000a; Song et al., 2005). Another method is restriction fragment length polymorphism, where the sample is first amplified by using the S gene as target. The PCR products containing genotype-specific regions are then digested by restriction enzymes, where the HBV genotype is differentiated based upon differences in the size of the digested fragments (Bartholomeusz & Schaefer 2004). Amplicons of the S gene are then hybridized to strips pre-coated with genotype-specific oliginucleotide probes for line probe assays. A simple method is ELISA, where monoclonal antibodies to genotype-specific epitopes of the pre-S2 region are employed. However, Kirschberg (2004) and Chen (2007) have suggested that the Multiplex-PCR method is of low-cost and suitable for large-scale epidemiological surveys and clinical studies (Mahtab et al., 2008).

3.6.2.3 Geographical distribution of HBV genotypes

The distribution of HBV genotypes varies across regions and population migration. HBV genotypes A and D have global distribution. Genotype A predominates in Western Europe, East and South Africa, Genotype B and C in Asia, genotype D in the Mediterranean and the Middle East, genotype E in Western Africa and genotype F and H in Central and South America (Kirschberg et al., 2004; Chen et al., 2007b). Genotype G has no specific geographical distributions but has been reported in France and USA (Norder et al., 1993;

Lunel et al., 2000; Simmonds 2001; Arauz-Ruiz et al., 2002; Sugauchi et al., 2002; Chu et al., 2003; Sugauchi et al., 2003; Westland et al., 2003; Kato et al., 2004; Janssen et al., 2005; Fung et al., 2006; Mahtab et al., 2008).

Up to now, HBV genotypes B and C are divided into at least five subgenotypes (Stuyver et al., 2000; Chu et al., 2003). The strains B1 and B2 (formerly named Bj and Ba, respectively) are predominant within genotype B, whereas B1 is mainly found in Japan but it does not have any recombination with genotype C, whereas B2 is prevalent all over Asia (Nagasaki et al., 2006; Sakamoto et al., 2006). Genomes encoding adw are found in genotypes A–C, F and G, while the genomes encoding both adr and ayr occur in genotype C, along with adw, as shown in table 3.12. However, these results are still incomplete because the number of isolates analyzed are small and were limited to certain geographic areas (Sugauchi et al., 2002). Also, subgenotypes C1 (Cs) and C2 (Ce) are the predominant strains amongst the five subgenotypes of HBV genotype C (Liu et al., 2002). HBV subgenotypes C1 and C2, B1 and B2 show virological and epidemiological differences that may lead to an altered clinical picture in patients infected with HBV of these serotypes.

A relationship has been reported between HBV genotype and ethnicity. A study from Spain reported a prevalence of genotype A with 52%, D 35% and F 7% (Huy et al., 2004; Chan et al., 2005; Sakamoto et al., 2006). The genotypes mostly prevalent in China are B and C (Sanchez-Tapias et al., 2002). Recently, data from India suggest that genotypes A and D are most prevalent (Zeng et al., 2005). In the United States, the prevalence of genotype A is 35%, B 22%, C 31%, D 10%, E <1%, F <1% and G 1% (Bamshad et al., 2001; Kumar et al., 2005; Thakur et al., 2005).

Table 3.12 Geographic distribution of HBV genotype and serotypes

HBV genotype	Geographic area	Serotypes
A	Northwest Europe, North America, Central America	adw_2, ayw
B	Indonesia, China, Vietnam	adw_2, ayw
C	East Asia, Korea, China, Japan, Polynesia, Vietnam	adw, ayr, adr
D	Mediterranean area, Middle East, India	ayw
E	Africa	ayw
F	Native Americans, Polynesia	adw
G	United States, France	adw
H	Central America	adw

3.6.2.4 Clinical outcomes of the HBV genotypes

In recent years, there has been an explosion of knowledge with respect to HBV genotypes and their association with viral latency, HBcAg, HBeAg seroconversion, pathogenesis of liver disease, immune escape, treatment response and resistance to antiviral drug therapy (Chu et al., 2003). But these data are not available from many parts of the world. In addition, HBV genotypes may influence the manifestation of clinical disease in the host (Kato et al., 2004). Kumar (2005) revealed that the genotype A is associated with chronic liver disease more frequently than genotype D in Europe (Arrais et al., 2008). Likewise, some studies from Asia have illustrated that genotype C has a higher disease-inducing capacity than genotype B. For example, they have shown that liver dysfunction due to liver cirrhosis and HCC were found with more frequent rate among HBV genotype C than with genotype B (Kumar et al., 2005). Since genotypes B and C are predominant in most of the Asian countries, results of comparisons were restricted to patients with only these genotypes. However, one study from Taiwan contradicts this finding and revealed that genotype B was more frequently identified than other HBV genotypes in less than 50-year-old HCC patients (Bollyky et al., 1996; Kao et al., 2000; Bamshad et al., 2001; Sakugawa et al., 2002; Sugauchi et al., 2003; Sumi et al., 2003).

Recent studies have shown that patients with genotype F are more likely to die from liver disease than those with genotypes A and D (Kao et al., 2000). A Swiss study demonstrated

that progression from acute to chronic hepatitis is more likely with genotype A than with genotype D, thus establishing a role of HBV genotype in the rate of recovery after acute infection (Mahtab et al., 2008). In addition, in USA genotype D was associated with an outbreak of fulminant hepatitis (Mayerat et al., 1999). However studies on the relationship between HBV genotypes and their clinical implication also need further investigation.

HBeAg seroconversion, has been inversely related with progression to cirrhosis and HCC among all the eight known HBV genotypes, however this rating is not yet complete (Garfein et al., 2004). A study from India had reported that genotype A is more often associated with ALT elevation, core antigen positivity and negative anti-HBe in patients aged 25 years and above and more frequently seen with liver cirrhosis (Mahtab et al., 2008). Recent studies have indicated an association between the HBV genotype B and HBeAg seroconversion at an earlier age, less active liver disease, slower progression to cirrhosis and a higher rate of HBeAg response to INF therapy compared with genotype C (Kumar et al., 2005). Moreover, studies from Taiwan and Hong Kong found that genotype B had a higher rate of HBeAg seroconversion compared to C (Chu & Lok 2002; Chan et al., 2003; Yuen et al., 2004; Janssen et al., 2005); whereas in Spain genotype A had a higher rate of seroconversion than D (Kao et al., 2000; Wai et al., 2002).

The relation between HBV genotypes and HBV DNA levels is still inconclusive. Whereas some studies show higher level of HBV DNA with genotype C, others show no difference, particularly with genotype D (Sanchez-Tapias et al., 2002). Most of the patients reported were tested at one point in time and therefore the relationship between HBV DNA level and HBV genotype cannot be excluded based on these studies alone (Kao et al., 2002; Chu et al., 2003; Sumi et al., 2003; Westland et al., 2003; Yuen et al., 2003).

Pre-core variation of HBV is also HBV genotype dependent. A precore variant is most common with genotypes B and D and less common with A and C (Chu et al., 2003). The common pre-core variant G1896 A is infrequently encountered in the USA and Northern

Europe, where genotype A predominates. Genotypes B, C and D, which frequently have T at nucleotide 1858, predominate in areas with a high prevalence of pre-core variants like Asia and the Mediterranean basin (Lindh et al., 1999; Grandjacques et al., 2000; Chu et al., 2003).

HBeAg seroconversion rates, mutational patterns in the precore/core promoter regions, and response to antiviral therapy varies with the genotype (Lindh et al., 1999). Hence, identification of HBV genotype is important for virus and disease surveillance worldwide. It has been demonstrated among Asian patients that such variants are more common in those infected with genotype C than those with B (Chu et al., 2003; Mahtab et al., 2008; Liaw & Chu 2009). A study from the USA had reported higher core promoter variants with genotypes C and D than A and B (Lindh et al., 1999). In Yemen, Sallam (2002) has studied the core promoter variants where two major groups were identified among blood donors with basic core promoter substitutions and major deletions (Chu et al., 2003).

With respect to anti-viral therapy, HBV genotypes have shown an influence on the response to interferon therapy (Sallam & Tong 2002). Recent studies with anti-hepatitis therapy have confirmed that HBeAg seroconversion occurs more often with genotypes A and B as compared to C and D (Wai et al., 2002); although, anti-viral therapy may result in a shift in the predominant genotype in such patients (Arankalle et al., 2003; Janssen et al., 2005).

The risk of recurrence of HBV infection is higher with genotype D following liver transplantation (Hannoun et al., 2002). However other studies have failed to demonstrate any increased risk of re-infection in post-transplant patients with genotypes A and D (Devarbhavi et al., 2002).

3.6.3 Method

All ELISA positive sera for HBsAg (15 samples) from each study [8 samples from community study (chapter 3) and 7 from clinical study (chapter 6)] were tested for HBV DNA by polymerase chain reaction (PCR) assay using two different methods, as described in

chapter 2. In the first method the 15 samples were tested using specific primers in a multiplex PCR amplification reaction. As the results of this test were puzzling, a second sequencing method was used to clarify the obtained findings. Only the positive samples (11 samples) obtained from the first PCR round were included in the second test.

3.6.4 Results

From a total of 15 samples, only 11 (73%) were shown to be positive for HBV DNA in the multiplex primer assay. Of the 11 cases, 6 (2 males and 4 females) were apparently healthy and 5 were known male cases of CLD. The age of the participants ranged from 25 to 59 years old (mean age= 46.3 years). Expression of genotypes showed genotypes A through E, as illustrated in table 3.13. The most frequent types found were B and D (5/11 for each), followed by C and E (3/11 for each) and type A (2/11). Genotype F was not found in this study.

Based on the results of the first method, multiple genotypes were found. For example, among healthy participants, 4 genotypes (A through D), were observed in one case. In cases with chronic liver diseases 2 genotypes were found in two cases (Band E, and B and D, respectively) and 3 genotypes (B, C and D) in another case. In addition, genotypes B and E were predominant in cases with chronic liver diseases; whereas genotype D was predominant among asymptomatic participants and with absence of genotype E. Genotype A was also absent in cases with CLD. As this method was flawed due to inexperience and laboratory technique, the results of the second method is used.

On the other hand, findings from the second assay showed only genotype D (7 from 11 tested samples). Some results of the first method did not match the results from the second method. For example, one asymptomatic participant and 2 cases of CLD did not have genotype D in the first assay (B and E, respectively) but showed only genotype D in the second assay. Three out of 6 (50%) asymptomatic individuals were found with genotype D and 4/5 (80%) of cases with CLD show the same genotype in the second assay.

Table 3.13 Characteristics of cases according to HBV genotypes

Participants	Age	Sex	First method					Second method
			A	B	C	D	E	D
Asymptomatic	50	m			+			
Asymptomatic	55	f		+				
Asymptomatic	36	f	+	+	+	+		+
Asymptomatic	42	m				+		+
Asymptomatic	45	f				+		+
Asymptomatic	59	f	+					
Cirrhoses	55	m					+	+
Chronic Hepatitis	45	m		+			+	+
Chronic Hepatitis	42	m		+	+	+		
HCC	55	m					+	+
Chronic Hepatitis	25	m		+		+		+
Total N (%)		(7: 4)	2 (18.2%)	5 (45.5%)	3 (27.3%)	5 (45.5%)	3 (27.3%)	7/11 (63.6%)

3.6 5 Discussion

Two types of HBV genotyping assays were used resulting in this study with products of mostly different genotype profiles. The first one implemented with Naito et al. (2001) method using type-specific primers (A-F) to detect the specific genotype (Arankalle et al., 2003; Girlanda et al., 2004). By this method, multiple-genotypes were identified for the same individual where it was not seen common in the second method. This variation includes the detection of 2 to 4 genotypes for the same individual from A through E. However, such variation may be interesting for the decision of selecting the adequate method in future genotyping work. This first method however, has puzzled the interpretation of the findings which argue the conduction of the second method.

Based on findings from the second method, genotype D was identified dominant in all the 7 positively reacted HBV DNA samples produced in the second method. This findings concurs with a previous study in Yemen where described the presence and dominance of genotype D among indigenous Yemeni blood donors while genotype A was found only in communities with continuing African links (Sallam & Tong 2004). In addition, our findings from the second method are concurs with reports from other parts of the Middle East like Egypt, Turkey, Iran, and Tunisia, all showing that genotype D is the most common genotype in this region (Saudy et al., 2003; Amini-Bavil-Olyaee et al., 2005; Abdo et al., 2006; Bahri et al., 2006).

Although the distribution of HBV genotypes varies across regions and with population migration, it is documented that genotype D is dominant in the Middle East and genotype A more common in Africa (Saudy et al., 2003; Amini-Bavil-Olyaee et al., 2005; Abdo et al., 2006; Bahri et al., 2006).

Although, the use of the second method have diagnosed the predicted genotype in the area with confidence and it confirms the dominance of genotype D in the studied sample,

exclusion of whether minor variant of viruses in the sample that could circulate in the same patient might explain the presence of some of the observed genotypes in the first genotyping method in this study. It is interesting also that some of the genotype readings from the first assay were not matched with the findings of the second method. This could be explained as that due to multiplex primers method could be just give multiple band readings rather than specific bands which could show different in the reading of the detected genotypes and thus it needs further check out to confirm the actual products.

Although in Yemen, very limited number of studies were conducted to identify HBV genotypes; there is no information on the correlation between HBV genotypes and the outcome of acute HBV infection. There is no published data comparing the rate of HBeAg seroconversion, activity of liver disease and rate of progression to cirrhosis and HCC among patients with HBV genotypes. The lack of such studies is probably related to the limited laboratory infrastructure to perform the required investigations

CHAPTER 4: RISK FACTORS FOR HBV IN HEALTHY INDIVIDUALS

4.1 Introduction

This chapter describes the risk factors for infection with hepatitis B virus among a population attending a primary health care setting in Aden Governorate. Based on the information obtained through a questionnaire, data were analysed to identify risk factors associated with HBV infection. Factors examined included those related to transmission of infection and social factors. This analysis would help to provide a better understanding of the risk factors for HBV infection in Aden. It also will help the local health authorities to develop preventive and control measures to avoid transmission.

4.2 Objective

To identify risk factors for HBV infection among the study population in Aden.

4.3 Literature review

HBV infection can develop into acute or chronic hepatitis and furthermore may lead to cirrhosis or hepatocellular cancer. These conditions may require short to long period hospitalisation, follow-up care with economic consequences to the patient, his family and the health system (Arauz-Ruiz et al., 2002; Sugauchi et al., 2003; Kato et al., 2004; Kimbi et al., 2004; Amini-Bavil-Olyaee et al., 2005; Hannoun et al., 2005; Fung et al., 2006; Mahtab et al., 2008). Recent studies have associated the development of progressive liver disease with past exposure of the individual to risk factors including among others infection with HBV in early childhood, high viral load; viral genotype, viral mutants and age of HBeAg seroconversion (Zuckerman & Zuckerman 2000; WHO 2002b).

4.3.1 Mode of transmission of HBV

The understanding of the modes of transmission of HBV infection is considered an important step for the application of safety measures including the development of an optimal

vaccination programme for the country (Chen et al., 2006; Iloeje et al., 2006). Two main mode of transmission exist, vertical (mother-child) and horizontal. The dominance of one over the other is dependent on the exposure to risk factors in each country. Communities with high HBV prevalence and high rate of HBsAg carriage, as a potential source of infection, are more prone to have high risk of HBV transmission (Mast et al., 1999; Zuckerman & Zuckerman 2000; Qirbi & Hall 2001; WHO 2002b). In the vertical mode of transmission, the opportunity of HBV intrauterine infection is low, unless there are concomitant factors such as a high HBV DNA concentration, the existence of viral mutations, a poor placental barrier and a low immune status of mothers (Andre 2000).

HBV is present in high titers in blood and serous fluids, ranging from a few virions to 10^9 virions per ml. It is also present in moderate in saliva, semen and vaginal secretions and at very low level in urine and faeces (Su et al., 2005). The probability of HBV transmission is dependent upon the concentration of infectious virions in the body fluids, the volume of material transferred and the route of inoculation, either percutaneous or mucosal (Beltrami et al., 2000; WHO 2002b; Hou et al., 2005).

In the Middle East, available studies on HBV infection show multiple modes of transmission (Beltrami et al., 2000; Chien 2007). Perinatal and child to child transmission are commonly reported (WHO 2002b). Parenteral transmission is considered the dominant mode of transmission, although it has recently been reduced due to routine screening of blood products (Toukan et al., 1990; Toukan 1996; Qirbi & Hall 2001). Limited information is available on sexual transmission and unsafe injection practices.

4.3.2 Risk factors for Hepatitis B infection

Risk factors for HBV infection include social and environmental factors, exposure to unsafe medical procedures and risky behaviour. These factors include intravenous drug use (IDU), men who have sex with men and heterosexual practices with multiple partners (Weild et al., 2000; Baklan et al., 2004; Budd et al., 2004; Garfein et al., 2004; Bialek et al., 2005). In

settings where these factors are prevalent, infection in adolescents and young adults are the commonest mode of transmission (Lamden et al., 1998; Hope et al., 2001). In the USA, 15%–20% of acute hepatitis cases are IDU related and this group is considered an important mode of transmission in UK (Gish & Gadano 2006). In developing countries, perinatal, child to child and parenteral transmission, transfusion of blood and its derivatives, haemodialysis, surgical interventions, dental extraction, tattooing, and wet cupping (Hijjamah) are considered important routes of transmission (Lamden et al., 1998; Bialek et al., 2005). In health care-settings, transmission occurs predominantly by percutaneous (needle-sticks or sharp injuries) or mucosal exposure of workers to the blood or body fluids of infected patients (Sagliocca et al., 1997; Mahoney 1999; Andre 2000; Qirbi & Hall 2001; Al-Faleh 2003; Khan et al., 2008; Ter Borg et al., 2008). Some health care-settings are also potentially high risk places. These include the risk of transmission in haemodialysis centres where high rate of HBV infection are reported either for their patients and health workers (Beltrami et al., 2000; Kermode 2004a; Jafri et al., 2006). Infection may also be transmitted between household contacts and between sexual partners, either homosexual or heterosexual (Beltrami et al., 2000).

There are variations in the factors associated with HBV infection and carrier status across the world. Studies have illustrated an association between HBV carriage and large family size, poor socioeconomic status, older age, low educational status and a history of past blood transfusion, surgery or contact with a jaundiced person (Baqi et al., 1999; Alter 2006).

Chowdhury et al., (2005), described that being older than 20 years of age, poverty, a low level of education, use of reusable glass syringes, being shaved by a community barber and being born at home are risk factors for HBV infection (Scott et al., 1990; Toukan et al., 1990; Toukan 1996; El-Sayed et al., 1997; Al-Nassiri & Raja'a 2001; Kuniholm et al., 2008). Unsafe practices of injecting and re-injecting either patient or even vials and saline bags are considered as a source of contamination by multiple-used needles/syringes to administer intravenous medications to multiple patients (Chowdhury et al., 2005).

There are no previous studies to describe risk factors for HBV in Aden. Studies in Sana'a have described that both vertical and horizontal occur and that these modes are the more frequent modes of transmission in this setting (Cadranel et al., 2007; Alter 2008). Risk factors in that setting included blood transfusion, surgery, hospitalisation, dental extraction and large family size (Scott et al., 1990; Al-Shamahy 2000). Some previous studies elsewhere, considered Qat as associated risk factor with liver damage (Al-Mamary et al., 2002). Also others have found that chewing Qat may cause certain disturbances to the health of the individual beside the social and economic damage (Halbach 1972) and it has a direct association with HBV infection (Fox et al., 1988) whereas not found significant association in the study of El-Sorori (1991) within the Yemeni community in Taiz (El-Sorori 1991).

4.3.3 Socioeconomic factors

Socioeconomic status was measured by a combination of elements constituting the social and economic background of the families enrolled to predict their association with HBV infection. Many studies on factors associated with HBV infection have included socioeconomic markers (Scott et al., 1990; Al-Nassiri & Raja'a 2001). These include employment, education, family size and overcrowding (Tiwari & Kumar 2005; CTC 2007). Other factors have included the availability of piped water, toilet facilities, electricity and ownership of a house, car, private or public insurance and household appliances (Stronks et al., 1997; Evans & Kantrowitz 2002). The economic status does not have a causal relationship but acts as a measure of exposure to suboptimal environmental conditions (Scott et al., 1990; Evans & Kantrowitz 2002; Topuzoglu et al., 2005).

4.3.4 Medical related factors

In medical care settings, percutaneous exposure has the main role of transmitting HBV infection. This include transfusion of unscreened blood or blood products, sharing unsterilized injection needles for I.V. drug use, haemodialysis, acupuncture, and injuries from contaminated sharp instruments sustained by hospital personnel (Evans & Kantrowitz 2002; Tiwari & Kumar 2005). Therefore, past medical history including blood transfusion, family

liver disease, and exposure to surgery or related condition such as circumcision are important factors associated with infection (Alter 2001; Hou et al., 2005; Karkar et al., 2006; Avazova et al., 2008).

A considerable improvement would be achieved if control and preventive measures are generally applied in health care settings such as those used for blood transfusion, medical procedures and awareness to avoid risk factors and the use of the HBV vaccine are implemented in the community (Brabin et al., 2002; Alter 2003b; Alter 2006).

4.4 Methods

This was a case-control analysis which enrolled 87 participants with positive HBV markers as cases and 451 participants who were free of HBV markers as controls. All the participants were enrolled using a cross-sectional design as described for objective one with a total of 538 participants. Most of the methodology used in this chapter was described in chapter two.

Given the epidemiological characteristics described in chapter 2, there seemed to be a biological change after 13 years of age and this cut-off was considered an influential element in predicting the association of anti-HBc marker with different risk factors. Cases and controls were therefore analysed in two separate groups. One analysis includes participants of ≤ 13 years old and a separate analysis was conducted for participants over 13 years old. The first group (≤ 13 years old) contained 24 cases infected with HBV and 317 controls giving a total of 341 participants. The second group was composed of 63 cases and 134 controls with a total of 197 participants.

4.4.1 Risk factors

The following potential risk factors were analysed: Socioeconomic and demographic factors including age, gender, education level, household size, employment, overcrowding index, availability of electricity, piped water and toilet facilities. Ownership of some appliances such

as radio, TV, fridge, satellite dish, landline or mobile telephone, bicycle, computer, car and having an internet service were also included.
Past medical history included blood transfusions, vaccination against HBV, hospitalization, surgery, receiving blood during surgical procedures, intravenous medications, visiting the dentist or having experienced dental extractions and acupunctural manipulations.

History of common/traditional practices and past exposures with other viruses, such as practicing of wet-cupping (Hijamah*), male circumcision, past hepatitis infection either for the participant or his/her family, malaria, schistosomiasis, tuberculosis, HAV and HEV were included. In addition variables related to the attendance to public or private clinics and their frequency were considered.
* (Hijamah: *is the name in Arab traditional medicine for wet cupping, where blood is drawn by vacuum from a superficial small skin incision for therapeutic purposes*)

Behavioural factors included multiple sexual partners, tattoos, sharing of razor blades, drinking alcohol, smoking and chewing Qat.

To assess the likelihood of association between the presence of HBV positive markers and the factors investigated, a univariate analyses followed by a logistic regression method were used. The odds ratios were estimated with 95% confidence intervals (CIs). Factors resulting from the univariate analysis with p-value < 0.2 were selected for logistic regression analysis using a backward multiple logistic regression model. All tests were 2-sided, and P values < 0.05 were considered statistically significant.

4.5 Results

4.5.1. Univariate analysis of factors associated with HBV infection in participants ≤ 13 years old

4.5.1.1 Socio-demographic factors

Twenty four cases and 317 controls ≤ 13 years old were analysed. Socio-demographic factors associated with HBV infection among ≤ 13 years old are described in table 4.1. The mean age of children infected with HBV was higher among cases than controls (6.6 and 5.4 years, respectively) but the p- value was = 0.2. Cases were more likely to be male than controls, but this was not statistically significant.

Educational status was classified into minors (not in education) and those already enrolled in education. There was no association between education and HBV infection.

Household size was stratified into three categories (< 5, 5-9 and > 9 members). Infection rates increased with increasing household size (from 8.3% to 54.2%). The odds ratio indicates an increased risk when there were more than 9 members in the household (OR= 4.7, 95% CI 1.02-21.9). Children with HBV were more likely to live in a rented house than controls (p = 0.06).

Cases with HBV infection were less likely to have received assistance by non-medical personnel during delivery, but this and the crowding index were not statistically significant.

4.5.1.2 Factors related to medical history

Analyses of factors related to medical history are shown in table 4.2. Cases were more likely to have received a blood transfusion than controls (13% and 3%, respectively, p = 0.05). All other risk factors, including vaccination, medical procedures such as surgery, intravenous medication, visit to the dentist, experiencing dental extractions, wet-cupping (Hijjamah) and circumcision by non-medical worker were not statistically significant with p values > 0.05. Similarly, hospitalisation, attendance to public/private clinics and the frequency of attendance were not statistically significant.

Table 4.1 Univariate analysis of socio-demographic factors associated with HBV infection in participants ≤ 13 years old

Variable		Cases =24 N (%)	Controls =317 N (%)	Odds ratio	95% C.I.	P-value
Age (years)	mean (±SD)	6.6 (4.0)	5.4 (4.1)	1.07	0.97-1.19	0.153
Gender	female	9 (37.5)	158 (49.8)	1	†	1
	male	15 (62.5)	159 (50.2)	1.66	0.70- 3.90	0.251
Education status	minor	11 (45.8)	187 (59)	1	†	1
	basic education	13 (54.2)	130 (41)	1.70	0.74- 3.91	0.283
Household size (members)	< 5	2 (8.3)	51 (16.1)	1	†	0.004
	5-9	9 (37.5)	196 (61.8)	1.17	0.25-5.59	0.843
	> 9	13 (54.2)	70 (22.1)	4.74	1.024-21.91	0.047
Living in	rented house	23 (77.7)	242 (76.3)	0.14	0.02- 1.06	0.057
Assisted during delivery by	medical	15 (62.5)	235 (74.1)	1	†	1
	non-medical	9 (37.5)	82 (25.9)	1.72	0.73-4.10	0.219
Crowdedness index	≤ 2.5 /room	13 (54.2)	125 (39.4)	1	†	0.256
	2.6-4.5 /room	7 (29.2)	91 (28.7)	0.74	0.28-1.93	0.537
	≥ 4.6 /room	4 (16.7)	101 (31.9)	0.38	0.12-1.20	0.100

† reference

Table 4.2 Univariate analysis of factors related to medical history associated with HBV in participant ≤ 13 years old

Variable		Cases =24 N (%)	Controls =317 N (%)	Odds ratio	95% C.I.	P-value
Blood transfusions		3 (12.5)	10 (3.2)	4.39	1.12-17.15	0.053
HBV vaccination		8 (33.3)	126 (39.7)	0.76	0.32-1.82	0.536
Surgery		2 (8.3)	8 (2.5)	3.51	0.70-17.54	0.141
Intravenous medication		15 (62.5)	179 (56.5)	1.29	0.55-3.02	0.671
Visit to a dentist		5 (20.8)	49 (15.5)	1.44	0.51-4.04	0.559
Dental extractions		3 (12.5)	27 (8.5)	1.53	0.43-5.48	0.456
Wet-cupping (Hijamah)		2 (8.3)	10 (3.2)	2.79	0.58-13.53	0.203
Circumcised ††	by a medical	7 (46.7)	87 (56.1)	1	†	1
	by a non-medical	8 (53.3)	68 (43.9)	1.46	0.51-4.23	0.484
Hospitalization		8 (33.3)	62 (19.6)	2.06	0.84 5.02	0.118
Public clinic attendance		19 (79.2)	247 (77.9)	1.07	0.38-2.96	0.990
Private clinic attendance		11 (45.8)	119 (37.5)	1.41	0.61-3.24	0.514
Frequency of attendance to public clinic*		2.5 (1.06)	2.3 (0.9)	1.33	0.87-2.05	0.191
Frequency of attendance to private clinic*		1.8 (1.02)	1.6 (0.8)	1.33	0.86- 2.07	0.199

† reference; †† (N=170, 15 cases and 155 controls); * mean (±standard deviation),

4.5.1.3 Factors related to previous diseases

History of hepatitis/jaundice in the participants or in a family member, history of tuberculosis, malaria, and schistosomiasis, HAV or HEV were not statistically significant as shown in table 4.3.

4.5.1.4 Factors related to the household environment

The availability of electricity, piped water and toilet facilities was seen more often among cases (100%) than controls (98%, 92% and 82%, respectively), as seen in table 4.4. However, the availability of a toilet in the household was statistically significant (p=0.02). The ownership of satellite and fridge were likely associated with HBV infection with p- value <0.05 for each (OR=0.4, 95% CI 0.2- 0.9 and OR = 7.6, 95% CI 1.0 – 57.5, respectively). None of the other selected personal appliances included (radio, television, etc.) were statistically significant (p > 0.05).

Table 4.3 Univariate analysis of factors related to previous disease associated with HBV infection in ≤ 13 years old

Variable	Cases =24 N (%)	Controls =317 N (%)	Odds ratio	95% C.I.	P-value
History of hepatitis	4 (16.7)	40 (12.7)	1.38	0.45- 4.25	0.531
Family history of hepatitis	9 (37.5)	104 (32.8)	1.23	0.52- 2.90	0.656
History of tuberculosis	1 (4.2)	1 (0.3)	13.74	0.83- 226.83	0.067
History of malaria	7 (29.2)	58 (18.3)	1.84	0.73- 4.64	0.197
History of schistosomiasis	1 (4.2)	3 (0.9)	4.55	0.46- 45.50	0.197
HAV positive	20 (83.3)	251 (79.2)	1.32	0.43-3.98	0.628
HEV positive *	0 (0)	13 (6.7)	†	†	0.999

† reference. * 13 cases and 207 controls were tested with a total of 220.

Table 4.4 Univariate analysis of factors related to household environment associated with HBV infection in ≤ 13 years old

Variable		Cases =24 N (%)	Controls=317 N (%)	Odds ratio	95% C.I.	P-value
Availability of	electricity	24 (100)	309 (97.5)	†	†	0.999
	piped water	24 (100)	290 (91.5)	†	†	0.238
	toilet	24 (100)	260 (82)	†	†	0.020
Ownership of	radio	15 (62.5)	239 (75.4)	0.41	0.13- 1.37	0.148
	television	23 (95.8)	270 (85.2)	4.00	0.53- 30.36	0.180
	satellite	16 (66.7)	135 (42.6)	0.37	0.15- 0.89	0.027
	fridge	23 (95.8)	238 (75.1)	7.63	1.02 – 57.45	0.045
	mobile phone	9 (37.5)	104 (32.8)	1.23	0.52- 2.90	0.638
	land line phone	10 (41.7)	112 (35.3)	1.31	0.56- 3.04	0.533
	computer	2 (8.3)	9 (2.8)	3.11	0.63- 15.28	0.163
	internet	0 (0)	6 (1.9)	†	†	0.999
	bicycle	2 (8.3)	20 (6.3)	1.35	0.30- 6.15	0.698
	car	5 (20.8)	44 (13.9)	1.63	0.58- 4.59	0.353

† not defined

4.5.2 Multivariate analysis of risk factors associated with HBV infection

Variables obtained from the univariate analysis with p values < 0.2 were entered in step one of the logistic regression backward analysis. Only a household size of > 9 members per house was associated with HBV infection (AOR = 5, 95% CI = 1.07- 23.3) after adjusted with other factors in the last step of the backward analysis (table 4.5).

Table 4.5 Multivariate analysis of factors associated with HBV infection in children ≤ 13 years old.

Variable		Adjusted Odds Ratio	95% C.I.	P-value
Household size	< 5	1	†	0.002
	5-9	1.07	0.22- 5.22	0.935
	> 9	4.99	1.07- 23.30	0.041

† reference

4.5.3 Univariate analysis of factors associated with HBV infection in participants > 13 years old

4.5.3.1 Sociodemographic factors

A group of 63 cases and 134 controls > 13 years old were analysed. The socio-demographic factors associated with HBV infection among participants > 13 years old are described in table 4.6. The mean age was higher for cases than controls (45.2 and 38 years, respectively, p = 0.002). Gender and educational status were not associated with HBV infection, however, cases were less likely to have primary education than controls (p = 0.05). All other factors related to this group such as household size, employed, crowdedness and residing in a rented house were not significant.

Table 4.6 Univariate analysis of sociodemographic factors associated with HBV infection in participants > 13 years old

Variable		Cases =63 N (%)	Controls =134 N (%)	Odds ratio	95% C.I.	P-value
Age		45.2 (13.1)*	38.0 (15.5)*	1.03	1.01- 1.06	0.002
Gender	female	27 (42.9)	36 (47.8)	1	†	1
	male	36 (57.1)	70 (52.2)	1.22	0.67- 2.23	0.520
Education	illiterate/read and write	31 (49.2)	47 (35.1)	1	†	0.108
	primary	10 (15.9)	35 (26.1)	0.43	0.19 -1.0	0.050
	secondary school	11 (17.5)	35 (26.1)	0.48	0.21- 1.08	0.075
	higher diploma	11 (17.5)	17 (12.7)	0.98	0.41- 2.37	0.966
Employed		34 (54.8)	68 (51.9)	0.89	0.49- 1.63	0.703
Household size (members)	< 5	8 (12.7)	25 (18.7)	1	†	0.179
	5-9	39 (61.9)	64 (47.8)	1.90	0.78- 4.64	0.156
	> 9	16 (25.4)	45 (33.6)	1.11	0.42- 2.96	0.833
Crowdedness	≤ 3 /room	20 (31.7)	47 (35.1)	1	†	0.425
	3.1-5 /room	25 (39.7)	60 (44.8)	0.98	0.49-1.97	0.953
	≥ 5.1/room	18 (28.6)	27 (20.1)	1.57	0.71-3.46	0.267
Rented house		10 (15.9)	16 (11.9)	0.719	0.31-1.69	0.448

* mean (±standard deviation), † not defined

4.5.3.2 Factors related to medical history

The analyses of factors related to medical history associated with HBV among participants > 13 years old are shown in table 4.7. Surprisingly, the proportion of cases who had received blood transfusion was lower than in controls (6% and 17%, respectively, $p< 0.05$).

All other factors, including vaccination status, exposure to surgery, blood transfusion during surgical intervention, intravenous medications, visits to dentists or experiencing dental extractions, circumcision, acupuncture, wet cupping, having been hospitalised or attending public/private clinics and frequency of attendance were not statistically significant.

4.5.3.3 Factors related to previous diseases

The variables included in this analysis were history of hepatitis/jaundice, tuberculosis, malaria, schistosomiasis, evidence of HAV and HEV infection. None of these variables was statistically significant as shown in table 4.8.

4.5.3.4 Life style and behavioural factors

The lifestyle and behavioural factors are shown in table 4.9. Cases were more likely to drink alcohol than controls ($p = 0.02$, OR = 2.9, 95% CI= 1.2-7.5). Other variables did not have statistically significant associations, including smoking, chewing Qat, use of tattoos, sharing of razor with others and multiple sexual partners.

4.5.3.5 Factors related to the household environment

Nearly all participants had electricity, television or a fridge as shown in table 4.10. Ownership of a landline telephone was associated with HBV infection (OR = 2.8, 95% C I= 1.3-5.9, $p < 0.01$).

All other factors such as the availability of piped water and toilet facilities were not statistically significant. In addition, those related to the ownership of personal appliances such as radio, television, satellites, fridges, mobile telephones, computers, bicycles and cars or use of the internet services were not statistically significant.

Table 4.7 Univariate analysis of factors related to medical history associated with HBV in participants > 13 years old

Variable	Cases =63 N (%)	Controls =134 N (%)	Odds ratios	95% C.I.	P-value
Blood transfusion	4 (6.3)	23 (17.2)	0.33	0.11-0.99	0.048
Vaccination status	56 (11.1)	125 (6.7)	1.74	0.62- 4.90	0.297
Surgery	15 (23.8)	34 (25.4)	0.92	0.46- 1.85	0.813
Blood with surgery	4 (6.3)	15 (11.2)	0.54	0.17- 1.69	0.289
Intravenous medication	47 (74.6)	98 (74.2)	1.08	0.55- 2.14	0.827
Visit to dentist	31 (49.2)	63 (47)	1.09	0.60-1.99	0.774
Dental extraction	15 (24.2)	35 (26.3)	0.88	0.44-1.77	0.728
Circumcised by †† by a medical	10 (27.8)	17 (24.3)	1	†	1
by a non-medical	26 (72.2)	53 (75.7)	1.19	0.48- 2.98	0.696
Acupuncture	4 (6.3)	6 (4.6)	1.45	0.39-5.32	0.579
Practiced wet- cupping (Hijjamah)	5 (8.2)	11 (8.2)	0.96	0.32- 2.90	0.948
Hospitalisation	26 (41.9)	59 (44)	0.89	0.50- 1.64	0.715
Public clinic attendance	53 (84.1)	115 (85.8)	0.88	0.38- 2.01	0.754
Private clinic attendance	17 (27.0)	46 (34.6)	0.68	0.35- 1.32	0.259
Frequency of attendance public clinic	2.26 (1.86)*	2.98 (3.08)*	1.11	0.78-1.58	0.566
Frequency of attendance private clinic	0.90 (1.35)*	0.81 (1.88)*	0.87	0.55-1.36	0.528

†† (N= 106, 36 cases and 70 controls), † not defined, * mean (±standard deviation)

Table 4.8 Univariate analysis of some practices and previous diseases associated with HBV infection in > 13 years old

Variable	Cases =63 N (%)	Controls =134 N (%)	Odds ratios	95% C.I.	P-value
History of hepatitis	14 (22.2)	23 (17.2)	1.38	0.66-2.90	0.398
Family history of hepatitis	15 (23.8)	45 (33.6)	0.62	0.31- 1.22	0.167
History of malaria	29 (46)	55 (41.7)	1.23	0.67-2.24	0.509
History of schistosomiasis	2 (3.2)	3 (2.3)	1.43	0.23-8.79	0.689
History of tuberculosis	2 (3.2)	1 (0.8)	4.36	0.39- 49.02	0.233
HAV infection	63 (100)	132 (98.5)	†	†	0.999
HEV infection *	7 (13.7)	18 (18)	0.73	0.28-1.87	0.505

† not defined. (* 50 cases and 86 controls were tested, total 136)

Table 4.9 Univariate analysis of life style and behavioural factors associated with HBV in participants > 13 years old

Variable	Cases =63 N (%)	Controls =134 N (%)	Odds ratios	95% C.I.	P-value
Drink alcohol	11 (18)	9 (6.8)	2.94	1.15- 7.51	0.024
Smoking cigarette	16 (25.4)	28 (21.4)	1.29	0.64- 2.61	0.480
Chewing Qat	21 (33.3)	41 (31.3)	1.13	0.59-2.15	0.700
Tattoos	3 (5)	3 (2.3)	2.18	0.43-11.14	0.348
Shared razor or shaving blades	0 (0)	2 (1.5)	†	†	0.999
Multiple sexual partners	5 (35.7)	9 (64.3)	0.82	0.26- 2.57	0.735

† not defined

Table 4.10 Univariate analysis of factors related to household environment associated with HBV infection in > 13 years old

Variable		Cases =63 N (%)	Controls =134 N (%)	Odds ratios	95% C.I.	P-value
Availability of	electricity	63 (100)	132 (98.5)	†	†	0.999
	piped water	60 (95.2)	128 (96.2)	0.94	0.23- 3.88	0.929
	toilet	57 (90.5)	122 (91)	0.93	0.33- 2.62	0.897
Ownership of	radio	58 (92.1)	118 (88.1)	1.57	0.55- 4.51	0.399
	television	63 (100)	127 (94.8)	†	†	0.999
	satellite	45 (71.4)	91 (67.9)	1.18	0.61- 2.28	0.619
	fridge	63 (100)	133 (99.3)	†	†	0.999
	mobile phone	40 (63.5)	81 (61.4)	1.14	0.61- 2.11	0.682
	land line phone	52 (82.5)	84 (63.2)	2.81	1.34-5.89	0.006
	computer	4 (6.3)	8 (6)	1.07	0.31- 3.69	0.917
	internet	0 (0)	7 (5.2)	†	†	0.999
	bicycle	9 (14.5)	22 (16.5)	0.85	0.37- 1.98	0.702
	car	13 (21)	38 (28.4)	0.66	0.32- 1.35	0.250

† not defined

4.5.4 Multivariate analysis of risk factors associated with HBV infection in > 13 years old.

Variables obtained from the univariate analysis with a reading of p value < 0.2 were entered in the logistic regression backward analysis. The following variables met the criteria for selection and were found at the last step of analysis which includes: age, household size, blood transfusion and possessions of land line telephone as shown in table 4.11.

The analysis identified that age increased the risk for HBV infection for each year (AOR=1.03, 95% CI=1.01-1.05), household size (5-9 members, AOR=2.9, 95% CI = 1.1-7.6); and ownership of a land line telephone (AOR = 2.8, 95% CI = 1.3-5.8) were associated with and increased risk of HBV infection while a history of blood transfusions (AOR = 0.3, 95% CI = 0.1-0.8) decreased the risk.

Table 4.11 Multivariate analysis of factors associated with HBV infection in participants > 13 years old

Variable		Adjusted Odds ratio	95% C.I.	P-value
Age		1.03	1.01-1.05	0.005
Household size	<5	1	†	0.053
	5-9	2.91	1.12 – 7.56	0.028
	>9	1.57	0.56 – 4.44	0.396
Blood transfusion		0.26	0.08 - 0.81	0.020
Land line phone		2.77	1.32 – 5.84	0.007

† reference

4.6 Discussion

To implement a prevention programme of hepatitis B requires at least baseline knowledge of the epidemiology of the disease in the target population. In Aden such specific information was not available. This is the first case-controlled study which investigated the risk factors for hepatitis B infection in Aden and its neighbouring governorates.

The initial findings suggested that age ≤ and > 13 years old had different seroprevalence of infection and their age was used as a cut-off point. The analysis of the risk factors was therefore analysed as two distinct groups, those under or above the age of 13 years.

Forty six variables were analysed in this study, of these, only four were significant after backward logistic regression analysis. The main risk factors associated with HBV infection were age, a large family size greater than 5 members, having received transfusions of blood-products and having a landline telephone.

Even after stratification into age groups ≤ and > 13years, age was still positively associated with HBV infections in adults. The mean age was higher in cases than in controls for participants ≤ and > 13 years old. The association of HBV infection with age was weaker in children than in adults. This is likely to be due to the cumulative effect of exposure over a longer period of time in adults, as the marker used (IgG) did not distinguish between recent and old infections. The logistic regression analysis, in this study confirms the increased seroprevalence of HBV infection with increasing age; with each year increasing the risk by a factor of 1.03.

Vertical transmission is unlikely to be a significant mode of transmission in children under 13 years of age, a very few young children were infected. However, in participants over 13, the increasing seroprevalence of HBV may reflect the absence of control methods in the past, particularly of blood transfusion, sterilisation of surgical

equipment and communal use of equipment. Similarly, it could reflect the absence of vaccination until 2000.

Our findings show disagreement with West Africa data where hepatitis B vaccine was not routinely used, and which highlighted that most HBV infections occurred in childhood (Zuckerman & Zuckerman 2000; WHO 2002b). In addition, studies from populations in other parts of Yemen have reported that children have a low prevalence. Scott (1990) and Al-Nassiri (2001) reported lower rates of infection among children < 15 years old than in adults (Whittle et al., 1990; Edmunds et al., 1993). The age cohort effect of the HBV infection has been reported in other Middle East countries, however, most of the data in the region were based on studies on adults whereas very few included children (Scott et al., 1990; Al-Nassiri & Raja'a 2001).

The provision of the HBV vaccine within the EPI since 2000 seems to have had a positive impact in the prevention of HBV infection among children. The low frequency of infection among children may also reflect a reduction in post-transfusion-related infections through the use of safer blood supplies and more widely practiced infection control procedures among medical personnel in recent years.

The household's characteristics are often quoted in the literature as increasing the risk of acquiring the infection (Andre 2000; Al-Faleh 2003). HBV infection is more likely to occur within large families suggesting that person to person transmission can occur in these conditions. It is possible that an infection within the household occurs through common utensils shared by the household members (Tiwari & Kumar 2005; Topuzoglu et al., 2005; CTC 2007). Our study also demonstrated that there is an increased risk of HBV infection when the household size increased over five members. Family size is often confounded by the presence of carriers in the household (particularly those with positive HBeAg), economic status of the family, education level of the parents and other members and the habit of sharing items like razors and toothbrushes (Bile et al., 1992;

Omer et al., 2001b; Alter 2003a). A study in New Zealand found that where there were more than five carriers in household this was major risk factor for HBV infection (Phoon et al., 1987; Arboleda et al., 1995). Similar results were found in another study showed that a household with many carriers results in an increased rate of infection (Milne et al., 1987; Phoon et al., 1987).

Poor housing quality and overcrowding are associated with poverty, specific ethnic groups and increased susceptibility to disease (Euler et al., 2003). In addition, the existence of HBV carriage or current/past history of hepatitis of member (s) in a household increase the prevalence of HBV infection (Alter 2003a). In Yemen however, Scott (1999) and Al-Nassiri (2001) showed no association between HBV infection and household size in their study population in Sana'a (Abdool Karim et al., 1991). This difference between our study and the study in Sana'a could relate to the differences in the study design and population settings.

Among medically related factors, surprisingly, blood transfusion had a reverse association with HBV infection in adult cases (AOR=0.2, 95 % CI=0.1-0.8, p= 0.04). This finding was inconsistent with findings reported by studies conducted in Sana'a where blood transfusion was an important factor for transmitting HBV infection (Scott et al., 1990; Al-Nassiri & Raja'a 2001). Sallam et al (2002) identified that there was a lower rate of HBV carriers among blood donors in Aden (6.7%) than in Sana'a (15%) (Scott et al., 1990; Al-Shamahy 2000) and also in comparison with populations residing Socotra Island or residents from an African ethnic minority in Sana'a (Sallam & Tong 2002).

The transmission of HBV via blood transfusion in Yemen has become less frequent than in the past because of the implementation of control measures used in blood transfusion facilities in the country (Sallam et al., 2003b). Recently, the Ministry of Health paid attention to the diagnostic laboratory services and facilities for blood transfusions

particularly in the medical laboratories and blood banks at the central level and in governorates. In 2000, the first national workshop was held to develop a strategy to separate blood transfusion services from diagnostic laboratories. In March 2005, training courses were held with Italian expertise for the staff of blood banks (Own 2005). According to the report of the director of the blood bank in Aden, "the blood bank is applying compulsory control measures in the field of blood transfusions in Aden's branch. These include measures of periodic check-up of hospitals' laboratories responsible for supplying the blood to patients" (Own 2005).

A study from the Middle East identified a reduction in the transmission of HBV via blood or blood-products in the last few years (Omer 2007). This reduction was associated with improvement of the control measures for blood transfusions in the medical care settings (Al-Faleh 2003; Ayoola et al., 2003; Arafa et al., 2005).

The ownership of a landline telephone (AOR=2.8, 95% CI = 1.32 – 5.84, p value <0.01) was found to be associated with HBV infection. This is also a marker of wealth in this setting, suggesting that cases were economically better than controls.

We found no significant association between medical factors, medical history and sexual behaviour. These findings are consistent with studies elsewhere which did not found association of HBV infection and these variables except dental surgery (Andre 2000; Ghavanini & Sabri 2000; Al-Faleh 2003; Hasan 2005; Panhotra et al., 2005; Al Awaidy et al., 2006; El Sherbini et al., 2006). Previous studies from Sana'a have reported associations between HBV infection and surgery, transfusion of blood or hospitalisation (Arboleda et al., 1995). In Lebanon, individuals who reported a history of hepatitis/jaundice had received blood transfusion or had been exposed to gastrointestinal endoscopy had a higher risk of HBV infection (Scott et al., 1990; Al-Shamahy 2000). Although circumcision has been reported as risk factor among Somali

households in Liverpool UK (Baddoura et al., 2002), it was not found as risk factor in this study.

Nosocomial transmission from patient to patient accounts for a substantial disease burden in countries with inadequate infection control, including the reuse of contaminated medical or dental equipment, failure to use appropriate disinfection and sterilization practices for equipment and environmental surfaces and improper use of multi-dose medication vials (Brabin et al., 2002).

However, there still remain several un-answered questions about the quality control of laboratories, emergency and blood transfusion units and their relevance to HBV transmission. Also, issues such as inappropriate practices of multiple uses of syringes and needles in hospitals and health centres should be investigated in-depth.

It is important to consider the limitations in this study. This was a cross sectional survey and thus it was not possible to account for infected individuals who did not survive, whether neonates, babies, children or adults, resulting in a selection bias to a healthier population. Also there are other shortcomings for collecting accurate information about sensitive practices. For example, information on sexual partners and the use of IV recreational drugs, alcohol consumption and others are highly sensitive in this environment. In addition, there must be an important recall bias for factors that had potentially occurred several decades earlier. The cumulative effect of HBV infection on the markers used would result in an attempt to correlate the characteristics of the patients at the time of the study with an infection that occurred at some point in the past. For economic reasons we were unable to test for some other markers of active HBV infection such as HBV DNA and/or hepatitis B e antigen (HBeAg), IgM anti-HBc an indicator of early acute HBV infection, and anti-HBs (antibodies to HBsAg) to exclude those who might have resolved past infection. Therefore, some participants in this category may have been missed since some cases with acute self-limited primary HBV infection never have detectable HBsAg in the blood. Despite the high sensitivity and

specificity of ELISA method, it is not without drawbacks. Since the method is based on coating of solid phase with recombinant antigens used to capture specific antibody. This may result in false positive reaction unrelated to the HBV. Therefore, for further studies in this field, an accurate and proper selection of markers of recent infection could allow taking into account the risk conditions temporarily associated with acquiring HBV infection.

HBV vaccines were introduced in the country and became used in practice since the year 2000. Because of the early acquisition of HBV infection, efforts were made to vaccinate children under one year of age within the EPI programme. However the vaccination programme does not include high risk groups of the population such as health care workers, partners or the family members of HBV infected patients. In addition, HBV screening is uncommonly performed at present; it could be offered routinely, for instance to women attending antenatal clinics.

Further educational programs should be targeted to both public and hospital personnel to increase awareness concerning this pathogen and the strict preventive measures required to avoid transmission of HBV.

CHAPTER 5: RISK FACTORS ASSOCIATED WITH HBV/HCV IN CHRONIC LIVER DISEASE

5.1 Introduction

This chapter describes the seroprevalence of HBV and HCV and the risk factors associated with these infections among patients with chronic hepatitis, cirrhosis or hepatocellular cancer in Aden.

Investigating the epidemiological characteristics of patients with chronic liver diseases will help in identifying potential factors associated with the transmission of HBV/HCV infection in the community. Understanding these factors will also provide an adequate knowledge for the application of preventive and control measures.

The literature review in this chapter focuses on the seroprevalence of HBV and HCV in patients with chronic liver diseases and the risk factors for infection in this population.

5.2 Objective

To identify the seroprevalence of HBV and HCV infection among patients with chronic liver disease.

To identify if infection with HBV and HCV are significant risk factors for chronic hepatitis, cirrhosis and hepatocellular cancer.

5.3 Literature review

Chronic liver disease is a term associated with a condition that tends to progressively destroy liver tissue over a long period of time (Mast et al., 1999). It often causes progressive hepatic fibrosis (Poynard et al., 2000; WHO 2002b). This can eventually become cirrhosis, causing hepatocellular carcinoma (HCC), liver failure, a need for liver transplant or death (Lin & Kirchner 2004; Vogt et al., 2006). Different reasons or causes lead to this condition, among them, HBV and or HCV infection (Zarski 2006; Bacchetti et al., 2007).

Chronic HBV infection is becoming prevalent worldwide and epidemiological studies have described it as a common cause of cirrhosis and HCC, particularly in endemic countries (WHO 2002b; Serin et al., 2005). Shortly after specific laboratory tests for markers of infection with HBV were developed, an association between hepatitis B, chronic liver diseases and primary hepatocellular carcinoma became apparent in different geographical sites (Yim & Lok 2006).

Large population-based cohort studies in areas of high HBV prevalence have provided the evidence establishing HBsAg carriage as a risk factor for HCC and liver disease (Zuckerman 1982; Donato et al., 1998; Raimondo et al., 2005a). Therefore, a strong correlation was established between the endemicity level of HBV infection and the frequency of HBV related chronic hepatitis and cirrhosis (Crook et al., 2003). These observations suggest that the current prevalence rate of chronic liver diseases found in the population actually reflects a previous endemicity level because the infection might have been acquired 10-20 years earlier (Deutsch & Hadziyannis 2008).

Three-quarters of patients with chronic hepatitis B in the world are in China, and sub Saharan Africa is also considered as a high endemic area (Khan et al., 2008). Prevalence of chronic hepatitis B in these regions reached up to 20%, with most infections occurring in the neonatal period or during early childhood (El-Serag 2001; Zeng et al., 2008). Many studies reported that the earlier in life the infection is acquired, the greatest

the risk of progression towards chronic liver disease in adulthood (Edmunds et al., 1993). A report from Taiwan showed that 30%-85% of babies born to HBsAg positive mothers become infected via a vertical route of transmission and they were prone to become chronically infected adults with high risk of developing chronic liver diseases and HCC (Beltrami et al., 2000; Zuckerman & Zuckerman 2000; WHO 2002b; Lai et al., 2003; WHO 2004c; Avazova et al., 2008; Ter Borg et al., 2008). Epidemiological studies from Mediterranean area have shown that up to 90% of children with chronic hepatitis were HBsAg positive (Yu et al., 2002)

Mast et al (1999), reported a relation between the occurrence of chronic liver diseases in families and the high transmission rate of HBsAg between siblings during early childhood (Gaeta & Giusti 1990). The higher prevalence of chronic HBV, as well as the longer period of exposure to infection largely explains the higher HBV related HCC risk in these endemic areas (Mast et al., 1999).

The prevalence of chronic liver disease in North America, northern, western, and central Europe, and in Australia is low (0.2%–0.5%), and most infections are transmitted during adolescence or adulthood through sexual contact or intravenous drug use (Bernier et al., 1982; Mast et al., 1999). Although, a low prevalence was seen in these countries, a trend of increasing chronic infections was also added with the migration of new families from endemic areas (Gaeta et al., 2000).

Previous study has suggested that HBV genotype B is associated with a earlier HBeAg seroconversion than genotype C but with low ALT levels after HBeAg seroconversion (Chu et al., 2002a), whereas other studies have found that more severe liver disease was found in carriers infected with HBV genotype C than in carriers with genotype B (Lindh et al., 1999; Chan et al., 2003) which may contribute to a higher risk of hepatocarcinogenesis. With regard to the administered drug, patients infected with HBV genotype C showed significantly better response to Pegylated IFN-a-2b than to

conventional IFN-a-2b and the rate of relapse was significantly greater in patients infected with genotype C than in those infected with genotype B (Zhao et al., 2007).

Based on epidemiological and geographical observations, HBV and HCV have been implicated in the pathogenesis of HCC. Hepatitis B antigens were present in the malignant tissue of patients with HCC (Chan et al., 2003; Yuen et al., 2004; Janssen et al., 2005). A higher incidence of this type of malignancy was reported among males compared with females and this is correlated with the higher rates of HBV carriages in males (El-Serag 2001; Parkin et al., 2001a; Liaw 2002; Fattovich et al., 2004). For example, in Hong Kong, HCC is the second most common cancer in males (WHO 2002b; Yu et al., 2002). In China, the high prevalence of HBsAg carrier (14%) and the high incidence of mortality by HCC (17 per 100,000 populations) are related (Lam et al., 2004).

In 1992, the International Association for Cancer Research reported that HCC was the fourth most common cancer in the world with a high incidence in Asian and Western Pacific populations, followed by African, European, and North and South American populations (Yu et al., 2002). The incidence rate found in Japanese was 5.5/100,000, African American 7.1/100,000, Hispanics 9.8/100,000 and Chinese 16.2/100,000 male populations (Parkin 1999; Marrero & Marrero 2007).

During the last two decades, increasing trends in the incidence of HCC have been noted in Australia, Central Europe, United Kingdom, Japan, North America and Italy (Parkin 1999). In countries with a low HBV prevalence, like the United Kingdom, mortality from HCC is 1 per 100,000. However, during the same two decades, there has been an increase in mortality due to liver cancer in all countries (Deuffic et al., 1998; Donato et al., 1998; El-Serag & Mason 1999; Andre 2000; Manno et al., 2004). A recent study in the U.S., has shown that over the last 10 years liver cancer has been the tumour with the highest increase in the incidence compared to other tumours (Parkin et al., 2001b). This

is probably due to the increase in the infection among adults particularly in those with risk behaviour.

Cirrhosis and HCC are considered the most frequent complications of HBV and HCV infections resulting in considerable morbidity and mortality and substantial social and economic burden (Marrero & Marrero 2007). Risk factors for HCC in patients with chronic HBV infection include male gender, family history of HBsAg or HCC, older age, presence of cirrhosis and past exposure with HCV (Lee et al., 2006). In Japan, occurrence of HCC was found associated with 25% of HBV and 57% of HCV infections (Serfaty et al., 1997; Mast et al., 1999; Lok & McMahon 2001; Lok & McMahon 2004). Akuta et al. (2001), has demonstrated the relationship between HCV infection and liver cirrhosis in young adults with a positive family history of similar or related liver disease (Nishioka et al., 1991). However, other studies have reported that, 30% to 50% of HCC associated with HBV, occurs in the absence of cirrhosis (Akuta et al., 2001).

Case-control studies have described the correlation between HCC and liver cirrhosis, chronic infection with HBV/HCV, aflatoxin B1exposure, sex, alcohol drinking, cigarette smoking, obesity, diabetes and elevated alpha-fetoproteins (Khan & Yatsuhashi 2000; Kuper et al., 2000; Yu et al., 2000; Trevisani et al., 2001; Nair et al., 2002; Sumi et al., 2003; Wu et al., 2003; El-Serag et al., 2004; Fattovich et al., 2004; Marrero et al., 2005; Zarski 2006; Zago et al., 2007).

Lok and McMahon (2004), found that persons who are chronically co-infected with HBV and HCV may have more rapid progression of liver disease and a higher risk of developing HCC than carriers of HBV alone (Lok & McMahon 2004). A meta-analysis has also demonstrated that the concomitant infection of HBV with HCV is associated with a higher risk of HCC development than either infection alone (Lok & McMahon 2004; Yim & Lok 2006).

In Saudi Arabia dual infection of both viruses was significantly associated with cirrhosis and HCC than controls (Donato et al., 1998); however, other study in other part of the country have not shown the same (Khan et al., 2001a). These findings are similar to studies in Italy and France (Ayoola & Gadour 2004).
High rates of HCV infection have been reported in patients with chronic hepatitis, cirrhosis and hepatocellular carcinoma (Zarski et al., 2006; Cadranel et al., 2007). About half of patients with HCV commonly progress to chronic hepatitis and another 20% progress to cirrhosis and probable HCC (Adachi et al., 2008). Cirrhosis occurs after 10 to 20 years in approximately 20% of patients with chronic HCV (Bacchetti et al., 2007). Approximately 26% of newly diagnosed cases of cirrhosis are caused by chronic HCV or HBV infection (Nishioka et al., 1991; Nowicki & Balistreri 1995). HCV infection is found in wide-ranging proportions among HCC cases in different populations. It has been found in 75%-90% of cases in Japan, 60-75% in Spain, 44%-76% in Italy, and 31%-47% of HCC cases in the U.S (Serfaty et al., 1997).

Most HBV-and HCV-related HCC cases occur in the presence of cirrhosis (Fattovich et al., 2002; Echevarria et al., 2005; Donato et al., 2006). In areas of high HBV endemicity such as Asian and African countries, persons with cirrhosis have an approximately 3-fold higher risk for HCC than those with chronic hepatitis but without cirrhosis (Chen et al., 2003a; Raimondo et al., 2005b; Marrero & Marrero 2007).

The WHO estimates that the prevalence of antibody to HCV ranges from 1% to 12% (about 21.3 million) in the Eastern Mediterranean countries (WHO 1999a). This is close to the estimated combined number of infected people in Americas (13.1 millions) and Europe (8.9 millions) (Ikeda et al., 2005). Moreover, HCC were also reported in association with HBV/HCV infection in Middle East countries such as Egypt (93%), in Lebanon (67%), Saudi Arabia (53%), and Sudan (53%) (WHO 1999a; Altaf 2001; Omer et al., 2001a; Shobokshi et al., 2003b; Ramia & Eid-Fares 2006; Yaghi et al., 2006).

Al-Moslih and Al-Huraibi (2001), reported that the prevalence of HCV among patients with CLD in Sana'a, Yemen reached 61.3% (OR=13.5, 95% CI=5.1-44.8) (Al-Moslih & Al-Huraibi 2001) and more similar to what was found by El Gunaid (1993) (75.9%) among patients with CLD (El Guneid et al., 1993). Both studies reported HBsAg in 33.6% and 24.1%, respectively (El Guneid et al., 1993; Al-Moslih & Al-Huraibi 2001). There are no data on the prevalence of HBV/HCV among chronic liver patients, cirrhosis or HCC in Aden. This study therefore, would inform the role that these viruses have in patients with chronic liver disease in Aden.

5.4 Method

The method used in this chapter was described in chapter two where the total of 49 adults with a clinical diagnosis of chronic hepatitis, liver cirrhosis or hepatocellular cancer were enrolled in the main hospitals in Aden. A comparison group was selected randomly from participants attending health care centres in Aden, as described for objective one. This group was from the same age distribution of the cases and were free of any apparent liver disease. The ratio of cases to the comparison group was approximately 1:3 for a total of 49 cases and 174 controls.

5.4.1 Inclusion criteria

A case of chronic liver disease (CLD), in the opinion of the resident specialist, was diagnosed on the basis of signs and symptoms of hepatitis for more than 6 months and persistently abnormal liver function test (ALT/AST) and/or histopathology evidence and /or ultrasound evidence. These CLD cases include chronic hepatitis, cirrhosis and hepatocellular cancer.

To measure the strength of factors associated with liver disease, ORs with 95% CIs were computed using univariate analysis. Backward logistic regression was used to obtain AOR.

5.5 Results

5.5.1. Characteristics of patients with liver disease and controls

5.5.1.1. Socio-demographic characteristics

A total of 49 patients diagnosed with liver disease admitted to the three main hospitals of Aden Governorate in the period from April through September 2005 and 174 participants attending the health centres were included in the study. The description of their socio-demographic characteristics is shown in table 5.1.

The mean age (±SD) of cases was 43.3 (±14.2) years compared to 43.7 (±12.7) years in controls, however, both having the same range of age from 19 to 79 years. There were more males (63.3%) among the cases than the controls (55%).

Twenty two (44.9%) cases had no formal or basic education and 27 (55.1%) had secondary and higher education. In the controls, 116 (66.7%) had no formal or basic education while 58 (33.3) had secondary and higher education with a statistical significant difference between both cases and controls ($p< 0.05$). Thirty two (65%) cases were employed compared to 102 (59%) controls. However, no statistical differences were seen between them. There was a median of 8 members per household in cases than 7 in controls with approximately similar crowding ratio of 3 individuals per room.

5.5.1.2 Medically related characteristics

Eight (16%) of the cases had a history of blood transfusions with a similar proportion among controls. Higher proportions were observed among cases who received intravenous medication, practiced acupuncture or cupping or attended private clinics (90%, 22%, 22% and 61%, respectively) compared to controls (73%, 6%, 9% and 31%, respectively) with a statistical significant difference between both cases and controls ($p < 0.01$). In addition, the mean frequency of attending private clinics was higher in cases (1.9) than controls (1.4) with a significant statistical difference ($p < 0.01$). However, rates of vaccination by HBV vaccine, attending public clinics and its mean frequency were higher in controls than cases ($P < 0.05$) as shown in table 5.2.

Table 5.1 Sociodemographic characteristics of cases with chronic liver disease and controls

Variables		Cases N= **49** (%)	Controls N=**174** (%)	P value
Age / years	mean (±S.D) [IQR]	43.3 (±14.2) [33 -55]	43.7 (±12.7) [34-53]	0.856
Gender	male: female (% male)	31 : 18 (63.3)	95 : 79 (54.6)	0.329
Education	basic or no education	22 (44.9)	116 (66.7)	0.008
	secondary and higher	27 (55.1)	58 (33.3)	-
Employment	employed	32 (65.3)	102 (58.6)	0.415
	unemployed	17 (34.7)	72 (41.4)	-
Number of residents in the household	media [IQR]	8 [6-10]	7 (6-10)	0.557
Median crowding ratio*	median [IQR]	3 [2 -5]	3.2 [2-5]	0.142
Availability of	electricity	42 (85.7)	173 (99.4)	0.000
	piped water	39 (79.6)	166 (95.4)	0.001
	toilet	33 (76.3)	158 (90.8)	0.000

±SD= standard deviation; IQR = inter quartile range; * calculated as number of resident/number of bed rooms

Table 5.2 Medical history of cases with chronic liver disease and controls

Variables	Cases N= **49** (%)	Controls N=**174** (%)	P-value
Blood transfusion	8 (16.3)	27 (15.5)	0.999
Vaccination	0 (0)	16 (9.2)	0.026
Surgery	14 (28.6)	44 (25.3)	0.713
Blood with surgery	3 (6.1)	19 (10.9)	0.422
Intravenous medication	44 (89.8)	127 (73)	0.000
Visit to dentist	24 (49)	83 (47.7)	0.999
Dental extraction	24 (49)	43 (24.7)	0.851
Acupuncture	11 (22.4)	10 (5.7)	0.001
Wet-Cupping (Hijamah)	11 (22.4)	15 (8.6)	0.012
Hospitalization	24 (49)	77 (44.3)	0.627
Attend public clinic	32 (65.3)	151 (86.8)	0.001
Frequency of attending public clinic*	1.7 (±1.7)	2.3 (±0.8)	0.001
Attend private clinic	30 (61.2)	45 (31)	0.000
Frequency of attending private clinic*	1.9 (±2.3)	1.4 (±0.7)	0.011
Mean time to arrive clinic *	18 (±15.4)	15.2 (±10.4)	0.135

* mean and ±standard deviation

5.5.1.3 Previous diseases and behavioural characteristics

A history of hepatitis/jaundice or in family members, schistosomiasis, tuberculosis smoking and chewing Qat were seen more frequently in cases (59%, 59% 12%, 8.2%, 53% and 69%, respectively) than controls (18%, 29%, 3%, 2%, 25% and 36%, respectively). The differences were statistically significant, as shown in table 5.3. Other variables were not statistically significant.

5.5.1.4 Characteristics related to household environment

Electricity, piped water and toilet facilities were available in 86%, 80% and 76% of the households of cases compared to 99%, 95% and 91%, respectively, in controls ($p < 0.01$), as shown in table 5.4.

Proportions of ownership of personal appliances such as television, satellites, fridges, mobile phones and landline phone were lower in cases (84%, 51%, 69%, 35%, 49%, respectively) compared to controls (97%, 70%, 99%, 61% and 71%, respectively) with statistically significant differences ($p < 0.05$). The other characteristics were not statistically significant.

Table 5.3 Previous diseases and behavioural characteristics of cases with chronic liver disease and controls

Variables	Cases N= **49** (%)	Controls N=**174** (%)	p- value
History of hepatitis	29 (59.2)	32 (18.4)	0.000
Family history of hepatitis	29 (59.2)	50 (28.7)	0.000
History of malaria	28 (57.1)	74 (42.5)	0.076
History of schistosomiasis	6 (12.2)	5 (2.9)	0.016
History of tuberculosis	4 (8.2)	3 (1.7)	0.043
Serological evidence of HBV	14 (28.6)	62 (35.6)	0.397
Serological evidence of HBsAg	5 (10.2)	8 (4.6)	0.771
Serological evidence of HCV	1 (2)	1 (1)	0.999
Tattoos	5 (10.2)	6 (3.4)	0.067
Shared shaving razor	0 (0)	1 (0.6)	0.999
Multiple sexual partners	1 (2)	14 (8)	0.200
Drink alcohol	7 (14.3)	20 (11.5)	0.622
Smoking cigarette	26 (53.1)	44 (25.3)	0.000
Chewing Qat	34 (69.4)	62 (35.6)	0.000

Table 5.4 Characteristics related to household environment of cases with chronic liver disease and controls

Variables		Cases N= **49** (%)	Controls N=**174** (%)	P- value
Ownership of	Radio	41 (83.7)	156 (89.7)	0.312
	Television	41 (83.7)	168 (96.6)	0.003
	Satellite	25 (51)	121 (69.5)	0.018
	Fridge	34 (69.4)	173 (99.4)	0.000
	mobile phone	17 (34.7)	106 (60.9)	0.002
	land line phone	24 (49)	123 (70.7)	0.006
	Computer	0 (0)	12 (6.9)	0.073
	Internet	0 (0)	7 (4)	0.352
	Bicycle	8 (16.3)	27 (15.5)	0.999
	Car	9 (18.4)	47 (27)	0.265

5.5.2.1 Distribution of HBV and HCV infection in cases and controls

Table 5.5 shows the seroprevalence of HBV infection in cases and controls. Although it was unexpected a lower rate of infection was found among cases where 14 (29%) were positive with anti-HBc compared to 62 (36%) in controls, the rate of HBsAg was higher in cases than controls (10% vs. 5%, respectively), whoever all these results have not shown statistical significance (p=0.4 and 0.8, respectively).

Table 5.5 Seroprevalence of HBV and HCV in cases of CLD and controls

Variables	Cases N= 49(%)	(95% CI)	Controls N=174(%)	(95% CI)	p-value
Anti-HBc	14 (28.6)	(16.6- 43.3)	62 (35.6)	(28.5-42.6)	0.397
HBsAg	5 (10.2)	(1.7 – 18.7)	8 (4.6)	(1.5-7.7)	0.771
HCV	1 (2.0)	(-1.9 – 6.0)	1 (0.6)	(-0.6-1.7)	0.999

The 95% CI was 17, 43 for cases and 29, 43 for controls, as illustrated in figure 5.1. Among the sub groups of the cases, a higher rate of anti-HBc was found in 9 (50%) cases diagnosed as cirrhosis followed by a case of HCC and 4 of chronic hepatitis (20% and 15%, respectively) with a statistical significant difference between the subgroups ($p<0.05$), as illustrated in table 5.6 and figure 5.2. There was only one case of HCV among both cases and controls.

Table 5.6 Distribution of Anti-HBc, HBsAg and HCV markers in CLD cases

Variables	All N=49 (%)	Anti-HBc† N (%)	(95% CI)	HBsAg N (%)	HCV N (%)
Chronic hepatitis	26 (53.1)	4 (15.4)	(1.5 –29.3)	3 (11.5)	1 (3.8)
Cirrhosis	18 (36.7)	*9 (50)	(27 – 73)	1 (5.6)	0 (0)
Hepatocellular carcinoma	5 (10.2)	1 (20)	(-15.1 –55.1)	1 (20)	0 (0)

*$p < 0.05$

Figure 5.1 Seroprevalence of HBV among case of chronic liver diseases and controls (95% confidence interval)

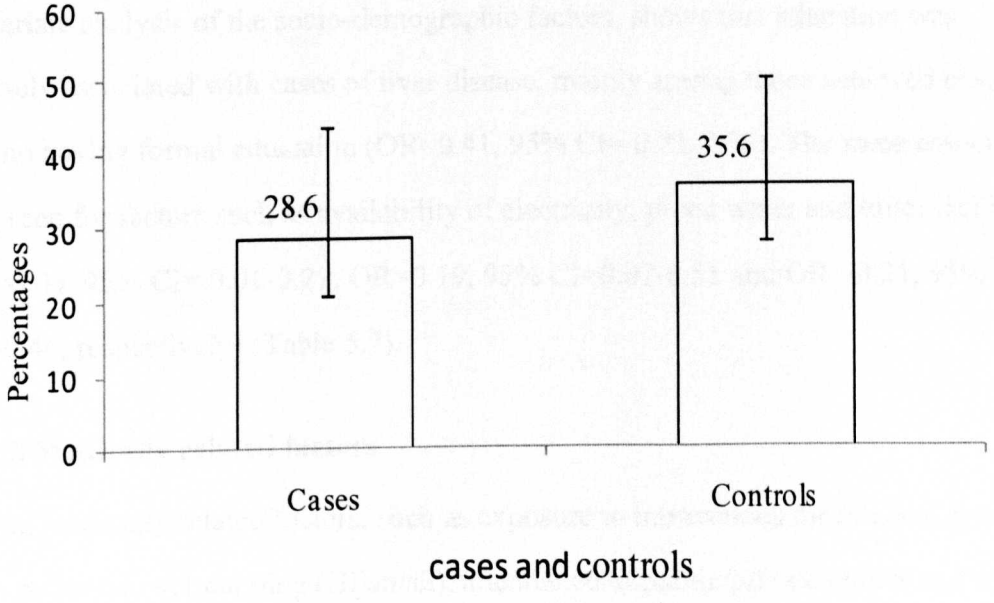
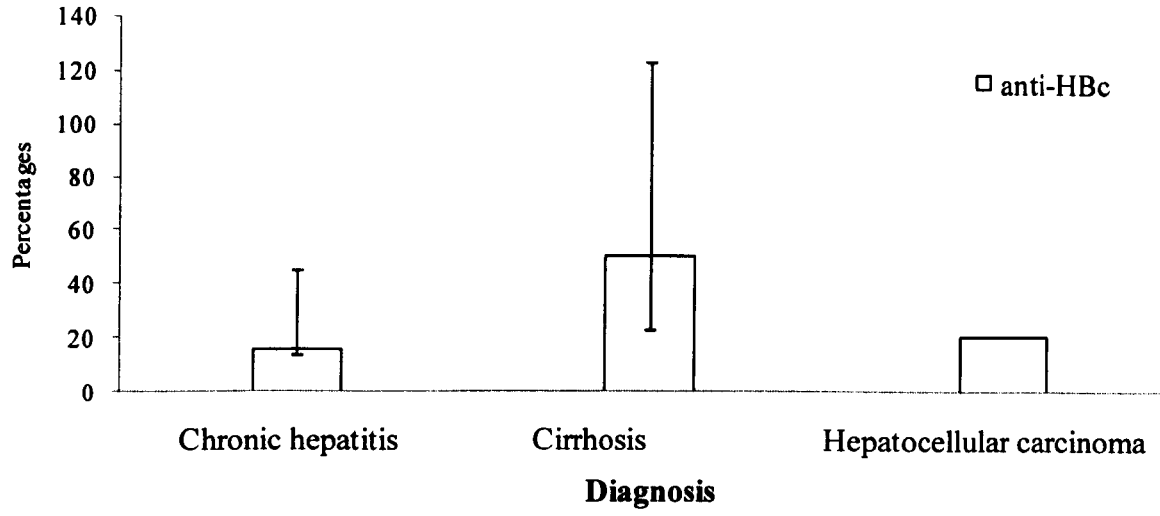


Figure 5.2 Seroprevalence of HBV infection in cases of CLD by diagnostic entity



5.5.3 Univariate analysis of risk factors for chronic liver diseases

All the 29 potential risk factors with p-value < 0.2 obtained from the data of characteristics of the patients and controls were admitted to the univariate analysis. However, essential factors such as age, sex and serologic evidences of HBV, HBsAg and HCV were also entered in the analysis.

5.5.3.1 Socio-demographic characteristics

Univariate analysis of the socio-demographic factors, shows that education was inversely associated with cases of liver disease, mainly among those achieved basic or with no having formal education (OR=0.41, 95% CI= 0.21-0.78). The same associations were seen for factors such as availability of electricity, piped water and toilet facilities (OR=0.04, 95% CI= 0.01-0.29; OR=0.19, 95% CI=0.07-0.51 and OR =0.21, 95% CI= 0.09-0.46, respectively) (Table 5.7).

5.5.3.2 Medically related factors

Among medically related factors, such as exposure to intravenous medication, practices of acupuncture, wet-cupping (Hijamah), attendance to public/private clinics and the

mean frequency of attending these clinics were seen with significant association with chronic liver diseases, as shown in table 5.8.

Table 5.7 Univariate analysis of study-subjects by socio-demographic factors.

Variables		OR	95% CI	p- value
Age / years		0.99	0.97 – 1.02	0.856
Sex		1.43	0.75 – 2.75	0.281
Education	basic or no education	0.41	0.21 – 0.78	0.006
	secondary and higher	†	†	1
Availability of	electricity	0.04	0.01 – 0.29	0.002
	piped water	0.19	0.07 – 0.51	0.001
	toilet	0.21	0.09 – 0.46	0.000

† Reference

Table 5.8 Univariate analysis of study-subjects by medical factors.

Variables	OR	95% CI	p-value
Intravenous medication	3.26	1.22 – 8.71	0.019
Acupuncture	4.75	1.88– 11.99	0.001
Wet-Cupping (Hijamah)	3.07	1.31 – 7.21	0.010
Attend public clinic	0.29	0.14 – 0.59	0.001
Attend private clinic	3.51	1.82 – 6.78	0.000
Frequency of attending public clinic *	0.58	0.43 – 0.80	0.001
Frequency of attending private clinic *	1.33	1.04 – 1.69	0.024
Mean time to arrive clinic *	1.02	0.99 – 1.05	0.142

* mean and ±standard deviation

5.5.3.3 Previous diseases and behavioural characteristics

Regarding factors of previous diseases like history of hepatitis, schistosomiasis and tuberculosis were seen associated with chronic liver disease, as shown in tables 5.9. Also variables related to behavioural factors include history of smoking and chewing Qat, were associated with chronic liver disease. Surprisingly, the serological evidence of HBV, HBsAg, and HCV has showed no association with chronic liver disease.

5.5.3.4 Factors related to household environment

As illustrated in table 5.10, the ownership of televisions, satellites, fridges, mobile and landline phones were seen less likely associated with the cases of chronic liver disease than controls.

Table 5. 9 Univariate analysis of study-subjects by previous diseases.

Variables	OR	95% CI	p-value
History of hepatitis	6.43	3.24 – 12.79	0.000
Family history of hepatitis	3.59	1.86 – 6.94	0.000
History of malaria	1.80	0.95 – 3.42	0.072
History of schistosomiasis	4.72	1.37 – 16.19	0.014
History of tuberculosis	5.07	1.09 – 23.46	0.038
Serological evidence of HBV	1.38	0.69 – 2.77	0.397
Serological evidence of HBsAg	1.28	0.39 – 4.19	0.683
Serological evidence of HCV	0.51	0.03 – 8.23	0.632
Tattooing	3.18	0.93 – 10.91	0.066
Smoking cigarette	3.34	1.73 – 6.44	0.000
Chewing Qat	4.10	2.07 – 8.10	0.000

Table 5.10 Univariate analysis of study-subjects by household environment

Variables		OR	95% CI	p-value
Ownership of	television	0.18	0.06 – 0.56	0.003
	satellite	0.46	0.24 – 0.87	0.017
	fridge	0.01	0.00 – 0.10	0.000
	mobile phone	0.34	0.18 – 0.66	0.001
	land line phone	0.39	0.21 – 0.76	0.005

5.5.3.5 Univariate analysis of case with chronic liver diseases

By the use of the univariate analysis, strong association was found between HBV and cirrhosis than other types of chronic liver diseases such as chronic hepatitis or HCC (table 5.11).

Table 5. 11 Univariate analysis of case with chronic liver diseases

Variables		HBV N (%)	Odds ratio	(95% CI)	p-value
chronic hepatitis	n = 26	4 (28.6)	reference	-	0.052
cirrhosis	n = 18	9 (64.3)	5.50	(1.34 – 22.53)	**0.018**
Hepatocellular carcinoma	n = 5	1 (7.1)	1.38	(0.12 – 15.72)	0.798

5.5. 4 Multivariate logistic regression analysis of risk factors for chronic liver disease

All the factors resulting from the univariate analysis with p value < 0.2 were admitted to the multivariate backward logistic regression analysis. Ten factors were independently

associated with liver disease. These included practices of acupuncture (AOR=7.24; 95% CI=1.86–28.16), wet-cupping (AOR= 3.66; 95% CI=1.20 -11.16) and attendance to private clinics (AOR= 3.33; 95% CI= 1.38 – 8.00) which were associated with chronic liver disease. Also, compared to controls, cases were 5 to 6 times more likely to have a history of hepatitis (AOR= 5.84; 95% CI= 2.40- 14.20) and a history of jaundice/hepatitis in their family member(s) (AOR= 4.03; 95% CI= 1.61 – 10.06), as shown in table 5.12.

Among other factors with a significant associations with chronic liver diseases, a history of tobacco smoking (AOR= 4.59; 95% CI= 1.81 – 11.60), chewing Qat (AOR= 6.14; 95% CI=2.01-18.80), education status (AOR= 0.41; 95%CI= 0.17 – 0.98) and ownership of refrigerators or landline phones (AOR= 0.01, 95%CI=0.00 - 0.07 and AOR=0.35, 95%CI= 0.14-0.86, respectively) were independently associated. However, the last three factors had an inverse association.

The use of intravenous medications had a positive association with chronic liver disease in the univariate analysis but this was not confirmed when fitted in the logistic regression model. Other covariates such age and sex as confounders as well as factors of serological evidence with anti-Anti-HBc, HBsAg and anti-HCV markers which were forced in the model, had no association with cases.

Table 5.12 Multivariate analysis for factors associated with chronic liver disease

Variables		OR	95% CI	P value
Acupuncture		7.24	1.86 - 28.16	0.004
Wet-Cupping		3.66	1.20 - 11.16	0.022
Attend private clinic		3.33	1.38 – 8.00	0.007
History of hepatitis		5.84	2.40 - 14.20	0.000
Family history of hepatitis		4.03	1.61 - 10.06	0.003
Tobacco smoking		4.59	1.81 - 11.60	0.001
Chewing Qat		6.14	2.01 - 18.80	0.001
Education	basic or no education	0.41	0.17 - 0.98	0.044
	secondary and higher	Reference	Reference	1
Ownership of fridge		0.01	0.00 - 0.07	0.000
Ownership of landline phone		0.35	0.14 - 0.86	0.023

5.6 Discussion

This is a first case-control analysis study in Aden City aiming to identify the seroprevalence of HBV and HCV infection among patients with chronic liver disease and the risk factors associated with cases and controls.

Surprisingly, a lower seroprevalence of HBV infection was found in cases than controls (Anti-HBc 29% vs. 36%) but carriage showed higher markers in cases than controls (HBsAg 10% vs. 5%), while approximately, a similar proportion for HCV infection (2% and 1%, respectively) was observed in both groups. In addition, neither HBV nor HCV could be identified as risk factors for cases or controls. These unexpected findings could be due to the small sample size of the study. In addition, the fact that there was low level of HBV (16% anti-HBc and 1.5% HBsAg) and HCV (0.4%) infections among participants attending primary health care facilities in Aden City, as illustrated in chapter 3. However, a number of studies across the world have reported a correlation between the rate of chronic liver disease and the endemicity of HBsAg in the population (El Guneid et al., 1993; Al-Moslih & Al-Huraibi 2001).

Most previous studies have shown correlation of only one hepatitis viruses, either HBV or HCV and sometimes jointly, with one clinical entity of chronic liver diseases such as HCC or cirrhosis. Very few studies have described the whole spectrum of chronic liver diseases which includes among them chronic hepatitis and hepatic autoimmune diseases. A study from Taiwan, where the incidence rate of HCC is among the highest in the world has shown that, chronic infection with HBV appears to account 80% of HCC cases and were co-infected with 10–30% of HCV (Yu et al., 2001; WHO 2002b; Yu et al., 2002). Another study among the U.S. military patients with chronic hepatitis has shown that HCV (46%) was more prevalent among cases than HBV (8%) (Lee et al., 1999). However, the findings of the two studies are inconsistent with our results, where the seroprevalence of HBV was 29% and HCV only one case of our studied subjects. It is assumed, as observed by the clinicians in the studied hospitals, that the

proportion of diagnosed autoimmune hepatitis is higher in Aden. Although to prove this hypothesis, additional laboratory tests are recommended such as NAAT, histopathologic confirmation and other biochemical investigation to exclude any involvement of blood-borne viral hepatitis. A further investigation with larger samples could be worth to determine the causative factors of chronic liver disease in this community.

Only 5 (10%) HCC cases were seen in our study compared to cirrhosis (37%) and chronic hepatitis (53%). From these HCC cases, only one was infected with HBV. Overall, few HCC cases were reported at the Aden Cancer Registry (official cancer registry for Aden and 3 adjacent governorates). Incidence data from 2002 through 2004, put HCC at the 10^{th} place of the most common reported cancer in males and number 14^{th} among females, with an age standardised rate of 1.9 and 1 per 100 000 population, respectively (Hyams 2000). The univariate analysis showed that cirrhosis was likely associated with HBV infection (OR= 5.5, 95%CI=1.3-22.5), but not for HCC or chronic hepatitis.

When analyzing medically related factors, the adjusted logistic analysis demonstrated that the practice of acupuncture and wet-cupping are independently associated risk factors for chronic liver diseases. In both cases, sharp instruments are used/reused and likely not adequately sterilized after each use. It was however, uncertain whether the reported acupuncture or wet-cupping treatments actually preceded the disease or if they were sought afterwards to relieve some of the symptoms of chronic liver disease.

In 2003 Ernst and Sheman conducted a Meta-Analysis on the association of acupuncture with HBV and HCV infections. No consistent relationships were found between the studies reviewed (ACC 2007). Some of these studies have suggested that acupuncture results in a modest increase in the risk for hepatitis C infections in populations where the seroprevalence is low. Others have reported a potential relationship between acupuncture and HBV infections (Ernst & Sherman 2003).

Positive associations were only ever found in communities where the use of disposable needles may not be fully established (Stryker et al., 1986).

Wet-cupping, a popular practice in the Middle East, where blood is drawn by creating a vacuum on top of a small skin incisions made by a sharp blade, performed at the site of an ache or pain in order to alleviate it. The probability of acquiring hepatitis viruses and with it chronic liver disease would greatly increase if the blade was reused without prior sterilisation. Again the use of disposable blades would greatly reduce any risk of infection. In this study, wet-cupping increased 4 fold the risk of CLD, although, the small sample of cases; still restrict the robustness of the findings.

The history of having hepatitis or jaundice in the individual or his/her contacted family members was a factor associated with chronic liver disease after adjustment with other independent factors. However, it is not clear in this study, that this reported jaundice is due HBV/HCV or other type of viral hepatitis. In addition, 99% of adults in Aden city had evidence of HAV infection and usually developed jaundice in young childhood or adulthood (seroevidence from findings of participants attending primary health care facilities in Aden Governorate during the time being of the study, as illustrated in chapter 3). Also the probability of horizontal transmission of HAV/HBV among the household members would be reasonable.

Most cases could not distinguish if the history of jaundice/hepatitis was caused by HAV or other types of viral hepatitis. Recall-bias could further underestimate the history of hepatitis among the family members. Ertekin and Selimoglu (2003) in a study of patients with chronic liver diseases demonstrated the relationship between liver diseases with patients who had a prior history of jaundice or hepatitis in their household (Kiyosawa et al., 1994; Sun et al., 1999; Shin et al., 2000). However, 25% of asymptomatic individuals infected during their infancy or early childhood with HBV

could develop chronic liver disease in their late life which would not be easily demonstrated.

Our study also provides evidence for other less recognized chronic liver disease risk factors. These include the use of private clinics (AOR=3.3, 95%CI= 1.4-8), a history of tobacco smoking (AOR=4.6, 95%CI=1.8-11.6), chewing Qat (AOR=6.1, 95% CI=2.0-18-8), low level of education (AOR=0.4, 95%CI=0.2-0.9) and economic markers such as the lack of household appliances such as refrigerators and landline phones (AOR=0.01, 95%CI=0.00 - 0.07and AOR= 0.35, 95%CI= 0.14 - 0.86, respectively).

Association of private clinics and CLD is understandable. These types of facilities are quickly expanding compared to the public clinics. The chronic nature of the disease and prolonged symptoms involved drive cases to select specialized private clinic.

In this study, tobacco smoking and chewing Qat were observed with a fourfold and six fold increase, respectively, in the association with CLD in cases compared to controls. Chewing Qat has not been found in the literature as a risk of chronic liver disease. Usually, chewing Qat sessions are associated with the use of tobacco smoking. Therefore, most of the Qat chewers are smokers (4chewers vs. 3 smokers) and the risk of chewing Qat could also attributed to the dominant effect of smoking, or both are markers of a risky behaviour. Qat and smoke is more prevalent among adult males which in turn have highly mobility and often engage in more risky behaviour for infection than females. Some studies in the past have suggested some harmful effect of Qat in liver function in experimental animals feeding with Qat leaves (Al-Mamary et al., 2002). El-Sorori (1991) has studied the relationship between the hepatitis carriage rate among Qat-users and non-users in Taiz, Yemen. No significant difference was seen between both groups in relation to the seroprevalence of hepatitis B carriage(El-Sorori 1991). In the contrary, Fox et al. (1988), in his study of viral hepatitis markers in Djiboutian population has found a significant association between the abuse of Qat and the chronic HBsAg carrier state (Fox et al., 1988). Besides certain disturbances to the

health of the individual, the chronic consumption of Qat can cause social and economic damage too (Halbach 1972).

A study of 53,000 Chinese individuals demonstrated that the use of tobacco increased the risk for acquiring HCC 2-fold (Chen et al., 2003b). Other studies across the world, confirm that smoking is an additional risk factor in developing cirrhosis or HCC, and that its relationship is stronger in the presence of an HBV and/or HCV infection (Kuper et al., 2000; Marrero & Marrero 2007). For HCC, both indirect and direct carcinogenic mechanisms are involved in its pathogenesis which is induced by chronic HBV/HCV infection (Tzonou et al., 1991; Donato et al., 2006). Our findings, however did not clarify the dose-effect of the tobacco or quantity of Qat used as well as the duration of the smoking and Qat history. To determine the relationship between length of use, number of cigarettes, quantity and frequency of Qat and their significance in developing HCC further prospective studies would be needed.
Participant's age, serological evidence of HBV, HBsAg and HCV infection were dropped from our model as they showed no independent association with chronic liver disease. Neither do they have an effect on the risk estimates of the other variables in the model.

The relationship between surgical or dental procedures and CLD did not show enough associations. Intravenous therapy was according to our univariate analysis associated with chronic liver disease although this was not demonstrated in our multivariate regression model. It was reported by some studies that, a large proportion of patients in developing countries, prefer injected medicines and considered these procedures as more efficacious than other routes of drug administration (Marrero & Marrero 2007). It is also documented that, the reuse of needles, syringes and other equipment used to inject medications can result in the transmission of blood borne pathogens, including hepatitis (Simonsen et al., 1999). In addition, iatrogenic transmission during anti-schistosomal therapy programs in Egypt in the 1980s has been recently linked to very

high levels of HCV infection (Sanchez et al., 2000; Talaat et al., 2003; Alter 2008). The risk of acquiring hepatitis and other liver diseases should thus be considered in such settings. These findings possibly support the significant association found in the univariate analysis between history of intravenous therapy and chronic liver disease. However, our small sample size also limits the findings of this factor and further study may be needed to illustrate this.

Our study is limited by its relatively small sample size which restricts the number of variables that can be examined by multivariate analysis. Also it was limited by lack of accurate classification of chronic liver disease as opposed only to chronic hepatitis, cirrhosis and HCC whereas autoimmune liver disease not yet clearly differentiated from the chronic hepatitis. This essentially may be due to the absence of advanced laboratory tests that was not available in these hospitals such as nucleic acid amplification test (NAAT) and poor utilisation of histopathologic tests. Additional limitations were seen in the collection of accurate information about sensitive practices (e.g. sexual partners, use of IV recreational drugs, alcohol consumption). Another restriction was the recall bias of events that had potentially occurred several decades earlier. The restriction on the use of only 3 serological markers (Anti-HBc, HBsAg and Anti-HCV) had added more limitation for the study and also to the none uses of the HBV DNA detection methods.

In conclusion, this study show a low seroprevalence of HBV/HCV in cases of chronic liver disease, however, HBV was associated with cases of cirrhosis. Some medically related factors such as the practice of acupuncture and cupping were identified among the risky practices associated with CLD where sharp instruments were used and reused. A history of being with hepatitis/jaundice was associated with CLD but a clear type of viral hepatitis was not identified. Other behaviours such as the use of tobacco and chewing Qat were seen associated with the CLD. The study also identified that the lack

of such as refrigerators or landline telephones among the household appliances were associated with presence of CLD.

It is anticipated that information yielded by this study whether on the seroprevalence of HBV and HCV infection on chronic liver disease cases or of their risk factors would likely have to improve our understanding of the role of these viruses in chronic liver diseases. It will also help in the application of the preventive and control measures of great relevance to the disease in the population. Furthermore, the control and prevention of the other risk factors will decrease the infection rate of HBV/HCV and, consequently, the occurrences of chronic liver disease. It is assumed that, maintaining the HBV vaccine within the EPI programme will have an impact, however, not earlier than 20 years from now.

CHAPTER 6: RISK FACTORS ASSOCIATED WITH HBV/HCV IN PATIENTS WITH POLYTRANSFUSED/HAEMODILAYSIS

6.1 Introduction

This chapter describes the seroprevalence of HBV and HCV and the association of these infections with a history of poly-transfusion and/or haemodialysis among patients in Aden.

A study of the risk of acquiring HBV and HCV in the health care facilities is an important element in developing approaches to avoid nosocomial infection. An analysis was undertaken to demonstrate the main factors associated with the transmission of these viruses either related to the host or to the health care environment. A greater understanding of these issues will help in developing recommendations for the use of preventive and control measures which should be then applied in the country.

6.2 Objective

To identify the seroprevalence of HBV and HCV infection among poly-transfused and/or haemodialysis patients enrolled from the health care facilities in Aden.

To identify if infection with HBV and HCV are significantly associated with poly-transfusion and haemodialysis practices in Aden.

6.3 Literature review

The high risk of HBV and HCV infection from medical and nonmedical parenteral exposures in developing countries is an important global public health problem. Blood transfusion is a route for transmission of blood-borne and hepatitis inducing viruses, and the haemodialysis sitting, where patients with chronic renal diseases are treated, facilitates transmission unless strict preventive measures are implemented.

6.3.1 Blood transfusion

Blood transfusion and its component therapies are an essential medical practice. The need for this type of therapy varies from one region to another. It is substantial for countries with high rates of malaria and anaemia in women and children (Talaat et al., 2003). According to the WHO reports, 30% to 42% of women living in developing countries have anaemia and about 24% of postpartum deaths are related to obstetric haemorrhage (Tagny et al., 2008). Children in these countries are also overwhelmed with high morbidity and mortality rates from anaemia due to malaria, hemoglobinopathies, malnutrition and other endemic factors (WHO 1999b; Mbanya et al., 2003). Therefore, transfusion becomes an emergency treatment for severe anaemia which may be fatal if not corrected. Safe transfusion equipment is thus essential to avoid the risk of transmission of blood-borne pathogens from donors.

Blood transfusion is a major source of HBV and HCV transmission in many developing countries (WHO 2002b; Weinbaum et al., 2005). Blood collected from large populations is inevitably associated with a risk of infectious pathogen transmission (Hoofnagle, 1990; Schreiber et al., 1996). The WHO reported in 2004 that up to 5% of the infections in Asia and Africa are caused by blood transfusions (Yotsuyanagi et al., 1998; Mast et al., 1999; Noborg et al., 2000) and that the prevalence of HBV and HCV is very high in these areas, especially among blood donors.

Serologic screening of blood for transfusion for HBV and HCV is the main method to reduce transfusion-transmitted infections, although the availability and quality of screening services varies across countries. Recent studies report the implementation of very sensitive screening assays in industrialised countries for the detection of blood-borne pathogens (WHO 2004b). These include the use of the nucleic acid amplification tests (NAAT) and others which has led to the detection of an occasional donor with an acute or recent HBV/HCV infection in industrialised countries (Niederhauser et al., 2008; Velati et al., 2008). Therefore, the risk of transfusion transmission of these viruses is becoming extremely low in these countries to approximately 11.1 per million blood units for HCV (Van der Bij et al., 2006; Niederhauser et al., 2008; O'Brien et al., 2008) and 15.3 per million blood units for HBV (Schreiber et al., 1996; Soldan et al., 1998; Glynn et al., 2000; O'Brien et al., 2007).

Unfortunately, the situation is quite different in most developing countries, where transfusion-transmission of HBV and HCV are still frequent and where the resources to routinely screen donors are not available (Donahue et al., 1992; Alter et al., 1997; Busch et al., 2003; Busch et al., 2005). The global survey of blood collection, screening, and transfusion practices conducted by the WHO program for transfusion medicine in 2002 showed a low rate (51%) of HCV screening among blood donors in 178 member states (Niederhauser et al., 2008).

The strategies used for testing blood donors vary greatly between countries where hepatitis viruses are high to those of low prevalence. For example, in low prevalence countries, like the USA and Japan, blood donors are screened for both HBsAg and anti-HBc (Lai et al., 2003). Individual positive for either are disqualified because of ongoing or potentially occult hepatitis B (OHB) (Allain et al., 2003; Busch et al., 2003). By contrast, in many developing countries in Asia and Africa where HBV is intermediately or highly endemic, about 16%-90% of adults may have either past or ongoing HBV infections (Niederhauser et al., 2008). Thus, the combined HBsAg and anti-HBc

screening strategy would disqualify most volunteer blood donors. An additional point is that, the incidence of HBV infection from HBsAg-negative and anti-HBc-positive donors with OHB is likely to be higher than in non-endemic areas (Chen et al., 2000; Alter 2003b).

In low-prevalence areas, no more than 5% of HBsAg negative and anti-HBc positive blood units contain HBV DNA (Allain et al., 1999; Kleinman et al., 2003). In contrast, in high-prevalence areas (such as India and Taiwan), serum HBV DNA is found in up to 25% of HBsAg negative and anti-HBc positive populations (Lai et al., 1989; Wang et al., 1991; Iizuka et al., 1992; Nagaraju et al., 1992; Minuk et al., 2005). This may account for the higher prevalence rate of OHB in anti-HBc-positive populations in these areas (Schneeberger et al., 2000).

Many efforts have been used to control such infections in different areas through the use of education, screening, the application of preventive safety measures in medical care and vaccination programmes for HBV (Reesink et al., 2008). Studies from the Middle East, for example, have identified a reduction in the transmission of HBV via blood or blood-products in recent years. This reduction is related to the remarkable improvement of the application of control measures dealing with blood transfusion in the medical care settings (Alexander & Kowdley 2006).

In Yemen, few studies have been performed in this field. Scott et al (1990) and Alshamahi et al (2001) have reported that blood transfusion is an important risk factor for transmitting HBV and HCV infection in Sana'a (Andre 2000; Ghavanini & Sabri 2000; Al-Faleh 2003; Hasan 2005; Panhotra et al., 2005; Al Awaidy et al., 2006; El Sherbini et al., 2006). In addition, Scott suggested that because assay reagents were not always readily available in Yemen in the 1980s, blood may not have been tested for hepatitis markers, resulting in a higher rate of hepatitis transmission from blood transfusions (Scott et al., 1990; Al-Nassiri & Raja'a 2001). Sallam and Tong identified a

lower rate of HBV carriers among blood donors in Aden (6.7%) than in Sana'a (15%) (Scott et al., 1990). In recent years transmission of HBV via blood transfusions is becoming less frequent because of the application of control measures in the blood transfusion facilities of the country. However, no studies are available, on quality assurance of the tests and processes to detect blood-borne agents, including HBV and HCV, in the health care facilities concerned with blood transfusion or haemodialysis and particularly in facilities in remote areas.

Blood services in Yemen are based mainly on volunteer blood donors. Blood is donated by friends, family member or relatives and sometimes through donation campaigns. Donors are not paid. A general medical examination for fitness is usually performed, followed by a serum investigation of HIV, HBV and HCV markers using ELISA 4th generation. Any positive person is excluded from the donation. The maximum quantity permitted to be donated is 450 cc per setting (Sallam & Tong 2002). However, individuals within the window period of HBV or during the late stage of infection (OB) where it could not be detected by this method; therefore, the possibility of transmitting the infection is still considered.

6.3.2 Haemodialysis (HD)

Patients receiving HD are considered at high risk of acquiring HBV/HCV infections and more vulnerable to blood-borne viral hepatitis than others because of frequent blood transfusions, injections, partial immunosuppression and, on some occasions kidney transplants (Omer 2007). The duration of haemodialysis treatment and nosocomial HBV/HCV transmission have also been suggested as contributing factors (Olmer et al., 1997). An additional concern for patients receiving dialysis is that, they are suffering from a partial impairment of their immune system resulting in an increased susceptibility to infections including hepatitis viruses. These infections, in turn, lead to the increased morbidity and mortality (Sandhu et al., 1999; Scotto et al., 1999; Carrilho et al., 2004; Amiri et al., 2005; Olut et al., 2005).

Recently, a marked decline in the incidence rate of HBV infection has been observed in patients treated in HD facilities in many countries across the world. This is a result of the application of specific control measures, including the use of HBV vaccines; whereas HCV rates are still reported to be high (Verkade et al., 2007). The prevalence of anti-HCV antibodies in dialysis patients has been reported to range from 20% to 81.6% across the world (Carrilho et al., 2004; Olut et al., 2005; Alter 2008). For example, in European countries, different rates of infection have been reported in the UK (3%), Netherlands (3.4%), Hungary (15%), Italy (16%), and Poland (44%) (Ramezani et al., 2007; Alter 2008). There are also different reported rates among HD patients across the Middle East such as Iraq (7%), Turkey (19%), Iran (24.8%), Jordan (34.6%), Syria (49%) and Saudi Arabia (55.7%) (Jadoul 2000; Schneeberger et al., 2000).

A dramatic decrease in the incidence of these viruses in HD patients has also been due to the application of other control measures such as universal precautionary measures, screening techniques, selection of blood donors, HBsAg positive patients' isolation during dialysis, routine vaccination of uraemic patients and the use of erythropoietin as a substitutive therapy for blood transfusion (Othman & Monem 2001; Bdour 2002; Shobokshi et al., 2003a; Amiri et al., 2005; Olut et al., 2005).

Currently in Aden, haemodialysis cases were undergoing pre- serological tests to identify the present of HIV, HBV and HCV infections. This is followed by separation of cases on the dialysis machines. Every six months, the tests are repeated for all patients admitted to the dialysis unit as part of the control measures. Reliable and valid data on HBV and HCV prevalence and incidence among blood recipients and patient under haemodialysis could be useful in assessing the magnitude of the problem in Aden. It will also illustrate the risk factors associated with infection to document the effectiveness of prevention programs.

6.4 Methods

The method used in this chapter was described in Chapter two.

6.4.1 Definition of cases

A total of 39 adults with a history of multiple transfusions or who had received haemodialysis were enrolled as cases from the main hospitals and haemodialysis facilities in Aden. Admitted cases to the hospital or to the haemodialysis centre who presented at least one of the following conditions were included: bleeding disorder such as haemophilia A; haemophilia B; Von Willebrand's disease; homozygous Thalassemia; homozygous Sickle Cell Disease; Congenital Methemoglobinemia and other bleeding disorders or a history of haemodialysis on more than 3 occasions, transfusions at least once with a total of at least ten units of allogeneic blood or other blood components (i.e. whole blood, plasma, red blood cells or platelets). The diagnosis was obtained from hospital records before enrolment.

6.4.2 Definition of controls

A comparison group was selected by randomly selecting participants attending the health care centres in Aden, as described for objective one. Controls were selected of their age distribution as was the same of the cases and had received or not blood or blood products, less than 3 pints and were not receiving haemodialysis. The ratio of cases to controls was approximately 1:4 with 39 cases and 174 controls.

To measure the strength of factors associated with HBV and HCV as HD or blood transfusion, ORs with 95% CIs were computed using univariate analysis. Backward logistic regression was used to obtain AOR.

6.5 Results

6.5.1 Characteristics of polytransfused/HD patients and controls

6.5.1.1 Socio-demographic characteristics

Thirty nine cases were admitted in the three main hospitals in Aden Governorate from April through September 2005 and were enrolled in the study. Of these, 31 were receiving haemodialysis and were polytransfused and 8 patients were polytransfused but were not receiving haemodialysis (4 with Haemophilia; 2 with Thalassemia; 2 with Sickle Cell Disease). The description of the socio-demographic characteristics of cases and controls is shown in table 6.1.

The mean age (±SD) of cases was 42.7 (±14.8) years compared to 34.7 (±12.7) years for controls. The male-to-female ratio was 1.3:1 in cases (M/F, 22/17) and 1.2:1 (M/F, 95/79) in controls. No significant differences were found in the age and gender ratios.

Thirty (77%) cases had basic or no formal education and 9 (23%) had secondary or higher education compared to 116 (67%) and 58 (33%) of the controls, respectively ($p< 0.05$). Seventeen (44%) cases were employed compared to 102 (59%) controls. There was a median of 8 members per household in cases and of 7 in controls, with approximately similar ratio of 4 individuals per room in their household. However, all of these variables did not show a statistically significant difference.
Electricity, piped water and toilet facilities were available in 82% vs. 99%, 56% vs. 95% and 31% vs. 91% respectively; in households of cases and controls, with $P < 0.001$ for each factor.

6.5.1.2 Medically related characteristics

Table 6.2 shows the medical characteristics of cases and controls. All 39 (100%) cases had received blood transfusions with an average of 14 ± 26 blood units compared to 27 (16%) of the controls with a mean of 0.2 ± 0.6 blood units. Thirty one (79.5%) cases had a history of haemodialysis with a mean frequency of 171 ±172 times per life and within an average of 2.7 ±2.9 years; All cases had been hospitalized (as per case

definition) compared to 77 (44%) of the controls. All the above results have shown significant statistical differences between cases and controls ($p< 0.001$).

Past visits to a dentist or a history of dental extractions were seen in 15 (39%) and 8 (21%) cases, and 83 (48%) and 43 (25%) controls, respectively and 3 (8%) cases and 15 (9%) controls practiced cupping, however, it was not statistically significant difference.

Twelve cases (31%) had undergone surgery and one case (3%) had received blood during surgery compared to 44 (25%) and 19 (11%) of the controls, respectively. Cases had attended public and/or private clinics more frequently (90% and 39%) than controls (87% and 31%, respectively) but these difference were not statistically significant. Cases reported requiring more time to arrive to the clinics (29 minutes) than controls (15 minutes) ($P < 0.001$).

Table 6.1 Sociodemographic characteristics of cases and controls

Variables		Cases = **39** N (%)	Controls =**174** N (%)	P value
Age / years	mean (S.D) [IQR]	42.7 (14.8) [34-50]	43.7 (12.7) [34-53]	0.691
Gender	male: female (% male)	22 : 17 (56.4)	95 : 79 (54.6)	0.861
Education	basic /no formal		116 (66.7)	0.255
	education	30 (76.9)		
	secondary and higher	9 (23.1)	58 (33.3)	-
Employment	employed	17 (43.6)	102 (58.6)	0.108
Number of residents in the household	media [IQR]	8 (6-10)	7 (6-10)	0.557
Median crowding ratio*	media [IQR]	3 (2-5)	3.2 (2-5)	0.142
Availability of	electricity	32 (82.1)	173 (99.4)	0.000
	piped water	22 (56.4)	166 (95.4)	0.000
	toilet	12 (30.8)	158 (90.8)	0.000

* mean; SD=± standard deviation

Table 6.2 Medical characteristics of cases and controls

Variables	Cases = 39 N (%)	Controls = 174 N (%)	P value
Blood transfusion	39 (100)	27 (15.5)	0.000
Frequency of transfusion *	13.8 (26.3)	0.2 (0.6)	0.000
Haemodialysis	31 (79.5)	0 (0)	0.000
Frequency of haemodialysis *	147.6 (168.6)	0 (0)	0.000
Period of being haemodialysis/years*	2.4 (2.9)	0 (0)	0.000
Hospitalisation	39 (100)	77 (44.3)	0.000
Surgery	12 (30.8)	44 (25.3)	0.547
Blood with surgery	1 (2.6)	19 (10.9)	0.134
Intravenous medication	39 (100)	127 (73)	0.000
Visit to dentist	15 (38.5)	83 (47.7)	0.375
Dental extraction	8 (20.5)	43 (24.7)	0.681
Wet cupping (Hijamah)	3 (7.7)	15 (8.6)	0.999
Attend public clinic	35 (89.7)	151 (86.8)	0.792
Attend private clinic	15 (38.5)	45 (31)	0.449
Frequency of public clinic attendance*	2.2 (1.3)	2.3 (0.8)	0.332
Frequency of private clinic attendance*	1.6 (2.2)	1.4 (0.7)	0.326
Mean time to arrive clinic *	28.5 (16.7)	15.2 (10.4)	0.000

* mean and ± standard deviation

6.5.1.3 Previous diseases and behavioural characteristics

Cases reported more frequently hepatitis/jaundice, malaria, schistosomiasis and tuberculosis than controls, as shown in table 6.3. Similarly cases were more likely to share shaving razor, smoking and chewing Qat than controls, but these differences were not statistically significant. The proportion of cases with a history of hepatitis in family members or who had received vaccines against the HBV was higher in controls (29% and 9%, respectively) than cases (10% and 0%, respectively $p < 0.05$, for both).

The prevalence of anti-HBc, HBsAg and anti-HCV antibodies in cases and controls is illustrated in the same table. From the 39 cases, 26 (67%) were anti-HBc positive compared to 62 (36%) of the 174 controls ($p<0.001$). The carrier rate (HBsAg positive) was lower (8%) in cases than controls (13%) but this was not statistically significant ($p=0.32$). For anti-HCV antibodies, 18 (46%) cases were positive compared to only one control ($p<0.001$).

Table 6.3 Previous diseases and behavioural characteristics of cases and controls

Variables	Cases = **39** N (%)	Controls =**174** N (%)	P value
History of hepatitis	10 (25.6)	32 (18.4)	0.372
Family history of hepatitis	4 (10.3)	50 (28.7)	0.015
Vaccination	0 (0)	16 (9.2)	0.048
Anti-HBc	26 (66.7)	62 (35.6)	0.001
HBsAg	3 (7.7)	8 (12.9)	0.319
Anti-HCV	18 (46.2)	1 (5.3)	0.001
History of malaria	25 (64.1)	74 (42.5)	0.020
History of schistosomiasis	3 (7.7)	5 (2.9)	0.164
History of tuberculosis	2 (5.1)	3 (1.7)	0.227
Multiple sexual partners	3 (7.7)	14 (8)	0.999
Tattoos	1 (2.6)	6 (3.4)	0.999
Shared shaving razor	3 (7.7)	1 (0.6)	0.020
Drink alcohol	5 (12.8)	20 (11.5)	0.786
Smoking cigarette	13 (33.3)	44 (25.3)	0.321
Chewing Qat	19 (48.7)	62 (35.6)	0.146

6.5.1.4 Characteristics related to household environment

Proportion of cases who own personal appliances such as television, satellites, fridges, mobile phones, landline phone and bicycle were lower in cases than controls with statistically significant differences for all ($p < 0.05$), as shown in table 6.4

Table 6.4 Characteristics related to the household environment of cases and controls

Variables		Cases = **39** N (%)	Controls =**174** N (%)	P value
Ownership of	radio	34 (87.2)	156 (89.7)	0.581
	television	26 (66.7)	168 (96.6)	0.000
	satellite	19 (48.7)	121 (69.5)	0.016
	fridge	21 (53.8)	173 (99.4)	0.000
	mobile phone	15 (38.5)	106 (60.9)	0.012
	landline phone	7 (17.9)	123 (70.7)	0.000
	computer	1 (2.6)	12 (6.9)	0.470
	internet	1 (2.6)	7 (4)	0.999
	bicycle	1 (2.6)	27 (15.5)	0.034
	car	8 (20.5)	47 (27)	0.426

6.5.2. Dual infections with HBV and HCV among cases and controls

Table 6.5 shows the frequency of dual infections among cases and controls, exposed to either blood transfusions, haemodialysis or both. Twelve (30.8%) cases had dual

infections with both HBV and HCV, 14 (35.9%) were infected with HBV only, 6 (15.4%) with HCV only and 7 (17.9%) were free from infection. Only one control (2.5%) was found with dual infection of HBV and HCV.

Table 6.5 Dual infection of HBV and HCV among cases

		HCV					
		Cases = 39			Controls = 96*		
		Positive N (%)	Negative N (%)	p-value	Positive N (%)	Negative N (%)	p-value
HBV	Positive N (%)	12 (30.8)	14 (35.9)	1.00	1 (1.0)	39 (40.6)	0.417
	Negative N (%)	6 (15.4)	7 (17.9)	-	0 (0)	56 (58.3)	-

* Only 96 of controls were tested for anti-HCV

6.5.3. Risk factors for anti-HBc and anti-HCV markers in polytransfused and haemodialysis cases with and without hepatitis markers.

An analysis of factors associated with HBV/HCV infections in patients with polytransfusion/HD was undertaken. These include factors under sociodemographic, past medical diseases or interventions, previous diseases, other related behaviours and ownership of household appliances. Table 6.6, only shows those factors produced from the analysis with p value < 0.2. In summary, findings from this table, illustrates that, the frequency and the time of being under haemodialysis sittings (years) was statistically associated with HBV and HCV infection ($p<0.05$ and <0.01, respectively). In addition, history of malaria ($p< 0.01$ for all) was also statistically associated with HCV infection. All the other factors were not statistically significant for either with HBV or HCV infections.

An analysis for HBsAg carriage was attempted; however, there were no any statistical significant differences.

Table 6.6 Risk factors for anti-HBc and anti-HCV infection in polytransfused/haemodialysis cases.

Variables		Anti-HBc			Anti-HCV		
		positive	negative	P value	positive	negative	P value
Blood transfusion		26 (66.7)	13 (33.3)	-	18 (46.2)	21 (53.8)	-
Frequency of transfusion †		11.4 (16.5)	18.5 (39.9)	0.431	16.1 (34.1)	11.7 (17.8)	0.613
Number of transfusions	<10 units	19 (70.4)	8 (29.6)	0.566	12 (42.9)	16 (57.1)	0.723
	≥10 units	7 (63.6)	4 (36.4)	-	6 (54.5)	4 (45.5)	-
Haemodialysis		22 (71.0)	9 (29)	0.402	16 (51.6)	15 (48.4)	0.247
Frequency of haemodialysis †		192.5 (183.6)	57.1 (80.9)	**0.016**	244.1 (170.9)	64.5 (116.4)	**0.000**
Haemodialysis sets	0	4 (50)	4 (50)	0.074	2 (25)	6 (75)	**0.006**
	< 100	7 (50)	7 (50)	-	3 (21.4)	11 (78.6)	-
	100 to < 200	4 (80)	1 (20)	-	3 (60)	2 (40)	-
	≥ 200	11 (91.7)	1 (8.3)	-	10 (83.3)	2 (16.7)	-
Period of being haemodialysis	1 year	7 (50)	7 (50)	**0.044**	3 (21.4)	11 (78.6)	**0.004**
	≥2 years	15 (88.2)	2 (11.8)	-	13 (76.5)	4 (23.5)	-
Number of used HD facilities	one	9 (56.2)	7 (43.8)	0.113	8 (50)	8 (50)	0.999
	two and more	13 (86.7)	2 (13.3)	-	8 (53.3)	7 (46.7)	-
Family history of hepatitis		1 ((25)	3 (75)	0.099	-	-	-
History of malaria		-	-	-	8 (32)	17 (68)	**0.024**
Frequency of private clinic attendance†		-	-	-	1.1 (2.1)	2.1 (2.2)	0.138
Availability of	toilet	5 (41.7)	7 (58.3)	0.062	-	-	-
Employment		9 (52.9)	8 (47.1)	0.172	-	-	-
Chewing Qat		-	-	-	6 (31.6)	13 (68.4)	0.111
Ownership of radio		21 (61.8)	13 (38.2)	0.149	-	-	-

† mean and SD (± standard deviation)

6.5.4. Multivariate logistic regression analysis of factors associated with HBV and HCV infection in polytransfusion and haemodialysis patients

All the factors with p value < 0.2 were admitted to the backward stepwise logistic regression analysis. One factor was independently associated with HBV infection and two factors for HCV infection among polytransfusion/HD patients. These included period of being haemodialysis/years for HBV and HCV (AOR=7.5; 95% CI=1.3 -45.8; p< 0.029 and AOR= 11.9; 95% CI= 2.2 – 65.2; p <0.004) and also a history of malaria for HCV (and AOR= 6.3; 95% CI 1.2-34.2; p<0.034), as shown in table 6.7.

Table 6.7 Multivariate logistic regression analysis for factors associated with HBV and HCV among polytransfused and haemodialysis cases

	HBV			HCV		
Variables	AOR	95% CI	P value	AOR	95% CI	P value
History of malaria	-	-	-	6.3	1.2 – 34.2	0.034
Period of being haemodialysis/years	7.50	1.23-45.81	0.028	11.92	2.18–65.15	0.004

6.6 Discussion

This is a first case control study in Aden City aiming to identify the seroprevalence of HBV and HCV infection among patients with polytransfused/HD and the risk factors associated with these infections. Two factors were independently associated with HBV and HCV infection among patients with polytransfused/HD, vis a vis the time of being under haemodialysis/years and a history of malaria.

Infections with HBV were two fold higher in patients with polytransfused/HD than in healthy controls (67% vs. 36%) and HCV was nine fold, higher (46% vs. 5%). In addition, both viruses were identified as being associated with the polytransfused/HD patients who underwent frequent blood transfusion or haemodialysis. Sallam et al (2003) found that the rate of HBV (24.1%) among blood donors in Aden City was higher than HCV (0.6%). Studies in other settings have shown that infection with HBV is less prevalent than HCV in haemodialysis units (Carrilho et al., 2004; Amiri et al., 2005; Olut et al., 2005). The high HBV prevalence may reflect the lack of preventive measures and the quality of blood services, which up to recently did not meet the international standards. In other countries that introduced vaccine against HBV in cases undergoing HD, isolation of HBV positive patients, use of dedicated dialysis machines and regular surveillance for HBV infection have dramatically reduced the spread of HBV in this setting (Oesterreicher et al., 1995; Baid-Agrawal et al., 2008; Toosi et al., 2008). On the other hand, the prevalence of HCV infection is still high among haemodialysis patients and varies between countries (2% to 60%) and between dialysis units within a single country (Reddy et al., 2005; Beran 2008; Toosi et al., 2008). Our findings on the seroprevalence of HCV are in agreement with other studies (Oesterreicher et al., 1995).

Dual infections of HBV and HCV were found in 31% of the cases in this study. These results were found in patients under polytransfusion and haemodialysis rather than only exposed to polytransfusion. There are very few reports on the seroprevalence of dual

infections in haemodialysis patients. However, the available reports show variation in the rate of dual infection in patients with polytransfusion/HD (Gul & Iqbal 2003; Jadoul et al., 2004; Baid-Agrawal et al., 2008; Toosi et al., 2008). For example, low proportion has been reported in countries such as Laos (Vientiane) and Saudi Arabia (0.12% and 3.4%, respectively) and a high rate in Greece (29.6%) (Reddy et al., 2005; Jutavijittum et al., 2007; Ramalingam et al., 2007). HBV and HCV share a common route of transmission and coexist simultaneously. Although, both routes of transmission are very important, the long duration and frequent sittings of haemodialysis rather than blood transfusions seems to be most important in our setting, however, due to the small sample size in this study, this assumption would require further investigation.

Our results are in agreement with previous studies that both HBV and HCV are associated with polytransfusion/HD (Elisaf et al., 1991; Ayoola & Gadour 2004; Jutavijittum et al., 2007). Studies from Sana'a reported that blood transfusion played a role in the transmission of HBV infection among the general population (Wang et al., 2002; WHO 2003b; Jadoul et al., 2004; Imarengiaye et al., 2006; Khattab 2008; Niederhauser et al., 2008; O'Brien et al., 2008). It was recently reported that transmission of HBV/HCV via blood transfusion has become less frequent because of the implementation of control measures in blood transfusion facilities in the country (Scott et al., 1990; Al-Shamahy 2000). The high rates are most likely found among older populations who acquired the infection through the previous absence of control measures in blood transfusion, unsafe use of needles and vaccination. Thus, these factors in the past might have contributed to the longstanding exposure of the cases in this study. Prior to 1992, there was no standard guideline for the screening of blood donors in the main hospitals. From 1992 to 1998 the Abbot's Latex test was used to detect HBV and in 2003 the ELISA method was introduced. More recently, a fourth generation ELISA analysis system has been utilized in the central referral laboratory (Own 2005).

Several recent reports indicate that, there is an association between occult HBV as a risk factor with blood transfusion as blood collected during the early seronegative window period of HBV or in late infection period when HBsAg is negative but the HBV DNA is still present, so it become highly infectious (Omer 2007). Therefore, to overcome this hazard, more sensitive screening methods have been developed to detect viral antigens or nucleic acids through Nucleic Acid Amplification Testing (NAAT). These are widely implemented in USA, Europe and Japan (Conjeevaram & Lok 2001; Raimondo et al., 2005b; Liu et al., 2006; Re et al., 2007; Hollinger 2008). NAAT testing methods aim to narrow the infectious window in the early stage of acute HBV infection and detect HBV-DNA in persistently infected individuals with the extremely low concentrations of HBV antigen and antibody (Gessoni et al., 2005; Liu et al., 2006; Re et al., 2007; Hollinger 2008).

Our findings have also illustrated that polytransfused patients exposed to haemodialysis have higher rates of HBV/HCV infection (71% and 52%, respectively). Usually, cases who undergo haemodialysis are persons at increased risk due to their frequent exposure to blood transfusions and exposure to contaminated medical equipment during haemodialysis or renal transplantation (Gessoni et al., 2005). However, a worldwide variation in the prevalence of HBV/HCV is recognised in dialysis units, which range from as low as 1% to as high as 63% (Meyers et al., 2003). For the HCV, it seroprevalence was found in range from 8–36% among dialysis patients in North America, 39% in South America, 1–54% in Europe, 17–51% in Asia, 1.2–10% in New Zealand and Australia (Al Traif et al., 2000; Pol et al., 2002). In the Middle East, the prevalence of HCV in Egypt (59%), Tunisia (46.5%), Saudi Arabia (30%), Pakistan (23.7%), Sudan (23.7%), and Iran (8.5%) have been reported (Mansour-Ghanaei et al., 2002; Jutavijittum et al., 2007; Khattab 2008; Nascimento et al., 2008; Toosi et al., 2008; WHO 2008b). The majority of these results were lower than in Aden (52%), with the exception of Egypt (59%). Nevertheless, our findings are in agreement with another study in Yemen (Hajjah), which reported an HCV prevalence of 40% among

haemodialysis patients (Al Traif et al., 2000; Hassan & Khalil 2000; Sassi et al., 2000; Reddy et al., 2005; Khattab 2008; Toosi et al., 2008).

Findings from this study also show that patients exposed to HD for more than one year were around 7 times and 12 times more likely to be at risk of acquiring HBV and HCV, respectively and positive anti-HBV and anti-HCV were higher in patients who had longer dialysis duration (more than one year). These findings are consistent with studies from Saudi Arabia (Haidar 2002) and Brazil (Khan & Khan 2001) where the seroprevalence of HBV and HCV were significantly associated with the duration of dialysis.

The Ministry of Health and population (Aden Branch), reported for the Haemodialysis Centre (Carrilho et al., 2004) identified that the haemodialysis filters were not reused and dialysis was performed with disposable kits, syringes and needles. Regular disinfection of the dialysis machines included rinsing between sessions by circulating hot water (80°C) for 25 minutes, and dedicated machines for anti-HCV/HBV negative patients were used. Therefore, the possibility of acquiring and transmitting nosocomial infections by the use of equipments, syringes and needles was considered to be low (Ahmed 2005). The quality control of the procedures in other centres in the country, where patients receive medical services is not known (e.g. Taiz, Mukalla and Qaten). Some authors suggest that the dissemination of HBV/HCV could be provoked accidently when the pressure testing device is used with inadequate disinfection (Ahmed 2005). In addition, the multiple parenteral exposures and the sharing of drugs (heparin) among patients may be involved or may play a role in the transmission of the viruses (Stuyver et al., 1996).

Recently, many studies across the world have reported a dramatic decrease in the incidence of HBV/HCV in haemodialysis patients (Pujol et al., 1996). This could be related to the improvement of standards in the selection of blood donors, use of

advanced diagnostic tests and routine vaccination of uraemic patients (Jadoul et al., 2004; Olut et al., 2005; Khattab 2008).

The prevention of transfusion-associated viral disease depends upon pre-donation evaluation followed by serologic testing for infectious pathogens, including HBV and HCV (Beran 2008). Its prevention has also become more feasible in developed countries where strict screening system for donors is being implemented with dramatic decline in the prevalence of these viruses (Blackmore et al., 1992; Niu et al., 1993; Pereira 1999). These steps are required to improve the screening process for blood transfusion in Yemen. This could be achieved by the use of sensitive screening tests, donor deferral, and more conservative use of blood. Vaccination against HBV in adults newly admitted to HD programme must be further added to reduce the HBV infection from polytransfused /HD patients who are free from infection. HBV vaccines were introduced in the country within the EPI programme since 2000. However this programme does not include high risk groups of the population such as patients under risk of exposure to multiple transfusions and those who undergo haemodialysis.

A history of malaria was significantly associated with HCV infection in patients undergoing HD. Malaria remains a devastating global health problem worldwide. Severe malaria leads to acute renal failure in endemic countries such as India (13%), Pakistan (19.4%) and Ethiopia (37.9%) where haemodialysis treatment is required (Seed et al., 2005; O'Brien et al., 2007). Acute renal failure occurs commonly in *Plasmodium falciparum* malaria, which it is the most frequent form of malaria in Yemen. In addition, cases with severe malaria could develop anaemia, which may require blood transfusions. Thus, the role of malaria could be via the causing of renal failure which could lead to haemodialysis or by causing anaemia and requiring polytransfusion. Further study is required to identify the role of malaria as a cause of renal failure in Yemen and its association with further hepatitis infection.

Our study is limited by its relatively small sample size which restricts the number of variables that can be examined by multivariate analysis and may not have enough power. For that reason, the interpretation of these results has to be taken carefully. Another limitation was the difficulty of collecting accurate information about sensitive practices (e.g. sexual partners, use of IV recreational drugs, alcohol consumption, etc.). In addition, there must be an important recall bias for factors that had potentially occurred several decades earlier. The cumulative effect of HBV/HCV infection on the markers used would result in an attempt to correlate the characteristics of the patients at the time of the study with an infection that occurred at some point in the past.

In conclusion, this study reveals a high seroprevalence of HBV/HCV in cases of polytransfusion/HD. A prolonged exposure to HD was associated with high seroprevalences of HBV or HCV. A history of malaria was associated with cases of polytransfusion/HD. In addition, the risk of dual infection with HBV and HCV was higher among patients undergoing polytransfusion or HD.

The information yielded in this study would be useful to improve our understanding of the role of these viruses in this environment. It will also help to monitor the application of preventive and control measure in these populations as the proper application of preventive and control measures could reduce the risk of acquiring these conditions.

CHAPTER 7: VACCINATION COVERAGE RATE OF HBV VACCINE IN CHILDREN < 5

7.1 Introduction

Childhood vaccinations have a major impact on the reduction and elimination of many causes of morbidity and mortality among children. One of these causes is HBV, which is prevalent in Yemen. Vaccination against HBV was introduced in the Expanded Programme of Immunization (EPI) of the country in 2000. Monitoring the vaccination coverage is necessary to characterize under vaccinated populations and to evaluate the effectiveness of efforts provided to improve the coverage rate of HBV vaccine in the targeted children < 5 years of age in Aden City.

The literature review focuses on the epidemiological dimensions and characteristics of vaccination coverage in different countries. The findings in this study were compared to those reported globally, regionally and at the local level. Through building on the current literature, this study contributes to the enhancement of prevention and control measures of the disease in this population.

7.2 Objectives

To determine the vaccination coverage rate of the HBV vaccine among children < 5 in Aden Governorate.

7.3 Literature review

Hepatitis B infection is one of the most widespread viral diseases, with over 2 billion population being infected and approximately 360 million being chronically infected worldwide (Zewdu 1994; Mehta et al., 2001; Abdul Manan et al., 2006). Of these, 4 million individuals develop acute disease, 25% become carriers and one million die from chronic active hepatitis, cirrhosis or primary liver cancer each year (Alter 2001; Chen et al., 2007a). Prevention of the infection and its consequences has become a high priority globally (WHO 2002a; Cohen et al., 2008). Vaccination against HBV infection is considered the most powerful tool for avoiding the occurrence of disease, disability and death (De Mitri & Bernardi 2008).

In the late 1960s, Krugman (1962) developed the earliest effective HBV vaccine (Faingezicht et al., 2002; WHO 2002b). Since 1982 commercial formulations of plasma-derived HBV vaccines have been produced using chemical methods (Krugman et al., 1962; Krugman 1974; Brown et al., 1986). Currently, the vaccine is produced by recombinant DNA technology (usually in yeast) (Szmuness et al., 1980; Brown et al., 1986; Jilg et al., 1989; Hipgrave et al., 2006). Both plasma-derived and recombinant vaccines have proved to be highly immunogenic, with 95% efficacy, and safe with uncommon serious adverse reactions (McAleer et al., 1984) and constituted the first vaccines against liver cancer (Dandolos et al., 1985; WHO 2002a). Recombinant vaccines replaced plasma-derived vaccines and the latter were withdrawn from the market.

The vaccine can be given at any time before or after other inactivated or live vaccines, because the HBV vaccines do not interfere with the immune responses to other vaccines (Parkin 1999). Most of the EPI vaccines require a cold chain to preserve their potency. The HBV vaccine however tolerates temperatures of up to 45°C for one week and of up to 37°C for one month without changes in its immunogenicity or reactogenicity (WHO 2001c). Freezing of the HBV vaccine however causes the HBsAg protein to dissociate

from the alum adjuvant and thus to lose its immunogenicity/ potency (Otto et al., 1999). Field studies in China, Indonesia, Vietnam and under experimental conditions (Van Damme et al., 1992) confirmed the heat stability of the vaccine (WHO 1993; Otto et al., 1999; Hipgrave et al., 2006; Wang et al., 2007) and the WHO considers that this vaccine is a candidate for removal from the cold chain because of its thermal stability and its vulnerability to freeze damage during refrigerated storage and transport (Sutanto et al., 1999). Giving its stability at high temperature, many attempts have been reported to improve the birth dose coverage of the vaccine in remote areas of developing countries, where refrigeration is not available (WHO 1993), as this would facilitate achieving higher rates of immunization.

Childhood vaccinations have a major impact on the reduction and elimination of morbidity and mortality in children (Otto et al., 1999; Sutanto et al., 1999; Hipgrave et al., 2006). Reports on HBV vaccination coverage show that coverage has steadily increased since 1990 due to the increasing number of countries introducing the vaccine into their routine immunization schedules (Salmaso et al., 1999; Talaat et al., 2003). Until 1992, only 31 countries had included this vaccine in their EPI, which caused the World Health Assembly to endorse recommendations urging all member states to integrate the vaccine in their immunization programmes by 1997 (WHO 2001a). This was later followed by another call to increase coverage rate to 90% on a national level with at least 80% coverage in every district by 2010 (WHO 1993; Bonanni et al., 2003). By the end of 2007, 171countries had included this vaccine in their EPI (WHO 2002a).

The main objective of HBV immunization is to prevent chronic HBV infections and the long term consequences of infection such as cirrhosis and HCC. Activities include routine infant vaccination, prevention of perinatal transmission and catch-up vaccination of older age groups (Da Villa et al., 2007). Universal infant immunization is considered the most effective preventive measure against HBV-induced disease (WHO 2001c). Moreover, successful HBV vaccination programmes have demonstrated a

gradual reduction of HBV-related chronic hepatitis, liver cirrhosis and HCC in endemic areas (WHO 2003a; Zuckerman 2006). In Taiwan, the rate of HBV infection decreased from 34% to 10% and the carriage rate fell from 11% to 4% before and after the implementation of vaccination (Ayoola et al., 2003; van der Sande et al., 2006; Chen et al., 2007a). In Italy, the prevalence of HBV has progressively decreased over the last 20 years, as a result of the national implementation of the vaccine (Chen et al., 2007a). In the Middle East, Ayoola (2003) illustrated a significant decline in the prevalence of HBV in the Saudi population after the integration of the vaccine into the national EPI. The overall prevalence of HBsAg among children decreased from 6.7% to 0.3 % through a 10 year period (Zanetti 2001).

A review of the long-term effect of early vaccination in USA, Italy, Singapore, Spain, Taiwan and Gambia have documented the prolonged efficacy of the vaccine for ≥15 years (Ayoola et al., 2003). Thus this vaccine has the long term ability of preventing HBV carriage and its sequelae (Whittle et al., 1995; Da Villa et al., 1997; WHO 2001c; Ayoola et al., 2003; Fitzsimons et al., 2005; van der Sande et al., 2006).

Vaccination coverage against HBV varies globally. Reports from Turkey, Italy, Belgium and USA revealed rates of 44%, 79% and 83% by the end of the last century, respectively (Whittle et al., 1995; WHO 2001c). Middle Eastern countries such as Oman, Saudi Arabia, Kuwait, Jordan, UAE, Qatar and Bahrain have similar rates across the region with 99%, 95%, 94%, 90%, 90%, 90% and 89% respectively (Da Villa et al., 1997; Salmaso et al., 1999; Yusuf et al., 2001; Vellinga et al., 2002).

In Yemen, the government has attempted to reach a vaccination uptake of between 80% and 100% over the last 5 years. WHO-UNICEF reported a vaccine coverage of 15% in 2000, the year vaccine was introduced, reaching 87% by 2007 (Qirbi & Hall 2001). However, consideration should be taken of coverage variations between governorates as well as districts within the same governorate. The EPI reported a coverage rate of HBV

vaccine for Aden Governorate (WHO 2008a) of 93% for the first dose and 87% for the third dose among children < one year old through 2006.

A variety of schedules can be introduced for HBV immunization depending on the local epidemiological situation (EPI/Aden 2007). In countries where a high proportion of HBV infections are acquired perinatally, the first dose of the vaccine should be given immediately after birth to achieve optimum efficacy in preventing infection (Mahoney 1999; Alter 2001; Hall 2003). In countries where a lower proportion of infections are acquired perinatally, the relative contribution of perinatal infections to the overall disease burden and the feasibility and cost-effectiveness of providing vaccination at birth should be considered before deciding the vaccination schedule (Da Villa et al., 1997). Following the administration of the scheduled doses, a high percentage of children will be protected, probably for life, without the need for booster injections (Fitzsimons et al., 2005).

In Yemen, vaccination is conducted almost exclusively in primary care centres using three postnatal doses of the vaccine. Currently, a pentavalent vaccine was introduced for routine vaccination (Whittle et al., 1995; Fitzsimons et al., 2005). This vaccine confers combined protection for diphtheria, tetanus, pertussis, HBV and *Haemophilus influenzae* type b. Three doses of the vaccine induce protective levels of HBsAg antibody in over 95% of healthy infants and 90% of children born to infected mothers (MOPHP 2005). Under the current schedule of immunisation, Yemeni-children receive their first dose at 6 weeks, followed by the 2nd and 3rd dose at 10 and 14 weeks of age. In the previous EPI schedule the 3rd dose was given at 6 months but a revised schedule was introduced with the expectation of a reduced number of injections and time, aiding further compliance (Yusuf et al., 2001; Talaat et al., 2003).

It is worth mentioning that the use of single monovalent vaccines for HBV is still required to vaccinate high risk groups such as those requiring haemodialysis or a frequent blood transfusion, and that these vaccines are still not provided in Yemen (see chapter 6).

7.4 Methods

A community-based cross-sectional cluster sampling household survey were conducted targeting children < 5 years old residing at the time of the study in Aden Governorate. The main objective was to assess the vaccination coverage rate for HBV. A total of 480 children < 5 years were studied from 8 districts. Calculation of the sample size, method and distribution of the clusters were described in chapter two.

7.4.1 Inclusion criteria

Any household with a child < 5 years of age at the time of the study was considered eligible for the study regardless of sex or vaccination status. Consent was obtained from the parent/guardian of each participating child.

To measure the strength of factors associated with a lack of vaccination coverage of HBV vaccine, backward logistic regression was used to obtain AOR and 95% CIs.

7.5 Results

7.5.1 Household characteristics of children participating in the survey.

7.5.1.1 Socio-demographic characteristics

A total of 480 children were enrolled from eight districts at Aden Governorate. The number of children enrolled from each district reflected the proportion of the children < 5 residing in each area and ranged from 8.3% to 19.6% per district.

The description of their socio-demographic characteristics is shown in table 7.1. The mean age (±SD) was 24 (±15) months. Both genders were represented, with 45% being male. There was a median of 6 members per household with a ratio of 3 individuals per room. Eighty six percent of participants lived in privately owned houses whilst 14% were in rented accommodation. Electricity, piped water and toilet facilities were available in 99%, 98% and 98% of the households, respectively.

Among the parents, 328 (68%) fathers had secondary or higher education compared to 212 (44%) mothers ($p < 0.001$). Also 449 (94%) fathers were employed compared to 91 (19%) of the mothers.

7.5.1.2 Vaccination related characteristics

A total of 395 (82.3%) children had a valid vaccination card, 21 (4%) parents claimed that their children were vaccinated but failed to show the card and were considered doubtful and 64 (13.7%) children were not vaccinated. Among the 395 children with vaccination cards who had received the vaccine, 337 (85.3%) received the 1st dose in the first two months after birth, 47 (11.9%) between the 3rd and 4th month and 11(2.8%) before the end of the first year, as illustrated in table 7.2.

Table 7.1 Sociodemographic characteristics of enrolled children and their parents

Variables		N= 480 (%)	95% CI
Age / months	mean (±S.D)	24.1 (±14.5)	23-25
Gender	male: female (% male)	216:264 (45.0)	-
Number of residents in the household	Median [IQR]	6 [4-7]	-
Median crowding ratio*	Median [IQR]	2.7 (1.7-4)	-
Household ownership	owned/family	414 (86.2)	83-89
	rented	66 (13.8)	11-17
Education (father)	basic or no education	152 (31.7)	28-36
	secondary and higher	328 (68.3)	64-72
Education (mother)	basic or no education	268 (55.8)	51- 60
	secondary and higher	212 (44.2)	40-49
Employment (father)	employed	449 (93.5)	91-96
	unemployed	31 (6.5)	5-9
Employment (mother)	employed	91 (19)	16-22
	unemployed	389 (81)	77-84
Districts of enrolment	Dar Saad	40 (8.3)	6-11
	Khormaksar	40 (8.3)	6 -11
	Tawahi	48 (10)	8-13
	Muala'a	48 (10)	8-13
	Buraika	56 (11.7)	9-15
	Crater	66 (13.8)	11-17
	Mansura	88 (18.3)	15-22
	Sheikh Othman	94 (19.6)	16-23
Availability of	electricity	473 (98.5)	97-99
	piped water	469 (97.7)	96- 99
	toilet	471 (97.7)	96-99

± SD= standard deviation

* calculated as number of resident/number of bed rooms

Table 7.2 Proportion of children with vaccination cards and time where the first dose was admitted

Variables		N=480(%)	95% CI
Proportion of children with vaccination card	Available	395 (82.3)	79- 85
	not available*	85 (17.7)	14-21
Age where first dose of the vaccine was received**	≤ 2 months	337 (85.3)	81-86
	3-4 months	47 (11.9)	8 - 16
	5-12 months	11 (2.8)	1-5

* The parents of 21 children without vaccination cards said their children had received the vaccine.

**Only includes children with vaccination cards.

7.5.1.3 Number of vaccine doses received

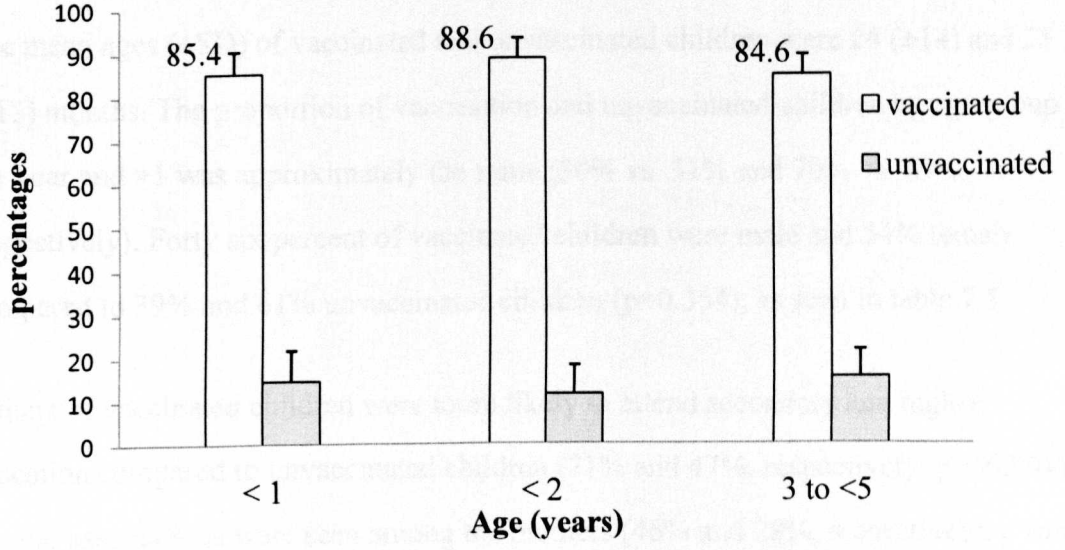
Table 7.3 shows the number of vaccine doses received by the children. Among children < 1 year old, 18 (15.4%) had received one dose, 66 (56.4%) two doses and 33 (28.2%) three doses. Among 1 - 5 years old children, 12 (4.3%) had received one dose, 51 (18.3%) two doses and 215 (77.3%) three doses. Among all vaccinated children < 5 years, 30 (7.6%) had received one dose, 117 (29.6%) two doses and 248 (62.8%) the three doses ($p < 0.01$).

The overall vaccination coverage rate of HBV vaccine was similar across the age groups if only the first dose is considered, with 85% (117/137), 88.6% (124/140) and 84.6% (154/182) of children <1, <2 and 3-5 years old receiving at least one dose of the vaccine, as shown in figure 7.1.

Table 7.3 Number of vaccine doses received by age

Vaccine doses received by age	1 dose N (%)	2 doses N (%)	3 doses N (%)	P value
6 to 12 months (117)	18 (15.4)	66 (56.4)	33 (28.2)	-
1 year (124)	3 (2.4)	28 (22.6)	93 (75)	-
2 years (76)	3 (3.9)	14 (18.4)	59 (77.6)	-
3 years (50)	3 (6)	7 (14)	40 (80)	-
4 years (28)	3 (10.7)	2 (7.1)	23 (82.1)	-
All (395)	30 (7.6)	117 (29.6)	248 (62.8)	0.001

Figure 7.1 Vaccination coverage rate by age among children < 5 years old (95% C.I.)



7.5.1.4 Knowledge and attitude toward vaccination

Most of the children's parents/guardians (89%) understood the importance of the vaccine, 10% did not consider the vaccine important and 1% did not know. Among the 64 unvaccinated children, the most frequent reason given was personal negligence (35.6%), social reasons (25%), fear of side effects (17%), lack of trust for the vaccines (13%) and the remaining (9%) cited that the baby was sick at the time of the vaccination (table 7.1.4).

Table 7.4 Knowledge and attitude toward vaccination

Variables		N (%)	95% CI
Understands the importance of vaccination (480)	yes	429 (89.4)	86 – 92
	no	46 (9.6)	7 - 13
	unknown	5 (1)	0.3 - 3
Reason for not being vaccinated (64)	negligence	23 (35.9)	24 - 49
	social reasons	16 (25)	15 - 38
	fear of side effect	11 (17.2)	9 - 29
	not trust on vaccine	8 (12.5)	6 - 24
	medical reasons	6 (9.4)	4 - 20
Walking distance to reach the clinic (minutes)	mean (±SD)	13.2 (6.7)	12.6-13.8

7.5.2 Factors related to vaccination coverage

A comparison between vaccinated and unvaccinated children was undertaken to illustrate the factors affecting vaccination uptake in the study population.

7.5.2.1 Socio-demographic factors

The mean ages (±SD) of vaccinated and unvaccinated children were 24 (±14) and 25 (±15) months. The proportion of vaccination and unvaccinated children by age group of < 1 year and >1 was approximately the same (30% vs. 31% and 70% vs. 69%, respectively). Forty six percent of vaccinated children were male and 54% female compared to 39% and 61% unvaccinated children (p=0.354), as seen in table 7.5.

Fathers of vaccinated children were more likely to attend secondary and higher education compared to unvaccinated children (71% and 47%, respectively; p < 0.001). Similar associations were seen among the mothers (46% and 28%, respectively, p value

< 0.01). A similar relationship was found between the fathers' employment and lack of vaccination (p< 0.01) but not among the mothers.

Of the 8 districts, Sheikh Othman and Mansura have the highest (21%) and Tawahi the lowest HBV vaccination coverage (7.1%; p <0.05).

Table 7.5 Sociodemographic characteristics of vaccinated and unvaccinated children

Variables		Vaccinated N 395 (%)	Unvaccinated N 64 (%)	P value
Age / months	mean (±S.D)	23.8 (14.3)	24.8 (15.4)	0.619
Vaccination rate by age	< 1 year	117 (29.6)	20 (31.2)	0.883
	1- < 5 years	278 (70.4)	44 (68.8)	-
Gender	male	181 (45.8)	25 (39.1)	0.345
	female	214 (54.2)	39 (60.9)	-
Education (father)	basic or no education	114 (28.9)	34 (53.1)	0.001
	secondary and higher	281 (71.1)	30 (46.9)	-
Education (mother)	basic or no education	213 (53.9)	46 (71.9)	0.007
	secondary and higher	182 (46.1)	18 (28.1)	-
Employment (father)	Employed	375 (94.9)	54 (84.4)	0.002
	Unemployed	20 (5.1)	10 (15.6)	-
Employment (mother)	Employed	78 (19.7)	9 (14.1)	0.282
	Unemployed	317 (80.3)	55 (85.9)	-
Districts of enrolment	Tawahi	28 (7.1)	12 (15.6)	0.003
	Muala'a	34 (8.6)	10 (22.7)	-
	Crater	53 (13.4)	13 (20.3)	-
	Khormaksar	30 (7.6)	5 (7.8)	-
	Sheikh Othman	83 (21.0)	11 (17.2)	-
	Buraika	46 (11.6)	6 (9.4)	-
	Mansura	83 (21.0)	5 (7.8)	-
	Dar Saad	38 (9.6)	2 (3.1)	-

(±SD) = ±standard deviation

7.5.2.2 Household characteristics and attitude toward HBV vaccination

There was a median of 6 members per household and 2.9 individuals per room in the households of vaccinated children compared to 7 members and 3.7 persons per room of unvaccinated children (p < 0.001 for both), as illustrated in table 7.6.

The availability of electricity, piped water and toilet facilities was approximately the same among vaccinated and unvaccinated participants. Similarly a high percentage of

vaccinated and unvaccinated children lived in privately owned houses than rented houses (86% vs. 14% and 88% vs. 13%, respectively). There was also no difference in the time taken to reach the clinics (13 and 15 minutes, respectively) for parents/guardian of vaccinated and unvaccinated children.

Ninety three percent of parents/guardians of vaccinated children understood the importance of the HBV vaccine compared to 66% for unvaccinated children ($p<0.001$). The overall proportion of parents who were aware that hepatitis was caused by a virus was higher (37%) in parents/guardians of vaccinated than in unvaccinated (22%; $p < 0.05$).

Table 7.6 Household characteristics and knowledge toward HBV vaccine

Variables		Vaccinated N 395 (%)	Unvaccinated N 64 (%)	P value
Number of residents in the household	median [IQR]	6 (4-7)	7 (2-9)	0.001
Crowding ratio*	median [IQR]	2.9 (1.7-4)	3.7 (1-5)	0.001
Availability of	electricity	390 (86.3)	62 (13.7)	0.254
	piped water	387 (86.4)	61 (13.6)	0.188
	toilet	388 (86.2)	62 (13.8)	0.364
Household ownership	owned/family	338 (85.6)	56 (87.5)	0.708
	rented	57 (14.4)	8 (12.5)	-
Average walking distance to reach the clinics/minutes	mean (±SD)	13.1 (6.6)	14.8 (6.9)	0.059
Understanding the importance of vaccination	yes	366 (92.7)	42 (65.6)	0.001
	no	24 (6.1)	22 (34.4)	-
	unknown	5 (1.3)	0 (0)	-
Causes of hepatitis	viruses	146 (37)	13 (20.3)	0.023
	fear/stress	78 (19.7)	14 (21.9)	-
	unknown	171 (43.3)	37 (57.8)	-

± SD = standard deviation, IQR = inter quartile range

* calculated as number of resident/number of bed rooms

7.5.2.3 Household ownership and appliances

Ownership of household appliances was used as surrogate markers of socioeconomic status. These included radio, motor cycle, televisions, fridges, computers, bicycle and

cars. The frequency of the latter five was statistically different between vaccinated and unvaccinated children ($p < 0.05$), as shown in table 7.7. In addition, the monthly family income was higher among families of vaccinated than unvaccinated children ($p < 0.05$),

Table 7.7 Characteristics related to the ownership of household appliances

Variables		Vaccinated N 395 (%)	Unvaccinated N 64 (%)	P value
Ownership of	radio	306 (77.5)	53 (82.8)	0.337
	television	373 (94.4)	55 (85.9)	0.012
	fridge	371 (93.9)	54 (84.4)	0.007
	computer	90 (22.8)	5 (7.8)	0.006
	bicycle	117 (29.6)	15 (15.6)	0.020
	motor cycle	6 (1.5)	2 (3)	0.360
	car	147 (37.2)	11 (17.2)	0.002
Family income / month (Y.R)*		19713 (14294)	158918 (12686)	0.041

* Mean and ± SD (standard deviation); Y.R = Yemeni Riyal

7.5.3. Multivariate logistic regression analysis for factors associated with HBV vaccine coverage rate in vaccinated and unvaccinated children < 5 years old.

An analysis of factors associated with the lack of vaccination was undertaken. These include factors with p values < 0.2, identified through the univariate analysis, as illustrated in table 7.8.

All the factors selected were entered into a backward stepwise logistic regression analysis. Two factors were independently associated with lack of vaccination. These included low education of the father and no ownership of a car (AOR= 2.7; 95% CI 1.6 – 4.6; $p < 0.01$ and AOR= 2.6; 95% CI 1.3 - 5.2; $p < 0.01$, respectively). All the other factors selected were not statistically significant after adjusting for confounding.

Table 7.8 Multivariate logistic regression analysis for factors associated with HBV vaccine coverage among vaccinated and unvaccinated children

Variables		OR	P value	AOR	95% CI
Education (father)	basic or no education	-	†	-	-
	secondary and higher	2.8	0.001	**2.66**	1.55 – 4.59
Education (mother)	basic or no education	-	†	-	-
	secondary and higher	2.2	0.009	-	1.22-3.89
Employment (father)	employed	0.29	0.004	-	0.13- 0.65
	unemployed	-	†	-	-
Districts of enrolment	Tawahi	8.14	0.001	-	1.69 -39.32
	Muala'a	5.59	0.034	-	1.14 - 27.32
	Crater	4.66	0.051	-	0.99 - 21.87
	Khormaksar	3.17	0.186	-	0.57 - 17.48
	Mansura	1.15	0.875	-	0.21 - 6.17
	Sheikh Othman	2.52	0.244	-	0.53 - 11.92
	Buraika	2.48	0.283	-	0.47 - 12.99
	Dar Saad	-	0.008	-	-
Number of residents in the household		1.20	0.001	-	1.09 – 1.33
Crowding ratio*		1.42	0.001	-	1.20 – 1.68
Average walking distance to reach the clinics/minutes		1.04	0.059	-	0.99 – 1.08
Understanding the importance of vaccination	yes	0.15	0.000	-	0.08 - 0.29
	no/un known	-	†	-	-
Causes of hepatitis	viruses	0.41	0.030	-	0.21 -0.80
	fear/stress	0.83	0.009	-	0.42 -1.62
	un known	-	0.585	-	-
Ownership of	television	0.36	0.018	-	0.16-0.82
	fridge	0.35	0.009	-	0.16-0.77
	computer	0.29	0.007	-	0.11-0.74
	bicycle	0.44	0.023	-	0.22-0.89
	car	2.86	0.006	**2.62**	1.32–5.20
Family income / month (Y.R)*		1.0	0.041	-	1.0 -1.1

† Reference

7.6 Discussion

This study provides an up to date estimate of HBV vaccine coverage in Aden. Sixty three percent of children < 5 years old participating in the survey were vaccinated with three doses and 82% received at least one dose of the vaccine. The three-dose vaccine coverage rate was lower than the target of the EPI for Yemen (85%), but higher than the rates reported for the country as a whole (49%) and for some of the Governorates with large population such as Sana'a (36%), Taiz (22%) and Al Mukalla (46%) (Dodoo et al., 2007). Despite Aden having higher rates than other governorates, coverage was lower than reported from other Middle East countries (MOPHP/EPI 2005).

The proportion of children received three doses of HBV vaccine varied by age. It was higher (77%) among 1-5 years olds children and lower (28%) in infants. Twenty three percent of 1-5 years old children had delayed vaccine up-take. The uptake rate of the first dose in infants reached 89% coverage. If all these children were fully vaccinated, this would have increased the proportion of children completing vaccination to above the EPI target.

It is not known why children > 1 year old have not completed their vaccination. Possible causes could include health service related factors, such as availability of vaccine, EPI policy of providing HBV vaccine free of charge only to children < 1 during the first three years of the programme (2000-2002), and individual factors such as the knowledge and attitude of parents toward those vaccines.

A delay in the up-take of vaccines among infants has been shown in studies from different countries. In China, 51% of children received the first dose of the vaccine by 3 months of age, 71% at 8 months and 86% by 12 months of age (Toukan 1996; Qirbi & Hall 2001). In Italy, 79% of children received the 3rd dose of HBV by the end of the first year, (Cui & Gofin 2007).

The coverage of the HBV vaccine at the time of the study was still at an unsatisfactory level. Therefore, it is important to increase the HBV vaccine uptake to achieve the target for the vaccine of 95%. This could be facilitated by the incorporation of the pentavalent vaccine (DPT, HBV and Haemophilus influenzae type b) available since March 2005 through the EPI programme of Yemen and facilitated by the Global Alliance for Vaccines and Immunisation (GAVI) (Salmaso et al., 1999).
Since the last two-to three decades, it has been possible to produce combined vaccines, of which a number are now available. These emerged from the idea that simultaneously targeting multiple diseases with one injections would increase their up-take (GAVI 2006). Thus, different methods were used to combine the vaccines. These comes in forms where the health care provider may mix vaccines from separate vials prior to administration, the manufacturer provides a dual-chambered syringe in which the vaccines are prefilled or vaccines are combined during manufacturing. The latter method is the most frequently employed method (Singh & O'Hagan 1999).

Some intervention trials across the world, have measured changes in the frequency of missed opportunities for immunization before and after the use of vaccine combinations (Goldenthal et al., 1995). A multicentre randomized clinical trial in Brazil, Mexico and Argentina showed that programs with fewer injections were most likely to have a positive impact on compliance. Furthermore, vaccination-associated costs decreased and acceptance to the vaccine increased (Hutchins et al., 1993). Other trials in five European countries (Spain, Belgium, The Netherlands, Switzerland and Lithuania) to assess the safety, reactogenicity and immunogenicity of the combined hepatitis A/hepatitis B vaccines showed that the use of combined vaccines were safe, well tolerated and highly immunogenic, with similar serological responses compared with monovalent vaccines (Ramonet et al., 2002). A recent study in Filipino infants using the combined DTP and HBV vaccine demonstrated that the vaccines was highly immunogenic for all antigens, well tolerated and safe when given to infants at 6, 10 and 14 weeks of age (Thoelen et al., 1999). These studies concluded that combination

vaccines are more convenient, may potentially result in better compliance and have often been found to be more economical when other associated costs are considered. There is also a large reduction of missed opportunities for immunization and enhanced protection of the community by facilitating the introduction of new antigens (Capeding et al., 2008) which are as safe and effective as the individual single-disease vaccines (Aggarwal et al., 2007).

It is expected in Yemen that by the use of this pentavalent vaccine would to reduce the number of injections and visits needed for children to complete all three doses of HBV vaccine. Children would be able to receive the 3 doses of the HBV vaccine according to the pentavalent vaccine schedule (6 weeks, 10 weeks and 14 weeks). In addition, the EPI in Yemen planned to achieve high coverage of the 3rd dose of the pentavalent vaccine at 87% and 90% in 2006 and 2007 respectively, then to reach 95% in 2008 and sustain it in future years (Capeding et al., 2008).

Two factors were likely to be associated with low coverage rate of HBV vaccine in this study. Low education of the parents (AOR = 2.7; 95% CI 1.6-4.6; $p < 0.01$) and not having a car (AOR = 2.6; 95%CI 1.3-5.2; $p < 0.01$). Uneducated fathers are likely to have less information about HBV transmission and the protective role of the vaccine. Although only 37% of parents of vaccinated children know about the real cause of the HBV, however, 90% of parents/guardians of vaccinated children were aware of the importance of immunizing their children with the vaccine. This, awareness could be developed through vaccination campaigns, as frequently conducted in the past for other vaccines such as Poliomyelitis, DTP and Measles. Messages for these vaccines are usually disseminated through the local media (radio, TV and newspapers) before and during vaccination campaigns, although unfortunately they are not broadcast constantly. Studies across the world have shown the importance of parent education in the timely compliance with the vaccines scheduled (MOPHP/EPI 2005).

Car ownership by parents/guardians was associated with higher vaccination coverage. HBV vaccine coverage was 2 to 3 fold higher among families with a car. This could be explained by better access to the health centres to receive their vaccines as public transport in Aden is insufficient. In addition, ownership of a car is an indirect marker of high income, as families with a higher income were more likely to have their children vaccinated. Similar results were found in urban areas of Guinea and Puerto Rico, where it was observed that children from economically better families were more likely to complete their vaccination once entered into the vaccination system (Waldhoer et al., 1997; Salmaso et al., 1999).

The review of the estimated vaccination coverage rates in Yemen highlighted some inconsistencies between the national, provincial and international estimate for 2003, 2004 and 2005. For example, in 2003 and 2005, the overall reported rates among children < 1 year by WHO/UNICEF was 42% and 86% (Cutts et al., 1990; Simonetti et al., 2002) while the Ministry of Health and Population/EPI reported 33% and 26%, respectively (WHO/UNICEF 2006) . In addition, a separate report from the EPI in Aden Governorate for the years 2004 and 2005, showed a coverage rate of 72% and 63% for the 3rd dose of the vaccine (MOPHP/EPI 2005) while the central level reported a coverage of 44% and 57%, respectively (EPI/Aden 2005) . These inconsistencies suggest a need to improve the accuracy of the system. Assessment of the immunization coverage is a critical component of any immunization programme. Recently, a pilot study to verify the quality and consistency of the immunisation monitoring system was conducted in selected health centres of four districts (Ba'adan, Bajel, Al-Misrakh and Jabal Ash Sharq) by the Liverpool Associates in Tropical Health (LATH) and Euro Health Group Consultants, supported by the GAVI. The study observed an improvement of the performance of the immunisation services in Yemen and very good data quality audit in the areas (MOPHP/EPI 2005). This pilot however was not representative of all facilities in the country.

Usually the main source for data on vaccination in Yemen would be the primary health care centres in each district. In some countries, parents have the choice to have their children immunised in public or private clinics. However, there is debate in Yemen on whether to include the private sector in the vaccination programme as many parents prefer private services to public clinics. Although, involvement of this sector could increase the coverage rate of HBV vaccine, private practioners are often reluctant to allow scrutiny of their facilities (e.g. cold chain integrity) and records and to participate in external quality assurance schemes. In high burden countries, private practitioners have a good reputation but their quality assurance may be poor and their standards questionable at best. This approach therefore is discouraged in most national EPI.

In order to reduce the risk of infections by blood borne viruses attributable to unsafe injection practices, the WHO, UNICEF, the United Nations Population Fund (UNFPA) and the International Federation of the Red Cross and Red Crescent Societies (IFRC) called for the exclusive use of auto-disable syringes in immunization programmes by the end of 2003, as a strategy for eliminating the re-use of injecting equipment (WHO-UNICEF-UNFPA 1999). This approach to achieving injection safety is not without its critics, who argue that the exclusive use of auto-disable syringes in immunization programmes in low-income countries will be difficult to sustain in the long-term (Battersby et al., 1999a; Battersby et al., 1999b).

In many developing countries and countries with economies in transition, health care injections are overused and are frequently administered in an unsafe manner (Simonsen et al., 1999; Hutin et al., 2003; Kermode 2004b). For example, it has been estimates that around 20 million new HBV infections, 2 million new HCV infections, and 260,000 new HIV infections are associated with unsafe injection practices each year worldwide (Kane et al., 1999). In addition, it has been estimated that unsafe injection practices could account for 30% of HBV infections, 31% of HCV infections, 28% of liver cancer, 24% of cirrhosis cases, 5% of HIV infections and 0.9% of deaths worldwide (Kermode

2004b). Singh et al. (1998) confirm the occurrence of an outbreak of viral hepatitis in an Indian village which was linked to the use of inadequately sterilised needles and syringes by an unqualified medical practitioner (Singh et al., 1998). However, to estimate the magnitude and patterns of such practices in Yemen, a rapid assessment of injection practices have to be conducted and to measure to what extent the injection practices is sticking on the WHO guidelines.

With regard to the reasons mentioned by the parents of unvaccinated children the main cause was the parents' negligence to take the child to the clinic. In addition some social reasons were quoted by parents in employment. Concurrent childhood illnesses during the time of the required dose of vaccine appear to be the least frequent barrier. For the first two reasons, EPI authorities have to act with the parents of unvaccinated children and to encourage them about the use of this service free of charge which aims to protect their children from HBV infection.

Some limitations of this study need to be accounted for in its interpretation. The first mentioned is the cross-sectional design of the study which only provides information on the rate of coverage at the time of the survey, some children < 1 year may not have reached the time of the appropriate schedule of the subsequent dose. Thus the vaccination coverage obtained may be an underestimate. Second, it was found that the 2nd and the 3rd dose of the vaccine were not reported with the date received or the age of the child. Thus, estimates of noncompliance were limited.

In conclusion, this study includes updates information for the health authorities at the EPI in Aden about the vaccination coverage rate of HBV vaccine and achievements for 2004. In addition, it has identified factors associated with the lack of vaccination among children < 5 in this population which could be considered in further plans.

CHAPTER 8: PAST EXPOSURES BETWEEN HERPES VIRUS AND HEPATITIS VIRUSES.

8.1 Introduction

To date, eight members of the human *Herpesviredae* family have been studied and three of them are discussed in this chapter to explore their association with hepatitis viruses. Epstein-Barr virus (EBV), cytomegalovirus (CMV) and human herpes virus 6 (HHV6) cause disease in man and share the ability to remain in a latent or persistent state in the host for life and to reactivate during periods of relative immunosuppression. Their prevalence in adults is usually high, although wide geographic variations exist and are better documented in industrialised countries. There is much more limited information regarding their prevalence in developing countries and whether transmission is increased in individuals receiving multiple blood transfusions or blood products.

There is no information regarding the seroprevalence of EBV, CMV of HHV6 in Yemen, and this chapter describes their seroprevalence in Aden and the frequency of past exposure with other hepatic viruses.

The literature review focuses on the epidemiology and characteristics of these viruses in different countries. The findings in this study are compared to that reported elsewhere and regionally.

8.2 Objectives

To describe past exposures between viral hepatitis viruses and Epstein-Barr, cytomegalovirus and human herpes virus 6 in Aden Governorate.

8.3 Literature review

There is a lot of evidences show that after a short acute, often asymptomatic episode, these lymphotropic viruses (EBV, CMV and HHV6) establish lifelong infections in the majority of humans. The viruses also remain latent until re-activated by immunosuppression (LATH/EuroHealthGroup. 2006). Although, the three viruses usually produce unapparent infection or transient immune compromise in healthy individuals, they are able to cause life-threatening primary or reactivated infections in individuals with congenital or acquired T-cell immunodeficiencies (Epstein & Achong 1973; Ward 1998; Griffiths et al., 2000; Kozireva et al., 2001; Michalek & Horvath 2002; Macsween & Crawford 2003; Williams & Crawford 2006). The spectrum of diseases caused by these viruses is documented in patients undergoing bone marrow or organ transplantation (Epstein & Achong 1973; Maltezou et al., 2000; Michalek & Horvath 2002; Varghese et al., 2008) and in individuals infected with human immunodeficiency virus (HIV) (Tong et al., 1998; Claviez et al., 2000; Griffiths et al., 2000; Maltezou et al., 2000; Michalek & Horvath 2002; Green & Webber 2007; Lim et al., 2007; Lim et al., 2009).

Liver involvement is nearly universal in healthy persons with EBV. Infection with EBV produces jaundice in 10-15% of cases with usually mild and self-limited and rarely results in hepatic failure or severe jaundice (Smith et al., 1996; Tsaparas et al., 2000). However recently, some studies illustrate an attribution between primary EBV infection and manifestations of hepatitis which resembles HAV infection (Hara et al., 2006) and thus suggests that many undiagnosed hepatitis cases could be caused by some of the herpesviruses (Massei et al., 2001; Prassouli et al., 2007). Few reports have studied the mechanism of EBV associated hepatitis (Prassouli et al., 2007). The major difference between EBV associated hepatitis and hepatitis caused by HAV, HBV, and HCV is that EBV does not appear to infect hepatocytes, billiary epithelium or vascular endothelium (Yuge et al., 2004). The pathogenesis of the hepatitis seen in infectious mononucleosis is unclear (Kimura et al., 2001). Similarly, symptoms of hepatitis have been

occasionally observed after primary HHV6 or CMV mainly after the use of immunosuppressive therapy (Kimura et al., 2001) or among pregnant women (Chan et al., 1995b; Humar et al., 2002; Tarhan et al., 2003; Eskild et al., 2005).

8.3.1 Epstein - Barr virus (EBV)

8.3.1.1 Epidemiology

EBV belongs to the *gamma-herpesvirus* family and it is one of the most successful viruses in humans (Suga et al., 2000; Ozaki et al., 2001; Csire et al., 2007). It causes infectious mononucleosis (IM), establishing latency in resting memory B lymphocytes, and it is involved in oncogenesis through poorly understood mechanisms (Epstein & Achong 1973; Bergallo et al., 2007).

It was first identified in 1964, in cultures from a biopsy of a Burkitt's lymphoma (Yoshizaki et al., 2007; Al Tabaa et al., 2009). Furthermore, in vitro experiments the virus has demonstrate a potent transforming ability in cell culture and it have shown that the virus induced blast transformation and uncontrolled proliferation of infected B lymphocytes (Epstein & Achong 1973). This new characteristic reflects the oncogenic potential of the virus and since there it has been associated with a number of malignancies such as Burkitt's lymphoma, Hodgkin disease, nasal T-cell lymphoma, gastric carcinoma and primary effusion lymphoma (Williams & Crawford 2006). Therefore, since its discovery, EBV has moved from its doubtful role as a causative agent for the African tumour to its present leading role as the prime example of a human tumour virus that is aetiologically linked to an unexpectedly diverse range of malignancies (Alexander et al., 2000; Spano et al., 2003; Pai et al., 2007; Yoshizaki et al., 2007).

EBV has co-evolved with human beings over millions of years and the virus life cycle has become supremely adapted to its human host (Young & Rickinson 2004; Young et al., 2008). The virus has a high infectivity with estimation of > 90% of the world's adult population being infected (Macsween & Crawford 2003). Like all herpes viruses, EBV

has latent and productive (lytic) phases in its life cycle. The former maintains the virus long term in its host and the latter effecting virus production and spread (Michalek & Horvath 2002; Macsween & Crawford 2003). Therefore, the virus is able to persist in the host for life in the vast majority of healthy carriers without causing disease (Macsween & Crawford 2003; Young & Rickinson 2004; Williams & Crawford 2006). This is because a delicate balance is maintained between the host immune system, which limits production of virus particles, and the virus, which persists and is successfully transmitted in the face of host antiviral immunity. Disruption of this balance, resulting from primary or acquired immunodeficiency, may lead to the development of EBV-associated disease (Crawford 2001).

The port of entry for EBV is also the port of exit, i.e., the oropharynx where it replicates in its epithelial cells and B cells and spreads through the body via infected B cells (Williams & Crawford 2006). The only reservoir of the virus is man from which it is intermittently shed from saliva. The main route of transmission thus is directly from person to person; however, transmission via blood products, transplantation, and sexual transmission have been reported (Borza & Hutt-Fletcher 2002; Macsween & Crawford 2003).

Epidemiological studies of EBV-seropositivity in the 1970s showed that primary infection occurs early in developing countries and is maintained throughout life, whereas in affluent societies seroconversion may be delayed until adolescence, when IgM develops in between 50% and 74% of adolescents (Crawford et al., 2002; Michalek & Horvath 2002; Macsween & Crawford 2003; Ebell 2004; Hess 2004; Williams & Crawford 2006; Higgins et al., 2007). Several studies have also reported an increasing risk of transmission through earlier sexual activity and multiple partners (Biggar et al., 1978; Babcock et al., 1998; Chan et al., 2001; White et al., 2001; Macsween & Crawford 2003; Takeuchi et al., 2006).

Early studies in Japan, Indonesia, Taipei, Hong Kong and Singapore describe seropositivities of 90% among 5–9-year-old children (Williams & Crawford 2006; Higgins et al., 2007) and similar rates were reported from Mexico and African countries (Kangro et al., 1994; Pancharoen et al., 2001; Takeuchi et al., 2006). More recently, Takeuchi (2006) demonstrated a decline of the rate of infection in Japan to < 50% (Young & Rickinson 2004; Adjei et al., 2008) and in other industrialised countries such as USA, England, France, Denmark and Australia, the rate remains around 50% in young children (Takeuchi et al., 2006).

Primary infection during early childhood is usually asymptomatic, while in young adults and older adolescents, nearly half of the primary infections result in an increase in IgM accompanied by strong signs and symptoms of late primary infection and with strong immunological reactions and develop what is known as infectious mononucleosis (Morris et al., 2002; Takeuchi et al., 2006).

EBV has a double-stranded DNA (186-kb) codes for a number of structural and non-structural genes (Macsween & Crawford 2003; Bergallo et al., 2007). The morphology of the virus shows immature particles about 75 to 80 mp in diameter occurring in both the cell nuclei and the cytoplasm (Borza & Hutt-Fletcher 2002). The particles frequently exhibit a hexagonal profile and are either empty or contain a ring-shaped or dense central nucleotide (Macsween & Crawford 2003).

The genome of EBV comprises over 70 open reading frames that allow for the transcription of genes for several different proteins. Different forms of infections are characterized by expression of different combinations of these proteins as antibodies to several antigen complexes which may be measured (Epstein & Achong 1973). These antigens are the viral capsid antigen (VCA), the early antigen, and the EBV determined nuclear antigen (EBNA) (Kuppers 2003; Bergallo et al., 2007). In addition, differentiation of immunoglobulin G and M subclasses to the viral capsid antigen

became helpful for confirmation (Kuppers 2003; Lu et al., 2008; De Paschale et al., 2009). In most cases, a distinction can be made as to whether a person is susceptible to EBV, has had a recent infection, has had infection in the past, or has a reactivated EBV infection (Fahmi et al., 2000). Several diagnostic assays are available and are routinely used for the diagnosis of EBV infection employing various techniques such as the indirect immunofluorescence assay (IFA); the enzyme immunoassay (EIA) technique (Kuppers 2003; Amon & Farrell 2005) or the Real-time PCR. The later, is also used as a rapid, sensitive, specific and reproducible technique for the detection and monitoring of EBV DNA levels in patients who carry EBV-associated diseases (Gartner et al., 2003; Callan 2004).

It is usually sufficient to use just three parameters (VCA IgG and IgM, EBNA IgG) to distinguish acute and past infections in immunocompetent patients by using serological tests. The optimal combination of serologic testing consists of the antibody titration of four markers: IgM and IgG to the viral capsid antigen (VCA), IgM to the early antigen, and antibody to EBNA (Maurmann et al., 2003; Bergallo et al., 2007; Hayden et al., 2008). In general, IgM antibodies to VCA disappear within 1to 2 months of the onset of IM, while IgG antibody to VCA persist for life and can be used to indicate immunity. In the same instance, lifelong presence of IgG antibodies for EBNA reflects latency of the virus (Bergallo et al., 2007; De Paschale et al., 2009). The presence of VCA IgG and VCA IgM without EBNA IgG indicate recent infection, whereas the presence of VCA IgG and EBNA-1 IgG without VCA IgM is typical of past infection (Okano et al., 1988).

With the increased understanding of the immune variables that control EBV infection, many attempts have been made to develop a vaccine for the control of the infection. These attempts were directed towards minimising the clinical consequences of primary infection with the virus, hence controlling the development of IM and post transplant lymphoproliferative disease rather than towards malignancies associated with the virus

such as Hodgkin's disease, nasopharyngeal carcinoma, and Burkitt's lymphoma (Okano et al., 1988; Gartner et al., 2003; Callan 2004; Young & Rickinson 2004). Clinical symptoms of IM are rare in developing countries, where primary infection typically occurs in the first few years of life which is in contrast to the pattern in Western countries (Moss et al., 1998).

Several trials are now under way with candidate vaccines against primary infection (Babcock et al., 1998). A clinical trial in China showed that a proportion (6/9) of children negative for EBV who were given recombinant vaccine virus gained protection from subsequent infection (Morgan 1992). Another clinical trial was developed using cytotoxic T cell epitope vaccines and has been well tolerated with no significant adverse reactions (Moss et al., 1998).

The development of an EBV vaccine is slow due to difficulties and the debate of what could be achieved by this vaccine in developing countries (Thomson et al., 1998). However, changing demography and life styles in the past four decades are likely to have altered this pattern, and new studies are required before a logical vaccine strategy can be planned.

8.3.2 Cytomegalovirus (CMV)

CMV belongs to the *beta-herpes viruses*, which are the largest viruses of the herpes family (Khanna et al., 2005; Balfour 2007; Hayden et al., 2008). The virus is considered an important cause of congenital infections, causing hearing loss, mental retardation and other central nervous system disorders (Sarov et al., 1982; Liu et al., 1990; Shen et al., 1992; Joseph et al., 2005). Its re-activation is also a major problem in immunocompromised patients including individuals with AIDS and post transplantation under immunosuppressive therapy (Liu et al., 1990; Joseph et al., 2005; Colugnati et al., 2007; Pang et al., 2008; Eggert-Kruse et al., 2009).

The virus was identified in animal studies in the 1920s and isolated in humans in the 1950s (Gilbert et al., 1998; Staras et al., 2006; Steininger 2007). Morphologically, CMV

is a ubiquitous human herpes virus with a double stranded DNA genome (Eggert-Kruse et al., 2009).

8.3.2.1 Epidemiology

The seroprevalence of CMV varies worldwide between 40% and 100% and increases with age (Braun et al., 1997; Wang et al., 2006; Liu et al., 2007). In the USA the seroprevalence increases from 36% in 6-11 years old to 91% in 80 year old (Golubjatnikov et al., 1973; Liu et al., 1990; Kozireva et al., 2001; Staras et al., 2006). In Canada, 57% of female educators working in day care centres were seropositive (Staras et al., 2006). In China (1968-1987), the prevalence was 52% in children < 1 year, 58% in 3 year old and 60% in children 4-7 years old (Joseph et al., 2005). In Taiwan, the seroprevalence among infants < 6 month old reached 70% and increased to 82% by 12 years of age (Liu et al., 1990). In Egypt, 96% of pregnant women have CMV antibodies (Shen et al., 1992).

Primary infection with CMV is usually asymptomatic in immunocompetent individuals (Hammouda et al., 1993; el-Nawawy et al., 1996). However it leads to serious complications and severe sequelae in immunesuppressed patients in the form of hepatitis, encephalitis, microcephaly, sensor neural deafness (Braun et al., 1997; Liu et al., 2007), pneumonitis, splenomegaly, enamel defects in teeth and retinitis (Boppana et al., 1999; Fowler et al., 1999; Oliveira et al., 2002; Pass & Burke 2002; Bradford et al., 2005).

In USA, from a total of around 40,000 infected infants each year, 9000 have symptoms and 2.2% develop congenital anomalies (Chan et al., 2000b; Gaytant et al., 2002; Westall et al., 2003; Williams et al., 2003). Generally, anomalies are shown in the form of visual and hearing loss, mental retardation and other neurological sequelae (Demmler 1991; Joseph et al., 2005; Staras et al., 2006). Young children are an important source of CMV infection due to high excretion rates and inadequate hygiene (Boppana et al., 1999; Bradford et al., 2005; Grosse et al., 2008).

Chronic CMV infection causes atherosclerosis and recurrent stenosis after balloon stenting and coronary bypass (Joseph et al., 2005). In addition, 60-100% of patients under immunosuppressive therapy develop primary or reactive CMV infection (Braun et al., 1997; Speir et al., 1998) and is a major threat in most solid organ transplants such as liver, heart, renal, pancreas and small bowel (Holma et al., 2000; Li et al., 2007). CMV is transmitted horizontally through direct contact with saliva, breast milk, urine, blood transfusions and vaginal secretions after an incubation period of 20-60 days (Tong et al., 1998; Rao et al., 2000; Sia & Patel 2000; Tong et al., 2001; Westall et al., 2003). Vertical transmission is the main source of congenital infections (Adler et al., 1983; Peckham et al., 1987; Liu et al., 1990; Kozireva et al., 2001; Revello & Gerna 2002; Joseph et al., 2005; Staras et al., 2006; Colugnati et al., 2007).

Blood transfusion and its products are also important risk factors of CMV transmission, especially in immunocompromised host and cancers patients (Chandler et al., 1985; Handsfield et al., 1985; Pass & Burke 2002; Revello & Gerna 2002; Joseph et al., 2005; Colugnati et al., 2007). A study from the Middle East shows a relationship between blood transfusion and CMV infection among employees working in neonatal intensive care units and health care workers (Michalek & Horvath 2002). In India, a study on the seroprevalence of CMV among voluntary blood donors revealed 95% positive infection (Morgan et al., 2003).

The prevention of CMV infection via blood products in the immunesuppressed or those undergoing solid organ transplantation is important due to its complications, morbidity, acute graft rejection and death (Dinand et al., 2007)

Commercial assays are available for the detection of CMV pp65 lower matrix protein (pp65 antigen [Ag]) and are widely used for diagnosis (Sia & Patel 2000; Kothari et al., 2002). Molecular assays based on quantitative PCR are now also routinely used to identify CMV DNA (al-Ali et al., 1999). Studies identified that CMV DNA was detected more frequently in whole blood (88.5%) than in the peripheral blood leukocytes (65.7% and $P < 0.0001$) or the plasma (55.2% and $P < 0.0001$) (Limaye et

al., 2001; Garrigue et al., 2008). Advances in technology with the availability of more sensitive tests means that it is possible to have positive laboratory assay results well before the onset of disease. However, laboratory diagnosis of active CMV infection is not necessarily always associated with symptomatic CMV disease.

8.3.3 Human herpes virus-6 (HHV 6)

The HHV 6 belongs to the beta group of the family herpesviredae with a linear double stranded DNA genome (Mengelle et al., 2003). Two variants (HHV-6A and HHV-6B) have been described based on genomic, antigenic and biological differences (Braun et al., 1997; De Bolle et al., 2005a; Ibrahim et al., 2005). Variant A is not reported to cause any major illnesses whereas variant B has been shown to be almost exclusively the cause of the complications resulting from reactivation of the virus (Braun et al., 1997; Zou et al., 1999; Ahlqvist et al., 2005; De Bolle et al., 2005a). The virion size ranges from 160 mm to 200 mm in diameter, encodes approximately 100 proteins and has five characteristic genes (DR3, U6, U22, U83 and U94) (Pascher et al., 2004; Karatas et al., 2008). The overall nucleotide sequence identity between HHV-6A and HHV-6B is approximately 90% (Isegawa et al., 1999).

Clinical disease due to primary HHV6 infection is extremely uncommon in young immunocompetent adults (Dominguez et al., 1999). Infection by the virus is often asymptomatic. Symptomatic children can develop Rosella infantum or exanthem subitum, accompanied by febrile convulsions (Braun et al., 1997; Tanaka et al., 2002; Merk et al., 2005). HHV-6 may also be a trigger of Pityriasis rosea in the presence of other aetiological factors (Hall et al., 1994; Braun et al., 1997; Tanaka et al., 2002; Zerr et al., 2005).

The virus has also been recognized as an opportunistic pathogen in bone marrow transplant recipients and is often reactivated in patients during their use of immunosuppressive therapy (Canpolat Kirac et al., 2009). In addition, the virus may cause severe complications in immunocompromised patients (Burd & Carrigan 1993;

Singh & Carrigan 1996; Sebelin-Wulf et al., 2007; Muramatsu et al., 2009). However in adults complications have been reported in the form of severe CNS disease such as encephalitis and fulminant multifocal disease associated with seizures and high fever (Asano et al., 1994; Braun et al., 1997; De Bolle et al., 2005a; Harris 2008).

Some studies have suggested that the virus plays a role in the development of multiple sclerosis and potentiates the auto-immunity which causes neurocellular destruction (Mackenzie et al., 1995). Moreover, an association has been reported between the virus and different types of neoplasias such as Hodgkin's and non Hodgkin's lymphomas and leukaemia (Fotheringham & Jacobson 2005). Recently, some authors have described a role of the virus in the chronic fatigue syndrome (Kondo et al., 1993; Hall et al., 1994; Barone et al., 1995; Cone et al., 1999; Yoshikawa & Asano 2000; Kato et al., 2003), mainly in patients receiving transfusion therapy (Braun et al., 1997; De Bolle et al., 2005a).

8.3.3.1 Epidemiology

Studies across the world have reported a seroprevalence > 95% in adults; however, its prevalence varies from 70% to 100% (Michalek & Horvath 2002). Seroconversion mostly occurs by 2 years of age with high seroprevalence rates in adulthood (Okuno et al., 1989; De Bolle et al., 2005a). In the USA, UK and Taiwan infection at early age range from 64% to 83% by 13 years of age (Hall et al., 1994) with 40% of infants being infected by the end of the first year of life and up to 77% at the end of two years of age (Braun et al., 1997).

Recent studies elsewhere, have found that HHV6 could integrated with the host genome where it infect the chromosomes of both the virus type A and B in 1-3% of immune competent subjects (Ward et al., 2005; Leong et al., 2007). It also demonstrated that the integration of the complete virus lead to expression of viral genes and passage in the germ line and noticed correspondingly high level of viral DNA in blood, cerebrospinal fluid and hair follicles (Ward 2009). The clinical relevance of such integration is

unknown and may be unique to HHV6. Furthermore, this integration could be inherited through different generations (Daibata et al., 1998; Clark et al., 2006).

The most common route of transmission is saliva through direct contact between mother and baby or between children (Zerr et al., 2005). Prenatal and perinatal transmission can occur as the virus is detected in vaginal secretions (De Bolle et al., 2005a). Breast feeding, faeco-oral and sexual transmission are considered routes of transmission (Hall et al., 1994). Diagnosis can be done by Western blot, immunoassays and PCR (Braun et al., 1997; De Bolle et al., 2005b; Zerr et al., 2005).

8.4 Methods

This is a cross sectional study to describe past exposures between EBV, CMV and HHV6 and hepatitis A, B, C and E viruses. All participants enrolled from the polyclinics were tested for EBV and CMV while HHV6 was tested exclusively in children <5 years. Laboratory methods are described in chapter 2 and sociodemographic characteristics in chapter 3.

Descriptive statistics were used to describe the characteristics of the viruses in the study population. A statistical analysis was used to estimate the proportion of past exposures with EBV, CMV, HHV6 and hepatitis viruses.

8.5 Results

8.5.1 Characteristics of the participants

8.5.1.1 Socio-demographic characteristics of the participants

A total of 538 participants selected from the participants attending the primary health care facilities of Aden Governorate were selected. The general characteristics of all the participants are described in chapter 3 (tables 3.1. and 3.2), however, selected variables of the participants are illustrated in table 8.1.

Two thirds of the population (364) were children (<18 years) (67.7%) and of these, 234 (64.3%) were under 7 years old. Both genders were represented, with 52% being male. The age of the participants ranged from one month to 79 years with a mean of 6 years for children and 44 for adults. Of 174 adults, 116 (67%) had no formal education or only primary education and 58 (33%) completed secondary or higher education (95% CI 59-74; 26-41, respectively). Around 59% of the adults were employed.

There was a median of 7 members per household and 3.4 individuals per room.

Electricity, piped water and toilet facilities were available in 98%, 94% and 87% of the households, respectively.

Table 8.1 Socio-demographic characteristics of the participants

		N= **538** (%)	95 % CI
Adults: children <18	(adults %)	174: 364 (32.3)	28 - 37
Age /years (children)	mean (S.D) [IQR]	6 (4.5) [2, 10]	-
(adults)	mean (S.D) [IQR	44 (13) [34, 53]	-
Gender	male: female (male %)	280: 258 (52)	44-52
Education (adults 174)	basic/ no formal education	116(66.7)	59-74
	secondary or higher	58(33.3)	26-41
Employment (adults 174)	employed	102 (58.6)	51-66
	unemployed	72 (42.4)	34-49
Number of household members	median [IQR]	7 [5-10]	-
Median crowding ratio*	median [IQR]	3.4 [2-4]	-
Availability of	electricity	528 (98.1)	96-99
	piped water	502 (93.8)	91-95
	toilet	463 (86.7)	83-89

±SD= standard deviation; IQR = inter quartile range

* calculated as number of resident/number of bed rooms

8.5.2 Seroprevalence of EBV, CMV and HHV6 according to characteristics of the participants

8.5.2.1 Overall seroprevalence of EBV, CMV and HHV6 among the participants

Out of 538 samples tested for EBV and CMV, 498 (92%, 95% CI 90%-95%) and 508 (94%, 95% CI 92%-96%), respectively were positive. HHV6 was tested in 182 children < 5 years and of these 137 (80%, 95% CI 75%-87%) were positive.

There was a strong association between EBV, CMV and HHV6 seroprevalence and age as shown in figure 8.2.1. Infants were less frequently infected with EBV, CMV and HHV6 than young adults/children (69% vs. 99%, 80% vs. 99% and 58% vs. 87%, respectively, p values <0.001 for all). Infection occurred from a very early age and 1-2 year old participants had already a high seroprevalence.

8.5.2.2 Seroprevalence of EBV, CMV and HHV6 according to the sociodemographic characteristics of the participants.

The seroprevalence of infection in males (92%, 95% and 77% for EBV, CMV and HHV6, respectively) was similar to the seroprevalence among females (93%, 94% and 73%, respectively). HHV6 cases were all children (minors) not in school and there was no significant difference in the seroprevalence of EBV and CMV by educational level, as infection by these viruses was nearly universal in adults, as shown in table 8.2 Adult participants either employed (98% and 97%) or not (99% and 100%) had similar rates of EBV and CMV infection.

Of the 8 districts, Crater had the highest (97%) and Mualla the lowest EBV infection seroprevalence (81%; p <0.05). For CMV and HHV6, no statistical significant differences were seen in the rate of infection among the 8 districts.

Figure 8.1 Seroprevalence of antibodies to EBV, CMV and HHV6 by age with 95% C.I.

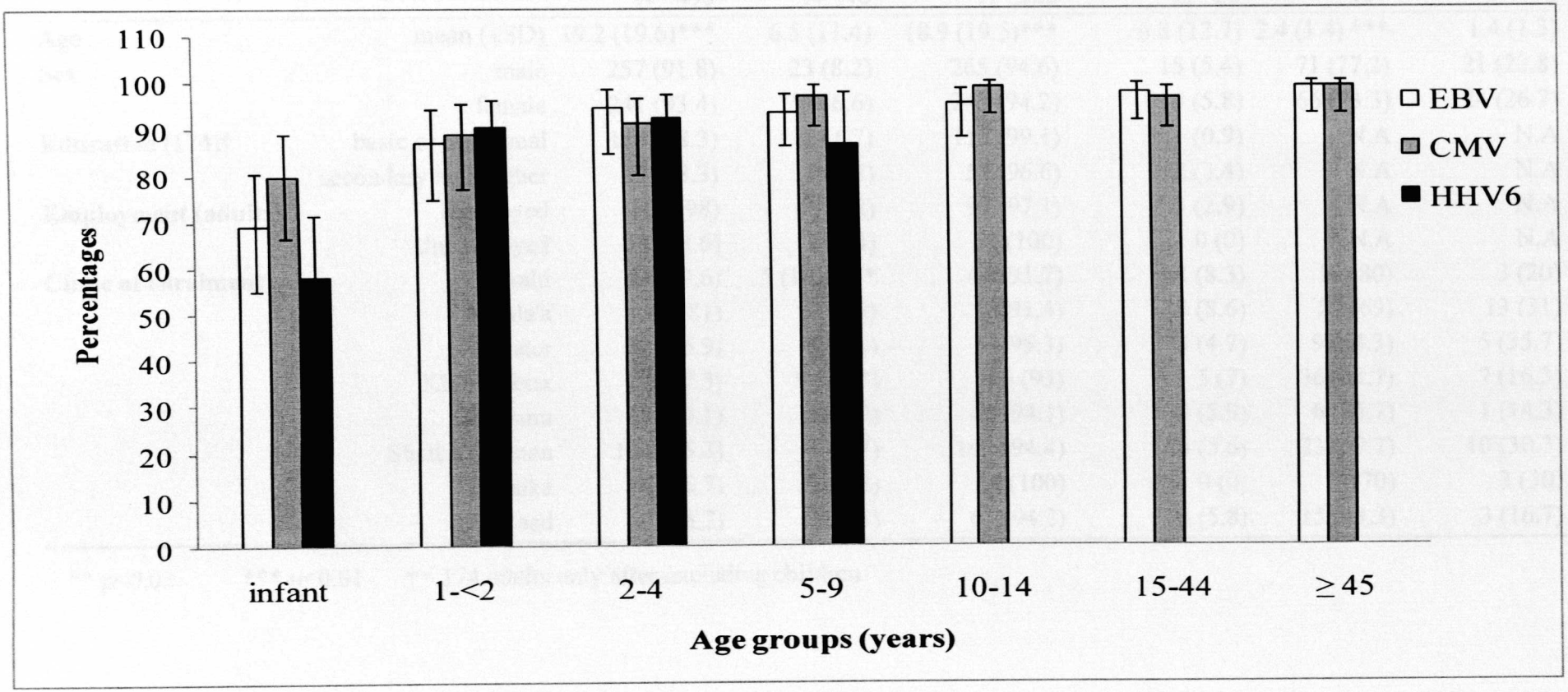


Table 8.2 Seroprevalence of EBV, CMV and HHV6 according to the socio-demographic characteristics of the participants.

		EBV N=538		CMV N=538		HHV6 N=182	
		Positive (%) N= 498	Negative (%) N= 40	Positive (%) N= 508	Negative (%) N= 30	Positive (%) N= 137	Negative (%) N= 45
Age	mean (±SD)	19.2 (19.6)***	6.5 (11.4)	18.9 (19.5)***	6.8 (12.7)	2.4 (1.4) ***	1.4 (1.3)
Sex	male	257 (91.8)	23 (8.2)	265 (94.6)	15 (5.4)	71 (77.2)	21 (22.8)
	female	241 (93.4)	17 (6.6)	243 (94.2)	15 (5.8)	66 (73.3)	24 (26.7)
Education (174)†	basic or no formal	114 (98.3)	2 (1.7)	115 (99.1)	1 (0.9)	N.A	N.A
	secondary and higher	57 (98.3)	1 (1.7)	57 (96.6)	2 (3.4)	N.A	N.A
Employment (adults)	Employed	100 (98)	2 (2)	99 (97.1)	3 (2.9)	N.A	N.A
	Unemployed	68 (98.6)	1 (1.4)	69 (100)	0 (0)	N.A	N.A
Clinic of enrolment	Tawahi	43 (89.6)	5 (10.4) **	44 (91.7)	4 (8.3)	12 (80)	3 (20)
	Muala'a	47 (81)	11 (19)	53(91.4)	5 (8.6)	29 (69)	13 (31)
	Crater	62 (96.9)	2 (3.1)	61(95.3)	3 (4.7)	9 (64.3)	5 (35.7)
	Khormaksar	62 (87.3)	9 (12.7)	66 (93)	5 (7)	36 (83.7)	7 (16.3)
	Mansura	48 (94.1)	3 (5.9)	48 (94.1)	3 (5.9)	6 (85.7)	1 (14.3)
	Sheikh Othman	104 (96.3)	4 (3.7)	102 (94.4)	6 (5.6)	23 (69.7)	10 (30.3)
	Buraika	66 (95.7)	3 (4.3)	69 (100)	0 (0)	7 (70)	3 (30)
	Dar Saad	66 (95.7)	3 (4.3)	65 (94.2)	4 (5.8)	15 (83.3)	3 (16.7)

** $p<0.05$ *** $p<0.01$ †= 174 adults only after excluding children

8.5.2.3 Seroprevalence of EBV, CMV and HHV6 according to medical-related, previous diseases and behavioural characteristics of the participants.

Table 8.3 shows the medical characteristics of participants infected with EBV, CMV and HHV6. There were no significant statistical differences between positive or negative infection in relation to any of these factors, with the exception of being received intravenous therapy and admission to a hospital, which was associated with EBV ($p < 0.01$ and 0.05, respectively).

The association between EBV, CMV and HHV6 viruses and history of hepatitis, malaria, schistosomiasis, tuberculosis or behavioural factors such as exposure to multiple sexual partners, drinking alcohol, smoking cigarettes and chewing Qat had no significant statistical differences between participants with positive or negative infection. However, individuals who indicated that they had experienced malaria had an association with EBV ($p < 0.05$).

8.5.3 Co-infection between EBV, CMV and HHV6

All 475 EBV positive were either co-infected with CMV alone (74%) or with both CMV and HHV6 (26%), as shown in table 8.4. Thirty one participant with CMV were not co-infected with any other viruses (6%) while 355 (70%) were co-infected with another virus (mainly EBV) and 122 (24%) were co-infected with two viruses (EBV and HHV6). For HHV6, 122 (89%) were co-infected with EBV and CMV simultaneously, 2 (2%) were co-infected with CMV alone and 13 (10%) did not have co-infection with other viruses. In general, co-infection between EBV and CMV ($p <0.01$), EBV and HHV6 ($p <0.001$) and CMV with HHV6 ($p<0.05$) were frequent, as shown in table 8.5.

Table 8.3 Seroprevalence of EBV, CMV and HHV6 according to medical history, previous diseases and behavioural characteristics of participants

Variables	EBV N=538		CMV N=538		HHV6 N=182	
	Positive (%) N= 498	Negative (%) N= 40	Positive (%) N= 508	Negative (%) N= 30	Positive (%) N= 137	Negative (%) N= 45
Blood transfusion	38 (7.6)	2 (5)	40 (7.9)	0 (0)	3 (2.2)	0 (0)
Surgery	56 (11.2)	3 (7.5)	57 (11.2)	2 (6.7)	1 (0.7)	0 (0)
Blood with surgery	21 (4.2)	1 (2.5)	22 (4.3)	0 (0)	0	0
Intravenous medication	322 (64.7)	17 (42.5)***	321 (63.2)	18 (60)	64 (46.7)	26 (57.8)
Dental extraction	76 (15.3)	4 (10)	79 (15.6)	1 (3.3)	1 (07)	1 (2.2)
Acupuncture	11 (2.2)	0 (0)	11 (2.2)	0 (0)	3 (2.2)	1 (2.2)
Wet-Cupping (Hijamah)	28 (5.6)	0 (0)	26 (5.1)	2 (6.7)	3 (2.2)	1 (2.2)
Hospitalization	149 (29.9)	5 (12.5)**	150 (29.5)	4 (13.3)	17 (12.4)	8 (17.8)
History of hepatitis	79 (15.9)	2 (5)	78 (15.4)	3 (10)	11 (8)	2 (4.4)
Family history of hepatitis	162 (32.5)	11(27.5)	163 (32.1)	10 (33.3)	40 (29.2)	18(40)
History of malaria	144 (28.9)	5 (12.5)**	142 (28)	7 (23.3)	9 (6.6)	3 (6.7)
History of schistosomiasis	8 (1.6)	1 (2.5)	9 (1.8)	0 (0)	0	0
History of tuberculosis	5 (1)	0 (0)	5 (1)	0 (0)	0	0
Multiple sexual partners	14 (2.9)	0 (0)	13 (2.6)	1 (3.3)	0	0
Drink alcohol	20 (4)	0 (0)	19 (3.7)	1 (3.3)	0	0
Smoking cigarette	34 (8.6)	1 (2.5)	43 (8.5)	1 (3.3)	0	0

** $p<0.05$ *** $p<0.01$

Table 8.4 Co-infections between EBV, CMV and HHV6

	single	+ one virus			+ two viruses	total
		EBV	CMV	HHV6	CMV/HHV6	
EBV (%)	0 (0)	NA	353 (74.3)	0 (0)	122 (25.7)	475
					EBV/HHV6	
CMV (%)	31 (6.1)	353 (69.5)	NA (-)	2 (0.4)	122 (24)	508
					EBV/CMV	
HHV6 (%)	13 (9.5)	0 (0)	2 (1.5)	NA	122 (89)	137

Table 8.5 Association of co-infection between EBV, CMV and HHV6

		CMV		HHV6	
		+ve N (%)	-ve N (%)	+ve N (%)	-ve N (%)
EBV	+ve N (%)	475 (95.4)	23 (4.6)***	122 (79.7)	31 (20.3) ***
	-ve N (%)	33 (82.5)	7 (17.5)	15 (51.7)	14 (48.3)
CMV	+ve N (%)	-	-	124 (78)	35 (22)**
	-ve N (%)	-	-	13 (56.5)	10 (43.5)

** p value < 0.05, *** p value < 0.01

8.5.4 Seroprevalence of EBV, CMV and HHV6 and positive hepatitis markers

A high proportion of infection with EBV and CMV (87% for each) was found among participants with serological evidence of HAV ($p< 0.01$ for EBV) and 70% for HHV6. In addition, participants with positive markers of anti-HBcore showed a rate of 17% infection for either EBV and CMV and 3% for HHV6 which was statically significant for the later ($p <0.05$). Similarly, those with positive markers of HEV were 11% infected with each EBV and CMV and 4% with HHV6. All those participants with positive markers for HBsAg and HCV were infected with EBV and CMV, as shown in table 8.6.

Table 8.6 Past exposure of herpesviruses with hepatitis viruses

Serological evidence of viral hepatitis		**EBV N=538**		**CMV N=538**		**HHV6 N=182**	
		Positive (%) N= 498	Negative (%) N= 40	Positive (%) N= 508	Negative (%) N= 30	Positive (%) N= 137	Negative (%) N= 45
HAV	positive	441 (88.6)	25 (62.5)***	442 (87)	24 (80)	93 (69.9)	31 (68.1)
	negative	57 (11.4)	15 (37.5)	66 (13)	6 (20)	44 (32.1)	14 (31.1)
HBV (anti-HBcore)	positive	82 (16.5)	5 (12.5)	85 (16.7)	2 (6.7)	4 (2.9)	5 (11.1)**
	negative	416 (83.5)	35 (87.5)	423 (83.3)	28 (93.3)	133 (97.1)	40 (88.9)
HBsAg	positive	8 (9.8)	0 (0)	8 (9.4)	0 (0)	0	0
	negative	74 (90.2)	5 (100)	77 (90.6)	2 (100)	0	0
Anti-HCV	positive	1 (0.4)	0 (0)	1 (0.4)	0 (0)	0	0
	negative	244 (99.6)	14 (100)	245 (99.6)	13 (100)	64 (100)	17 (100)
Anti-HEV	positive	38 (11.1)	0 (0)	35 (10.5)	3 (13.6)	3 (3.8)	1 (3.7)
	negative	304 (88.9)	14 (100)	299 (89.5)	19 (86.4)	77 (96.2)	26 (96.3)

** $p<0.05$ *** $p<0.01$

8.5.5 Multiple past exposures between EBV, CMV and HHV6 with hepatitis A, B, C and E viruses.

Multiple past exposures were also found between hepatitis viruses and the three herpesviruses. Generally, multiple past exposure was frequently identified with 4 viruses for HCV, 5 viruses for HEV and HHV6 and 6 viruses with HAV, HBV, EBV and CMV. A high proportion (81%) of patients with HBV was simultaneously past exposure with HAV, EBV and CMV. Around half of patients with HAV, EBV and CMV were co-infected with two of these three viruses (table 8.7 and 8.8).

The expected frequency of past exposure for 2 viruses was calculated by multiplying the prevalence of one virus by the prevalence of the second virus. Variations were found between the observed and expected rates of past exposure. Observed rates were higher than expected rates in past exposure between HAV, EBV, CMV and HHV6. The seroprevalence of these four viruses is high in this community. For example, the observed and expected past exposure rates between HAV and the other 6 viruses were 89% vs. 81%, 95% vs. 82%, 85% vs. 65%, 18 vs. 14%, 12% vs. 10% and 0.4 vs. 0.4 for EBV, CMV, HHV6, HBV, HEV and HCV, respectively (table 8.9)

Table 8.7 Multiple past exposures between herpesviruses and hepatitis viruses

	Multiple past exposure							
	Single	1 virus	2 virus	3 virus	4 virus	5 virus	6 virus	Total
CMV (%)	33 (6.5)	23 (4.5)	207 (40.7)	124 (24.4)	85 (16.7)	35 (6.9)	1 (0.2)	508
EBV (%)	23 (4.6)	0 (0)	232 (46.6)	122 (24.5)	82 (16.5)	38 (7.6)	1 (0.2)	498
HAV (%)	24 (5.2)	1 (0.2)	227 (48.7)	93 (20.0)	83 (17.8)	37 (7.9)	1 (0.2)	466
HHV6 (%)	13 (9.5)	2 (1.5)	22 (16.1)	93 (67.9)	4 (2.9)	3 (2.2)	0 (0.0)	137
HBV (%)	2 (2.3)	2 (2.3)	1 (1.1)	70 (80.5)	7 (8.0)	4 (4.6)	1 (1.1)	87
HEV (%)	0 (0.0)	1 (2.6)	2 (5.3)	25 (65.8)	7 (18.4)	3 (7.9)	0 (0.0)	38
HCV (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100)	0 (0.0)	0 (0.0)	1

Table 8.8 Distribution of past exposures in herpesviruses and hepatitis viruses

EBV (%)		EBV	CMV	HAV	HHV6	HBV	HEV	HCV
Single	23 (4.6)							
1 virus	0 (0)							
2 viruses	232 (46.6)		√	√				
3 viruses	122 (24.5)		√	√	√			
4 viruses	82 (16.5)		√	√	√	√		
5 viruses	38 (7.6)		√	√	√	√	√	
6 viruses	1 (0.2)		√	√	√	√	√	√
CMV (%)								
Single	(33 (6.5)							
1 virus	23 (4.5)	√						
2 viruses	207 (40.7)	√		√				
3 viruses	124 (24.4)	√		√	√			
4 viruses	85 (16.7)	√		√	√	√		
5 viruses	35 (6.9)	√		√	√	√	√	
6 viruses	1 (0.2)	√		√	√	√	√	√
HHV6 (%)								
Single	13 (9.5)							
1 viruses	2 (1.5)		√					
2 viruses	22 (16.1)	√	√					
3 viruses	93 (67.9)	√	√	√				
4 viruses	4 (2.9)	√	√	√		√		
5 viruses	3 (2.2)	√	√	√		√	√	
6 viruses	0 (0.0)							

HAV (%)	CMV	EBV	HAV	HHV6	HBV	HEV	HCV
24 (5.2)							
1 (0.2)	√						
227 (48.7)	√	√					
93 (20.0)	√	√		√			
83 (17.8)	√	√		√	√		
37 (7.9)	√	√		√	√	√	
1 (0.2)	√	√		√	√	√	√
HBV (%)							
2 (2.3)							
2 (2.3)	√						
1 (1.1)	√		√				
70 (80.5)	√	√	√				
7 (8.0)	√	√	√	√			
4 (4.6)	√	√	√	√			
1 (1.1)	√	√	√	√		√	√
HEV (%)							
0 (0)							
1 (2.6)							
2 (5.3)	√						
25 (65.8)	√	√					
7 (18.4)	√	√	√				
3 (7.9)	√	√	√		√		
0 (0.0)							

* One patient infected with HCV was co-infected with 4 viruses (CMV, EBV, HAV and HBV)

Table 8.9 Expected and observed past exposure rates (%) in herpes and hepatitis viruses

	HAV		EBV		CMV		HHV6		HBV		HEV		HCV	
	E	O	E	O	E	O	E	O	E	O	E	O	E	O
HAV	-	-	81	89	82	87	65	70	14	18	10	97	0.4	100
EBV	81	89	-	-	87	94	70	89	15	18	10	100	0.4	100
CMV	82	95	87	95	-	-	71	89	15	16	10	92	0.4	100
HHV6	65	85	70	88	71	84	-	-	12	2	8	100	0.3	0
HBV	14	18	15	17	15	17	12	3	-	-	2	18	0.01	100
HEV	10	12	10	11	10	11	8	4	2	11	-	-	0.04	0
HCV	0.4	0.4	0.4	0.4	0.4	0.4	0.3	0	0.1	2	0.04	0	-	-

E=expected; O=observed

8.6 Discussion

This study was undertaken to characterize the seroepidemiology of EBV, CMV and HHV6 infections in Aden Governorate of Yemen and their frequency of past exposure with hepatitis viruses. The overall seroprevalence of infection was 93%, 94% and 75%, respectively, which is consistent with seroprevalences reported worldwide (Black et al., 1996; Zerr et al., 2005). These seroprevalences were seen to progressively increase with age, similar to the pattern found in most studies across the world, in particular with those of developing countries (Macsween & Crawford 2003; De Bolle et al., 2005a; Zerr et al., 2005; Takeuchi et al., 2006). Most of these studies have also identified a lower seroprevalence of EBV, CMV and HHV6 in children compared to adults (50% vs. 100%, 60% vs. 98% and 57% vs. 83%, respectively) (Arcenas & Widen 2002; De Bolle et al., 2005a; Takeuchi et al., 2006; Zadeh et al., 2006).

This is the first study of the seroprevalence of these viruses in Yemen with a wide range of age groups including children and infants. There were no significant differences in the seroprevalence of CMV and HHV6 among the participants across the districts of Aden, although participants enrolled from Crater and Mualla had the highest and lowest seroprevalence of EBV ($p < 0.05$). However, there is no reason to expect differences in the seroprevalence by district, as living conditions do not have marked differences and the mode of transmission of these viruses is mostly the same.

A significant proportion of participants infected with EBV had a history of being hospitalized or contracting malaria ($p<0.05$ for both) which was not seen among participants infected with CMV and HHV6. Feorino (1974) and Kataaha (1984) have suggested that malaria facilitates EBV replication (Okuno et al., 1989; Liu et al., 1990; Bhattarakosol et al., 2001; de Ory et al., 2004; Figueira-Silva & Pereira 2004). Other studies have shown a special importance of malaria as a co-factor in the development of Burkitt's lymphoma, particularly where malaria transmission is holoendemic (Feorino & Mathews 1974; Kataaha et al., 1984). Moreover, several studies have illustrated that

patients who developed Burkitt's lymphoma have significantly higher titres of antibodies against EBV viral capsid antigen than controls (Moormann et al., 2005; Rasti et al., 2005; Lubega 2007). Although malaria and EBV infection are recognized cofactors for Burkitt's lymphoma, their relative contribution is not understood. A recent study identified an association between acute malaria and sustained increase in EBV load and a transient decrease in EBV-specific T cells (Parkin et al., 2000; Moormann et al., 2005). Given the cross sectional characteristics of the study and the retrospective ascertainment of malaria, without parasitological confirmation, it is uncertain whether the malaria reported preceded EBV infection and recall-bias could further underestimate or overestimate the history of malaria among the participants, which needs cautious interpretation.

Many studies across the world have suggested the oncogenic potential of EBV infection to develop Hodgkin lymphoma (Njie et al., 2009). However there is a considerable diversity in the incidence, age and sex distribution, as well as morphology of this tumour in different populations which argues for a major impact of environmental and/or genetic factors in the development of the disease (Chan et al., 1995a; Glaser et al., 1997; Weiss 2000; Spano et al., 2003; Mautner et al., 2004; Young & Rickinson 2004; Pai et al., 2007; Young et al., 2008). For example, Weinreb (1996) demonstrated the active involvement of EBV in the pathogenesis of Hodgkin's disease in children, but with significant variation, from 50% of the cases reported from the United Kingdom, South Africa, Egypt, and Jordan to 100% from Kenya (Gulley et al., 1994; Quintanilla-Martinez et al., 1995; Glaser et al., 1997; Engel et al., 2000; Al-Kuraya et al., 2006; Lindner & Sugden 2007). Weiss (2000) reported EBV in the neoplastic element of Hodgkin's disease in about 40% to 50% of cases in Western populations (Weinreb et al., 1996). Other report however have identified lowest rates of EBV-associated Hodgkin lymphoma in young adults (Weiss 2000) and recent studies do not support that EBV is associated with paediatric Hodgkin lymphoma and have suggested a correlation with a

genetic susceptibility for Hodgkin lymphoma between different ethnic groups (Yung & Linch 2003).

In Aden, the overall annual incidence of Hodgkin lymphoma, as reported from 2002 to 2004, was 1.9 per 100.000 populations (Al-Kuraya et al., 2006; Chabay et al., 2008; Hassan et al., 2008; Hassan et al., 2008a). Variations were also observed in the incidence between children and adults, and between males and females. For example, Hodgkin lymphoma was higher in male than female children (1.1 and 0.6 per 100.000 population), respectively (ACC 2007). This incidence is not far from those reported in neighbouring countries such as Bahrain, Saudi Arabia, Kuwait, Oman and Qatar for males (1.2, 2.1, 4, 3.1 and 6.1 per 100.000 population, respectively) and females (1.3, 1.2, 0.6, 5.9 and 3.5 per 100.000 population), respectively (ACC 2007). However, few or no studies describing the prevalence of the EBV are available from these countries.

Past exposures were found between the three herpes and the hepatitis viruses (HAV, HBV, HCV and HEV). A high proportion of HAV-positive participants were co-infected with EBV and CMV, whereas 75% of the children with HAV were co-infected with HHV6. The seroprevalence of HAV per se is high in this community with rates of 81% and 99% among children and adults, respectively (see chapter 3). As the seroprevalence of infection of these viruses is high, the high past exposure rate is expected. The differences in the seroprevalence of the four viruses (HAV, EBV, CMV and HHV6) were significantly different between children and adults. HBV was found to be co-infected with the 3 herpesviruses although the association with HHV6 was only significant.

Some authors have grouped the three herpesviruses (EBV, CMV and HHV6) and some of the hepatitis viruses (mainly B and C) as transfusion- related viruses that can cause hepatitis (al-Lawati et al., 1999; Kandil et al., 2001; Al-Kuraya et al., 2006; GCCR 2006), with overlapping clinical presentation (Kaur & Basu 2005). Altered liver function enzymes have been reported during the course of EBV infection; although, overt clinical hepatitis with icteric state is rarely encountered (Yuge et al., 2004; Crum

2006). In addition, it has been suggested that this mild hepatitis is usually caused by primary EBV infection (Barlow et al., 2000; Macsween & Crawford 2003; Yuge et al., 2004; Dogan et al., 2007). Previous studies have related infection of HHV-6 with transfusion of blood or its components, as the virus has white blood cell tropism (Al-Faleh et al., 1995; Karagoz et al., 2005; Hara et al., 2006).

The majority of HHV6 children were co-infected with EBV and CMV. This picture is quite expected as the last two viruses were highly prevalent in adults and attained a high seroprevalence in children. In general, few studies have investigated whether there is any clinical intermingling between CMV reactivation with EBV or HHV6 in patients especially patients with immunesuppressed conditions. EBV and CMV can reactivate independently in patients under immunosuppressive therapy or AIDS (Chamberland et al., 2001; Pascher et al., 2004). Some studies have identified an association in the reactivation stage between these viruses (Stevens et al., 2001; Wagner et al., 2002). HHV-6 infection has been proposed as a co-factor for EBV or CMV disease, leading to increases of CMV replication in blood (Arcenas & Widen 2002; Zadeh et al., 2006; Yoshizaki et al., 2007).

No association has been found in this study between cases reporting a history of hepatitis or jaundice and infection with any of the three herpesviruses. Some studies elsewhere have shown that symptoms of hepatitis have been occasionally observed after primary HHV6 not only in infants but also in adults (Humar et al., 2000; Cainelli & Vento 2002; Jacobs et al., 2003; Bauer et al., 2007). Recently, several case-report studies have attributed manifestations of hepatitis to primary EBV infection (Mendel et al., 1995; Suga et al., 2000; Aita et al., 2001; Csire et al., 2007) which resemble HAV infection. This development raised a question of how many serologically unproven cases of jaundice could be due to primary EBV (Massei et al., 2001; Prassouli et al., 2007). Therefore, EBV, CMV and HHV6 should be included in the differential of patients presenting with liver abnormalities. It is known that EBV infects the liver and

occasionally causes an acute state; however, this later stage was observed commonly associated with the high EBV viral load in the peripheral blood mainly in patients under immunosuppressive therapy or with AIDS (Prassouli et al., 2007). Sometimes, this virus may trigger autoimmune mechanisms which present with diffuse involvement of the liver associated with disseminated intravascular coagulation (Whitley 1994; Mason et al., 1996; Yuge et al., 2004; Hara et al., 2006).

Some authors have reported that cases of chronic active EBV could lead to death due to hepatic failure (Cermelli et al., 1999; Hinedi & Koff 2003). Post-transplant CMV disease is a significant cause of morbidity and graft loss. CMV hepatitis has been described as the most frequent manifestation of CMV tissue invasive disease after liver or renal transplantation and among pregnant women (Barlow et al., 2000; Okamura et al., 2000). However, the advancement in the rapid and accurate early molecular diagnosis and the use of antiviral agents through the pre-emptive therapy, could contribute positively toward its prevention in these situations (Chan et al., 1995b; Humar et al., 2002; Seehofer et al., 2002; Tarhan et al., 2003; Eskild et al., 2005).

It is anticipated that the information yielded by this study, whether on the seroprevalence of EBV, CMV or HHV6 infection and of their past exposure with other hepatitis viruses can improve our understanding of the role of these viruses in other related diseases. This study can help in the application of the preventive and control measures relevant to some related diseases in the population such as IM, Burkitt's lymphomas and organ transplantations.

CHAPTER 9: GENERAL DISCUSSION

9.1 Introduction

This chapter summarises the main findings related to the seroprevalence of the four hepatitis viruses (A, B, C and E) among a population attending primary health care facilities in Aden Governorate; the risk factors of HBV transmission in this community; the dominant HBV genotypes; the seroprevalence and risk factors of HBV and HCV among chronic liver disease patients and among those exposed to multiple blood transfusion or undergoing haemodialysis. The factors enhancing or affecting HBV vaccine coverage in children < 5 years old in Aden City and past exposure between three herpesviruses (EBV, CMV and HHV6) and hepatitis viruses also discussed. The study limitations are highlighted and questions for further research are proposed.

9.2 Epidemiology of viral hepatitis infection.

9.2.1 Seroprevalence in healthy individuals

The findings in Aden City coincide with the worldwide epidemiological features of hepatitis viruses (A, B, C and E) from very high rates of infection in HAV and very low HCV.

9.2.1.1 Enterically transmitted viruses (HAV and HEV)

A significant difference in seroprevalence of HAV and HEV was observed in this study between children <18 years and adults. The seroprevalence of HAV increased steadily from 53% among infants, 82% in pre-school children to 99% amongst adults. Although the overall seroprevalence was very high (87%), it was lower than reported for the northern governorates of Yemen (Scott et al., 1990). The mean age of participants in the later study was higher than in the participants in this study and the resulting difference of 13% could be an artefact of population structure. However, data for Scott's study is not reported by age and is not possible to investigate whether the effect is due to this confounder.

The high seroprevalence of HAV infection in pre-school children (82%) was similar to other studies on children of the same age group in developing countries (Abdool Karim & Coutsoudis 1993; Acharya et al., 2003), but higher than other Middle East countries (≤ 50%) (Arif M 1995; MOH-SO 1999; Sacy et al., 2005), and far higher than the seroprevalences currently reported from Industrialised countries (≤ 13%) (Sohn et al., 2000; Mossong et al., 2006; Kiyohara et al., 2007; Ansaldi et al., 2008). In adults, the seroprevalence in Aden was similar to the seroprevalence in other Middle East countries (> 95%) (MOH-SO 1999; Fathalla et al., 2000; Tufenkeji 2000; Jacobsen & Koopman 2004). No data are available from community based studies in Yemen, however, a hospital-based study in 1988 in northern governorates showed similar prevalence as this study (Scott et al., 1990). This is the first study in Yemen to show differences in the rate of infection between children and adults.

HEV in Yemen has barely been studied before. One study based on patients with acute hepatitis reported a rate of 14% (Gunaid et al., 1997). The seroprevalence rate of 11% obtained in this study would place Yemen in the high seroprevalence zone (>5%) which is similar to the seroprevalence in other developing countries (7.2% to 24.5%) (Krawczynski et al., 2000; Sookoian 2006). Population-based studies in Middle East countries suggest that the virus is highly endemic in the region (>15%), although waterborne outbreaks have not been reported (Abdelaal et al., 1998; Irshad 1999; Fix et al., 2000; Kumar et al., 2001; Meky et al., 2006; Stoszek et al., 2006c).

Yemen still has a low availability of potable water and sanitation (WorldBank 2006). According to the 2005 Ministry of Planning and International Cooperation (MOPIC) report, access to water and sanitation services was available for 65% and 35% of the population, respectively (MOPIC 2006) . Similarly, there is poor control of food hygiene, low level of health awareness, high illiteracy and poverty rates and high infection rates among young children (WorldBank 2006; Worldbank 2007).

These factors contribute to an increasing spread of HAV and HEV in the community. In comparing Aden with other cities in Yemen, piped water and sanitary toilet services are available for 94% and 87%, respectively, of the population in Aden which could not found in other city in the country particularly in main cities such as Sana'a and Taiz. To confront the water crisis in these cities residents have been forced to buy untreated water from private sources. A microbiological examinations study was conducted in Sana'a and Taiz (main cities in northern governorates) to assessed the quality of partially treated drinking water in private establishments in the City have shown that 83%-90% of the samples were contaminated; 50% with faecal coliforms and 33% with total coliforms (Raja'a et al., 2001; Metwali 2003). This indicates probably a problem of gross contamination with viruses (including HAV and HEV) or other parasites. Therefore, substantial changes would be needed to achieve the control of HAV and HEV infection in Yemen with priority on improving the water quality, sanitation coverage, public food hygiene and awareness of the risk of infection and prevention.

9.2.1.2 Blood-borne viruses (HBV and HCV)

The overall seroprevalence of HBV infection in this study was 16% with variation in the seroprevalence by age. Low rates were observed in children <18 years (7%) and high rates in adults (35%). HBV carriage had a similar trend with an overall rate of 1.5% and none of the children <18 years old being carriers compared to 4.6% of the adults. This age variation reflects the cumulative exposure of adults to hepatitis. It may also reflect lack of education, inadequate blood transfusion safety measures, unsafe use of needles and syringes and lack of vaccination against HBV in previous decades. The seroprevalence found therefore is a mixture of the cohort effect of the markers of infection used, with a probably decreasing rate of infection in younger cohorts which might be protected by the vaccine. To our knowledge there is no previous study of the seroprevalence of HBV among children in Aden to enable a valid comparison with the current situation. Most studies conducted in Yemen have reported high seroprevalence of HBV in adults in tertiary health care settings in Sana'a and have included hospitalised

patients with acute or chronic hepatitis (Abdel Raheem et al., 1991; Gunaid et al., 1997; Al-Moslih & Al-Huraibi 2001; Al-Nassiri & Raja'a 2001; Haidar 2002) or blood donors (Sallam & Tong 2002). Community surveys however have rarely been conducted and are on a small scale (Scott et al., 1992; Sallam et al., 2003b).

Such findings therefore suggest the following. First, HBV infection is of low seroprevalence rate in the population, which would place Aden in the low seroprevalence zone rather than high endemic zone, as categorized in previous studies in the Middle East (Andre 2000; Qirbi & Hall 2001; WHO 2002b). However, further representative studies in other areas of the country with emphasis to include all age groups, could reveal clear evidence on the country's HBV endemicity in Yemen. Second, age is an important confounder in the picture of HBV infection in this community. Previous studies conducted among hospital employees (Al-Jarba & Al-Sayyari 2003) and blood donors (Sallam et al., 2003a) in Aden City reported high rates of infection among adults and resembled the findings of this study. Third, the lower seroprevalence among children and increase in early adolescence support the hypothesis that HBV infection is more likely to be acquired in early adulthood rather than by vertical transmission. However, recent studies on pregnant women in the perinatal period attending primary health care facilities in Aden City (Yousef Khalidah 2003), showed a carriage rate of 2.8% (Al-Shamahy 2000) and similar rates were reported from Sana'a, where 4% of healthy mothers were identified to be carriers. Vertical transmission may not be a rare event.

It is likely that the implementation of the HBV vaccine would lead to a reduction in the seroprevalence of HBV infection in the population, although data on the seroprevalence of HBV before 1990 are not available. The effectiveness of vaccination programmes against HBV has been clearly demonstrated in some countries in which the rate of HBV infection decreased from 34% to 10% and the carriage rate fell from 11% to 4%, before and after implementing the vaccination programme (Chen et al., 2007a).

The seroprevalence of HCV is very low, with only 0.4% of the population infected and only one adult participant being anti-HCV positive. This rate is at variance from those found in other studies in Yemen, namely in a Soqotrian population (5.1%), Sana'a blood donors (5.2%) (Sallam et al., 2003b) and in Mukalla City (1.6%) (Bahaj 2003). Al-Jarba and Al Sayyari (2003) study of 1.3% HCV seroprevalence was conducted among hospital employees in Aden (Al-Jarba & Al-Sayyari 2003). Globally, it is reported that the transmission of HCV is less common than that of HBV in the general population (WHO 2003b). Some risk factors such as multiple partners, intravenous drug users and commercial sex workers are not common in Yemen. However, further study could be needed to develop a comprehensive picture of the HCV infection in the country looking in-depth at the underling risk factors in different communities.

9.2.2 Seroprevalence of HBV and HCV among risk groups

9.2.2.1 Patients with chronic liver disease

The seroprevalence of HBV and HCV in patients with chronic liver diseases and in those who received polytransfusions or individuals under haemodialysis was studied for the first time in Aden. Unexpectedly, there was a low seroprevalence of HBV markers in patients with chronic liver disease compared to controls (29% and 36%), even though, the rate of HBsAg was higher in cases than controls. For HCV, equal proportion for HCV infection was observed in cases and controls (1 each). These unexpected findings could be due to the small sample size of the study. A number of studies across the world have reported a relationship between the rate of chronic liver disease and the endemicity of HBsAg in the population (Yu et al., 2001; WHO 2002b; Yu et al., 2002). It is assumed, as observed by clinicians in the studied hospitals, that the proportion of autoimmune hepatitis diagnosed might be higher in Aden. However to prove this hypothesis, additional laboratory tests are recommended such as nucleic acid amplification test and other biochemical investigations to exclude any involvement of

blood-borne viral hepatitis. A further investigation with larger sample size could help to determine the causative factors of chronic liver disease in this community.

9.2.2.2 Polytransfused and haemodialysed patients

Infections with HBV were two fold higher in patients with polytransfused/HD than in healthy controls (67% and 36%, respectively) and HCV was nine fold (46% and 5%, respectively) higher. The higher seroprevalence of HBV than HCV was described previously in blood donors at Aden city (Sallam et al., 2003b). The high HBV seroprevalence may reflect the lack of preventive measures and quality of blood services, which up to recently did not meet international standards. Several studies across the world have documented a lower rate of HBV compared to HCV infection in patients receiving HD; however the seroprevalence of HCV in this study was higher than reported elsewhere (Jadoul et al., 2004; Ramezani et al., 2007; Alter 2008). Nevertheless, our findings are in agreement with another study in Yemen (Hajjah), which reported a high seroprevalence of the HCV infection among haemodialysis patients (Haidar 2002). Other countries that have introduced the HBV vaccine for patients undergoing HD together with the isolation of HBV positive patients, the use of dedicated dialysis machines and regular surveillance for HBV infection, have dramatically reduced the spread of HBV among this high risk population (Reddy et al., 2005; Beran 2008; Toosi et al., 2008).

9.2.3 Risk factors for HBV and HCV infection

9.2.3.1 Risk factors for HBV and HCV infection in healthy participants

This is the first case-control analysis of risk factors for HBV and HCV infection among a population attending primary health care settings in Aden Governorate. Four main factors were significantly associated with HBV infection. These include age, a family size > 5, having received transfusions of blood or blood products and having a landline telephone. Even after stratification into age groups ≤ and > 13 years, age was still positively associated with HBV infections in adults; suggesting that new infection can occur in adults. HBV infection being more likely to occur within large families suggest

that person to person transmission can occur in these conditions through common utensils shared by the household members (Omer et al., 2001b; Alter 2003a). Similar studies across the world have shown that households with many carriers results in an increased rate of infection (Milne et al., 1987; Phoon et al., 1987; Euler et al., 2003; Tiwari & Kumar 2005; Topuzoglu et al., 2005; CTC 2007). In Yemen however, Scott (1999) and Al-Nassiri (2001) found no association between HBV infection and household size in Sana'a and this could be due to differences in the study design and population settings (Scott et al., 1990; Al-Nassiri & Raja'a 2001).

Surprisingly, a history of having received blood transfusion had an inverse association with HBV infection in adult cases. This finding was inconsistent with findings of previous studies from Sana'a (Scott et al., 1990; Al-Shamahy 2000; Al-Nassiri & Raja'a 2001). However, a study on HBV markers among blood donors identified a lower rate of carriers in Aden than in Sana'a and may explain these paradoxical findings (Sallam & Tong 2002).

Blood services in Yemen are based mainly on volunteer blood donation, usually by friends or relatives and sometimes through donation campaigns. The transmission of HBV via blood transfusion in Yemen has become less frequent than in the past due to the implementation of control measures in blood transfusion facilities. Recently, the Ministry of Health paid attention to the diagnostic laboratory services and blood transfusions facilities particularly medical laboratories and blood banks at the central level and governorates (Own 2005), and this may indicate a reduction in the rate of transmission of HBV. However further investigations would be required to elucidate this assumption and to assess the quality of laboratories, emergency and blood transfusion services. Other issues, such as inappropriate multiple use of syringes and needles should also be investigated.

The association between ownership of a landline telephone and HBV infection is difficult to explain. This is a surrogate marker of wealth in this setting, suggesting that cases were economically better off than controls.

9.2.3.2 Risk factors for HBV and HCV in patients with chronic liver disease

This study demonstrated that the practice of acupuncture and wet- cupping (Hijamah) are factors independently associated with chronic liver disease. In both cases, sharp instruments are used/reused and probably not adequately sterilized after each use. It has been shown that cupping increased 4 fold the risk of CLD, although, the small sample of cases; still restricts the robustness of the findings. It was however, uncertain whether the reported acupuncture and cupping treatments actually preceded the disease or if they were sought afterwards to relieve some of the symptoms of chronic liver disease. Studies across the world have found contradictory findings of practiced acupuncture or cupping and CLD (Kiyosawa et al., 1994; Sun et al., 1999; Shin et al., 2000).
A personal or family history of hepatitis or jaundice was associated with CLD after adjustment with other independent factors. However, it is not clear whether this was due to HBV/HCV or other viral hepatitis. The fact that almost all adults in Aden have evidence of HAV infection and usually developed jaundices in young childhood or adulthood (see chapter 3); suggest that horizontal transmission of HAV/HBV among the household members could occur. Recall-bias further underestimates the history of hepatitis among the family members.

Our study identified other less frequently recognized CLD risk factors. These include a history of chewing Qat, smoking tobacco, lack of education, the use of private clinics and economic markers such as the lack of household appliances such as refrigerators.

Chewing Qat is associated with smoking tobacco. Cigarette smoking is commonly used in Qat sessions (Griffiths et al., 1997). Smoking is a risk factor for cirrhosis or HCC (Kuper et al., 2000; Chen et al., 2003b; Marrero & Marrero 2007), and that it's relationship is stronger in the presence of HBV and/or HCV infection (Tzonou et al.,

1991; Donato et al., 2006). Our study, however did not seek to describe if there was a dose-effect of Qat or tobacco or the duration of these habits and further studies would be needed.

9.2.3.3 Risk factors for HBV and HCV in polytransfusion and/or haemodialysis

Although, a study of the risk of acquiring HBV and HCV in the health care facilities is an important element in developing approaches to avoid nosocomial infection, the analysis in this study was undertaken to demonstrate the main factors associated with the transmission of HBV and HCV viruses either related to the host or to the health care environment. Two factors were independently associated with HBV and HCV infection among patients with polytransfused/HD, namely the duration along of being under haemodialysis/years and a history of malaria. The former is in agreement with the medical literature (Wang et al., 2002; WHO 2003b; Jadoul et al., 2004; Imarengiaye et al., 2006; Khattab 2008; Niederhauser et al., 2008; O'Brien et al., 2008). Patients on HD for more than one year were around 7 and 12 times more likely to acquire HBV and HCV. These findings are consistent with studies from Saudi Arabia (Khan & Khan 2001) and Brazil (Carrilho et al., 2004) where the seroprevalence of HBV and HCV were significantly associated with the duration of dialysis.

Recently, studies across the world have reported a decrease in the incidence of HBV/HCV in haemodialysis patients (Jadoul et al., 2004; Olut et al., 2005; Khattab 2008). This could be related to the improvement of standards in the selection of blood donors, use of advanced diagnostic tests and routine vaccination of uraemic patients (Beran 2008).

A history of malaria was associated with HCV infection in patients undergoing HD. Malaria remains a devastating global health problem worldwide (Zewdu 1994; Mehta et al., 2001; Abdul Manan et al., 2006). Acute renal failure commonly occurs in sever *Plasmodium falciparum* malaria, which is the most frequent form of malaria in Yemen (Amran 2006). In addition, cases with severe malaria can develop anaemia, which may

require blood transfusions. Thus, the role of malaria could be via its role in renal failure leading to haemodialysis or by causing anaemia requiring transfusions. Further studies are required to identify the role of malaria as a cause of renal failure in Yemen and its association with further hepatitis infection.

9.2.4 Past exposure of hepatitis viruses

9.2.4.1 Past exposure in healthy participants

Past exposure between the hepatitis viruses was frequently found and a maximum of 4 viruses were identified per person. For instance, HAV was co-infected with HBV, HCV and HEV. An association was found between HAV and HBV however, their route of transmission is different, probably due to the high rate of HAV infection.

9.2.4.2 Past exposure in patients with CLD or individuals at risk of polytransfusion and/or haemodialysis

Only one case with dual infection of HBV and HCV was identified among patients with CLD compared to one third of the individuals under polytransfusion or haemodialysis. HBV and HCV share a common route of transmission and coexist simultaneously, which may explain their likelihood to show the past exposure. Global reports show variation in the rate of dual infection in patients with polytransfusion/HD from low to high rate (Reddy et al., 2005; Jutavijittum et al., 2007; Ramalingam et al., 2007).

9.2.4.3 Past exposure of hepatitis viruses with herpesviruses

This is the first study of the seroprevalence of EBV, CMV and HHV6 in Yemen identifying the frequency of past exposure with hepatitis viruses. The overall high seroprevalence of infection is consistent with seroprevalences reported from developing countries (Macsween & Crawford 2003; De Bolle et al., 2005a; Zerr et al., 2005; Takeuchi et al., 2006) with an early increase with age (Arcenas & Widen 2002; De Bolle et al., 2005a; Takeuchi et al., 2006; Zadeh et al., 2006).

A significant proportion of participants with EBV had a history of being hospitalized or contracting malaria, which was not seen among participants infected with CMV and

HHV6. Several studies have suggested that malaria facilitates EBV replication and both are recognised as co-factors in the development of Burkitt's lymphoma, however, their relative contribution is not understood (Feorino & Mathews 1974; Kataaha et al., 1984; Moormann et al., 2005; Rasti et al., 2005; Lubega 2007). Given the cross sectional characteristics of the study and the retrospective ascertainment of malaria, without parasitological confirmation, it is uncertain whether the malaria reported preceded EBV infection and recall-bias could further underestimate or overestimate these findings and need cautious interpretation.

Past exposures were found between the three herpes and the four hepatitis viruses. A high proportion of HAV-positive participants were co-infected with EBV and CMV, whereas 70% of the children with HAV were co-infected with HHV6. The seroprevalence of HAV per se is high in this community, thus the high past exposure rate is expected.

A high proportion of participants with HAV and HBV were co-infected with CMV. Patients with HBV were found to be co-infected with the 3 herpesviruses, although only the association with HHV6 was statistically significant. Some authors grouped the herpesviruses EBV, CMV and HHV6 with the hepatitis HBV and HCV as transfusion-related viruses that can cause hepatitis (Kaur & Basu 2005), with overlapping clinical presentation (Yuge et al., 2004; Crum 2006).

The majority of HHV6 children were co-infected with HAV, EBV and CMV. This picture is quite expected as these viruses were highly prevalent in adults and attained a high seroprevalence in children. Recently, several case reports have attributed the manifestations of hepatitis to primary EBV infection (Massei et al., 2001; Prassouli et al., 2007) which resemble HAV infection. CMV hepatitis has been described as the most frequent manifestation of CMV tissue invasive disease after liver transplantation and among pregnant women (Chan et al., 1995b; Humar et al., 2002; Seehofer et al.,

2002; Tarhan et al., 2003; Eskild et al., 2005). Therefore, EBV, CMV and HHV6 should be included in the differential diagnosis of patients presenting with liver abnormalities in this setting.

9.3 The role of HBV vaccine in the EPI programme

Sixty three percent of children < 5 years old were vaccinated in Aden with three doses and 82% received at least one dose of the vaccine. The three-dose vaccine coverage rate was lower than the target of the EPI for Yemen (85%), but higher than the rates reported for the country as a whole (49%) and for some of the Governorates with large populations such as Sana'a (36%), Taiz (22%) and Al Mukalla (46%) (MOPHP/EPI 2005). Despite the higher rate in Aden, this rate is lower than reported from other Middle East countries (Toukan 1996; Qirbi & Hall 2001).

This study illustrated that the lack of parental education and not having a car were associated with HBV vaccine uptake. Uneducated fathers are likely to have less information about HBV transmission and the protective role of the vaccine. Nearly all parents/guardians of vaccinated children were aware of the importance of immunizing their children. Studies across the world have shown the importance of parental education in the timely compliance with the vaccines schedule (Waldhoer et al., 1997; Salmaso et al., 1999).

HBV vaccine coverage was 2 to 3 fold higher among families with a car. This could be explained by better access to the health centres to receive their vaccines as public transport in Aden is inadequate. In addition, ownership of a car is a marker of income, as families with higher income are more likely to have their children vaccinated.

It is not known why children > 1 year old have not completed their vaccination. Possible causes could include health service related factors (such as availability of vaccine, EPI policy of providing HBV vaccine free of charge only to children < 1 during the first three years of the programme (2000-2002), and individual factors such

as the knowledge and attitude of parents toward those vaccines. A delay in the up-take of vaccines among infants has been shown in studies from different countries (Salmaso et al., 1999; Cui & Gofin 2007).

Despite the coverage of the HBV vaccine at the time of the study still being at unsatisfactory levels, the current incorporation of the pentavalent vaccine, available since March 2005 through the EPI programme of Yemen, could increase the rate up to 95% as targeted.

9.4 Limitations

Over the course of the work, some limitations were considered, which are summarised in this section. As most of the studies were cross sectional surveys it was not possible to account for infected individuals who did not survive, whether neonates, babies, children or adults, resulting in a selection bias of a healthier population. A further shortcoming is the difficulty in collecting information for sensitive issues. For example, information on multiple sexual partners, the use of recreational drugs and alcohol are highly sensitive in this environment. In addition, recall bias of factors that had potentially occurred several decades earlier is likely to play a significant role. The cumulative effect of HBV infection on the markers used would result in an attempt to correlate the characteristics of the patients at the time of the study with an infection that occurred at some point in the past. For financial reasons we were unable to test for other markers of recent or active HBV infection such as the presence of HBV DNA and/or hepatitis B e antigen (HBeAg), IgM anti-HBc an indicator of early infection and anti-HBs (antibodies to HBsAg) to exclude those who might have resolved past infections or had been vaccinated. Therefore, some participants in this category may have been missed as some cases with self-limited primary HBV infection never have detectable HBsAg in blood. Although HBV vaccine is a protective factor against infection, the participants' vaccination history was not recorded, which would unavoidably miss some important information on vaccination failure.

Some limitations are also pertinent to the case-control analysis, as the sample size was small and restricted the number of variables that could be examined by multivariate analysis and may not have enough power. Also the classification of chronic liver disease was limited by the lack of objective diagnosis accuracy as opposed to cirrhosis and HCC. Furthermore, autoimmune liver disease is a diagnosis that not clearly differentiated from chronic hepatitis. This was due to the unavailability of diagnostic laboratory tests such as NAAT tests. Only 3 serological markers (Anti-HBc, HBsAg

and Anti-HCV) were used in the case-control studies and the HBV DNA detection method was not used.

The cross-sectional design of the vaccination coverage survey for HBV vaccine only provides information on the coverage rate at the time of the survey, some children < 1 year may not have reached the appropriate time for the subsequent dose. Thus the vaccination coverage obtained may underestimate the vaccination coverage rate. Also, it was found that the 2nd and the 3rd doses of the vaccine were not reported with the date the vaccine was received or the age of the child. Thus, estimates of noncompliance were limited.

9.5 Conclusions

The overall findings in this study indicate that viral hepatitis is an important public health problem in this community. However, the trend of infection with HBV and HCV seems to have improved with the reduction in blood transmitted infections and increase in immunisation in the last decades.

The seroprevalence of viruses causing hepatitis varied from hyperendemic (HAV) to fairly low (HCV); and is associated with increasing age. None of the children < 18 years had evidence of HBV carriage or HCV infection.

This is the first study to estimate the seroprevalence of HEV in Yemen revealing it to be a significant problem. This study is the first in Yemen to show obvious differences in the rates of infection between children and adults.

The overall seroprevalence of HBV observed would place Aden in the low endemic zone category. Further studies in other areas of the country with inclusion of all age groups, could provide further evidence on HBV endemicity.

Perinatal transmission is not a major mode of transmission for HBV in this sitting, which may be the result of a low seroprevalence of carriers and the absence of carriers in children < 18 years old.

Beside vaccination, the low frequency of infection among children may also reflect a reduction in post-transfusion-related infections through the use of safer blood supplies and more widely practiced infection control procedures among medical personnel in recent years.

Acupuncture and cupping are independently associated risk factors for chronic liver diseases. Therefore, close supervision may be needed to control these practices for preventing viral hepatitis infection and their complications.

Polytransfusion and HD are important risk factors for contracting HBV and HCV infection. The duration of the haemodialysis and a history of malaria were associated with increased HBV and HCV infection among patients with polytransfused/HD.

The provision of the HBV vaccine within the EPI since 2000 seems to have had a positive impact on the prevention of HBV infection among children. HBV vaccination coverage in Aden has achieved a rate of 63% in children < 5 years old, which was lower than the EPI target for Yemen (85%), but higher than elsewhere in the country.

The low education of the parents or lack of accessibility to health care facilities was associated with the non-achievement of the required vaccination target, which has to be considered in the future.

This is the first description of the seroprevalence of EBV, CMV and HHV6 in Yemen and high seroprevalence rates and past exposures either within the same herpes group or viral hepatitis viruses were observed.

Information on the seroprevalence of EBV, CMV or HHV6 infection and of their past exposure with other hepatitis viruses could improve our understanding of the role of these viruses in other related diseases and the application of appropriate preventive and control measures.

The information yielded in this study can help to improve our understanding of the role of these viruses in this environment. It will also help to monitor the application of preventive and control measures to reduce the risk of infection.

9.6 Recommendations

(1) Initiate educational programs to target both public and hospital personnel to increased awareness concerning the transmission and the risk of hepatitis viruses and the method of their prevention.

(2) Substantial changes are needed to achieve the control of HAV and HEV infection in Yemen. Priority should be given to improving water quality, sanitation coverage, and food hygiene and health awareness.

(3) Maintain and improve the current methods of pre-and post assessment of patients under haemodialysis. Also any strict adherence to universal precautions combined with proper disinfection procedures are very important and together with keeping new machines for newly diagnosed anti-HCV negative patients and hepatitis B vaccination as early as possible might contain the spread of hepatitis C and B in patients with polytransfusion or haemodialysis and the use of NAAT test.

(4) Further studies would be needed to develop a comprehensive picture of HCV infection in the country and to look for the underling risk factors in these communities as also the dominant genotype.

(5) It is recommended that the use of the pentavalent vaccine within the EPI programme of Yemen may increase the coverage rate of HBV vaccines up to 95% by reducing the number of visits required.

(6) Most cases with CLD had a clinical diagnosis. Efforts are needed to confirm whether this diagnosis is accurate and would require access to more advanced diagnostic tests.

(7) Factors such as multiple sexual partners, intravenous drug use and commercial sex workers are not frequently reported in Yemen. However; further qualitative studies

could be needed to obtain a more reliable picture of these factors and their association with HBV and HCV infection in the country.

(8) The findings of high HBV and HCV seroprevalence among blood recipients and patients under haemodialysis are useful in assessing the magnitude of the problem in Aden. A further study with larger sample is required to illustrate other risk factors probably associated with the occurrence of these infections.

(9) A history of malaria was associated with haemodialysis; emphasising the need of malaria control to prevent associated chronic diseases and its consequences in this community.

(10) Further study is required to identify the role of malaria as a cause of renal failure in Yemen and its association with further hepatitis infection.

(11) The three herpesviruses reported in this study should be included in the differential of patients presenting with liver abnormalities in clinical sites. Therefore, diagnostic facilities should be available at these clinical sites.

10 REFERENCES

Abdel Raheem, S. M., Abou-Lohum, T. S., el-Didy, H., el-Eriani, H., Mansour, S., Hafez, A. S. (1991). Hepatitis B infection in Sana'a City, Republic of Yemen. Prevalence among pregnant women and materno-foetal transmission. *J Egypt Public Health Assoc*, **66**, 491-503.

Abdelaal, M., Rowbottom, D., Zawawi, T., Scott, T., Gilpin, C. (1994). Epidemiology of hepatitis C virus: a study of male blood donors in Saudi Arabia. *Transfusion*, **34**, 135-137.

Abdelaal, M., Zawawi, T. H., al Sobhi, E., Jeje, O., Gilpin, C., Kinsara, A., Osoba, A., Oni, G. A. (1998). Epidemiology of hepatitis E virus in male blood donors in Jeddah, Saudi Arabia. *Ir J Med Sci*, **167**, 94-96.

Abdo, A. A., Al-Jarallah, B. M., Sanai, F. M., Hersi, A. S., Al-Swat, K., Azzam, N. A., Al-Dukhayil, M., Al-Maarik, A., Al-Faleh, F. Z. (2006). Hepatitis B genotypes: relation to clinical outcome in patients with chronic hepatitis B in Saudi Arabia. *World J Gastroenterol*, **12**, 7019-7024.

Abdool Karim, S. S., Coutsoudis, A. (1993). Sero-epidemiology of hepatitis A in black South African children. *S Afr Med J*, **83**, 748-750.

Abdool Karim, S. S., Thejpal, R., Coovadia, H. M. (1991). Household clustering and intra-household transmission patterns of hepatitis B virus infection in South Africa. *Int J Epidemiol*, **20**, 495-503.

Abdul Manan, J., Ali, H., Lal, M. (2006). Acute renal failure associated with malaria. *J Ayub Med Coll Abbottabad*, **18**, 47-52.

Abdulkarim, A. S., Zein, N. N., Germer, J. J., Kolbert, C. P., Kabbani, L., Krajnik, K. L., Hola, A., Agha, M. N., Tourogman, M., Persing, D. H. (1998). Hepatitis C virus genotypes and hepatitis G virus in hemodialysis patients from Syria: identification of two novel hepatitis C virus subtypes. *Am J Trop Med Hyg*, **59**, 571-576.

Abe, K., Hayakawa, E., Sminov, A. V., Rossina, A. L., Ding, X., Huy, T. T., Sata, T., Uchaikin, V. F. (2004). Molecular epidemiology of hepatitis B, C, D and E viruses among children in Moscow, Russia. *J Clin Virol*, **30**, 57-61.

Abe, K., Li, T. C., Ding, X., Win, K. M., Shrestha, P. K., Quang, V. X., Ngoc, T. T., Taltavull, T. C., Smirnov, A. V., Uchaikin, V. F., Luengrojanakul, P., Gu, H., El-Zayadi, A. R., Prince, A. M., Kikuchi, K., Masaki, N., Inui, A., Sata, T., Takeda, N. (2006). International collaborative survey on epidemiology of hepatitis E virus in 11 countries. *Southeast Asian J Trop Med Public Health*, **37**, 90-95.

ACC. (2007). Cancer incidence report 2002-2004., 1, p. 21. Aden Cancer Centre, Faculty of Medicine and health Sciences. University of Aden. Yemen.

Acharya, S. K., Batra, Y., Bhatkal, B., Ojha, B., Kaur, K., Hazari, S., Saraya, A., Panda, S. K. (2003). Seroepidemiology of hepatitis A virus infection among school children in Delhi and north Indian patients with chronic liver disease: implications for HAV vaccination. *J Gastroenterol Hepatol*, **18**, 822-827.

Adachi, S., Shibuya, A., Miura, Y., Takeuchi, A., Nakazawa, T., Saigenji, K. (2008). Impact of occult hepatitis B virus infection and prior hepatitis B virus infection on development of hepatocellular carcinoma in patients with liver cirrhosis due to hepatitis C virus. *Scand J Gastroenterol*, **43**, 849-856.

Adjei, A. A., Armah, H. B., Gbagbo, F., Boamah, I., Adu-Gyamfi, C., Asare, I. (2008). Seroprevalence of HHV-8, CMV, and EBV among the general population in Ghana, West Africa. *BMC Infect Dis*, **8**, 111.

Adler, S. P., Chandrika, T., Lawrence, L., Baggett, J. (1983). Cytomegalovirus infections in neonates acquired by blood transfusions. *Pediatr Infect Dis*, **2**, 114-118.

Aggarwal, R., Balachandran, A., Menon, U., Nagpal, R., Pokharna, G., Rao, S., Shah, D., Srinivasa, K., Datta, S., Bock, H. (2007). Reactogenicity of a combined

hepatitis A and hepatitis B vaccine in healthy Indian children and adults. *Indian J Gastroenterol*, **26**, 248-249.

Aggarwal, R., Krawczynski, K. (2000). Hepatitis E: an overview and recent advances in clinical and laboratory research. *J Gastroenterol Hepatol*, **15**, 9-20.

Aguilera Guirao, A., Romero Yuste, S., Regueiro, B. J. (2006a). Epidemiology and clinical manifestations of viral hepatitis. *Enferm Infecc Microbiol Clin*, **24**, 264-276.

Aguilera Guirao, A., Romero Yuste, S., Regueiro, B. J. (2006b). [Epidemiology and clinical manifestations of viral hepatitis]. *Enferm Infecc Microbiol Clin*, **24**, 264-276.

Agunos, A. C., Yoo, D., Youssef, S. A., Ran, D., Binnington, B., Hunter, D. B. (2006). Avian hepatitis E virus in an outbreak of hepatitis--splenomegaly syndrome and fatty liver haemorrhage syndrome in two flaxseed-fed layer flocks in Ontario. *Avian Pathol*, **35**, 404-412.

Ahlqvist, J., Fotheringham, J., Akhyani, N., Yao, K., Fogdell-Hahn, A., Jacobson, S. (2005). Differential tropism of human herpesvirus 6 (HHV-6) variants and induction of latency by HHV-6A in oligodendrocytes. *J Neurovirol*, **11**, 384-394.

Ahmed, A. A. (2005). Situation of the Haemodialysis unit at Algamhoria Teaching Hospital. Al Gamhoria T Hospital, Haemodialysis Unit. Ministry of Health and Population, Aden.

AHSR/Aden. (2003). Annual Health Statistical Report, of the Ministry of Public Health and Population /Aden Branch. Yemen. The Ministry of Public Health and Population.

Aita, K., Jin, Y., Irie, H., Takahashi, I., Kobori, K., Nakasato, Y., Kodama, H., Yanagawa, Y., Yoshikawa, T., Shiga, J. (2001). Are there histopathologic characteristics particular to fulminant hepatic failure caused by human herpesvirus-6 infection? A case report and discussion. *Hum Pathol*, **32**, 887-889.

Akuta, N., Chayama, K., Suzuki, F., Someya, T., Kobayashi, M., Tsubota, A., Suzuki, Y., Saitoh, S., Arase, Y., Ikeda, K., Kumada, H. (2001). Risk factors of hepatitis C virus-related liver cirrhosis in young adults: positive family history of liver disease and transporter associated with antigen processing 2(TAP2)*0201 Allele. *J Med Virol*, **64**, 109-116.

Al-Ahdal, M. N., Kessie, G. (1997). Serological diagnosis of hepatitis C virus in patients with liver disease in Saudi Arabia. Evaluation of antibody determination by recombinant immunoblot assays in relation to RNA detection by polymerase chain reaction. *Diagn Microbiol Infect Dis*, **27**, 69-73.

al-Ali, H. Y., Yasseen, S. A., Raof, T. Y. (1999). Follow-up of pregnant women with active cytomegalovirus infection. *East Mediterr Health J*, **5**, 949-954.

Al-Faleh, F. Z. (2003). Changing pattern of hepatitis viral infection in Saudi Arabia in the last two decades. *Ann Saudi Med*, **23**, 367-371.

Al-Faleh, F. Z., Ramia, S., Arif, M., Ayoola, E. A., al-Rashed, R. S., al-Jeffry, M., Hossain, A., el-Hazmi, M. (1995). Profile of hepatitis C virus and the possible modes of transmission of the virus in the Gizan area of Saudi Arabia: a community-based study. *Ann Trop Med Parasitol*, **89**, 431-437.

Al-Jarba, A. S., Al-Sayyari, W. M. (2003). Prevalence of hepatitis B virus and hepatitis C virus in health workers in 3 major hospitals in Aden, Republic of Yemen. *Saudi Med J*, **24**, 1031-1032.

Al-Kuraya, K., Narayanappa, R., Al-Dayel, F., El-Solh, H., Ezzat, A., Ismail, H., Belgaumi, A., Bavi, P., Atizado, V., Sauter, G., Simon, R. (2006). Epstein-Barr virus infection is not the sole cause of high prevalence for Hodgkin's lymphoma in Saudi Arabia. *Leuk Lymphoma*, **47**, 707-713.

al-Lawati, J. A., Santhosh-Kumar, C. R., Mohammed, A. J., Jaffer, M. A. (1999). Cancer incidence in Oman, 1993-1997. *East Mediterr Health J*, **5**, 1035-1041.

Al-Mamary, M., Al-Habori, M., Al-Aghbari, A. M., Baker, M. M. (2002). Investigation into the toxicological effects of Catha edulis leaves: a short term study in animals. *Phytother Res*, **16**, 127-132.

Al-Moslih, M. I., Al-Huraibi, M. A. (2001). Prevalence of hepatitis C virus among patients with liver disease in the Republic of Yemen. *East Mediterr Health J*, **7**, 771-778.

Al-Nassiri, K. A., Raja'a, Y. A. (2001). Hepatitis B infection in Yemenis in Sana'a: pattern and risk factors. *East Mediterr Health J*, **7**, 147-152.

Al-Shamahy, H. (2000). Prevalence of Hepatitis B Surface Antigen and Risk Factors of HBV Infection in a Sample of Healthy Mothers and their Infants in Sana'a, Yemen. *Ann Saudi Med*, **20**, 464-467.

Al-Shamahy, H. A., Rabbad, I. A., Al-Hababy, A. (2003). Hepatitis B virus serum markers among pregnant women in Sana'a, Yemen. *Ann Saudi Med*, **23**, 87-89.

Al Awaidy, S., Abu-Elyazeed, R., Al Hosani, H., Al Mulla, A., Al Busaiedy, S., Al Amiry, A., Farah, Z., Al Marrie, A., Bock, H. L., Al-Shaar, I., Shah, S. (2006). Sero-epidemiology of hepatitis B infection in pregnant women in Oman, Qatar and the United Arab Emirates. *J Infect*, **52**, 202-206.

Al Rashed, R. S. (1997). Prevalence of hepatitis A virus among Saudi Arabian children: A community-based study. *Ann Saudi Med*, **17**, 200-203.

Al Tabaa, Y., Tuaillon, E., Bollore, K., Foulongne, V., Petitjean, G., Seigneurin, J. M., Duperray, C., Desgranges, C., Vendrell, J. P. (2009). Functional Epstein-Barr virus reservoir in plasma cells derived from infected peripheral blood memory B cells. *Blood*, **113**, 604-611.

Al Traif, I., Huraib, S., Abdullah, A., Nondo, H., Said, R., Duarte, R., Quadri, K., Alghamdi, G., Lombardi, S., Wolfe, L. (2000). Anti-HCV positive hemodialysis patients: clinical, biochemical, histologic and virologic study and a proposed management scheme. *Ann Saudi Med*, **20**, 307-309.

Alexander, F. E., Jarrett, R. F., Lawrence, D., Armstrong, A. A., Freeland, J., Gokhale, D. A., Kane, E., Taylor, G. M., Wright, D. H., Cartwright, R. A. (2000). Risk factors for Hodgkin's disease by Epstein-Barr virus (EBV) status: prior infection by EBV and other agents. *Br J Cancer*, **82**, 1117-1121.

Alexander, J., Kowdley, K. V. (2006). Epidemiology of hepatitis B, clinical implications. *MedGenMed*, **8**, 13.

Allain, J. P., Candotti, D., Soldan, K., Sarkodie, F., Phelps, B., Giachetti, C., Shyamala, V., Yeboah, F., Anokwa, M., Owusu-Ofori, S., Opare-Sem, O. (2003). The risk of hepatitis B virus infection by transfusion in Kumasi, Ghana. *Blood*, **101**, 2419-2425.

Almuneef, M. A., Memish, Z. A., Balkhy, H. H., Qahtani, M., Alotaibi, B., Hajeer, A., Qasim, L., Al Knawy, B. (2006). Epidemiologic shift in the prevalence of Hepatitis A virus in Saudi Arabia: a case for routine Hepatitis A vaccination. *Vaccine*, **24**, 5599-5603.

Altaf, F. J. (2001). Hepatocellular carcinoma. *Saudi Med J*, **22**, 416-418.

Alter, M. J. (2001). Protecting future generations through immunization against hepatitis B. *Ann Intern Med*, **135**, 835-836.

Alter, M. J. (2003a). Epidemiology and prevention of hepatitis B. *Semin Liver Dis*, **23**, 39-46.

Alter, M. J. (2003b). Epidemiology of hepatitis B in Europe and worldwide. *J Hepatol*, **39 Suppl 1**, S64-69.

Alter, M. J. (2006). Epidemiology of viral hepatitis and HIV co-infection. *J Hepatol*, **44**, S6-9.

Alter, M. J. (2008). Healthcare should not be a vehicle for transmission of hepatitis C virus. *J Hepatol*, **48**, 2-4.

Alter, M. J., Coleman, P. J., Alexander, W. J., Kramer, E., Miller, J. K., Mandel, E., Hadler, S. C., Margolis, H. S. (1989). Importance of heterosexual activity in the transmission of hepatitis B and non-A, non-B hepatitis. *Jama*, **262**, 1201-1205.

Alter, M. J., Kruszon-Moran, D., Nainan, O. V., McQuillan, G. M., Gao, F., Moyer, L. A., Kaslow, R. A., Margolis, H. S. (1999). The prevalence of hepatitis C virus infection in the United States, 1988 through 1994. *N Engl J Med*, **341**, 556-562.

Alter, M. J., Nakatsuji, Y., Melpolder, J., Wages, J., Wesley, R., Shih, J. W., Kim, J. P. (1997). The incidence of transfusion-associated hepatitis G virus infection and its relation to liver disease. *N Engl J Med*, **336**, 747-754.

Ameen, R., Sanad, N., Al-Shemmari, S., Siddique, I., Chowdhury, R. I., Al-Hamdan, S., Al-Bashir, A. (2005). Prevalence of viral markers among first-time Arab blood donors in Kuwait. *Transfusion*, **45**, 1973-1980.

Amini-Bavil-Olyaee, S., Sarrami-Forooshani, R., Mahboudi, F., Sabahi, F., Adeli, A., Noorinayer, B., Azizi, M., Reza Zali, M. (2005). Genotype characterization and phylogenetic analysis of hepatitis B virus isolates from Iranian patients. *J Med Virol*, **75**, 227-234.

Amiri, Z. M., Shakib, A. J., Toorchi, M. (2005). Seroprevalence of hepatitis C and risk factors in haemodialysis patients in Guilan, Islamic Republic of Iran. *East Mediterr Health J*, **11**, 372-376.

Amon, W., Farrell, P. J. (2005). Reactivation of Epstein-Barr virus from latency. *Rev Med Virol*, **15**, 149-156.

Amran, J. (2006). Progress of NMCP Implementation in Yemen. Ministry of Health and Population. , Sana'a.

Anderson, D. A., Li, F., Riddell, M., Howard, T., Seow, H. F., Torresi, J., Perry, G., Sumarsidi, D., Shrestha, S. M., Shrestha, I. L. (1999). ELISA for IgG-class antibody to hepatitis E virus based on a highly conserved, conformational epitope expressed in Escherichia coli. *J Virol Methods*, **81**, 131-142.

Andre, F. (2000). Hepatitis B epidemiology in Asia, the Middle East and Africa. *Vaccine*, **18 Suppl 1**, S20-22.

Andre, F. E., Zuckerman, A. J. (1994). Review: protective efficacy of hepatitis B vaccines in neonates. *J Med Virol*, **44**, 144-151.

Ansaldi, F., Bruzzone, B., Rota, M. C., Bella, A., Ciofi Degli Atti, M., Durando, P., Gasparini, R., Icardi, G. (2008). Hepatitis A incidence and hospital-based seroprevalence in Italy: a nation-wide study. *Eur J Epidemiol*, **23**, 45-53.

Ansaldi, F., Bruzzone, B., Salmaso, S., Rota, M. C., Durando, P., Gasparini, R., Icardi, G. (2005). Different seroprevalence and molecular epidemiology patterns of hepatitis C virus infection in Italy. *J Med Virol*, **76**, 327-332.

Arafa, N., El Hoseiny, M., Rekacewicz, C., Bakr, I., El-Kafrawy, S., El Daly, M., Aoun, S., Marzouk, D., Mohamed, M. K., Fontanet, A. (2005). Changing pattern of hepatitis C virus spread in rural areas of Egypt. *J Hepatol*, **43**, 418-424.

Arankalle, V. A., Chadha, M. S., Chitambar, S. D., Walimbe, A. M., Chobe, L. P., Gandhe, S. S. (2001a). Changing epidemiology of hepatitis A and hepatitis E in urban and rural India (1982-98). *J Viral Hepat*, **8**, 293-303.

Arankalle, V. A., Joshi, M. V., Kulkarni, A. M., Gandhe, S. S., Chobe, L. P., Rautmare, S. S., Mishra, A. C., Padbidri, V. S. (2001b). Prevalence of anti-hepatitis E virus antibodies in different Indian animal species. *J Viral Hepat*, **8**, 223-227.

Arankalle, V. A., Murhekar, K. M., Gandhe, S. S., Murhekar, M. V., Ramdasi, A. Y., Padbidri, V. S., Sehgal, S. C. (2003). Hepatitis B virus: predominance of genotype D in primitive tribes of the Andaman and Nicobar islands, India (1989-1999). *J Gen Virol*, **84**, 1915-1920.

Arauz-Ruiz, P., Norder, H., Robertson, B. H., Magnius, L. O. (2002). Genotype H: a new Amerindian genotype of hepatitis B virus revealed in Central America. *J Gen Virol*, **83**, 2059-2073.

Arboleda, M., Castilho, M. C., Fonseca, J. C., Albuquerque, B. C., Saboia, R. C., Yoshida, C. F. (1995). Epidemiological aspects of hepatitis B and D virus infection in the northern region of Amazonas, Brazil. *Trans R Soc Trop Med Hyg*, **89**, 481-483.

Arcenas, R., Widen, R. H. (2002). Epstein-Barr virus reactivation after superinfection of the BJAB-B1 and P3HR-1 cell lines with cytomegalovirus. *BMC Microbiol*, **2**, 20.

Arif M, A.-F. F., Al-Frayh AR, Ramia S. (1995). Reduction in the prevalence of antibody to hepatitis A virus among young Saudi adults: Implications for hepatitis A vaccine. *Saudi Journal of Gastroenterology*, **1**, 93-96.

Arora, N. K., Nanda, S. K., Gulati, S., Ansari, I. H., Chawla, M. K., Gupta, S. D., Panda, S. K. (1996). Acute viral hepatitis types E, A, and B singly and in combination in acute liver failure in children in north India. *J Med Virol*, **48**, 215-221.

Arrais, T. C., Van Dooren, S., Vandamme, A. M., Brechot, C., Rimlinger, F., Silva, A. E., Perez, R. M., Ferraz, M. L., Thiers, V. (2008). Change in hepatitis C virus genotype in hemodialysis patients after end-of-treatment response to interferon monotherapy--relapse or re-infection? *J Med Virol*, **80**, 80-86.

Arthur, R. R., Hassan, N. F., Abdallah, M. Y., el-Sharkawy, M. S., Saad, M. D., Hackbart, B. G., Imam, I. Z. (1997). Hepatitis C antibody prevalence in blood donors in different governorates in Egypt. *Trans R Soc Trop Med Hyg*, **91**, 271-274.

Asano, Y., Yoshikawa, T., Suga, S., Kobayashi, I., Nakashima, T., Yazaki, T., Kajita, Y., Ozaki, T. (1994). Clinical features of infants with primary human herpesvirus 6 infection (exanthem subitum, roseola infantum). *Pediatrics*, **93**, 104-108.

Avazova, D., Kurbanov, F., Tanaka, Y., Sugiyama, M., Radchenko, I., Ruziev, D., Musabaev, E., Mizokami, M. (2008). Hepatitis B virus transmission pattern and vaccination efficiency in Uzbekistan. *J Med Virol*, **80**, 217-224.

Ayele, W., Nokes, D. J., Abebe, A., Messele, T., Dejene, A., Enquselassie, F., Rinke de Wit, T. F., Fontanet, A. L. (2002). Higher prevalence of anti-HCV antibodies among HIV-positive compared to HIV-negative inhabitants of Addis Ababa, Ethiopia. *J Med Virol*, **68**, 12-17.

Ayoola, A. E., Tobaigy, M. S., Gadour, M. O., Ahmad, B. S., Hamza, M. K., Ageel, A. M. (2003). The decline of hepatitis B viral infection in South-Western Saudi Arabia. *Saudi Med J*, **24**, 991-995.

Ayoola, E. A., Gadour, M. O. (2004). Hepatocellular carcinoma in Saudi Arabia: role of hepatitis B and C infection. *J Gastroenterol Hepatol*, **19**, 665-669.

Ayoola, E. A., Want, M. A., Gadour, M. O., Al-Hazmi, M. H., Hamza, M. K. (2002). Hepatitis E virus infection in haemodialysis patients: a case-control study in Saudi Arabia. *J Med Virol*, **66**, 329-334.

Babcock, G. J., Decker, L. L., Volk, M., Thorley-Lawson, D. A. (1998). EBV persistence in memory B cells in vivo. *Immunity*, **9**, 395-404.

Bacchetti, P., Tien, P. C., Seaberg, E. C., O'Brien, T. R., Augenbraun, M. H., Kral, A. H., Busch, M. P., Edlin, B. R. (2007). Estimating past hepatitis C infection risk from reported risk factor histories: implications for imputing age of infection and modeling fibrosis progression. *BMC Infect Dis*, **7**, 145.

Baddoura, R., Haddad, C., Germanos, M. (2002). Hepatitis B and C seroprevalence in the Lebanese population. *East Mediterr Health J*, **8**, 150-156.

Bahaj, A. (2003). Prevalence of hepatitis B surface antigen and hepatitis C antibodies among blood donors in Hadhramout region, Yemen. (1999-2002). *Yemeni Journal of Medical and Health Research*, **2**, 171-176.

Bahri, O., Cheikh, I., Hajji, N., Djebbi, A., Maamouri, N., Sadraoui, A., Mami, N. B., Triki, H. (2006). Hepatitis B genotypes, precore and core promoter mutants circulating in Tunisia. *J Med Virol*, **78**, 353-357.

Baid-Agrawal, S., Pascual, M., Moradpour, D., Frei, U., Tolkoff-Rubin, N. (2008). Hepatitis C virus infection in haemodialysis and kidney transplant patients. *Rev Med Virol*, **18**, 97-115.

Baklan, Z., Gorisek, J. R., Poljak, M., Pisec, A. (2004). Prevalence of HIV, hepatitis B, C and G virus infections among injecting drug users on methadone maintenance treatment in Maribor. *Wien Klin Wochenschr*, **116 Suppl 2**, 5-7.

Balayan, M. S. (1993). Hepatitis E virus infection in Europe: regional situation regarding laboratory diagnosis and epidemiology. *Clin Diagn Virol*, **1**, 1-9.

Balayan, M. S. (1997). Epidemiology of hepatitis E virus infection. *J Viral Hepat*, **4**, 155-165.

Balayan, M. S., Zamyatina, N. A., Mikhailov, M. I., Sychev, A. V., Usmanov, R. K., Dvoynikova, O. V., Nelga, I. V., Grishina, G. K., Ankhundinova, L. A. (1994).

Serological survey on hepatitis E virus infection in an endemic area: diagnosis potential of enzyme immunoassay for detection of IgG antibody. *Clin Diagn Virol*, **2**, 297-304.

Balfour, H. H., Jr. (2007). Epstein-Barr virus vaccine for the prevention of infectious mononucleosis--and what else? *J Infect Dis*, **196**, 1724-1726.

Bamshad, M., Kivisild, T., Watkins, W. S., Dixon, M. E., Ricker, C. E., Rao, B. B., Naidu, J. M., Prasad, B. V., Reddy, P. G., Rasanayagam, A., Papiha, S. S., Villems, R., Redd, A. J., Hammer, M. F., Nguyen, S. V., Carroll, M. L., Batzer, M. A., Jorde, L. B. (2001). Genetic evidence on the origins of Indian caste populations. *Genome Res*, **11**, 994-1004.

Baqi, S., Shah, S. A., Baig, M. A., Mujeeb, S. A., Memon, A. (1999). Seroprevalence of HIV, HBV, and syphilis and associated risk behaviours in male transvestites (Hijras) in Karachi, Pakistan. *Int J STD AIDS*, **10**, 300-304.

Barlow, G., Kilding, R., Green, S. T. (2000). Epstein-Barr virus infection mimicking extrahepatic biliary obstruction. *J R Soc Med*, **93**, 316-318.

Barone, S. R., Kaplan, M. H., Krilov, L. R. (1995). Human herpesvirus-6 infection in children with first febrile seizures. *J Pediatr*, **127**, 95-97.

Bartholomeusz, A., Schaefer, S. (2004). Hepatitis B virus genotypes: comparison of genotyping methods. *Rev Med Virol*, **14**, 3-16.

Battersby, A., Feilden, R., Nelson, C. (1999a). Sterilizable syringes: excessive risk or cost-effective option? *Bull World Health Organ*, **77**, 812-819.

Battersby, A., Feilden, R., Stoeckel, P., Da Silva, A., Nelson, C., Bass, A. (1999b). Strategies for safe injections. *Bull World Health Organ*, **77**, 996-1000.

Bauer, C. C., Jaksch, P., Aberle, S. W., Haber, H., Lang, G., Klepetko, W., Hofmann, H., Puchhammer-Stockl, E. (2007). Relationship between cytomegalovirus DNA load in epithelial lining fluid and plasma of lung transplant recipients and analysis of coinfection with Epstein-Barr virus and human herpesvirus 6 in the lung compartment. *J Clin Microbiol*, **45**, 324-328.

Bdour, S. (2002). Hepatitis C virus infection in Jordanian haemodialysis units: serological diagnosis and genotyping. *J Med Microbiol*, **51**, 700-704.

Beasley, R. P. (1988). Hepatitis B virus. The major etiology of hepatocellular carcinoma. *Cancer*, **61**, 1942-1956.

Beasley, R. P., Hwang, L. Y., Lin, C. C., Chien, C. S. (1981). Hepatocellular carcinoma and hepatitis B virus. A prospective study of 22 707 men in Taiwan. *Lancet*, **2**, 1129-1133.

Bellentani, S., Miglioli, L., Masutti, F., Saccoccio, G., Tiribelli, C. (2000). Epidemiology of hepatitis C virus infection in Italy: the slowly unraveling mystery. *Microbes Infect*, **2**, 1757-1763.

Bellentani, S., Pozzato, G., Saccoccio, G., Crovatto, M., Croce, L. S., Mazzoran, L., Masutti, F., Cristianini, G., Tiribelli, C. (1999). Clinical course and risk factors of hepatitis C virus related liver disease in the general population: report from the Dionysos study. *Gut*, **44**, 874-880.

Beltrami, E. M., Williams, I. T., Shapiro, C. N., Chamberland, M. E. (2000). Risk and management of blood-borne infections in health care workers. *Clin Microbiol Rev*, **13**, 385-407.

Bendall, R., Ellis, V., Ijaz, S., Thurairajah, P., Dalton, H. R. (2008). Serological response to hepatitis E virus genotype 3 infection: IgG quantitation, avidity, and IgM response. *J Med Virol*, **80**, 95-101.

Beran, J. (2008). Safety and immunogenicity of a new hepatitis B vaccine for the protection of patients with renal insufficiency including pre-haemodialysis and haemodialysis patients. *Expert Opin Biol Ther*, **8**, 235-247.

Bergallo, M., Costa, C., Baro, S., Musso, T., Balbo, L., Merlino, C., Cavallo, R. (2007). Multiplex-nested RT-PCR to evaluate latent and lytic Epstein Barr virus gene expression. *J Biotechnol*, **128**, 462-476.

Bernier, R. H., Sampliner, R., Gerety, R., Tabor, E., Hamilton, F., Nathanson, N. (1982). Hepatitis B infection in households of chronic carriers of hepatitis B

surface antigen: factors associated with prevalence of infection. *Am J Epidemiol*, **116**, 199-211.

Beutels, M., Van Damme, P., Aelvoet, W., Desmyter, J., Dondeyne, F., Goilav, C., Mak, R., Muylle, L., Pierard, D., Stroobant, A., Van Loock, F., Waumans, P., Vranckx, R. (1997). Prevalence of hepatitis A, B and C in the Flemish population. *Eur J Epidemiol*, **13**, 275-280.

Bhattarakosol, P., Pancharoen, C., Mekmullica, J. (2001). Seroprevalence of anti-human herpes virus-6 IgG antibody in children of Bangkok, Thailand. *Southeast Asian J Trop Med Public Health*, **32**, 143-147.

Bialek, S. R., Bower, W. A., Mottram, K., Purchase, D., Nakano, T., Nainan, O., Williams, I. T., Bell, B. P. (2005). Risk factors for hepatitis B in an outbreak of hepatitis B and D among injection drug users. *J Urban Health*, **82**, 468-478.

Biggar, R. J., Henle, G., Bocker, J., Lennette, E. T., Fleisher, G., Henle, W. (1978). Primary Epstein-Barr virus infections in African infants. II. Clinical and serological observations during seroconversion. *Int J Cancer*, **22**, 244-250.

Bile, K., Mohamud, O., Aden, C., Isse, A., Norder, H., Nilsson, L., Magnius, L. (1992). The risk for hepatitis A, B, and C at two institutions for children in Somalia with different socioeconomic conditions. *Am J Trop Med Hyg*, **47**, 357-364.

Black, J. B., Schwarz, T. F., Patton, J. L., Kite-Powell, K., Pellett, P. E., Wiersbitzky, S., Bruns, R., Muller, C., Jager, G., Stewart, J. A. (1996). Evaluation of immunoassays for detection of antibodies to human herpesvirus 7. *Clin Diagn Lab Immunol*, **3**, 79-83.

Blackmore, T. K., Stace, N. H., Maddocks, P., Hatfield, P. (1992). Prevalence of antibodies to hepatitis C virus in patients receiving renal replacement therapy, and in the staff caring for them. *Aust N Z J Med*, **22**, 353-357.

Blumberg, B. S. (1977). Australia antigen and the biology of hepatitis B. *Science*, **197**, 17-25.

Bollyky, P. L., Rambaut, A., Harvey, P. H., Holmes, E. C. (1996). Recombination between sequences of hepatitis B virus from different genotypes. *J Mol Evol*, **42**, 97-102.

Bonanni, P., Colombai, R., Franchi, G., Lo Nostro, A., Comodo, N., Tiscione, E. (1998). Experience of hepatitis A vaccination during an outbreak in a nursery school of Tuscany, Italy. *Epidemiol Infect*, **121**, 377-380.

Bonanni, P., Pesavento, G., Bechini, A., Tiscione, E., Mannelli, F., Benucci, C., Nostro, A. L. (2003). Impact of universal vaccination programmes on the epidemiology of hepatitis B: 10 years of experience in Italy. *Vaccine*, **21**, 685-691.

Boppana, S. B., Fowler, K. B., Britt, W. J., Stagno, S., Pass, R. F. (1999). Symptomatic congenital cytomegalovirus infection in infants born to mothers with preexisting immunity to cytomegalovirus. *Pediatrics*, **104**, 55-60.

Bortolotti, F., Resti, M., Marcellini, M., Giacchino, R., Verucchi, G., Nebbia, G., Zancan, L., Marazzi, M. G., Barbera, C., Maccabruni, A., Zuin, G., Maggiore, G., Balli, F., Vajro, P., Lepore, L., Molesini, M., Guido, M., Bartolacci, S., Noventa, F. (2005). Hepatitis C virus (HCV) genotypes in 373 Italian children with HCV infection: changing distribution and correlation with clinical features and outcome. *Gut*, **54**, 852-857.

Borza, C. M., Hutt-Fletcher, L. M. (2002). Alternate replication in B cells and epithelial cells switches tropism of Epstein-Barr virus. *Nat Med*, **8**, 594-599.

Bowyer, S. M., van Staden, L., Kew, M. C., Sim, J. G. (1997). A unique segment of the hepatitis B virus group A genotype identified in isolates from South Africa. *J Gen Virol*, **78 (Pt 7)**, 1719-1729.

Brabin, B., Beeching, N. J., Bunn, J. E., Cooper, C., Gardner, K., Hart, C. A. (2002). Hepatitis B prevalence among Somali households in Liverpool. *Arch Dis Child*, **86**, 67-68.

Bradford, R. D., Cloud, G., Lakeman, A. D., Boppana, S., Kimberlin, D. W., Jacobs, R., Demmler, G., Sanchez, P., Britt, W., Soong, S. J., Whitley, R. J. (2005). Detection of cytomegalovirus (CMV) DNA by polymerase chain reaction is

associated with hearing loss in newborns with symptomatic congenital CMV infection involving the central nervous system. *J Infect Dis*, **191**, 227-233.
Branda, M., Wands, J. R. (2006). Signal transduction cascades and hepatitis B and C related hepatocellular carcinoma. *Hepatology*, **43**, 891-902.
Braun, D. K., Dominguez, G., Pellett, P. E. (1997). Human herpesvirus 6. *Clin Microbiol Rev*, **10**, 521-567.
Brown, S. E., Stanley, C., Howard, C. R., Zuckerman, A. J., Steward, M. W. (1986). Antibody responses to recombinant and plasma derived hepatitis B vaccines. *Br Med J*, **292**, 159-161.
Bryan, J. P., Iqbal, M., Tsarev, S., Malik, I. A., Duncan, J. F., Ahmed, A., Khan, A., Rafiqui, A. R., Purcell, R. H., Legters, L. J. (2002). Epidemic of hepatitis E in a military unit in Abbotrabad, Pakistan. *Am J Trop Med Hyg*, **67**, 662-668.
Bryan, J. P., Tsarev, S. A., Iqbal, M., Ticehurst, J., Emerson, S., Ahmed, A., Duncan, J., Rafiqui, A. R., Malik, I. A., Purcell, R. H., et al. (1994). Epidemic hepatitis E in Pakistan: patterns of serologic response and evidence that antibody to hepatitis E virus protects against disease. *J Infect Dis*, **170**, 517-521.
Budd, J., Robertson, R., Elton, R. (2004). Hepatitis B vaccination and injecting drug users. *Br J Gen Pract*, **54**, 444-447.
Bukh, J., Purcell, R. H., Miller, R. H. (1993). At least 12 genotypes of hepatitis C virus predicted by sequence analysis of the putative E1 gene of isolates collected worldwide. *Proc Natl Acad Sci U S A*, **90**, 8234-8238.
Burd, E. M., Carrigan, D. R. (1993). Human herpesvirus 6 (HHV-6)-associated dysfunction of blood monocytes. *Virus Res*, **29**, 79-90.
Busch, M. P., Glynn, S. A., Stramer, S. L., Strong, D. M., Caglioti, S., Wright, D. J., Pappalardo, B., Kleinman, S. H. (2005). A new strategy for estimating risks of transfusion-transmitted viral infections based on rates of detection of recently infected donors. *Transfusion*, **45**, 254-264.
Busch, M. P., Kleinman, S. H., Nemo, G. J. (2003). Current and emerging infectious risks of blood transfusions. *Jama*, **289**, 959-962.
Cadranel, J. F., Lahmek, P., Causse, X., Bellaiche, G., Bettan, L., Fontanges, T., Medini, A., Henrion, J., Chousterman, M., Condat, B., Hervio, P., Periac, P., Eugene, C., Moindrot, H., Grasset, D., Nouel, O., Pilette, C., Szostak-Talbodec, N., Cayla, J. M., Si-Ahmed, S. N., Dumouchel, P., Pariente, A., Lesgourgues, B., Denis, J. (2007). Epidemiology of chronic hepatitis B infection in France: risk factors for significant fibrosis results of a nationwide survey. *Aliment Pharmacol Ther*, **26**, 565-576.
Cainelli, F., Vento, S. (2002). Infections and solid organ transplant rejection: a cause-and-effect relationship? *Lancet Infect Dis*, **2**, 539-549.
Callan, M. F. (2004). The immune response to Epstein-Barr virus. *Microbes Infect*, **6**, 937-945.
Canpolat Kirac, B., Adisen, E., Bozdayi, G., Yucel, A., Fidan, I., Aksakal, N., Gurer, M. A. (2009). The role of human herpesvirus 6, human herpesvirus 7, Epstein-Barr virus and cytomegalovirus in the aetiology of pityriasis rosea. *J Eur Acad Dermatol Venereol*, **23**, 16-21.
Capeding, M. R., Cadorna-Carlos, J., Book-Montellano, M., Ortiz, E. (2008). Immunogenicity and safety of a DTaP-IPV/PRP approximately T combination vaccine given with hepatitis B vaccine: a randomized open-label trial. *Bull World Health Organ*, **86**, 443-451.
Caron, M., Enouf, V., Than, S. C., Dellamonica, L., Buisson, Y., Nicand, E. (2006). Identification of genotype 1 hepatitis E virus in samples from swine in Cambodia. *J Clin Microbiol*, **44**, 3440-3442.
Carrilho, F. J., Moraes, C. R., Pinho, J. R., Mello, I. M., Bertolini, D. A., Lemos, M. F., Moreira, R. C., Bassit, L. C., Cardoso, R. A., Ribeiro-dos-Santos, G., Da Silva, L. C. (2004). Hepatitis B virus infection in Haemodialysis Centres from Santa Catarina State, Southern Brazil. Predictive risk factors for infection and molecular epidemiology. *BMC Public Health*, **4**, 13.

Castera, L., Pawlotsky, J. M. (2001). Hepatitis E virus infection: epidemiology and prevention. *Gastroenterol Clin Biol*, **25**, 521-537.

CDC. (2006). Viral hepatitist, glossary. In: STD, and TB Prevention, april. CDC, Atlanta.

Cendoroglo Neto, M., Draibe, S. A., Silva, A. E., Ferraz, M. L., Granato, C., Pereira, C. A., Sesso, R. C., Gaspar, A. M., Ajzen, H. (1995). Incidence of and risk factors for hepatitis B virus and hepatitis C virus infection among haemodialysis and CAPD patients: evidence for environmental transmission. *Nephrol Dial Transplant*, **10**, 240-246.

Cermelli, C., Concari, M., Pietrosemoli, P., Meacci, M., Sabbatini, A. M., Divincenzo, A., Carubbi, F., Loria, P., Bagni, A., Carulli, N., Portolani, M. (1999). Herpesvirus DNA is frequently detected in liver tissue from hepatitis C patients. *J Clin Virol*, **14**, 9-16.

Chabay, P. A., Barros, M. H., Hassan, R., De Matteo, E., Rey, G., Carrico, M. K., Renault, I. Z., Preciado, M. V. (2008). Pediatric Hodgkin lymphoma in 2 South American series: a distinctive epidemiologic pattern and lack of association of Epstein-Barr virus with clinical outcome. *J Pediatr Hematol Oncol*, **30**, 285-291.

Chamberland, M. E., Alter, H. J., Busch, M. P., Nemo, G., Ricketts, M. (2001). Emerging infectious disease issues in blood safety. *Emerg Infect Dis*, **7**, 552-553.

Chan, H. L., Leung, N. W., Lau, T. C., Wong, M. L., Sung, J. J. (2000a). Comparison of three different sensitive assays for hepatitis B virus DNA in monitoring of responses to antiviral therapy. *J Clin Microbiol*, **38**, 3205-3208.

Chan, H. L., Tsui, S. K., Tse, C. H., Ng, E. Y., Au, T. C., Yuen, L., Bartholomeusz, A., Leung, K. S., Lee, K. H., Locarnini, S., Sung, J. J. (2005). Epidemiological and virological characteristics of 2 subgroups of hepatitis B virus genotype C. *J Infect Dis*, **191**, 2022-2032.

Chan, H. L., Wong, M. L., Hui, A. Y., Hung, L. C., Chan, F. K., Sung, J. J. (2003). Hepatitis B virus genotype C takes a more aggressive disease course than hepatitis B virus genotype B in hepatitis B e antigen-positive patients. *J Clin Microbiol*, **41**, 1277-1279.

Chan, J. K., Yip, T. T., Tsang, W. Y., Lau, W. H., Wong, C. S., Ma, V. W. (1995a). Detection of Epstein-Barr virus in Hodgkin's disease occurring in an Oriental population. *Hum Pathol*, **26**, 314-318.

Chan, K. H., Tam, J. S., Peiris, J. S., Seto, W. H., Ng, M. H. (2001). Epstein-Barr virus (EBV) infection in infancy. *J Clin Virol*, **21**, 57-62.

Chan, L., Stefanac, T., Turcotte, N., Hu, Z., Chen, Y., Bedard, J., May, S., Jin, H. (2000b). Design and evaluation of dihydroisoquinolines as potent and orally bioavailable human cytomegalovirus inhibitors. *Bioorg Med Chem Lett*, **10**, 1477-1480.

Chan, Y., Kean-Chong, M., Gonzalez, D., Apuzzio, J. (1995b). Cytomegalovirus hepatitis during pregnancy. *Infect Dis Obstet Gynecol*, **3**, 164-165.

Chandler, J. D., Riddell, M. A., Li, F., Love, R. J., Anderson, D. A. (1999). Serological evidence for swine hepatitis E virus infection in Australian pig herds. *Vet Microbiol*, **68**, 95-105.

Chandler, S. H., Holmes, K. K., Wentworth, B. B., Gutman, L. T., Wiesner, P. J., Alexander, E. R., Handsfield, H. H. (1985). The epidemiology of cytomegaloviral infection in women attending a sexually transmitted disease clinic. *J Infect Dis*, **152**, 597-605.

Chang, H. C., Yu, M. W., Lu, C. F., Chiu, Y. H., Chen, C. J. (2001). Risk factors associated with hepatitis C virus infection in Taiwanese government employees. *Epidemiol Infect*, **126**, 291-299.

Chanpong, G. F., Laras, K., Sulaiman, H. A., Soeprapto, W., Purnamawati, S., Sukri, N., Sie, A., Tan, R., Campbell, J. R., Corwin, A. L. (2002). Hepatitis C among child transfusion and adult renal dialysis patients in Indonesia. *Am J Trop Med Hyg*, **66**, 317-320.

Chau, T. N., Lai, S. T., Tse, C., Ng, T. K., Leung, V. K., Lim, W., Ng, M. H. (2006). Epidemiology and clinical features of sporadic hepatitis E as compared with hepatitis A. *Am J Gastroenterol*, **101**, 292-296.

Chen, C. C., Yen, C. H., Wu, W. Y., Hu, S. W., Chen, S. C., Bell, W. R., Lee, M. C. (2007a). Epidemiology of hepatitis B virus infection among young adults in Taiwan, China after public vaccination program. *Chin Med J (Engl)*, **120**, 1155-1158.

Chen, C. J., Yang, H. I., Su, J., Jen, C. L., You, S. L., Lu, S. N., Huang, G. T., Iloeje, U. H. (2006). Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. *Jama*, **295**, 65-73.

Chen, J., Yin, J., Tan, X., Zhang, H., Chen, B., Chang, W., Schaefer, S., Cao, G. (2007b). Improved multiplex-PCR to identify hepatitis B virus genotypes A-F and subgenotypes B1, B2, C1 and C2. *J Clin Virol*, **38**, 238-243.

Chen, Y. P., Feng, X. R., Dai, L., Ding, H. B., Zhang, L. (2003a). Screening and evaluation of non-invasive diagnosis markers for compensated liver cirrhosis in patients with chronic hepatitis B. *Zhonghua Gan Zang Bing Za Zhi*, **11**, 225-227.

Chen, Y. P., Liang, W. F., Zhang, L., He, H. T., Luo, K. X. (2000). Transfusion transmitted virus infection in general populations and patients with various liver diseases in south China. *World J Gastroenterol*, **6**, 738-741.

Chen, Z. M., Liu, B. Q., Boreham, J., Wu, Y. P., Chen, J. S., Peto, R. (2003b). Smoking and liver cancer in China: case-control comparison of 36,000 liver cancer deaths vs. 17,000 cirrhosis deaths. *Int J Cancer*, **107**, 106-112.

Chien, R. N. (2007). A great deal of learning from the natural course of chronic hepatitis B virus infection. *J Gastroenterol Hepatol*, **22**, 962-964.

Choo, Q. L., Kuo, G., Weiner, A. J., Overby, L. R., Bradley, D. W., Houghton, M. (1989). Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science*, **244**, 359-362.

Chowdhury, A., Santra, A., Chakravorty, R., Banerji, A., Pal, S., Dhali, G. K., Datta, S., Banerji, S., Manna, B., Chowdhury, S. R., Bhattacharya, S. K., Mazumder, D. G. (2005). Community-based epidemiology of hepatitis B virus infection in West Bengal, India: prevalence of hepatitis B e antigen-negative infection and associated viral variants. *J Gastroenterol Hepatol*, **20**, 1712-1720.

Chu, C. J., Hussain, M., Lok, A. S. (2002a). Hepatitis B virus genotype B is associated with earlier HBeAg seroconversion compared with hepatitis B virus genotype C. *Gastroenterology*, **122**, 1756-1762.

Chu, C. J., Keeffe, E. B., Han, S. H., Perrillo, R. P., Min, A. D., Soldevila-Pico, C., Carey, W., Brown, R. S., Jr., Luketic, V. A., Terrault, N., Lok, A. S. (2003). Hepatitis B virus genotypes in the United States: results of a nationwide study. *Gastroenterology*, **125**, 444-451.

Chu, C. J., Lok, A. S. (2002). Clinical significance of hepatitis B virus genotypes. *Hepatology*, **35**, 1274-1276.

Chu, C. M., Liaw, Y. F. (2005). Genotype C hepatitis B virus infection is associated with a higher risk of reactivation of hepatitis B and progression to cirrhosis than genotype B: a longitudinal study of hepatitis B e antigen-positive patients with normal aminotransferase levels at baseline. *J Hepatol*, **43**, 411-417.

Chu, C. M., Yeh, C. T., Sheen, I. S., Liaw, Y. F. (2002b). Acute hepatitis C virus (HCV) infection in chronic carriers of hepatitis B virus (HBV): the impact of underlying active HBV replication on persistence of HCV infection and antibody responses to HCV. *Gut*, **51**, 95-99.

Clark, D. A., Nacheva, E. P., Leong, H. N., Brazma, D., Li, Y. T., Tsao, E. H., Buyck, H. C., Atkinson, C. E., Lawson, H. M., Potter, M. N., Griffiths, P. D. (2006). Transmission of integrated human herpesvirus 6 through stem cell transplantation: implications for laboratory diagnosis. *J Infect Dis*, **193**, 912-916.

Claviez, A., Tiemann, M., Wagner, H. J., Dreger, P., Suttorp, M. (2000). Epstein-Barr virus-associated post-transplant lymphoproliferative disease after bone marrow transplantation mimicking graft-versus-host disease. *Pediatr Transplant*, **4**, 151-155.

Clayson, E. T., Innis, B. L., Myint, K. S., Narupiti, S., Vaughn, D. W., Giri, S., Ranabhat, P., Shrestha, M. P. (1995). Detection of hepatitis E virus infections among domestic swine in the Kathmandu Valley of Nepal. *Am J Trop Med Hyg*, **53**, 228-232.

Clemente-Casares, P., Pina, S., Buti, M., Jardi, R., MartIn, M., Bofill-Mas, S., Girones, R. (2003). Hepatitis E virus epidemiology in industrialized countries. *Emerg Infect Dis*, **9**, 448-454.

Cockayne, E. A. (1912). Catarrhal jaundice, sporadic and epidemic, and its relation to acute yellow atrophy of the liver. *Q. J. Med.*, **6**, 1-29.

Cohen, C., Evans, A. A., London, W. T., Block, J., Conti, M., Block, T. (2008). Underestimation of chronic hepatitis B virus infection in the United States of America. *J Viral Hepat*, **15**, 12-13.

Colugnati, F. A., Staras, S. A., Dollard, S. C., Cannon, M. J. (2007). Incidence of cytomegalovirus infection among the general population and pregnant women in the United States. *BMC Infect Dis*, **7**, 71.

Cone, R. W., Huang, M. L., Corey, L., Zeh, J., Ashley, R., Bowden, R. (1999). Human herpesvirus 6 infections after bone marrow transplantation: clinical and virologic manifestations. *J Infect Dis*, **179**, 311-318.

Conjeevaram, H. S., Lok, A. S. (2001). Occult hepatitis B virus infection: a hidden menace? *Hepatology*, **34**, 204-206.

Corbet, S., Bukh, J., Heinsen, A., Fomsgaard, A. (2003). Hepatitis C virus subtyping by a core-envelope 1-based reverse transcriptase PCR assay with sequencing and its use in determining subtype distribution among Danish patients. *J Clin Microbiol*, **41**, 1091-1100.

Costa-Mattioli, M., Monpoeho, S., Nicand, E., Aleman, M. H., Billaudel, S., Ferre, V. (2002). Quantification and duration of viraemia during hepatitis A infection as determined by real-time RT-PCR. *J Viral Hepat*, **9**, 101-106.

Craig, A. S., Schaffner, W. (2004). Prevention of hepatitis A with the hepatitis A vaccine. *N Engl J Med*, **350**, 476-481.

Crawford, D. H. (2001). Biology and disease associations of Epstein-Barr virus. *Philos Trans R Soc Lond B Biol Sci*, **356**, 461-473.

Crawford, D. H., Swerdlow, A. J., Higgins, C., McAulay, K., Harrison, N., Williams, H., Britton, K., Macsween, K. F. (2002). Sexual history and Epstein-Barr virus infection. *J Infect Dis*, **186**, 731-736.

Crook, P. D., Jones, M. E., Hall, A. J. (2003). Mortality of hepatitis B surface antigen-positive blood donors in England and Wales. *Int J Epidemiol*, **32**, 118-124.

Crum, N. F. (2006). Epstein Barr virus hepatitis: case series and review. *South Med J*, **99**, 544-547.

Csire, M., Mikala, G., Jako, J., Masszi, T., Janosi, J., Dolgos, J., Fule, T., Tordai, A., Berencsi, G., Valyi-Nagy, I. (2007). Persistent long-term human herpesvirus 6 (HHV-6) infection in a patient with langerhans cell histiocytosis. *Pathol Oncol Res*, **13**, 157-160.

CTC. (2007). Housing conditions that serve as risk factors for tuberculosis infection and disease. *Can Commun Dis Rep.*, **33**, 1-13.

Cui, F. Q., Gofin, R. (2007). Immunization coverage and its determinants in children aged 12-23 months in Gansu, China. *Vaccine*, **25**, 664-671.

Cuthbert, J. A. (2001). Hepatitis A: old and new. *Clin Microbiol Rev*, **14**, 38-58.

Cutts, F. T., Glik, D. C., Gordon, A., Parker, K., Diallo, S., Haba, F., Stone, R. (1990). Application of multiple methods to study the immunization programme in an urban area of Guinea. *Bulletin of the World Health Organization*, **68**, 769-776.

Da Villa, G., Pelliccia, M. G., Peluso, F., Ricciardi, E., Sepe, A. (1997). Anti-HBs responses in children vaccinated with different schedules of either plasma-derived or HBV DNA recombinant vaccine. *Res Virol*, **148**, 109-114.

Da Villa, G., Romano, L., Sepe, A., Iorio, R., Paribello, N., Zappa, A., Zanetti, A. R. (2007). Impact of hepatitis B vaccination in a highly endemic area of south Italy and long-term duration of anti-HBs antibody in two cohorts of vaccinated individuals. *Vaccine*, **25**, 3133-3136.

Daibata, M., Taguchi, T., Sawada, T., Taguchi, H., Miyoshi, I. (1998). Chromosomal transmission of human herpesvirus 6 DNA in acute lymphoblastic leukaemia. *Lancet*, **352**, 543-544.

Dal Molin, G., Ansaldi, F., Biagi, C., D'Agaro, P., Comar, M., Croce, L., Tiribelli, C., Campello, C. (2002). Changing molecular epidemiology of hepatitis C virus infection in Northeast Italy. *J Med Virol*, **68**, 352-356.

Dalton, H. R., Thurairajah, P. H., Fellows, H. J., Hussaini, H. S., Mitchell, J., Bendall, R., Banks, M., Ijaz, S., Teo, C. G., Levine, D. F. (2007). Autochthonous hepatitis E in southwest England. *J Viral Hepat*, **14**, 304-309.

Dandolos, E., Roumeliotou-Karayannis, A., Richardson, S. C., Papaevangelou, G. (1985). Safety and immunogenicity of a recombinant hepatitis B vaccine. *J Med Virol*, **17**, 57-62.

Dawson, G. J., Chau, K. H., Cabal, C. M., Yarbough, P. O., Reyes, G. R., Mushahwar, I. K. (1992). Solid-phase enzyme-linked immunosorbent assay for hepatitis E virus IgG and IgM antibodies utilizing recombinant antigens and synthetic peptides. *J Virol Methods*, **38**, 175-186.

De Bolle, L., Naesens, L., De Clercq, E. (2005a). Update on human herpesvirus 6 biology, clinical features, and therapy. *Clin Microbiol Rev*, **18**, 217-245.

De Bolle, L., Van Loon, J., De Clercq, E., Naesens, L. (2005b). Quantitative analysis of human herpesvirus 6 cell tropism. *J Med Virol*, **75**, 76-85.

De Deus, N., Seminati, C., Pina, S., Mateu, E., Martin, M., Segales, J. (2006). Detection of hepatitis E virus in liver, mesenteric lymph node, serum, bile and faeces of naturally infected pigs affected by different pathological conditions. *Vet Microbiol*, **(2-4)**, 105-114.

de la Hoz, F., Perez, L., de Neira, M., Hall, A. J. (2008). Eight years of hepatitis B vaccination in Colombia with a recombinant vaccine: factors influencing hepatitis B virus infection and effectiveness. *Int J Infect Dis*, **12**, 183-189.

De Mitri, M. S., Bernardi, M. (2008). Predicting the development of hepatocellular cancer in hepatitis B carriers. *Gut*, **57**, 12-15.

de Ory, F., Ramirez, R., Garcia Comas, L., Leon, P., Sagues, M. J., Sanz, J. C. (2004). Is there a change in cytomegalovirus seroepidemiology in Spain? *Eur J Epidemiol*, **19**, 85-89.

De Paschale, M., Agrappi, C., Manco, M. T., Mirri, P., Vigano, E. F., Clerici, P. (2009). Seroepidemiology of EBV and interpretation of the "isolated VCA IgG" pattern. *J Med Virol*, **81**, 325-331.

Demmler, G. J. (1991). Infectious Diseases Society of America and Centers for Disease Control. Summary of a workshop on surveillance for congenital cytomegalovirus disease. *Rev Infect Dis*, **13**, 315-329.

Deuffic, S., Poynard, T., Buffat, L., Valleron, A. J. (1998). Trends in primary liver cancer. *Lancet*, **351**, 214-215.

Deutsch, M., Hadziyannis, S. J. (2008). Old and emerging therapies in chronic hepatitis C: an update. *J Viral Hepat*, **15**, 2-11.

Devarbhavi, H. C., Cohen, A. J., Patel, R., Wiesner, R. H., Dickson, R. C., Ishitani, M. B. (2002). Preliminary results: outcome of liver transplantation for hepatitis B virus varies by hepatitis B virus genotype. *Liver Transpl*, **8**, 550-555.

Dinand, V., Dawar, R., Arya, L. S., Unni, R., Mohanty, B., Singh, R. (2007). Hodgkin's lymphoma in Indian children: prevalence and significance of Epstein-Barr virus detection in Hodgkin's and Reed-Sternberg cells. *Eur J Cancer*, **43**, 161-168.

Ding, X., Mizokami, M., Ge, X., Orito, E., Iino, S., Ueda, R., Nakanishi, M. (2002). Different hepatitis B virus genotype distributions among asymptomatic carriers and patients with liver diseases in Nanning, southern China. *Hepatol Res*, **22**, 37-44.

Ding, X., Mizokami, M., Yao, G., Xu, B., Orito, E., Ueda, R., Nakanishi, M. (2001). Hepatitis B virus genotype distribution among chronic hepatitis B virus carriers in Shanghai, China. *Intervirology*, **44**, 43-47.

Djebbi, A., Triki, H., Bahri, O., Cheikh, I., Sadraoui, A., Ben Ammar, A., Dellagi, K. (2003). Genotypes of hepatitis C virus circulating in Tunisia. *Epidemiol Infect*, **130**, 501-505.

Dodoo, A. N., Renner, L., van Grootheest, A. C., Labadie, J., Antwi-Agyei, K. O., Hayibor, S., Addison, J., Pappoe, V., Appiah-Danquah, A. (2007). Safety monitoring of a new pentavalent vaccine in the expanded programme on immunisation in Ghana. *Drug Saf*, **30**, 347-356.

Dogan, I., Ergun, M., Cindoruk, M., Unal, S. (2007). Acute hepatitis induced by Epstein-Barr virus infection: a case report. *Turk J Gastroenterol*, **18**, 119-121.

Dominguez, A., Bruguera, M., Plans, P., Costa, J., Salleras, L. (2004). Prevalence of hepatitis A antibodies in schoolchildren in Catalonia (Spain) after the introduction of universal hepatitis A immunization. *J Med Virol*, **73**, 172-176.

Dominguez, G., Dambaugh, T. R., Stamey, F. R., Dewhurst, S., Inoue, N., Pellett, P. E. (1999). Human herpesvirus 6B genome sequence: coding content and comparison with human herpesvirus 6A. *J Virol*, **73**, 8040-8052.

Donahue, J. G., Munoz, A., Ness, P. M., Brown, D. E., Jr., Yawn, D. H., McAllister, H. A., Jr., Reitz, B. A., Nelson, K. E. (1992). The declining risk of post-transfusion hepatitis C virus infection. *N Engl J Med*, **327**, 369-373.

Donato, F., Boffetta, P., Puoti, M. (1998). A meta-analysis of epidemiological studies on the combined effect of hepatitis B and C virus infections in causing hepatocellular carcinoma. *Int J Cancer*, **75**, 347-354.

Donato, F., Gelatti, U., Chiesa, R., Albertini, A., Bucella, E., Boffetta, P., Tagger, A., Ribero, M. L., Portera, G., Fasola, M., Nardi, G. (1999). A case-control study on family history of liver cancer as a risk factor for hepatocellular carcinoma in North Italy. Brescia HCC Study. *Cancer Causes Control*, **10**, 417-421.

Donato, F., Gelatti, U., Limina, R. M., Fattovich, G. (2006). Southern Europe as an example of interaction between various environmental factors: a systematic review of the epidemiologic evidence. *Oncogene*, **25**, 3756-3770.

Drobeniuc, J., Favorov, M. O., Shapiro, C. N., Bell, B. P., Mast, E. E., Dadu, A., Culver, D., Iarovoi, P., Robertson, B. H., Margolis, H. S. (2001). Hepatitis E virus antibody prevalence among persons who work with swine. *J Infect Dis*, **184**, 1594-1597.

Dusheiko, G., Schmilovitz-Weiss, H., Brown, D., McOmish, F., Yap, P. L., Sherlock, S., McIntyre, N., Simmonds, P. (1994). Hepatitis C virus genotypes: an investigation of type-specific differences in geographic origin and disease. *Hepatology*, **19**, 13-18.

Ebell, M. H. (2004). Epstein-Barr virus infectious mononucleosis. *Am Fam Physician*, **70**, 1279-1287.

Echevarria, J. M., Avellon, A., Magnius, L. O. (2005). Molecular epidemiology of hepatitis B virus in Spain: identification of viral genotypes and prediction of antigenic subtypes by limited sequencing. *J Med Virol*, **76**, 176-184.

Edmunds, W. J., Medley, G. F., Nokes, D. J., Hall, A. J., Whittle, H. C. (1993). The influence of age on the development of the hepatitis B carrier state. *Proc Biol Sci*, **253**, 197-201.

Eggert-Kruse, W., Reuland, M., Johannsen, W., Strowitzki, T., Schlehofer, J. R. (2009). Cytomegalovirus (CMV) infection--related to male and/or female infertility factors? *Fertil Steril*, **91**, 67-82.

El-Hazmi, M. M. (2004). Prevalence of HBV, HCV, HIV-1, 2 and HTLV-I/II infections among blood donors in a teaching hospital in the Central region of Saudi Arabia. *Saudi Med J*, **25**, 26-33.

el-Nawawy, A., Soliman, A. T., el Azzouni, O., Amer el, S., Karim, M. A., Demian, S., el Sayed, M. (1996). Maternal and neonatal prevalence of toxoplasma and cytomegalovirus (CMV) antibodies and hepatitis-B antigens in an Egyptian rural area. *J Trop Pediatr*, **42**, 154-157.

el-Refaie, A., Savage, K., Bhattacharya, S., Khakoo, S., Harrison, T. J., el-Batanony, M., Soliman el, S., Nasr, S., Mokhtar, N., Amer, K., Scheuer, P. J., Dhillon, A.

P. (1996). HCV-associated hepatocellular carcinoma without cirrhosis. *J Hepatol*, **24**, 277-285.
El-Sayed, H. F., Abaza, S. M., Mehanna, S., Winch, P. J. (1997). The prevalence of hepatitis B and C infections among immigrants to a newly reclaimed area endemic for Schistosoma mansoni in Sinai, Egypt. *Acta Trop*, **68**, 229-237.
El-Serag, H. B. (2001). Epidemiology of hepatocellular carcinoma. *Clin Liver Dis*, **5**, 87-107, vi.
El-Serag, H. B., Mason, A. C. (1999). Rising incidence of hepatocellular carcinoma in the United States. *N Engl J Med*, **340**, 745-750.
El-Serag, H. B., Tran, T., Everhart, J. E. (2004). Diabetes increases the risk of chronic liver disease and hepatocellular carcinoma. *Gastroenterology*, **126**, 460-468.
El-Sorori, A. A. (1991). Knowledge, attitude and practices about Qat in a rural community in the Yemen Republic and the prevalence of hepatitis B carriage rate among Qat-users and non-users. In: Liverpool School of Tropical Medicine, MSc, p. 88. University of Liverpool, Liverpool.
El Gohary, A., Hassan, A., Nooman, Z., Lavanchy, D., Mayerat, C., el Ayat, A., Fawaz, N., Gobran, F., Ahmed, M., Kawano, F., et al. (1995). High prevalence of hepatitis C virus among urban and rural population groups in Egypt. *Acta Trop*, **59**, 155-161.
El Guneid, A. M., Gunaid, A. A., O'Neill, A. M., Zureikat, N. I., Coleman, J. C., Murray-Lyon, I. M. (1993). Prevalence of hepatitis B, C, and D virus markers in Yemeni patients with chronic liver disease. *J Med Virol*, **40**, 330-333.
El Sherbini, A., Mohsen, S. A., Seleem, Z., Ghany, A. A., Moneib, A., Abaza, A. H. (2006). Hepatitis B virus among schoolchildren in an endemic area in Egypt over a decade: Impact of hepatitis B vaccine. *Am J Infect Control*, **34**, 600-602.
Elisaf, M., Tsianos, E., Mavridis, A., Dardamanis, M., Pappas, M., Siamopoulos, K. C. (1991). Antibodies against hepatitis C virus (anti-HCV) in haemodialysis patients: association with hepatitis B serological markers. *Nephrol Dial Transplant*, **6**, 476-479.
Emerson, S. U., Clemente-Casares, P., Moiduddin, N., Arankalle, V. A., Torian, U., Purcell, R. H. (2006). Putative neutralization epitopes and broad cross-genotype neutralization of Hepatitis E virus confirmed by a quantitative cell-culture assay. *J Gen Virol*, **87**, 697-704.
Emerson, S. U., Purcell, R. H. (2003). Hepatitis E virus. *Rev Med Virol*, **13**, 145-154.
Emerson, S. U., Purcell, R. H. (2004). Running like water--the omnipresence of hepatitis E. *N Engl J Med*, **351**, 2367-2368.
Engel, M., Essop, M. F., Close, P., Hartley, P., Pallesen, G., Sinclair-Smith, C. (2000). Improved prognosis of Epstein-Barr virus associated childhood Hodgkin's lymphoma: study of 47 South African cases. *J Clin Pathol*, **53**, 182-186.
Engle, R. E., Yu, C., Emerson, S. U., Meng, X. J., Purcell, R. H. (2002). Hepatitis E virus (HEV) capsid antigens derived from viruses of human and swine origin are equally efficient for detecting anti-HEV by enzyme immunoassay. *J Clin Microbiol*, **40**, 4576-4580.
Enouf, V., Dos Reis, G., Guthmann, J. P., Guerin, P. J., Caron, M., Marechal, V., Nicand, E. (2006). Validation of single real-time TaqMan PCR assay for the detection and quantitation of four major genotypes of hepatitis E virus in clinical specimens. *J Med Virol*, **78**, 1076-1082.
EPI/Aden. (2007). Annual Report on Expanded Programm of Immunization for the year 2006. p. 13. Ministry of Public health and Population/ Aden, Aden, Yemen.
EPI/Aden, M. (2005). Annaual Statistical repaort on Health Status., p. 85. Ministry Of Public Health and Population/Aden Branch. Republic of Yemen.
Epstein, M. A., Achong, B. G. (1973). The EB virus. *Annu Rev Microbiol*, **27**, 413-436.
Erker, J. C., Desai, S. M., Schlauder, G. G., Dawson, G. J., Mushahwar, I. K. (1999). A hepatitis E virus variant from the United States: molecular characterization and transmission in cynomolgus macaques. *J Gen Virol*, **80 (Pt 3)**, 681-690.
Ernst, E., Sherman, K. J. (2003). Is acupuncture a risk factor for hepatitis? Systematic review of epidemiological studies. *J Gastroenterol Hepatol*, **18**, 1231-1236.

Eskild, A., Bruu, A. L., Stray-Pedersen, B., Jenum, P. (2005). Epstein-Barr virus infection during pregnancy and the risk of adverse pregnancy outcome. *BJOG*, **112**, 1620-1624.

Euler, G. L., Copeland, J., Williams, W. W. (2003). Impact of four urban perinatal hepatitis B prevention programs on screening and vaccination of infants and household members. *Am J Epidemiol*, **157**, 747-753.

Evans, G. W., Kantrowitz, E. (2002). Socioeconomic status and health: the potential role of environmental risk exposure. *Annu Rev Public Health*, **23**, 303-331.

Fahmi, H., Cochet, C., Hmama, Z., Opolon, P., Joab, I. (2000). Transforming growth factor beta 1 stimulates expression of the Epstein-Barr virus BZLF1 immediate-early gene product ZEBRA by an indirect mechanism which requires the MAPK kinase pathway. *J Virol*, **74**, 5810-5818.

Faingezicht, I., Avila-Aguerro, M. L., Cervantes, Y., Fourneau, M., Clemens, S. A. (2002). Primary and booster vaccination with DTPw-HB/Hib pentavalent vaccine in Costa Rican children who had received a birth dose of hepatitis B vaccine. *Rev Panam Salud Publica*, **12**, 247-257.

Fathalla, S. E., Al-Jama, A. A., Al-Sheikh, I. H., Islam, S. I. (2000). Seroprevalence of Hepatitis a virus markers in Eastern Saudi Arabia. *Saudi Med J*, **21**, 945-949.

Fattovich, G., Pantalena, M., Zagni, I., Realdi, G., Schalm, S. W., Christensen, E. (2002). Effect of hepatitis B and C virus infections on the natural history of compensated cirrhosis: a cohort study of 297 patients. *Am J Gastroenterol*, **97**, 2886-2895.

Fattovich, G., Stroffolini, T., Zagni, I., Donato, F. (2004). Hepatocellular carcinoma in cirrhosis: incidence and risk factors. *Gastroenterology*, **127**, S35-50.

Favorov, M. O., Khudyakov, Y. E., Mast, E. E., Yashina, T. L., Shapiro, C. N., Khudyakova, N. S., Jue, D. L., Onischenko, G. G., Margolis, H. S., Fields, H. A. (1996). IgM and IgG antibodies to hepatitis E virus (HEV) detected by an enzyme immunoassay based on an HEV-specific artificial recombinant mosaic protein. *J Med Virol*, **50**, 50-58.

Favorov, M. O., Kosoy, M. Y., Tsarev, S. A., Childs, J. E., Margolis, H. S. (2000). Prevalence of antibody to hepatitis E virus among rodents in the United States. *J Infect Dis*, **181**, 449-455.

Feorino, P. M., Mathews, H. M. (1974). Malaria antibody levels in patients with Burkitt's lymphoma. *Am J Trop Med Hyg*, **23**, 574-576.

Figueira-Silva, C. M., Pereira, F. E. (2004). Prevalence of Epstein-Barr virus antibodies in healthy children and adolescents in Vitoria, State of Espirito Santo, Brazil. *Rev Soc Bras Med Trop*, **37**, 409-412.

Fitzsimons, D., Francois, G., Hall, A., McMahon, B., Meheus, A., Zanetti, A., Duval, B., Jilg, W., Bocher, W. O., Lu, S. N., Akarca, U., Lavanchy, D., Goldstein, S., Banatvala, J., Damme, P. V. (2005). Long-term efficacy of hepatitis B vaccine, booster policy, and impact of hepatitis B virus mutants. *Vaccine*, **23**, 4158-4166.

Fix, A. D., Abdel-Hamid, M., Purcell, R. H., Shehata, M. H., Abdel-Aziz, F., Mikhail, N., el Sebai, H., Nafeh, M., Habib, M., Arthur, R. R., Emerson, S. U., Strickland, G. T. (2000). Prevalence of antibodies to hepatitis E in two rural Egyptian communities. *Am J Trop Med Hyg*, **62**, 519-523.

Fotheringham, J., Jacobson, S. (2005). Human herpesvirus 6 and multiple sclerosis: potential mechanisms for virus-induced disease. *Herpes*, **12**, 4-9.

Fowler, K. B., Dahle, A. J., Boppana, S. B., Pass, R. F. (1999). Newborn hearing screening: will children with hearing loss caused by congenital cytomegalovirus infection be missed? *J Pediatr*, **135**, 60-64.

Fox, E., Abbatte, E. A., Said, S., Constantine, N. T., Wassef, H. H., Woody, J. N. (1988). Viral hepatitis markers in Djibouti: an epidemiological survey. *Trans R Soc Trop Med Hyg*, **82**, 750-752.

Franco, S., Tural, C., Clotet, B., Martinez, M. A. (2006). Complete nucleotide sequence of genotype 4 hepatitis C viruses isolated from patients co-infected with human immunodeficiency virus type 1. *Virus Res*.

Frank, C., Mohamed, M. K., Strickland, G. T., Lavanchy, D., Arthur, R. R., Magder, L. S., El Khoby, T., Abdel-Wahab, Y., Aly Ohn, E. S., Anwar, W., Sallam, I. (2000). The role of parenteral antischistosomal therapy in the spread of hepatitis C virus in Egypt. *Lancet*, **355**, 887-891.

Fujiwara, K., Yokosuka, O., Ehata, T., Saisho, H., Saotome, N., Suzuki, K., Okita, K., Kiyosawa, K., Omata, M. (2002). Association between severity of type A hepatitis and nucleotide variations in the 5' non-translated region of hepatitis A virus RNA: strains from fulminant hepatitis have fewer nucleotide substitutions. *Gut*, **51**, 82-88.

Fung, S. K., Lok, A. S. (2004). Hepatitis B virus genotypes: do they play a role in the outcome of HBV infection? *Hepatology*, **40**, 790-792.

Fung, S. K., Wong, F. S., Wong, D. K., Hussain, M. T., Lok, A. S. (2006). Hepatitis B virus genotypes, precore and core promoter variants among predominantly Asian patients with chronic HBV infection in a Canadian center. *Liver Int*, **26**, 796-804.

Gaeta, G. B., Giusti, G. (1990). Epidemiology of chronic viral hepatitis in the Mediterranean area: present status and trends. *Infection*, **18**, 21-25.

Gaeta, G. B., Stroffolini, T., Chiaramonte, M., Ascione, T., Stornaiuolo, G., Lobello, S., Sagnelli, E., Brunetto, M. R., Rizzetto, M. (2000). Chronic hepatitis D: a vanishing Disease? An Italian multicenter study. *Hepatology*, **32**, 824-827.

Garfein, R. S., Bower, W. A., Loney, C. M., Hutin, Y. J., Xia, G. L., Jawanda, J., Groom, A. V., Nainan, O. V., Murphy, J. S., Bell, B. P. (2004). Factors associated with fulminant liver failure during an outbreak among injection drug users with acute hepatitis B. *Hepatology*, **40**, 865-873.

Garfein, R. S., Vlahov, D., Galai, N., Doherty, M. C., Nelson, K. E. (1996). Viral infections in short-term injection drug users: the prevalence of the hepatitis C, hepatitis B, human immunodeficiency, and human T-lymphotropic viruses. *Am J Public Health*, **86**, 655-661.

Garrigue, I., Doussau, A., Asselineau, J., Bricout, H., Couzi, L., Rio, C., Merville, P., Fleury, H., Lafon, M. E., Thiebaut, R. (2008). Prediction of cytomegalovirus (CMV) plasma load from evaluation of CMV whole-blood load in samples from renal transplant recipients. *J Clin Microbiol*, **46**, 493-498.

Garson, J. A., Tuke, P. W., Makris, M., Briggs, M., Machin, S. J., Preston, F. E., Tedder, R. S. (1990). Demonstration of viraemia patterns in haemophiliacs treated with hepatitis-C-virus-contaminated factor VIII concentrates. *Lancet*, **336**, 1022-1025.

Gartner, B. C., Hess, R. D., Bandt, D., Kruse, A., Rethwilm, A., Roemer, K., Mueller-Lantzsch, N. (2003). Evaluation of four commercially available Epstein-Barr virus enzyme immunoassays with an immunofluorescence assay as the reference method. *Clin Diagn Lab Immunol*, **10**, 78-82.

GAVI. (2006). Application form for country proposals: For Support to Immunization Services Yemen. Global Alliance for Vaccines and Immunisations, Geneva, Switzerland. .

Gaytant, M. A., Steegers, E. A., Semmekrot, B. A., Merkus, H. M., Galama, J. M. (2002). Congenital cytomegalovirus infection: review of the epidemiology and outcome. *Obstet Gynecol Surv*, **57**, 245-256.

GCCR, G. C. f. C. R. (2006). The Five-Year Cancer Incidence. Gulf Center for Cancer Registration. 1998-2002 Report. .

George, S. L., Varmaz, D., Stapleton, J. T. (2006). GB virus C replicates in primary T and B lymphocytes. *J Infect Dis*, **193**, 451-454.

Germer, J. J., Majewski, D. W., Yung, B., Mitchell, P. S., Yao, J. D. (2006). Evaluation of the invader assay for genotyping hepatitis C virus. *J Clin Microbiol*, **44**, 318-323.

Gessoni, G., Barin, P., Marchiori, G., Pisani, G., Gentili, G., Bressan, F., Aprili, G. (2005). Nucleic acid amplification technology screening for HBV DNA in Italy: routine application. Report on the first HBV DNA-positive HBsAG-negative donor. *Transfus Med*, **15**, 251-253.

Ghabrah, T. M., Tsarev, S., Yarbough, P. O., Emerson, S. U., Strickland, G. T., Purcell, R. H. (1998). Comparison of tests for antibody to hepatitis E virus. *J Med Virol*, **55**, 134-137.

Ghavanini, A. A., Sabri, M. R. (2000). Hepatitis B surface antigen and anti-hepatitis C antibodies among blood donors in the Islamic Republic of Iran. *East Mediterr Health J*, **6**, 1114-1116.

Gilbert, C., Handfield, J., Toma, E., Lalonde, R., Bergeron, M. G., Boivin, G. (1998). Emergence and prevalence of cytomegalovirus UL97 mutations associated with ganciclovir resistance in AIDS patients. *Aids*, **12**, 125-129.

Girlanda, R., Mohsen, A. H., Smith, H., Sablon, E., Yuen, M. F., O'Grady, J., Muiesan, P., Rela, M., Heaton, N., Norris, S. (2004). Hepatitis B virus genotype A and D and clinical outcomes of liver transplantation for HBV-related disease. *Liver Transpl*, **10**, 58-64.

Gish, R. G., Gadano, A. C. (2006). Chronic hepatitis B: current epidemiology in the Americas and implications for management. *J Viral Hepat*, **13**, 787-798.

Gitlin, N. (1997). Hepatitis B: diagnosis, prevention, and treatment. *Clin Chem*, **43**, 1500-1506.

Glas, C., Hotz, P., Steffen, R. (2001). Hepatitis A in workers exposed to sewage: a systematic review. *Occup Environ Med*, **58**, 762-768.

Glaser, S. L., Lin, R. J., Stewart, S. L., Ambinder, R. F., Jarrett, R. F., Brousset, P., Pallesen, G., Gulley, M. L., Khan, G., O'Grady, J., Hummel, M., Preciado, M. V., Knecht, H., Chan, J. K., Claviez, A. (1997). Epstein-Barr virus-associated Hodgkin's disease: epidemiologic characteristics in international data. *Int J Cancer*, **70**, 375-382.

Glynn, S. A., Kleinman, S. H., Schreiber, G. B., Busch, M. P., Wright, D. J., Smith, J. W., Nass, C. C., Williams, A. E. (2000). Trends in incidence and prevalence of major transfusion-transmissible viral infections in US blood donors, 1991 to 1996. Retrovirus Epidemiology Donor Study (REDS). *Jama*, **284**, 229-235.

Godshall, S. E., Kirchner, J. T. (2000). Infectious mononucleosis. Complexities of a common syndrome. *Postgrad Med*, **107**, 175-179, 183-174, 186.

Goldenthal, K. L., Burns, D. L., McVittie, L. D., Lewis, B. P., Jr., Williams, J. C. (1995). Overview--combination vaccines and simultaneous administration. Past, present, and future. *Ann N Y Acad Sci*, **754**, xi-xv.

Goldsmith, R., Yarbough, P. O., Reyes, G. R., Fry, K. E., Gabor, K. A., Kamel, M., Zakaria, S., Amer, S., Gaffar, Y. (1992). Enzyme-linked immunosorbent assay for diagnosis of acute sporadic hepatitis E in Egyptian children. *Lancet*, **339**, 328-331.

Goldstein, S. T., Zhou, F., Hadler, S. C., Bell, B. P., Mast, E. E., Margolis, H. S. (2005). A mathematical model to estimate global hepatitis B disease burden and vaccination impact. *Int J Epidemiol*, **34**, 1329-1339.

Golubjatnikov, R., Allen, V. D., Steadman, M., Del Pilar Olmos, B., Inhorn, S. L. (1973). Prevalence of antibodies to Epstein-Barr virus, cytomegalovirus and Toxoplasma in a Mexican highland community. *Am J Epidemiol*, **97**, 116-124.

Goswami, B. B., Burkhardt, W., 3rd, Cebula, T. A. (1997). Identification of genetic variants of hepatitis A virus. *J Virol Methods*, **65**, 95-103.

Grandjacques, C., Pradat, P., Stuyver, L., Chevallier, M., Chevallier, P., Pichoud, C., Maisonnas, M., Trepo, C., Zoulim, F. (2000). Rapid detection of genotypes and mutations in the pre-core promoter and the pre-core region of hepatitis B virus genome: correlation with viral persistence and disease severity. *J Hepatol*, **33**, 430-439.

Grebely, J., Conway, B., Raffa, J. D., Lai, C., Krajden, M., Tyndall, M. W. (2006). Hepatitis C virus reinfection in injection drug users. *Hepatology*, **44**, 1139-1145.

Green, M., Webber, S. A. (2007). Persistent increased Epstein-Barr virus loads after solid organ transplantation: Truth and consequences? *Liver Transpl*, **13**, 321-322.

Griffiths, P., Gossop, M., Wickenden, S., Dunworth, J., Harris, K., Lloyd, C. (1997). A transcultural pattern of drug use: qat (khat) in the UK. *Br J Psychiatry*, **170**, 281-284.

Griffiths, P. D., Clark, D. A., Emery, V. C. (2000). Betaherpesviruses in transplant recipients. *J Antimicrob Chemother*, **45 Suppl T3**, 29-34.

Grosse, S. D., Ross, D. S., Dollard, S. C. (2008). Congenital cytomegalovirus (CMV) infection as a cause of permanent bilateral hearing loss: a quantitative assessment. *J Clin Virol*, **41**, 57-62.

Grumbach, I. M., Heermann, K., Figulla, H. R. (1998). Low prevalence of hepatitis C virus antibodies and RNA in patients with myocarditis and dilated cardiomyopathy. *Cardiology*, **90**, 75-78.

Gul, A., Iqbal, F. (2003). Prevalence of hepatitis C in patients on maintenance haemodialysis. *J Coll Physicians Surg Pak*, **13**, 15-18.

Gulley, M. L., Eagan, P. A., Quintanilla-Martinez, L., Picado, A. L., Smir, B. N., Childs, C., Dunn, C. D., Craig, F. E., Williams, J. W., Jr., Banks, P. M. (1994). Epstein-Barr virus DNA is abundant and monoclonal in the Reed-Sternberg cells of Hodgkin's disease: association with mixed cellularity subtype and Hispanic American ethnicity. *Blood*, **83**, 1595-1602.

Gunaid, A. A., Nasher, T. M., el-Guneid, A. M., Hill, M., Dayton, R., Pal, A., Skidmore, S. J., Coleman, J. C., Murray-Lyon, I. M. (1997). Acute sporadic hepatitis in the Republic of Yemen. *J Med Virol*, **51**, 64-66.

Haagsma, E. B., van den Berg, A. P., Porte, R. J., Benne, C. A., Vennema, H., Reimerink, J. H., Koopmans, M. P. (2008). Chronic hepatitis E virus infection in liver transplant recipients. *Liver Transpl*, **14**, 547-553.

Hagiwara, S., Kudo, M., Minami, Y., Chung, H., Nakatani, T., Fukunaga, T., Osaki, Y., Yamashita, Y., Kajimura, K. (2006). Clinical significance of the genotype and core promoter/pre-core mutations in hepatitis B virus carriers. *Intervirology*, **49**, 200-206.

Haidar, N. A. (2002). Prevalence of hepatitis B and hepatitis C in blood donors and high risk groups in Hajjah, Yemen Republic. *Saudi Med J*, **23**, 1090-1094.

Halbach, H. (1972). Medical aspects of the chewing of khat leaves. *Bull World Health Organ*, **47**, 21-29.

Hall, A. J. (1993). Hepatitis in travellers: epidemiology and prevention. *Br Med Bull*, **49**, 382-393.

Hall, A. J. (2003). Vaccination politics. *Trop Med Int Health*, **8**, 1-2.

Hall, C. B., Long, C. E., Schnabel, K. C., Caserta, M. T., McIntyre, K. M., Costanzo, M. A., Knott, A., Dewhurst, S., Insel, R. A., Epstein, L. G. (1994). Human herpesvirus-6 infection in children. A prospective study of complications and reactivation. *N Engl J Med*, **331**, 432-438.

Hammouda, N. A., el-Gebaly, W. M., Sadaka, S. M. (1993). Seroprevalence of Toxoplasma and cytomegalovirus in complicated pregnancies. *J Egypt Soc Parasitol*, **23**, 865-870.

Handsfield, H. H., Chandler, S. H., Caine, V. A., Meyers, J. D., Corey, L., Medeiros, E., McDougall, J. K. (1985). Cytomegalovirus infection in sex partners: evidence for sexual transmission. *J Infect Dis*, **151**, 344-348.

Hannoun, C., Krogsgaard, K., Horal, P., Lindh, M. (2002). Genotype mixtures of hepatitis B virus in patients treated with interferon. *J Infect Dis*, **186**, 752-759.

Hannoun, C., Soderstrom, A., Norkrans, G., Lindh, M. (2005). Phylogeny of African complete genomes reveals a West African genotype A subtype of hepatitis B virus and relatedness between Somali and Asian A1 sequences. *J Gen Virol*, **86**, 2163-2167.

Haqshenas, G., Shivaprasad, H. L., Woolcock, P. R., Read, D. H., Meng, X. J. (2001). Genetic identification and characterization of a novel virus related to human hepatitis E virus from chickens with hepatitis-splenomegaly syndrome in the United States. *J Gen Virol*, **82**, 2449-2462.

Hara, S., Hoshino, Y., Naitou, T., Nagano, K., Iwai, M., Suzuki, K., Yamamoto, K., Nagasaka, T., Morishima, T., Kimura, H. (2006). Association of virus infected-T

cell in severe hepatitis caused by primary Epstein-Barr virus infection. *J Clin Virol*, **35**, 250-256.
Hardie, D. R., Williamson, C. (1997). Analysis of the preS1 gene of hepatitis B virus (HBV) to define epidemiologically linked and un-linked infections in South Africa. *Arch Virol*, **142**, 1829-1841.
Harris, R. C. (2008). Long-term effects of human herpesvirus 6 infection. *Pediatrics*, **122**, 679.
Harrison, T. J. (1999). Hepatitis E virus an update. *Liver*, **19**, 171-176.
Hasan, I. (2005). Epidemiology of hepatitis B. *Acta Med Indones*, **37**, 231-234.
Hassan, A. A., Khalil, R. (2000). Hepatitis C in dialysis patients in egypt: relationship to dialysis duration, blood transfusion, and liver disease. *Saudi J Kidney Dis Transpl*, **11**, 72-73.
Hassan, M. M., Zaghloul, A. S., El-Serag, H. B., Soliman, O., Patt, Y. Z., Chappell, C. L., Beasley, R. P., Hwang, L. Y. (2001). The role of hepatitis C in hepatocellular carcinoma: A case control study among egyptian patients. *J Clin Gastroenterol*, **33**, 123-126.
Hassan, R., Klumb, C. E., Felisbino, F. E., Guiretti, D. M., White, L. R., Stefanoff, C. G., Barros, M. H., Seuanez, H. N., Zalcberg, I. R. (2008a). Clinical and demographic characteristics of Epstein-Barr virus-associated childhood Burkitt's lymphoma in Southeastern Brazil: epidemiological insights from an intermediate risk region. *Haematologica*, **93**, 780-783.
Hassan, R., Stefanoff, C. G., Felisbino, F., Barros, M. H., Zalcberg, I. R., Klumb, C. E., Bigni, R. S., Seuanez, H. N. (2008). Second Epstein-Barr virus-associated Burkitt's lymphoma of the CNS in a child with progressive renal failure. *J Clin Oncol*, **26**, 3085-3087.
Hayashi, J. (1992). Epidemiology of the hepatitis C virus. *Fukuoka Igaku Zasshi*, **83**, 1-5.
Hayashi, J., Nakashima, K., Kajiyama, W., Noguchi, A., Morofuji, M., Maeda, Y., Kashiwagi, S. (1991). Prevalence of antibody to hepatitis C virus in hemodialysis patients. *Am J Epidemiol*, **134**, 651-657.
Hayden, R. T., Hokanson, K. M., Pounds, S. B., Bankowski, M. J., Belzer, S. W., Carr, J., Diorio, D., Forman, M. S., Joshi, Y., Hillyard, D., Hodinka, R. L., Nikiforova, M. N., Romain, C. A., Stevenson, J., Valsamakis, A., Balfour, H. H., Jr. (2008). Multicenter comparison of different real-time PCR assays for quantitative detection of Epstein-Barr virus. *J Clin Microbiol*, **46**, 157-163.
He, J. (2006). Molecular detection and sequence analysis of a new hepatitis E virus isolate from Pakistan. *J Viral Hepat*, **13**, 840-844.
He, J., Innis, B. L., Shrestha, M. P., Clayson, E. T., Scott, R. M., Linthicum, K. J., Musser, G. G., Gigliotti, S. C., Binn, L. N., Kuschner, R. A., Vaughn, D. W. (2006). Evidence that rodents are a reservoir of hepatitis E virus for humans in Nepal. *J Clin Microbiol*, **44**, 1208.
He, J., Tam, A. W., Yarbough, P. O., Reyes, G. R., Carl, M. (1993). Expression and diagnostic utility of hepatitis E virus putative structural proteins expressed in insect cells. *J Clin Microbiol*, **31**, 2167-2173.
Hens, N., Aerts, M., Shkedy, Z., Kung'u Kimani, P., Kojouhorova, M., P, V. A. N. D., Beutels, P. (2007). Estimating the impact of vaccination using age-time-dependent incidence rates of hepatitis B. *Epidemiol Infect*, 1-11.
Hess, R. D. (2004). Routine Epstein-Barr virus diagnostics from the laboratory perspective: still challenging after 35 years. *J Clin Microbiol*, **42**, 3381-3387.
Higgins, C. D., Swerdlow, A. J., Macsween, K. F., Harrison, N., Williams, H., McAulay, K., Thomas, R., Reid, S., Conacher, M., Britton, K., Crawford, D. H. (2007). A study of risk factors for acquisition of Epstein-Barr virus and its subtypes. *J Infect Dis*, **195**, 474-482.
Higuchi, M., Tanaka, E., Kiyosawa, K. (2002). Epidemiology and clinical aspects on hepatitis C. *Jpn J Infect Dis*, **55**, 69-77.
Hinedi, T. B., Koff, R. S. (2003). Cholestatic hepatitis induced by Epstein-Barr virus infection in an adult. *Dig Dis Sci*, **48**, 539-541.

Hinrichsen, H., Leimenstoll, G., Stegen, G., Schrader, H., Folsch, U. R., Schmidt, W. E. (2002). Prevalence and risk factors of hepatitis C virus infection in haemodialysis patients: a multicentre study in 2796 patients. *Gut*, **51**, 429-433.

Hipgrave, D. B., Maynard, J. E., Biggs, B. A. (2006). Improving birth dose coverage of hepatitis B vaccine. *Bull World Health Organ*, **84**, 65-71.

Hollinger, F. B. (2008). Hepatitis B virus infection and transfusion medicine: science and the occult. *Transfusion*, **48**, 1001-1026.

Holma, K., Tornroth, T., Gronhagen-Riska, C., Lautenschlager, I. (2000). Expression of the cytomegalovirus genome in kidney allografts during active and latent infection. *Transpl Int*, **13 Suppl 1**, S363-365.

Hope, V. D., Judd, A., Hickman, M., Lamagni, T., Hunter, G., Stimson, G. V., Jones, S., Donovan, L., Parry, J. V., Gill, O. N. (2001). Prevalence of hepatitis C among injection drug users in England and Wales: is harm reduction working? *Am J Public Health*, **91**, 38-42.

Horn, T., Learned, J. (2006). Viral Hepatitis and HIV. (ed. by Milano, M), p. 29. ACRIA, New York.

Hou, J., Liu, Z., Gu, F. (2005). Epidemiology and Prevention of Hepatitis B Virus Infection. *Int J Med Sci*, **2**, 50-57.

Hsieh, S. Y., Meng, X. J., Wu, Y. H., Liu, S. T., Tam, A. W., Lin, D. Y., Liaw, Y. F. (1999). Identity of a novel swine hepatitis E virus in Taiwan forming a monophyletic group with Taiwan isolates of human hepatitis E virus. *J Clin Microbiol*, **37**, 3828-3834.

Humar, A., Kumar, D., Raboud, J., Caliendo, A. M., Moussa, G., Levy, G., Mazzulli, T. (2002). Interactions between cytomegalovirus, human herpesvirus-6, and the recurrence of hepatitis C after liver transplantation. *Am J Transplant*, **2**, 461-466.

Humar, A., Malkan, G., Moussa, G., Greig, P., Levy, G., Mazzulli, T. (2000). Human herpesvirus-6 is associated with cytomegalovirus reactivation in liver transplant recipients. *J Infect Dis*, **181**, 1450-1453.

Huraib, S., al-Rashed, R., Aldrees, A., Aljefry, M., Arif, M., al-Faleh, F. A. (1995). High prevalence of and risk factors for hepatitis C in haemodialysis patients in Saudi Arabia: a need for new dialysis strategies. *Nephrol Dial Transplant*, **10**, 470-474.

Hutchins, S. S., Jansen, H. A., Robertson, S. E., Evans, P., Kim-Farley, R. J. (1993). Studies of missed opportunities for immunization in developing and industrialized countries. *Bull World Health Organ*, **71**, 549-560.

Hutin, Y. J., Hauri, A. M., Armstrong, G. L. (2003). Use of injections in healthcare settings worldwide, 2000: literature review and regional estimates. *Bmj*, **327**, 1075.

Hutin, Y. J., Pool, V., Cramer, E. H., Nainan, O. V., Weth, J., Williams, I. T., Goldstein, S. T., Gensheimer, K. F., Bell, B. P., Shapiro, C. N., Alter, M. J., Margolis, H. S. (1999). A multistate, foodborne outbreak of hepatitis A. National Hepatitis A Investigation Team. *N Engl J Med*, **340**, 595-602.

Huy, T. T., Ushijima, H., Quang, V. X., Win, K. M., Luengrojanakul, P., Kikuchi, K., Sata, T., Abe, K. (2004). Genotype C of hepatitis B virus can be classified into at least two subgroups. *J Gen Virol*, **85**, 283-292.

Hyams, K. C. (2000). Chronic liver disease among U.S. military patients: the role of hepatitis C and G virus infection. *Mil Med*, **165**, 178-179.

Ibrahim, A. I., Obeid, M. T., Jouma, M. J., Roemer, K., Mueller-Lantzsch, N., Gartner, B. C. (2005). Prevalence of herpes simplex virus (types 1 and 2), varicella-zoster virus, cytomegalovirus, and human herpesvirus 6 and 7 DNA in cerebrospinal fluid of Middle Eastern patients with encephalitis. *J Clin Microbiol*, **43**, 4172-4174.

Ikeda, K., Kobayashi, M., Saitoh, S., Someya, T., Hosaka, T., Akuta, N., Suzuki, Y., Suzuki, F., Tsubota, A., Arase, Y., Kumada, H. (2005). Significance of hepatitis B virus DNA clearance and early prediction of hepatocellular carcinogenesis in

patients with cirrhosis undergoing interferon therapy: long-term follow up of a pilot study. *J Gastroenterol Hepatol*, **20**, 95-102.
Iloeje, U. H., Yang, H. I., Su, J., Jen, C. L., You, S. L., Chen, C. J. (2006). Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. *Gastroenterology*, **130**, 678-686.
Imarengiaye, C. O., Enosolease, M. E., Iribhogbe, P. E., Ehigiegba, A. E. (2006). Risk of transfusion-transmitted hepatitis C virus in a tertiary hospital in Nigeria. *Public Health*, **120**, 274-278.
Irshad, M. (1999). Hepatitis E virus: an update on its molecular, clinical and epidemiological characteristics. *Intervirology*, **42**, 252-262.
Isegawa, Y., Mukai, T., Nakano, K., Kagawa, M., Chen, J., Mori, Y., Sunagawa, T., Kawanishi, K., Sashihara, J., Hata, A., Zou, P., Kosuge, H., Yamanishi, K. (1999). Comparison of the complete DNA sequences of human herpesvirus 6 variants A and B. *J Virol*, **73**, 8053-8063.
Jacobs, F., Knoop, C., Brancart, F., Gilot, P., Melot, C., Byl, B., Delforge, M. L., Estenne, M., Liesnard, C. (2003). Human herpesvirus-6 infection after lung and heart-lung transplantation: a prospective longitudinal study. *Transplantation*, **75**, 1996-2001.
Jacobsen, K. H., Koopman, J. S. (2004). Declining hepatitis A seroprevalence: a global review and analysis. *Epidemiol Infect*, **132**, 1005-1022.
Jadoul, M. (2000). Epidemiology and mechanisms of transmission of the hepatitis C virus in haemodialysis. *Nephrol Dial Transplant*, **15 Suppl 8**, 39-41.
Jadoul, M., Poignet, J. L., Geddes, C., Locatelli, F., Medin, C., Krajewska, M., Barril, G., Scheuermann, E., Sonkodi, S., Goubau, P. (2004). The changing epidemiology of hepatitis C virus (HCV) infection in haemodialysis: European multicentre study. *Nephrol Dial Transplant*, **19**, 904-909.
Jaffray, C. E., Flint, L. M. (2003). Blood-borne viral diseases and the surgeon. *Curr Probl Surg*, **40**, 195-251.
Jafri, W., Jafri, N., Yakoob, J., Islam, M., Ali Tirmizi, S. F., Jafar, T., Akhtar, S., Hamid, S., Shah, H. A., Nizami, S. Q. (2006). Hepatitis B and C: Prevalence and risk factors associated with seropositivity among children in Karachi, Pakistan. *BMC Infectious Diseases*, **6**, 1-10.
Janssen, H. L., van Zonneveld, M., Senturk, H., Zeuzem, S., Akarca, U. S., Cakaloglu, Y., Simon, C., So, T. M., Gerken, G., de Man, R. A., Niesters, H. G., Zondervan, P., Hansen, B., Schalm, S. W. (2005). Pegylated interferon alfa-2b alone or in combination with lamivudine for HBeAg-positive chronic hepatitis B: a randomised trial. *Lancet*, **365**, 123-129.
Jilg, W., Schmidt, M., Deinhardt, F. (1989). Four-year experience with a recombinant hepatitis B vaccine. *Infection*, **17**, 70-76.
Joseph, S. A., Beliveau, C., Muecke, C. J., Rahme, E., Soto, J. C., Flowerdew, G., Johnston, L., Langille, D., Gyorkos, T. W. (2005). Risk factors for cytomegalovirus seropositivity in a population of day care educators in Montreal, Canada. *Occup Med (Lond)*, **55**, 564-567.
Jothikumar, N., Cromeans, T. L., Robertson, B. H., Meng, X. J., Hill, V. R. (2006). A broadly reactive one-step real-time RT-PCR assay for rapid and sensitive detection of hepatitis E virus. *J Virol Methods*, **131**, 65-71.
Judd, A., Hickman, M., Jones, S., McDonald, T., Parry, J. V., Stimson, G. V., Hall, A. J. (2005). Incidence of hepatitis C virus and HIV among new injecting drug users in London: prospective cohort study. *Bmj*, **330**, 24-25.
Jutavijittum, P., Yousukh, A., Samountry, B., Samountry, K., Ounavong, A., Thammavong, T., Keokhamphue, J., Toriyama, K. (2007). Seroprevalence of hepatitis B and C virus infections among Lao blood donors. *Southeast Asian J Trop Med Public Health*, **38**, 674-679.
Kabrane-Lazizi, Y., Fine, J. B., Elm, J., Glass, G. E., Higa, H., Diwan, A., Gibbs, C. J., Jr., Meng, X. J., Emerson, S. U., Purcell, R. H. (1999). Evidence for widespread infection of wild rats with hepatitis E virus in the United States. *Am J Trop Med Hyg*, **61**, 331-335.

Kamar, N., Selves, J., Mansuy, J. M., Ouezzani, L., Peron, J. M., Guitard, J., Cointault, O., Esposito, L., Abravanel, F., Danjoux, M., Durand, D., Vinel, J. P., Izopet, J., Rostaing, L. (2008). Hepatitis E virus and chronic hepatitis in organ-transplant recipients. *N Engl J Med*, **358**, 811-817.

Kandil, A., Bazarbashi, S., Mourad, W. A. (2001). The correlation of Epstein-Barr virus expression and lymphocyte subsets with the clinical presentation of nodular sclerosing Hodgkin disease. *Cancer*, **91**, 1957-1963.

Kane, A., Lloyd, J., Zaffran, M., Simonsen, L., Kane, M. (1999). Transmission of hepatitis B, hepatitis C and human immunodeficiency viruses through unsafe injections in the developing world: model-based regional estimates. *Bull World Health Organ*, **77**, 801-807.

Kane, M. A. (2003). Global control of primary hepatocellular carcinoma with hepatitis B vaccine: the contributions of research in Taiwan. *Cancer Epidemiol Biomarkers Prev*, **12**, 2-3.

Kangro, H. O., Osman, H. K., Lau, Y. L., Heath, R. B., Yeung, C. Y., Ng, M. H. (1994). Seroprevalence of antibodies to human herpesviruses in England and Hong Kong. *J Med Virol*, **43**, 91-96.

Kao, J. H., Chen, P. J., Lai, M. Y., Chen, D. S. (2000). Hepatitis B genotypes correlate with clinical outcomes in patients with chronic hepatitis B. *Gastroenterology*, **118**, 554-559.

Kao, J. H., Chen, P. J., Lai, M. Y., Chen, D. S. (2004). Hepatitis B virus genotypes and spontaneous hepatitis B e antigen seroconversion in Taiwanese hepatitis B carriers. *J Med Virol*, **72**, 363-369.

Kao, J. H., Liu, C. J., Chen, D. S. (2002). Hepatitis B viral genotypes and lamivudine resistance. *J Hepatol*, **36**, 303-304.

Kar, P. (2005). Hepatitis B virus genotyping: will it stand the test of time? *Indian J Gastroenterol*, **24**, 4-5.

Kar, P., Jilani, N., Husain, S. A., Pasha, S. T., Anand, R., Rai, A., Das, B. C. (2008). Does hepatitis E viral load and genotypes influence the final outcome of acute liver failure during pregnancy? *Am J Gastroenterol*, **103**, 2495-2501.

Karagoz, G., Ak, O., Ozer, S. (2005). The coexistence of hepatitis A and infectious mononucleosis. *Turk J Gastroenterol*, **16**, 102-104.

Karatas, H., Gurer, G., Pinar, A., Soylemezoglu, F., Tezel, G. G., Hascelik, G., Akalan, N., Tuncer, S., Ciger, A., Saygi, S. (2008). Investigation of HSV-1, HSV-2, CMV, HHV-6 and HHV-8 DNA by real-time PCR in surgical resection materials of epilepsy patients with mesial temporal lobe sclerosis. *J Neurol Sci*, **264**, 151-156.

Karkar, A., Abdelrahman, M., Ghacha, R., Malik, T. Q. (2006). Prevention of viral transmission in HD units: the value of isolation. *Saudi J Kidney Dis Transpl*, **17**, 183-188.

Kataaha, P. K., Facer, C. A., Holborow, E. J. (1984). Plasmodium falciparum products enhance human lymphocyte transformation by Epstein-Barr virus. *Clin Exp Immunol*, **56**, 371-376.

Kato, H., Gish, R. G., Bzowej, N., Newsom, M., Sugauchi, F., Tanaka, Y., Kato, T., Orito, E., Usuda, S., Ueda, R., Miyakawa, Y., Mizokami, M. (2004). Eight genotypes (A-H) of hepatitis B virus infecting patients from San Francisco and their demographic, clinical, and virological characteristics. *J Med Virol*, **73**, 516-521.

Kato, Z., Kozawa, R., Teramoto, T., Hashimoto, K., Shinoda, S., Kondo, N. (2003). Acute cerebellitis in primary human herpesvirus-6 infection. *Eur J Pediatr*, **162**, 801-803.

Kaur, P., Basu, S. (2005). Transfusion-transmitted infections: existing and emerging pathogens. *J Postgrad Med*, **51**, 146-151.

Kermode, M. (2004a). Healthcare worker safety is a pre-requisite for injection safety in developing countries. *Int J Infect Dis*, **8**, 325-327.

Kermode, M. (2004b). Unsafe injections in low-income country health settings: need for injection safety promotion to prevent the spread of blood-borne viruses. *Health Promot Int*, **19**, 95-103.

Keystone, J. S., Hershey, J. H. (2008). The underestimated risk of hepatitis A and hepatitis B: benefits of an accelerated vaccination schedule. *Int J Infect Dis*, **12**, 3-11.

Khan, A., Kurbanov, F., Tanaka, Y., Elkady, A., Sugiyama, M., Dustov, A., Mizokami, M. (2008). Epidemiological and clinical evaluation of hepatitis B, hepatitis C, and delta hepatitis viruses in Tajikistan. *J Med Virol*, **80**, 268-276.

Khan, K. N., Yatsuhashi, H. (2000). Effect of alcohol consumption on the progression of hepatitis C virus infection and risk of hepatocellular carcinoma in Japanese patients. *Alcohol Alcohol*, **35**, 286-295.

Khan, L. A., Khan, S. A. (2001). Prevalence of hepatitis B and C markers in patients on maintenance hemodialysis in Najran. *Saudi Med J*, **22**, 641-642.

Khan, L. A., Khan, S. A., Al-Hateeti, H. (2001a). Hepatocellular carcinoma in Najran. *Saudi Med J*, **22**, 280-281.

Khanna, R., Moss, D., Gandhi, M. (2005). Technology insight: Applications of emerging immunotherapeutic strategies for Epstein-Barr virus-associated malignancies. *Nat Clin Pract Oncol*, **2**, 138-149.

Khattab, O. S. (2008). Prevalence and risk factors for hepatitis C virus infection in hemodialysis patients in an Iraqi renal transplant center. *Saudi J Kidney Dis Transpl*, **19**, 110-115.

Khuroo, M. S., Kamili, S., Dar, M. Y., Moecklii, R., Jameel, S. (1993). Hepatitis E and long-term antibody status. *Lancet*, **341**, 1355.

Kimbi, G. C., Kramvis, A., Kew, M. C. (2004). Distinctive sequence characteristics of subgenotype A1 isolates of hepatitis B virus from South Africa. *J Gen Virol*, **85**, 1211-1220.

Kimura, H., Nagasaka, T., Hoshino, Y., Hayashi, N., Tanaka, N., Xu, J. L., Kuzushima, K., Morishima, T. (2001). Severe hepatitis caused by Epstein-Barr virus without infection of hepatocytes. *Hum Pathol*, **32**, 757-762.

Kirschberg, O., Schuttler, C., Repp, R., Schaefer, S. (2004). A multiplex-PCR to identify hepatitis B virus--enotypes A-F. *J Clin Virol*, **29**, 39-43.

Kiyohara, T., Sato, T., Totsuka, A., Miyamura, T., Ito, T., Yoneyama, T. (2007). Shifting seroepidemiology of hepatitis A in Japan, 1973-2003. *Microbiol Immunol*, **51**, 185-191.

Kiyosawa, K., Tanaka, E., Sodeyama, T., Yoshizawa, K., Yabu, K., Furuta, K., Imai, H., Nakano, Y., Usuda, S., Uemura, K., et al. (1994). Transmission of hepatitis C in an isolated area in Japan: community-acquired infection. The South Kiso Hepatitis Study Group. *Gastroenterology*, **106**, 1596-1602.

Knisely, A. S., Strautnieks, S. S., Meier, Y., Stieger, B., Byrne, J. A., Portmann, B. C., Bull, L. N., Pawlikowska, L., Bilezikci, B., Ozcay, F., Laszlo, A., Tiszlavicz, L., Moore, L., Raftos, J., Arnell, H., Fischler, B., Nemeth, A., Papadogiannakis, N., Cielecka-Kuszyk, J., Jankowska, I., Pawlowska, J., Melin-Aldana, H., Emerick, K. M., Whitington, P. F., Mieli-Vergani, G., Thompson, R. J. (2006). Hepatocellular carcinoma in ten children under five years of age with bile salt export pump deficiency. *Hepatology*, **44**, 478-486.

Koff, R. S. (1998). Hepatitis A. *Lancet*, **351**, 1643-1649.

Kondo, K., Nagafuji, H., Hata, A., Tomomori, C., Yamanishi, K. (1993). Association of human herpesvirus 6 infection of the central nervous system with recurrence of febrile convulsions. *J Infect Dis*, **167**, 1197-1200.

Kothari, A., Ramachandran, V. G., Gupta, P., Singh, B., Talwar, V. (2002). Seroprevalence of cytomegalovirus among voluntary blood donors in Delhi, India. *J Health Popul Nutr*, **20**, 348-351.

Kozireva, S., Nemceva, G., Danilane, I., Pavlova, O., Blomberg, J., Murovska, M. (2001). Prevalence of blood-borne viral infections (cytomegalovirus, human herpesvirus-6, human herpesvirus-7, human herpesvirus-8, human T-cell

lymphotropic virus-I/II, human retrovirus-5) among blood donors in Latvia. *Ann Hematol*, **80**, 669-673.
Kramvis, A., Kew, M., Francois, G. (2005). Hepatitis B virus genotypes. *Vaccine*, **23**, 2409-2423.
Krawczynski, K. (2007). Hepatitis E vaccine--ready for prime time? *N Engl J Med*, **356**, 949-951.
Krawczynski, K., Aggarwal, R., Kamili, S. (2000). Hepatitis E. *Infect Dis Clin North Am*, **14**, 669-687.
Krawczynski, K., Kamili, S., Aggarwal, R. (2001). Global epidemiology and medical aspects of hepatitis E. *Forum*, **11**, 166-179.
Krugman, S. (1974). Viral hepatitis and hepatitis B antigen: recent advances. *Postgrad Med J*, **50**, 327-333.
Krugman, S., Ward, R., Giles, J. P. (1962). The natural history of infectious hepatitis. *Am J Med*, **32**, 717-728.
Kumar, A., Kumar, S. I., Pandey, R., Naik, S., Aggarwal, R. (2005). Hepatitis B virus genotype A is more often associated with severe liver disease in northern India than is genotype D. *Indian J Gastroenterol*, **24**, 19-22.
Kumar, R. M., Uduman, S., Rana, S., Kochiyil, J. K., Usmani, A., Thomas, L. (2001). Sero-prevalence and mother-to-infant transmission of hepatitis E virus among pregnant women in the United Arab Emirates. *Eur J Obstet Gynecol Reprod Biol*, **100**, 9-15.
Kuniholm, M. H., Mark, J., Aladashvili, M., Shubladze, N., Khechinashvili, G., Tsertsvadze, T., del Rio, C., Nelson, K. E. (2008). Risk factors and algorithms to identify hepatitis C, hepatitis B, and HIV among Georgian tuberculosis patients. *Int J Infect Dis*, **12**, 51-56.
Kuper, H., Tzonou, A., Kaklamani, E., Hsieh, C. C., Lagiou, P., Adami, H. O., Trichopoulos, D., Stuver, S. O. (2000). Tobacco smoking, alcohol consumption and their interaction in the causation of hepatocellular carcinoma. *Int J Cancer*, **85**, 498-502.
Kuppers, R. (2003). B cells under influence: transformation of B cells by Epstein-Barr virus. *Nat Rev Immunol*, **3**, 801-812.
Kurbanov, F., Tanaka, Y., Sugauchi, F., Kato, H., Ruzibakiev, R., Zalyalieva, M., Yunusova, Z., Mizokami, M. (2003). Hepatitis C virus molecular epidemiology in Uzbekistan. *J Med Virol*, **69**, 367-375.
Labrique, A. B., Thomas, D. L., Stoszek, S. K., Nelson, K. E. (1999). Hepatitis E: an emerging infectious disease. *Epidemiol Rev*, **21**, 162-179.
Lai, C. L., Ratziu, V., Yuen, M. F., Poynard, T. (2003). Viral hepatitis B. *Lancet*, **362**, 2089-2094.
Lam, C. M., Chan, A. O., Ho, P., Ng, I. O., Lo, C. M., Liu, C. L., Poon, R. T., Fan, S. T. (2004). Different presentation of hepatitis B-related hepatocellular carcinoma in a cohort of 1863 young and old patients - implications for screening. *Aliment Pharmacol Ther*, **19**, 771-777.
Lamden, K. H., Kennedy, N., Beeching, N. J., Lowe, D., Morrison, C. L., Mallinson, H., Mutton, K. J., Syed, Q. (1998). Hepatitis B and hepatitis C virus infections: risk factors among drug users in Northwest England. *J Infect*, **37**, 260-269.
Laskus, T., Radkowski, M., Wang, L. F., Vargas, H., Rakela, J. (1997). Lack of evidence for hepatitis G virus replication in the livers of patients coinfected with hepatitis C and G viruses. *J Virol*, **71**, 7804-7806.
LATH/EuroHealthGroup. (2006). Data Quality Audit-Yemen. 25th November to 14th December 2006. (ed. by Liverpool Associates in Tropical Health LATH, E H G), Liverpool.
Lau, G. K. (2009). A new magic bullet for chronic hepatitis B infection- Is this the end of the story? *Gastroenterology*.
Lauer, G. M., Walker, B. D. (2001). Hepatitis C virus infection. *N Engl J Med*, **345**, 41-52.
Laurent, C., Henzel, D., Mulanga-Kabeya, C., Maertens, G., Larouze, B., Delaporte, E. (2001). Seroepidemiological survey of hepatitis C virus among commercial sex

workers and pregnant women in Kinshasa, Democratic Republic of Congo. *Int J Epidemiol*, **30**, 872-877.
Lavanchy, D. (2002). Public health measures in the control of viral hepatitis: a World Health Organization perspective for the next millennium. *J Gastroenterol Hepatol*, **17 Suppl**, S452-459.
Lee, C. M., Lu, S. N., Changchien, C. S., Yeh, C. T., Hsu, T. T., Tang, J. H., Wang, J. H., Lin, D. Y., Chen, C. L., Chen, W. J. (1999). Age, gender, and local geographic variations of viral etiology of hepatocellular carcinoma in a hyperendemic area for hepatitis B virus infection. *Cancer*, **86**, 1143-1150.
Lee, C. M., Lu, S. N., Hung, C. H., Tung, W. C., Wang, J. H., Tung, H. D., Chen, C. H., Hu, T. H., Changchien, C. S., Chen, W. J. (2006). Hepatitis C virus genotypes in southern Taiwan: prevalence and clinical implications. *Trans R Soc Trop Med Hyg*, **100**, 767-774.
Lefrere, J. J., Roudot-Thoraval, F., Morand-Joubert, L., Brossard, Y., Parnet-Mathieu, F., Mariotti, M., Agis, F., Rouet, G., Lerable, J., Lefevre, G., Girot, R., Loiseau, P. (1999). Prevalence of GB virus type C/hepatitis G virus RNA and of anti-E2 in individuals at high or low risk for blood-borne or sexually transmitted viruses: evidence of sexual and parenteral transmission. *Transfusion*, **39**, 83-94.
Lemon, S. M. (1997). Type A viral hepatitis: epidemiology, diagnosis, and prevention. *Clin Chem*, **43**, 1494-1499.
Leong, H. N., Tuke, P. W., Tedder, R. S., Khanom, A. B., Eglin, R. P., Atkinson, C. E., Ward, K. N., Griffiths, P. D., Clark, D. A. (2007). The prevalence of chromosomally integrated human herpesvirus 6 genomes in the blood of UK blood donors. *J Med Virol*, **79**, 45-51.
Lewis, H., Morgan, D., Ijaz, S., Boxall, E. (2006). Indigenous hepatitis E virus infection in England and Wales. *Bmj*, **332**, 1509-1510.
Li, L., Chaudhuri, A., Weintraub, L. A., Hsieh, F., Shah, S., Alexander, S., Salvatierra, O., Jr., Sarwal, M. M. (2007). Subclinical cytomegalovirus and Epstein-Barr virus viremia are associated with adverse outcomes in pediatric renal transplantation. *Pediatr Transplant*, **11**, 187-195.
Li, T. C., Saito, M., Ogura, G., Ishibashi, O., Miyamura, T., Takeda, N. (2006). Serologic evidence for hepatitis E virus infection in mongoose. *Am J Trop Med Hyg*, **74**, 932-936.
Li, T. C., Zhang, J., Shinzawa, H., Ishibashi, M., Sata, M., Mast, E. E., Kim, K., Miyamura, T., Takeda, N. (2000). Empty virus-like particle-based enzyme-linked immunosorbent assay for antibodies to hepatitis E virus. *J Med Virol*, **62**, 327-333.
Li, X. M., Ma, L., Yang, Y. B., Shi, Z. J., Zhou, S. S. (2005). Clinical characteristics of fulminant hepatitis in pregnancy. *World J Gastroenterol*, **11**, 4600-4603.
Liaw, Y. F. (2002). Chronic hepatitis B guidelines: east versus west. *Hepatology*, **35**, 979-981.
Liaw, Y. F., Chu, C. M. (2009). Hepatitis B virus infection. *Lancet*, **373**, 582-592.
Lim, Z., Cassells, R., Giles, A., Cheow, H. K., Mir, N. (2007). Diffuse large B-cell lymphoma developing following treatment of Waldenstrom's macroglobulinaemia: spontaneous resolution upon cessation of fludarabine. *Leuk Lymphoma*, **48**, 1638-1640.
Lim, Z. Y., Cook, G., Johnson, P. R., Parker, A., Zuckerman, M., Marks, D., Wiltshire, H., Mufti, G. J., Pagliuca, A. (2009). Results of a phase I/II British Society of Bone Marrow Transplantation study on PCR-based pre-emptive therapy with valganciclovir or ganciclovir for active CMV infection following alemtuzumab-based reduced intensity allogeneic stem cell transplantation. *Leuk Res*, **33**, 244-249.
Limaye, A. P., Huang, M. L., Leisenring, W., Stensland, L., Corey, L., Boeckh, M. (2001). Cytomegalovirus (CMV) DNA load in plasma for the diagnosis of CMV disease before engraftment in hematopoietic stem-cell transplant recipients. *J Infect Dis*, **183**, 377-382.
Lin, K. W., Kirchner, J. T. (2004). Hepatitis B. *Am Fam Physician*, **69**, 75-82.

Lindh, M., Andersson, A. S., Gusdal, A. (1997). Genotypes, nt 1858 variants, and geographic origin of hepatitis B virus--large-scale analysis using a new genotyping method. *J Infect Dis*, **175**, 1285-1293.

Lindh, M., Hannoun, C., Dhillon, A. P., Norkrans, G., Horal, P. (1999). Core promoter mutations and genotypes in relation to viral replication and liver damage in East Asian hepatitis B virus carriers. *J Infect Dis*, **179**, 775-782.

Lindner, S. E., Sugden, B. (2007). The plasmid replicon of Epstein-Barr virus: mechanistic insights into efficient, licensed, extrachromosomal replication in human cells. *Plasmid*, **58**, 1-12.

Liu, C. J., Chen, D. S., Chen, P. J. (2006). Epidemiology of HBV infection in Asian blood donors: emphasis on occult HBV infection and the role of NAT. *J Clin Virol*, **36 Suppl 1**, S33-44.

Liu, C. J., Kao, J. H., Chen, P. J., Lai, M. Y., Chen, D. S. (2002). Molecular epidemiology of hepatitis B viral serotypes and genotypes in taiwan. *J Biomed Sci*, **9**, 166-170.

Liu, H. D., Zheng, H., Li, M., Hu, D. S., Tang, M., Cao, Y. (2007). Upregulated expression of kappa light chain by Epstein-Barr virus encoded latent membrane protein 1 in nasopharyngeal carcinoma cells via NF-kappaB and AP-1 pathways. *Cell Signal*, **19**, 419-427.

Liu, Z., Wang, E., Taylor, W., Yu, H., Wu, T., Wan, Z., Huang, Y., Ni, Z., Sackett, D. (1990). Prevalence survey of cytomegalovirus infection in children in Chengdu. *Am J Epidemiol*, **131**, 143-150.

Lobato, C., Tavares-Neto, J., Rios-Leite, M., Trepo, C., Vitvitski, L., Parvaz, P., Zoulim, F., D'Oliveira, A., Jr., Parana, R. (2006). Intrafamilial prevalence of hepatitis B virus in Western Brazilian Amazon region: epidemiologic and biomolecular study. *J Gastroenterol Hepatol*, **21**, 863-868.

Loftis, J. M., Matthews, A. M., Hauser, P. (2006). Psychiatric and substance use disorders in individuals with hepatitis C: epidemiology and management. *Drugs*, **66**, 155-174.

Lok, A. S., McMahon, B. J. (2001). Chronic hepatitis B. *Hepatology*, **34**, 1225-1241.

Lok, A. S., McMahon, B. J. (2004). Chronic hepatitis B: update of recommendations. *Hepatology*, **39**, 857-861.

London, W. T., Evans, A. A. (1996). The epidemiology of hepatitis viruses B, C, and D. *Clin Lab Med*, **16**, 251-271.

Lu, L., Li, C., Hagedorn, C. H. (2006). Phylogenetic analysis of global hepatitis E virus sequences: genetic diversity, subtypes and zoonosis. *Rev Med Virol*, **16**, 5-36.

Lu, Z. X., Ma, X. Q., Yang, L. F., Wang, Z. L., Zeng, L., Li, Z. J., Li, X. N., Tang, M., Yi, W., Gong, J. P., Sun, L. Q., Cao, Y. (2008). DNAzymes targeted to EBV-encoded latent membrane protein-1 induce apoptosis and enhance radiosensitivity in nasopharyngeal carcinoma. *Cancer Lett*, **265**, 226-238.

Lubega, J. (2007). T-helper 1 versus T-helper 2 lymphocyte immunodysregulation is the central factor in genesis of Burkitt lymphoma: hypothesis. *Infect Agent Cancer*, **2**, 10.

Lunel, F., Cadranel, J. F., Rosenheim, M., Dorent, R., Di-Martino, V., Payan, C., Fretz, C., Ghoussoub, J. J., Bernard, B., Dumont, B., Perrin, M., Gandjbachkh, I., Huraux, J. M., Stuyver, L., Opolon, P. (2000). Hepatitis virus infections in heart transplant recipients: epidemiology, natural history, characteristics, and impact on survival. *Gastroenterology*, **119**, 1064-1074.

Mackenzie, I. R., Carrigan, D. R., Wiley, C. A. (1995). Chronic myelopathy associated with human herpesvirus-6. *Neurology*, **45**, 2015-2017.

Macsween, K. F., Crawford, D. H. (2003). Epstein-Barr virus-recent advances. *Lancet Infect Dis*, **3**, 131-140.

Magnius, L. O., Norder, H. (1995). Subtypes, genotypes and molecular epidemiology of the hepatitis B virus as reflected by sequence variability of the S-gene. *Intervirology*, **38**, 24-34.

Mahoney, F. J. (1999). Update on diagnosis, management, and prevention of hepatitis B virus infection. *Clin Microbiol Rev*, **12**, 351-366.

Mahtab, M. A., Rahman, S., Khan, M., Karim, F. (2008). Hepatitis B virus genotypes: an overview. *Hepatobiliary Pancreat Dis Int*, **7**, 457-464.

Maltezou, H. C., Kafetzis, D. A., Abisaid, D., Mantzouranis, E. C., Chan, K. W., Rolston, K. V. (2000). Viral infections in children undergoing hematopoietic stem cell transplant. *Pediatr Infect Dis J*, **19**, 307-312.

Manno, M., Camma, C., Schepis, F., Bassi, F., Gelmini, R., Giannini, F., Miselli, F., Grottola, A., Ferretti, I., Vecchi, C., De Palma, M., Villa, E. (2004). Natural history of chronic HBV carriers in northern Italy: morbidity and mortality after 30 years. *Gastroenterology*, **127**, 756-763.

Mansour-Ghanaei, F., Fallah, M. S., Shafaghi, A., Yousefi-Mashhoor, M., Ramezani, N., Farzaneh, F., Nassiri, R. (2002). Prevalence of hepatitis B and C seromarkers and abnormal liver function tests among hemophiliacs in Guilan (northern province of Iran). *Med Sci Monit*, **8**, CR797-800.

Mansuy, J. M., Legrand-Abravanel, F., Calot, J. P., Peron, J. M., Alric, L., Agudo, S., Rech, H., Destruel, F., Izopet, J. (2008). High prevalence of anti-hepatitis E virus antibodies in blood donors from South West France. *J Med Virol*, **80**, 289-293.

Marrero, C. R., Marrero, J. A. (2007). Viral hepatitis and hepatocellular carcinoma. *Arch Med Res*, **38**, 612-620.

Marrero, J. A., Fontana, R. J., Fu, S., Conjeevaram, H. S., Su, G. L., Lok, A. S. (2005). Alcohol, tobacco and obesity are synergistic risk factors for hepatocellular carcinoma. *J Hepatol*, **42**, 218-224.

Martin, A., Lemon, S. M. (2006). Hepatitis A virus: from discovery to vaccines. *Hepatology*, **43**, S164-172.

Mason, A., Sallie, R., Perrillo, R., Rayner, A., Xu, L., Dohner, D. E., Dehner, M., Naoumov, N., Gelb, L., Saha, B., O'Grady, J., Williams, R. (1996). Prevalence of herpesviridae and hepatitis B virus DNA in the liver of patients with non-A, non-B fulminant hepatic failure. *Hepatology*, **24**, 1361-1365.

Massei, F., Palla, G., Ughi, C., Macchia, P., Maggiore, G. (2001). Cholestasis as a presenting feature of acute Epstein-Barr virus infection. *Pediatr Infect Dis J*, **20**, 721-722.

Mast, E. E., Alter, M. J., Holland, P. V., Purcell, R. H. (1998). Evaluation of assays for antibody to hepatitis E virus by a serum panel. Hepatitis E Virus Antibody Serum Panel Evaluation Group. *Hepatology*, **27**, 857-861.

Mast, E. E., Alter, M. J., Margolis, H. S. (1999). Strategies to prevent and control hepatitis B and C virus infections: a global perspective. *Vaccine*, **17**, 1730-1733.

Maurmann, S., Fricke, L., Wagner, H. J., Schlenke, P., Hennig, H., Steinhoff, J., Jabs, W. J. (2003). Molecular parameters for precise diagnosis of asymptomatic Epstein-Barr virus reactivation in healthy carriers. *J Clin Microbiol*, **41**, 5419-5428.

Mautner, J., Pich, D., Nimmerjahn, F., Milosevic, S., Adhikary, D., Christoph, H., Witter, K., Bornkamm, G. W., Hammerschmidt, W., Behrends, U. (2004). Epstein-Barr virus nuclear antigen 1 evades direct immune recognition by CD4+ T helper cells. *Eur J Immunol*, **34**, 2500-2509.

Mayerat, C., Mantegani, A., Frei, P. C. (1999). Does hepatitis B virus (HBV) genotype influence the clinical outcome of HBV infection? *J Viral Hepat*, **6**, 299-304.

Mbanya, D. N., Takam, D., Ndumbe, P. M. (2003). Serological findings amongst first-time blood donors in Yaounde, Cameroon: is safe donation a reality or a myth? *Transfus Med*, **13**, 267-273.

McAleer, W. J., Buynak, E. B., Maigetter, R. Z., Wampler, D. E., Miller, W. J., Hilleman, M. R. (1984). Human hepatitis B vaccine from recombinant yeast. *Nature*, **307**, 178-180.

McCrudden, R., O'Connell, S., Farrant, T., Beaton, S., Iredale, J. P., Fine, D. (2000). Sporadic acute hepatitis E in the united kingdom: an underdiagnosed phenomenon? *Gut*, **46**, 732-733.

Mehta, K. S., Halankar, A. R., Makwana, P. D., Torane, P. P., Satija, P. S., Shah, V. B. (2001). Severe acute renal failure in malaria. *J Postgrad Med*, **47**, 24-26.

Meky, F. A., Stoszek, S. K., Abdel-Hamid, M., Selim, S., Abdel-Wahab, A., Mikhail, N., El-Kafrawy, S., El-Daly, M., Abdel-Aziz, F., Sharaf, S., Mohamed, M. K., Engle, R. E., Emerson, S. U., Purcell, R. H., Fix, A. D., Strickland, G. T. (2006). Active surveillance for acute viral hepatitis in rural villages in the Nile Delta. *Clin Infect Dis*, **42**, 628-633.

Mellor, J., Holmes, E. C., Jarvis, L. M., Yap, P. L., Simmonds, P. (1995). Investigation of the pattern of hepatitis C virus sequence diversity in different geographical regions: implications for virus classification. The International HCV Collaborative Study Group. *J Gen Virol*, **76 (Pt 10)**, 2493-2507.

Mendel, I., de Matteis, M., Bertin, C., Delaporte, B., Maguer, D., Collandre, H., Buffet-Janvresse, C. (1995). Fulminant hepatitis in neonates with human herpesvirus 6 infection. *Pediatr Infect Dis J*, **14**, 993-997.

Meng, J., Dubreuil, P., Pillot, J. (1997). A new PCR-based seroneutralization assay in cell culture for diagnosis of hepatitis E. *J Clin Microbiol*, **35**, 1373-1377.

Meng, X. J., Dea, S., Engle, R. E., Friendship, R., Lyoo, Y. S., Sirinarumitr, T., Urairong, K., Wang, D., Wong, D., Yoo, D., Zhang, Y., Purcell, R. H., Emerson, S. U. (1999). Prevalence of antibodies to the hepatitis E virus in pigs from countries where hepatitis E is common or is rare in the human population. *J Med Virol*, **59**, 297-302.

Meng, X. J., Wiseman, B., Elvinger, F., Guenette, D. K., Toth, T. E., Engle, R. E., Emerson, S. U., Purcell, R. H. (2002). Prevalence of antibodies to hepatitis E virus in veterinarians working with swine and in normal blood donors in the United States and other countries. *J Clin Microbiol*, **40**, 117-122.

Mengelle, C., Sandres-Saune, K., Pasquier, C., Rostaing, L., Mansuy, J. M., Marty, M., Da Silva, I., Attal, M., Massip, P., Izopet, J. (2003). Automated extraction and quantification of human cytomegalovirus DNA in whole blood by real-time PCR assay. *J Clin Microbiol*, **41**, 3840-3845.

Merk, J., Schmid, F. X., Fleck, M., Schwarz, S., Lehane, C., Boehm, S., Salzberger, B., Birnbaum, D. E. (2005). Fatal pulmonary failure attributable to viral pneumonia with human herpes virus 6 (HHV6) in a young immunocompetent woman. *J Intensive Care Med*, **20**, 302-306.

Metwali, R. M. (2003). Water quality of some wells in Taiz City (Yemen Republic) and its surroundings. *Folia Microbiol (Praha)*, **48**, 90-94.

Meyers, C. M., Seeff, L. B., Stehman-Breen, C. O., Hoofnagle, J. H. (2003). Hepatitis C and renal disease: an update. *Am J Kidney Dis*, **42**, 631-657.

Michalek, J., Horvath, R. (2002). High incidence of Epstein-Barr virus, cytomegalovirus and human herpesvirus 6 infections in children with cancer. *BMC Pediatr*, **2**.

Michel, M. L., Pol, S., Brechot, C., Tiollais, P. (2001). Immunotherapy of chronic hepatitis B by anti HBV vaccine: from present to future. *Vaccine*, **19**, 2395-2399.

Michos, A., Terzidis, A., Kalampoki, V., Pantelakis, K., Spanos, T., Petridou, E. T. (2008). Seroprevalence and risk factors for hepatitis A, B, and C among roma and non-roma children in a deprived area of Athens, Greece. *J Med Virol*, **80**, 791-797.

Milne, A., Allwood, G. K., Moyes, C. D., Pearce, N. E., Newell, K. (1987). A seroepidemiological study of the prevalence of hepatitis B infections in a hyperendemic New Zealand community. *Int J Epidemiol*, **16**, 84-90.

Moenkemeyer, M., Schmidt, R. E., Wedemeyer, H., Tillmann, H. L., Heiken, H. (2008). GBV-C coinfection is negatively correlated to Fas expression and Fas-mediated apoptosis in HIV-1 infected patients. *J Med Virol*, **80**, 1933-1940.

Moghaddam, A., Reinton, N., Dalgard, O. (2006). A rapid real-time PCR assay for determination of hepatitis C virus genotypes 1, 2 and 3a. *J Viral Hepat*, **13**, 222-229.

MOH-SO. (1999). Viral Hepatitis A in S. Batinah. *Community Health and Disease Surveillance Newsletter*, **VIII**, 1-6.

Mohsen, A. H. (2001). The epidemiology of hepatitis C in a UK health regional population of 5.12 million. *Gut*, **48**, 707-713.

Montgemry S, Jennifer A. Brown , Matthew Kuehnert , Theresa L. Smith , Nicholas Crall , Robert S. Lanciotti , Alexandre Macedo de Oliveira , Thomas Boo , Anthony A. Marfin , Team, a. t. W. N. V. T.-A. T. I. (2006). Transfusion-associated transmission of West Nile virus, United States 2003 through 2005. *Transfusion*, **46**, 2038 - 2046.

Moormann, A. M., Chelimo, K., Sumba, O. P., Lutzke, M. L., Ploutz-Snyder, R., Newton, D., Kazura, J., Rochford, R. (2005). Exposure to holoendemic malaria results in elevated Epstein-Barr virus loads in children. *J Infect Dis*, **191**, 1233-1238.

MOPHP. (2005). Expanded Program on Immunization: Financial Sustainability Plan/Jan 05 /Yemen. 1-55. Ministry of Public Health & Population, Primary Health Care Sector, Sana'a.

MOPHP/EPI. (2005). Annual Statistical Report, Yemen. 1-32. Ministry of Public Health & Population, Primary Health Care Sector, Sana'a.

MOPIC, Y. (2005). Prelimanary report on the 2004 population census. Yemen. . Sana'a.

MOPIC, Y. (2006). Annual Progress Report of the Poverty Reduction Strategy for 2005. 1. Ministry of Planning and international cooperation, Sana'a.

Morgan, A. J. (1992). Epstein-Barr virus vaccines. *Vaccine*, **10**, 563-571.

Morgan, M. A., el-Ghany el, S. M., Khalifa, N. A., Sherif, A., Rasslan, L. R. (2003). Prevalence of cytomegalovirus (CMV) infection among neonatal intensive care unit (NICU) and healthcare workers. *Egypt J Immunol*, **10**, 1-8.

Morris, M. C., Edmunds, W. J., Hesketh, L. M., Vyse, A. J., Miller, E., Morgan-Capner, P., Brown, D. W. (2002). Sero-epidemiological patterns of Epstein-Barr and herpes simplex (HSV-1 and HSV-2) viruses in England and Wales. *J Med Virol*, **67**, 522-527.

Moss, D. J., Suhrbier, A., Elliott, S. L. (1998). Candidate vaccines for Epstein-Barr virus. *BMJ*, **317**, 423-424.

Mossong, J., Putz, L., Patiny, S., Schneider, F. (2006). Seroepidemiology of hepatitis A and hepatitis B virus in Luxembourg. *Epidemiol Infect*, **134**, 808-813.

MPDiagnostics. (2007). HEV ELISA Instruction Manual. Singapore.

Muramatsu, H., Watanabe, N., Matsumoto, K., Ito, M., Yoshikawa, T., Kato, K., Kojima, S. (2009). Primary infection of human herpesvirus-6 in an infant who received cord blood SCT. *Bone Marrow Transplant*, **43**, 83-84.

Mushahwar, I. K. (2008). Hepatitis E virus: molecular virology, clinical features, diagnosis, transmission, epidemiology, and prevention. *J Med Virol*, **80**, 646-658.

Myint, K. S., Endy, T. P., Gibbons, R. V., Laras, K., Mammen, M. P., Jr., Sedyaningsih, E. R., Seriwatana, J., Glass, J. S., Narupiti, S., Corwin, A. L. (2006a). Evaluation of diagnostic assays for hepatitis E virus in outbreak settings. *J Clin Microbiol*, **44**, 1581-1583.

Myint, K. S., Endy, T. P., Shrestha, M. P., Shrestha, S. K., Vaughn, D. W., Innis, B. L., Gibbons, R. V., Kuschner, R. A., Seriwatana, J., Scott, R. M. (2006b). Hepatitis E antibody kinetics in Nepalese patients. *Trans R Soc Trop Med Hyg*, **100**, 938-941.

Myint, K. S., Gibbons, R. V. (2008). Hepatitis E: a neglected threat. *Trans R Soc Trop Med Hyg*, **102**, 211-212.

Nafeh, M. A., Medhat, A., Shehata, M., Mikhail, N. N., Swifee, Y., Abdel-Hamid, M., Watts, S., Fix, A. D., Strickland, G. T., Anwar, W., Sallam, I. (2000). Hepatitis C in a community in Upper Egypt: I. Cross-sectional survey. *Am J Trop Med Hyg*, **63**, 236-241.

Nagasaki, F., Niitsuma, H., Cervantes, J. G., Chiba, M., Hong, S., Ojima, T., Ueno, Y., Bondoc, E., Kobayashi, K., Ishii, M., Shimosegawa, T. (2006). Analysis of the entire nucleotide sequence of hepatitis B virus genotype B in the Philippines reveals a new subgenotype of genotype B. *J Gen Virol*, **87**, 1175-1180.

Nainan, O. V., Xia, G., Vaughan, G., Margolis, H. S. (2006). Diagnosis of hepatitis (A) virus infection: a molecular approach. *Clin Microbiol Rev*, **19**, 63-79.
Nair, S., Mason, A., Eason, J., Loss, G., Perrillo, R. P. (2002). Is obesity an independent risk factor for hepatocellular carcinoma in cirrhosis? *Hepatology*, **36**, 150-155.
Naito, H., Hayashi, S., Abe, K. (2001). Rapid and specific genotyping system for hepatitis B virus corresponding to six major genotypes by PCR using type-specific primers. *J Clin Microbiol*, **39**, 362-364.
Naoumov, N. V. (2007). Hepatitis A and E. *MEDICINE*, **35**, 35-38.
Nascimento, M. C., Mayaud, P., Sabino, E. C., Torres, K. L., Franceschi, S. (2008). Prevalence of hepatitis B and C serological markers among first-time blood donors in Brazil: a multi-center serosurvey. *J Med Virol*, **80**, 53-57.
Niederhauser, C., Mansouri Taleghani, B., Graziani, M., Stolz, M., Tinguely, C., Schneider, P. (2008). Blood donor screening: how to decrease the risk of transfusion-transmitted hepatitis B virus? *Swiss Med Wkly*, **138**, 134-141.
Nishioka, K., Watanabe, J., Furuta, S., Tanaka, E., Iino, S., Suzuki, H., Tsuji, T., Yano, M., Kuo, G., Choo, Q. L., et al. (1991). A high prevalence of antibody to the hepatitis C virus in patients with hepatocellular carcinoma in Japan. *Cancer*, **67**, 429-433.
Niu, M. T., Coleman, P. J., Alter, M. J. (1993). Multicenter study of hepatitis C virus infection in chronic hemodialysis patients and hemodialysis center staff members. *Am J Kidney Dis*, **22**, 568-573.
Njie, R., Bell, A. I., Jia, H., Croom-Carter, D., Chaganti, S., Hislop, A. D., Whittle, H., Rickinson, A. B. (2009). The effects of acute malaria on Epstein-Barr virus (EBV) load and EBV-specific T cell immunity in Gambian children. *J Infect Dis*, **199**, 31-38.
Noborg, U., Gusdal, A., Horal, P., Lindh, M. (2000). Levels of viraemia in subjects with serological markers of past or chronic hepatitis B virus infection. *Scand J Infect Dis*, **32**, 249-252.
Norder, H., Courouce, A. M., Coursaget, P., Echevarria, J. M., Lee, S. D., Mushahwar, I. K., Robertson, B. H., Locarnini, S., Magnius, L. O. (2004). Genetic diversity of hepatitis B virus strains derived worldwide: genotypes, subgenotypes, and HBsAg subtypes. *Intervirology*, **47**, 289-309.
Norder, H., Courouce, A. M., Magnius, L. O. (1994). Complete genomes, phylogenetic relatedness, and structural proteins of six strains of the hepatitis B virus, four of which represent two new genotypes. *Virology*, **198**, 489-503.
Norder, H., Hammas, B., Lee, S. D., Bile, K., Courouce, A. M., Mushahwar, I. K., Magnius, L. O. (1993). Genetic relatedness of hepatitis B viral strains of diverse geographical origin and natural variations in the primary structure of the surface antigen. *J Gen Virol*, **74 (Pt 7)**, 1341-1348.
Nousbaum, J. B. (1998). Genomic subtypes of hepatitis C virus: epidemiology, diagnosis and clinical consequences. *Bull Soc Pathol Exot*, **91**, 29-33.
Nowicki, M. J., Balistreri, W. F. (1995). The hepatitis C virus: identification, epidemiology, and clinical controversies. *J Pediatr Gastroenterol Nutr*, **20**, 248-274.
O'Brien, S. F., Xi, G., Fan, W., Yi, Q. L., Fearon, M. A., Scalia, V., Goldman, M. (2008). Epidemiology of hepatitis B in Canadian blood donors. *Transfusion*.
O'Brien, S. F., Yi, Q. L., Fan, W., Scalia, V., Kleinman, S. H., Vamvakas, E. C. (2007). Current incidence and estimated residual risk of transfusion-transmitted infections in donations made to Canadian Blood Services. *Transfusion*, **47**, 316-325.
Oesterreicher, C., Hammer, J., Koch, U., Pfeffel, F., Sunder-Plassmann, G., Petermann, D., Muller, C. (1995). HBV and HCV genome in peripheral blood mononuclear cells in patients undergoing chronic hemodialysis. *Kidney Int*, **48**, 1967-1971.
Okada, S., Sato, T., Okusaka, T., Ishii, H., Ikeda, M., Nakasuka, H., Kosakamoto, H., Yoshimori, M., Wakabayashi, K. (1998). Past exposure to hepatitis B virus as a risk factor for hepatocellular carcinoma in patients with chronic liver disease. *Br J Cancer*, **77**, 2028-2031.

Okamura, T., Hatsukawa, Y., Arai, H., Inoue, M., Kawa, K. (2000). Blood stem-cell transplantation for chronic active Epstein-Barr virus with lymphoproliferation. *Lancet*, **356**, 223-224.

Okano, M., Thiele, G. M., Davis, J. R., Grierson, H. L., Purtilo, D. T. (1988). Epstein-Barr virus and human diseases: recent advances in diagnosis. *Clin Microbiol Rev*, **1**, 300-312.

Okuno, T., Takahashi, K., Balachandra, K., Shiraki, K., Yamanishi, K., Takahashi, M., Baba, K. (1989). Seroepidemiology of human herpesvirus 6 infection in normal children and adults. *J Clin Microbiol*, **27**, 651-653.

Oliveira, N. L., Kanawaty, F. R., Costa, S. C., Hessel, G. (2002). Infection by cytomegalovirus in patients with neonatal cholestasis. *Arq Gastroenterol*, **39**, 132-136.

Olmer, M., Bouchouareb, D., Zandotti, C., de Micco, P., de Lamballerie, X. (1997). Transmission of the hepatitis C virus in an hemodialysis unit: evidence for nosocomial infection. *Clin Nephrol*, **47**, 263-270.

Olut, A. I., Ozsakarya, F., Dilek, M. (2005). Seroprevalence of hepatitis C virus infection and evaluation of serum aminotransferase levels among haemodialysis patients in Izmir, Turkey. *J Int Med Res*, **33**, 641-646.

Omer, H. H. (2007). Past and current state of blood control at blood banks, Aden Hospitals. p. 7. Cntral Laboratory of the Ministry of Public Health and Population/Aden, Aden.

Omer, R. E., Van't Veer, P., Kadaru, A. M., Kampman, E., el Khidir, I. M., Fedail, S. S., Kok, F. J. (2001a). The role of hepatitis B and hepatitis C viral infections in the incidence of hepatocellular carcinoma in Sudan. *Trans R Soc Trop Med Hyg*, **95**, 487-491.

Omer, R. E., Verhoef, L., Van't Veer, P., Idris, M. O., Kadaru, A. M., Kampman, E., Bunschoten, A., Kok, F. J. (2001b). Peanut butter intake, GSTM1 genotype and hepatocellular carcinoma: a case-control study in Sudan. *Cancer Causes Control*, **12**, 23-32.

Osoba, A. O. (2002). Hepatitis C virus genotypes in Saudi Arabia. *Saudi Med J*, **23**, 7-12.

Othman, B., Monem, F. (2001). Prevalence of antibodies to hepatitis C virus among hemodialysis patients in Damascus, Syria. *Infection*, **29**, 262-265.

Othman, S. B., Trabelsi, A., Monnet, A., Bouzgarrou, N., Grattard, F., Beyou, A., Bourlet, T., Pozzetto, B. (2004). Evaluation of a prototype HCV NS5b assay for typing strains of hepatitis C virus isolated from Tunisian haemodialysis patients. *J Virol Methods*, **119**, 177-181.

Otto, B. F., Suarnawa, I. M., Stewart, T., Nelson, C., Ruff, T. A., Widjaya, A., Maynard, J. E. (1999). At-birth immunisation against hepatitis B using a novel pre-filled immunisation device stored outside the cold chain. *Vaccine*, **18**, 498-502.

Own, A. (2005). Blood banks and its importance in Yemen *Yemen Times*, **13 , June**.

Ozaki, Y., Tajiri, H., Tanaka-Taya, K., Mushiake, S., Kimoto, A., Yamanishi, K., Okada, S. (2001). Frequent detection of the human herpesvirus 6-specific genomes in the livers of children with various liver diseases. *J Clin Microbiol*, **39**, 2173-2177.

Pai, S., O'Sullivan, B., Abdul-Jabbar, I., Peng, J., Connoly, G., Khanna, R., Thomas, R. (2007). Nasopharyngeal carcinoma-associated Epstein-Barr virus-encoded oncogene latent membrane protein 1 potentiates regulatory T-cell function. *Immunol Cell Biol*.

Palmer, S., Brown, D., Morgan, D. (2005). Early qualitative risk assessment of the emerging zoonotic potential of animal diseases. *Bmj*, **331**, 1256-1260.

Pancharoen, C., Mekmullica, J., Chinratanapisit, S., Bhattarakosol, P., Thisyakorn, U. (2001). Seroprevalence of Epstein-Barr virus antibody among children in various age groups in Bangkok, Thailand. *Asian Pac J Allergy Immunol*, **19**, 135-137.

Panda, S. K., Thakral, D., Rehman, S. (2007). Hepatitis E virus. *Rev Med Virol*, **17**, 151-180.

Pang, X., Humar, A., Preiksaitis, J. K. (2008). Concurrent genotyping and quantitation of cytomegalovirus gB genotypes in solid-organ-transplant recipients by use of a real-time PCR assay. *J Clin Microbiol*, **46**, 4004-4010.

Panhotra, B. R., Al-Bahrani, A., Ul-Hassan, Z. (2005). Epidemiology of antibody to hepatitis B core antigen screening among blood donors in Eastern Saudi Arabia. Need to replace the test by HBV DNA testing. *Saudi Med J*, **26**, 270-273.

Parkin, D. M., Bray, F., Ferlay, J., Pisani, P. (2001a). Estimating the world cancer burden: Globocan 2000. *Int J Cancer*, **94**, 153-156.

Parkin, D. M., Bray, F. I., Devesa, S. S. (2001b). Cancer burden in the year 2000. The global picture. *Eur J Cancer*, **37 Suppl 8**, S4-66.

Parkin, D. M., Garcia-Giannoli, H., Raphael, M., Martin, A., Katangole-Mbidde, E., Wabinga, H., Ziegler, J. (2000). Non-Hodgkin lymphoma in Uganda: a case-control study. *Aids*, **14**, 2929-2936.

Parkin, D. M. W., S. L. , Ferlay, J. et al.,. (1999). Cancer incidence in five continents. *Journal Annals of Oncology*, **10**, 254

Pascher, A., Klupp, J., Schulz, R. J., Dignass, A., Neuhaus, P. (2004). CMV, EBV, HHV6, and HHV7 infections after intestinal transplantation without specific antiviral prophylaxis. *Transplant Proc*, **36**, 381-382.

Pass, R. F., Burke, R. L. (2002). Development of cytomegalovirus vaccines: prospects for prevention of congenital CMV infection. *Semin Pediatr Infect Dis*, **13**, 196-204.

Payne, C. J., Ellis, T. M., Plant, S. L., Gregory, A. R., Wilcox, G. E. (1999). Sequence data suggests big liver and spleen disease virus (BLSV) is genetically related to hepatitis E virus. *Vet Microbiol*, **68**, 119-125.

Peckham, C. S., Johnson, C., Ades, A., Pearl, K., Chin, K. S. (1987). Early acquisition of cytomegalovirus infection. *Arch Dis Child*, **62**, 780-785.

Peralta, B., Biarnes, M., Ordonez, G., Porta, R., Martin, M., Mateu, E., Pina, S., Meng, X. J. (2009). Evidence of widespread infection of avian hepatitis E virus (avian HEV) in chickens from Spain. *Vet Microbiol*, **137**, 31-36.

Pereira, B. J. (1999). Hepatitis C virus infection in dialysis: a continuing problem. *Artif Organs*, **23**, 51-60.

Perez, C. M., Suarez, E., Torres, E. A., Roman, K., Colon, V. (2005). Seroprevalence of hepatitis C virus and associated risk behaviours: a population-based study in San Juan, Puerto Rico. *Int J Epidemiol*, **34**, 593-599.

Phoon, W. O., Fong, N. P., Lee, J., Leong, H. K. (1987). A study on the prevalence of hepatitis B surface antigen among Chinese adult males in Singapore. *International Journal of Epidemiology*, **16**, 74-78.

Pina, S., Buti, M., Cotrina, M., Piella, J., Girones, R. (2000). HEV identified in serum from humans with acute hepatitis and in sewage of animal origin in Spain. *J Hepatol*, **33**, 826-833.

Pol, S., Vallet-Pichard, A., Fontaine, H., Lebray, P. (2002). HCV infection and hemodialysis. *Semin Nephrol*, **22**, 331-339.

Poynard, T., Ratziu, V., Benhamou, Y., Opolon, P., Cacoub, P., Bedossa, P. (2000). Natural history of HCV infection. *Baillieres Best Pract Res Clin Gastroenterol*, **14**, 211-228.

Prassouli, A., Panagiotou, J., Vakaki, M., Giannatou, I., Atilakos, A., Garoufi, A., Papaevangelou, V. (2007). Acute acalculous cholecystitis as the initial presentation of primary Epstein-Barr virus infection. *J Pediatr Surg*, **42**, E11-13.

Prince, A. M. (1968). An antigen detected in the blood during the incubation period of serum hepatitis. *Proc Natl Acad Sci U S A*, **60**, 814-821.

Prince, A. M., Ikram, H., Hopp, T. P. (1982). Hepatitis B virus vaccine: identification of HBsAg/a and HBsAg/d but not HBsAg/y subtype antigenic determinants on a synthetic immunogenic peptide. *Proc Natl Acad Sci U S A*, **79**, 579-582.

Probst, C., Jecht, M., Gauss-Muller, V. (1999). Intrinsic signals for the assembly of hepatitis A virus particles. Role of structural proteins VP4 and 2A. *J Biol Chem*, **274**, 4527-4531.

Pujol, F. H., Ponce, J. G., Lema, M. G., Capriles, F., Devesa, M., Sirit, F., Salazar, M., Vasquez, G., Monsalve, F., Blitz-Dorfman, L. (1996). High incidence of hepatitis C virus infection in hemodialysis patients in units with high prevalence. *J Clin Microbiol*, **34**, 1633-1636.

Qirbi, N., Hall, A. J. (2001). Epidemiology of hepatitis B virus infection in the Middle East. *East Mediterr Health J*, **7**, 1034-1045.

Quintanilla-Martinez, L., Gamboa-Domnquez, A., Gamez-Ledesma, I., Angeles-Angeles, A., Mohar, A. (1995). Association of Epstein-Barr virus latent membrane protein and Hodgkin's disease in Mexico. *Mod Pathol*, **8**, 675-679.

Raimondo, G., Cacciamo, G., Saitta, C. (2005a). Hepatitis B virus and hepatitis C virus co-infection: additive players in chronic liver disease? *Ann Hepatol*, **4**, 100-106.

Raimondo, G., Pollicino, T., Squadrito, G. (2005b). What is the clinical impact of occult hepatitis B virus infection? *Lancet*, **365**, 638-640.

Raja'a, Y. A., Al-Ashwal, M. Y., Al-Ghaili, A. A. (2001). The quality of partially treated drinking-water produced in Sana'a City. *East Mediterr Health J*, **7**, 247-254.

Ramalingam, S., Leung, T., Cairns, H., Sibley, P., Smith, M., Ijaz, S., Tedder, R., Zuckerman, M. (2007). Transmission of hepatitis B virus (genotype E) in a haemodialysis unit. *J Clin Virol*, **40**, 105-109.

Ramezani, A., Eslamifar, A., Banifazl, M., Ahmadi, F., Maziar, S., Razeghi, E., Kalantar, E., Amirkhani, A., Aghakhani, A. (2007). Efficacy and long-term immunogenicity of hepatitis B vaccine in haemodialysis patients. *Int J Clin Pract*.

Ramia, S., Eid-Fares, J. (2006). Distribution of hepatitis C virus genotypes in the Middle East. *Int J Infect Dis*, **10**, 272-277.

Ramonet, M., da Silveira, T. R., Lisker-Melman, M., Ruttimann, R., Pernambuco, E., Cervantes, Y., Canero-Velasco, M. C., Falleiros-Carvalho, L. E., Azevedo, T., Targa-Ferreira, C., Clemens, S. A. (2002). A two-dose combined vaccine against hepatitis A and hepatitis B in healthy children and adolescents compared to the corresponding monovalent vaccines. *Arch Med Res*, **33**, 67-73.

Rao, M., Finny, G. J., Abraham, P., Juneja, R., Thomas, P. P., Jacob, C. K., Sridharan, G. (2000). Cytomegalovirus infection in a seroendemic renal transplant population: a longitudinal study of virological markers. *Nephron*, **84**, 367-373.

Raptopoulou, M., Papatheodoridis, G., Antoniou, A., Ketikoglou, J., Tzourmakliotis, D., Vasiliadis, T., Manolaki, N., Nikolopoulou, G., Manesis, E., Pierroutsakos, I. (2009). Epidemiology, course and disease burden of chronic hepatitis B virus infection. HEPNET study for chronic hepatitis B: a multicentre Greek study. *J Viral Hepat*, **16**, 195-202.

Rasti, N., Falk, K. I., Donati, D., Gyan, B. A., Goka, B. Q., Troye-Blomberg, M., Akanmori, B. D., Kurtzhals, J. A., Dodoo, D., Consolini, R., Linde, A., Wahlgren, M., Bejarano, M. T. (2005). Circulating epstein-barr virus in children living in malaria-endemic areas. *Scand J Immunol*, **61**, 461-465.

Re, V. L., 3rd, Frank, I., Gross, R., Dockter, J., Linnen, J. M., Giachetti, C., Tebas, P., Stern, J., Synnestvedt, M., Localio, A. R., Kostman, J. R., Strom, B. L. (2007). Prevalence, risk factors, and outcomes for occult hepatitis B virus infection among HIV-infected patients. *J Acquir Immune Defic Syndr*, **44**, 315-320.

Reda, A. A., Arafa, M. A., Youssry, A. A., Wandan, E. H., Ab de Ati, M., Daebees, H. (2003). Epidemiologic evaluation of the immunity against hepatitis B in Alexandria, Egypt. *Eur J Epidemiol*, **18**, 1007-1011.

Reddy, G. A., Dakshinamurthy, K. V., Neelaprasad, P., Gangadhar, T., Lakshmi, V. (2005). Prevalence of HBV and HCV dual infection in patients on haemodialysis. *Indian J Med Microbiol*, **23**, 41-43.

Reesink, H. W., Engelfriet, C. P., Henn, G., Mayr, W. R., Delage, G., Bernier, F., Krusius, T., Assal, A., Gallian, P., Corbi, C., Morel, P., David, B., De Micco, P.,

Murokawa, H., Yugi, H., Hino, S., Tadokoro, K., Flesland, O., Brojer, E., Letowska, M., Olim, G., Nascimento, F., Goncalves, H., Castro, L., Morais, M., Stezinar, S. L., Alvarez, M., Sauleda, S., Gonzalez, R., Niederhauser, C., Stolz, M., Allain, J. P., Owusu-Ofori, S., Eglin, R., Stramer, S., Busch, M., Strong, D. M., Epstein, J., Biswas, R. (2008). Occult hepatitis B infection in blood donors. *Vox Sang*, **94**, 153-166.

Reimer, J., Lorenzen, J., Baetz, B., Fischer, B., Rehm, J., Haasen, C., Backmund, M. (2007). Multiple viral hepatitis in injection drug users and associated risk factors. *J Gastroenterol Hepatol*, **22**, 80-85.

Remy, A. J., Verdier, E., Perney, P., Ducos, J., Blanc, P., Blanc, F., Larrey, D. (1998). Route of infection, liver histology and response to interferon in patients with chronic hepatitis caused by genotype 4 HCV infection in a Western country. *J Hepatol*, **29**, 169.

Revello, M. G., Gerna, G. (2002). Diagnosis and management of human cytomegalovirus infection in the mother, fetus, and newborn infant. *Clin Microbiol Rev*, **15**, 680-715.

Reyes, G. R. (1993). Hepatitis E virus (HEV): molecular biology and emerging epidemiology. *Prog Liver Dis*, **11**, 203-213.

Rezende, G., Roque-Afonso, A. M., Samuel, D., Gigou, M., Nicand, E., Ferre, V., Dussaix, E., Bismuth, H., Feray, C. (2003). Viral and clinical factors associated with the fulminant course of hepatitis A infection. *Hepatology*, **38**, 613-618.

Rivkina, A., Rybalov, S. (2002). Chronic hepatitis B: current and future treatment options. *Pharmacotherapy*, **22**, 721-737.

Robertson, B. H. (2001). Viral hepatitis and primates: historical and molecular analysis of human and nonhuman primate hepatitis A, B, and the GB-related viruses. *J Viral Hepat*, **8**, 233-242.

Romano, L., Paladini, S., Tagliacarne, C., Zappa, A., Zanetti, A. R. (2009). The changing face of the epidemiology of type A, B, and D viral hepatitis in Italy, following the implementation of vaccination. *Vaccine*, **27**, 3439-3442.

Ruiz, E., Almonte, M. M., Pizarro, R., Celis, J., Montalbelti, J. A., Urbano, R. (1998). Hepatitis B and C Virus Infection as Risk Factors Forhepatocarcinoma in Peru: Case and Control Study. *Rev Gastroenterol Peru*, **18**, 199-212.

Sacy, R. G., Haddad, M., Baasiri, G., Khoriati, A., Gerbaka, B. J., Abu-Elyazeed, R. (2005). Hepatitis A in Lebanon: a changing epidemiological pattern. *Am J Trop Med Hyg*, **73**, 453-456.

Sagliocca, L., Stroffolini, T., Amoroso, P., Manzillo, G., Ferrigno, L., Converti, F., Palumbo, F., Izzo, E., Mele, A. (1997). Risk factors for acute hepatitis B: a case-control study. *J Viral Hepat*, **4**, 63-66.

Sakamoto, T., Tanaka, Y., Orito, E., Co, J., Clavio, J., Sugauchi, F., Ito, K., Ozasa, A., Quino, A., Ueda, R., Sollano, J., Mizokami, M. (2006). Novel subtypes (subgenotypes) of hepatitis B virus genotypes B and C among chronic liver disease patients in the Philippines. *J Gen Virol*, **87**, 1873-1882.

Sakugawa, H., Nakasone, H., Nakayoshi, T., Orito, E., Mizokami, M., Yamashiro, T., Maeshiro, T., Kinjo, F., Saito, A., Miyagi, Y. (2002). Preponderance of hepatitis B virus genotype B contributes to a better prognosis of chronic HBV infection in Okinawa, Japan. *J Med Virol*, **67**, 484-489.

Saldanha, J. (1999). Sensitivity of PCR assays for the determination of hepatitis A virus RNA in plasma pools. A collaborative study. *Vox Sang*, **76**, 163-165.

Sallam, T. A., Cuevas, L. E., Tong, C. Y. (2003a). Increase in susceptibility of young adults to hepatitis B infection in the Republic of Yemen. *Trans R Soc Trop Med Hyg*, **97**, 302-304.

Sallam, T. A., Tong, C. Y. (2002). Two distinct types of hepatitis B virus core promoter variants in Yemeni blood donors. *J Med Virol*, **68**, 328-334.

Sallam, T. A., Tong, C. Y. (2004). African links and hepatitis B virus genotypes in the Republic of Yemen. *J Med Virol*, **73**, 23-28.

Sallam, T. A., Tong, C. Y., Cuevas, L. E., Raja'a, Y. A., Othman, A. M., Al-Kharsa, K. R. (2003b). Prevalence of blood-borne viral hepatitis in different communities in Yemen. *Epidemiol Infect*, **131**, 771-775.

Salmaso, S., Rota, M. C., Ciofi Degli Atti, M. L., Tozzi, A. E., Kreidl, P. (1999). Infant vaccination coverage in Italy: Estimates by simultaneous EPI cluster survey of regions. *Bull. World Health Org.*, **77**, 845-851.

Sanchez-Tapias, J. M., Costa, J., Mas, A., Bruguera, M., Rodes, J. (2002). Influence of hepatitis B virus genotype on the long-term outcome of chronic hepatitis B in western patients. *Gastroenterology*, **123**, 1848-1856.

Sanchez, J. L., Sjogren, M. H., Callahan, J. D., Watts, D. M., Lucas, C., Abdel-Hamid, M., Constantine, N. T., Hyams, K. C., Hinostroza, S., Figueroa-Barrios, R., Cuthie, J. C. (2000). Hepatitis C in Peru: risk factors for infection, potential iatrogenic transmission, and genotype distribution. *Am J Trop Med Hyg*, **63**, 242-248.

Sandhu, J., Preiksaitis, J. K., Campbell, P. M., Carriere, K. C., Hessel, P. A. (1999). Hepatitis C prevalence and risk factors in the northern Alberta dialysis population. *Am J Epidemiol*, **150**, 58-66.

Sarov, B., Naggan, L., Rosenzveig, R., Katz, S., Haikin, H., Sarov, I. (1982). Prevalence of antibodies to human cytomegalovirus in urban, kibbutz, and Bedouin children in southern Israel. *J Med Virol*, **10**, 195-201.

Sassi, F., Gorgi, Y., Ayed, K., Abdallah, T. B., Lamouchi, A., Maiz, H. B. (2000). Hepatitis C virus antibodies in dialysis patients in Tunisia: a single center study. *Saudi J Kidney Dis Transpl*, **11**, 218-222.

Saudy, N., Sugauchi, F., Tanaka, Y., Suzuki, S., Aal, A. A., Zaid, M. A., Agha, S., Mizokami, M. (2003). Genotypes and phylogenetic characterization of hepatitis B and delta viruses in Egypt. *J Med Virol*, **70**, 529-536.

Schildgen, O., Muller, A., Simon, A. (2008). Chronic hepatitis E and organ transplants. *N Engl J Med*, **358**, 2521-2522; author reply 2522.

Schlauder, G. G., Dawson, G. J., Erker, J. C., Kwo, P. Y., Knigge, M. F., Smalley, D. L., Rosenblatt, J. E., Desai, S. M., Mushahwar, I. K. (1998). The sequence and phylogenetic analysis of a novel hepatitis E virus isolated from a patient with acute hepatitis reported in the United States. *J Gen Virol*, **79 (Pt 3)**, 447-456.

Schlauder, G. G., Frider, B., Sookoian, S., Castano, G. C., Mushahwar, I. K. (2000). Identification of 2 novel isolates of hepatitis E virus in Argentina. *J Infect Dis*, **182**, 294-297.

Schlauder, G. G., Mushahwar, I. K. (2001). Genetic heterogeneity of hepatitis E virus. *J Med Virol*, **65**, 282-292.

Schneeberger, P. M., Keur, I., van Loon, A. M., Mortier, D., de Coul, K. O., van Haperen, A. V., Sanna, R., van Der Heijden, T. G., van Den Hoven, H., van Hamersvelt, H. W., Quint, W., van Doorn, L. J. (2000). The prevalence and incidence of hepatitis C virus infections among dialysis patients in the Netherlands: a nationwide prospective study. *J Infect Dis*, **182**, 1291-1299.

Schreiber, G. B., Busch, M. P., Kleinman, S. H., Korelitz, J. J. (1996). The risk of transfusion-transmitted viral infections. The Retrovirus Epidemiology Donor Study. *N Engl J Med*, **334**, 1685-1690.

Scott, D. A., Burans, J. P., al-Ouzeib, H. D., Arunkumar, B. K., al-Fadeel, M., Nigad, Y. R., al-Hadad, A., Elyazeed, R. R., Hyams, K. C., Woody, J. N. (1990). A seroepidemiological survey of viral hepatitis in the Yemen Arab Republic. *Trans R Soc Trop Med Hyg*, **84**, 288-291.

Scott, D. A., Constantine, N. T., Callahan, J., Burans, J. P., Olson, J. G., al-Fadeel, M., al-Ozieb, H., Arunkumer, H., Hyams, K. C. (1992). The epidemiology of hepatitis C virus antibody in Yemen. *Am J Trop Med Hyg*, **46**, 63-68.

Scotto, G., Avcella, F., Panunzio, M., Savastano, A. M., Ktena, M., Forcella, M., Fazio, V., Calzone, G., Passione, A., Procaccini, D., Demin, A., Stallone, C. (1999). Hepatitis C virus infection in four haemodialysis units of southern Italy: epidemiological report. *Eur J Epidemiol*, **15**, 217-223.

Seaberg, E. C., Belle, S. H., Beringer, K. C., Schivins, J. L., Detre, K. M. (1998). Liver transplantation in the United States from 1987-1998: updated results from the Pitt-UNOS Liver Transplant Registry. *Clin Transpl*, 17-37.

Sebelin-Wulf, K., Nguyen, T. D., Oertel, S., Papp-Vary, M., Trappe, R. U., Schulzki, A., Pezzutto, A., Riess, H., Subklewe, M. (2007). Quantitative analysis of EBV-specific CD4/CD8 T cell numbers, absolute CD4/CD8 T cell numbers and EBV load in solid organ transplant recipients with PLTD. *Transpl Immunol*, **17**, 203-210.

Seed, C. R., Kiely, P., Keller, A. J. (2005). Residual risk of transfusion transmitted human immunodeficiency virus, hepatitis B virus, hepatitis C virus and human T lymphotrophic virus. *Intern Med J*, **35**, 592-598.

Seehofer, D., Rayes, N., Tullius, S. G., Schmidt, C. A., Neumann, U. P., Radke, C., Settmacher, U., Muller, A. R., Steinmuller, T., Neuhaus, P. (2002). CMV hepatitis after liver transplantation: incidence, clinical course, and long-term follow-up. *Liver Transpl*, **8**, 1138-1146.

Serfaty, L., Chazouilleres, O., Poujol-Robert, A., Morand-Joubert, L., Dubois, C., Chretien, Y., Poupon, R. E., Petit, J. C., Poupon, R. (1997). Risk factors for cirrhosis in patients with chronic hepatitis C virus infection: results of a case-control study. *Hepatology*, **26**, 776-779.

Serin, M. S., Akkiz, H., Abayli, B., Oksuz, M., Aslan, G., Emekdas, G. (2005). Genotyping of hepatitis B virus isolated from chronic hepatitis B patients in the south of Turkey by DNA cycle-sequencing method. *Diagn Microbiol Infect Dis*, **53**, 57-60.

Shen, C. Y., Chang, W. W., Chang, S. F., Chao, M. F., Huang, E. S., Wu, C. W. (1992). Seroepidemiology of cytomegalovirus infection among children between the ages of 4 and 12 years in Taiwan. *J Med Virol*, **37**, 72-75.

Shepard, C. W., Finelli, L., Alter, M. J. (2005). Global epidemiology of hepatitis C virus infection. *Lancet Infect Dis*, **5**, 558-567.

Shin, H. R., Kim, J. Y., Ohno, T., Cao, K., Mizokami, M., Risch, H., Kim, S. R. (2000). Prevalence and risk factors of hepatitis C virus infection among Koreans in rural area of Korea. *Hepatol Res*, **17**, 185-196.

Shiratori, Y., Shiina, S., Imamura, M., Kato, N., Kanai, F., Okudaira, T., Teratani, T., Tohgo, G., Toda, N., Ohashi, M., et al. (1995). Characteristic difference of hepatocellular carcinoma between hepatitis B- and C- viral infection in Japan. *Hepatology*, **22**, 1027-1033.

Shobokshi, O. A. (2003). Hepatis C virus genotype 4. *Saudi Med J*, **24 Suppl 2**, S79-80.

Shobokshi, O. A., Serebour, F. E., Al-Drees, A. Z., Mitwalli, A. H., Qahtani, A., Skakni, L. I. (2003a). Hepatitis C virus seroprevalence rate among Saudis. *Saudi Med J*, **24 Suppl 2**, S81-86.

Shobokshi, O. A., Serebour, F. E., Skakni, L., Al-Saffy, Y. H., Ahdal, M. N. (1999). Hepatitis C genotypes and subtypes in Saudi Arabia. *J Med Virol*, **58**, 44-48.

Shobokshi, O. A., Serebour, F. E., Skakni, L. I. (2003b). Hepatitis C genotypes/subtypes among chronic hepatitis patients in Saudi Arabia. *Saudi Med J*, **24 Suppl 2**, S87-91.

Shrestha, M. P., Scott, R. M., Joshi, D. M., Mammen, M. P., Jr., Thapa, G. B., Thapa, N., Myint, K. S., Fourneau, M., Kuschner, R. A., Shrestha, S. K., David, M. P., Seriwatana, J., Vaughn, D. W., Safary, A., Endy, T. P., Innis, B. L. (2007). Safety and efficacy of a recombinant hepatitis E vaccine. *N Engl J Med*, **356**, 895-903.

Sia, I. G., Patel, R. (2000). New strategies for prevention and therapy of cytomegalovirus infection and disease in solid-organ transplant recipients. *Clin Microbiol Rev*, **13**, 83-121, table of contents.

Simmonds, P. (2001). The origin and evolution of hepatitis viruses in humans. *J Gen Virol*, **82**, 693-712.

Simmonds, P., Holmes, E. C., Cha, T. A., Chan, S. W., McOmish, F., Irvine, B., Beall, E., Yap, P. L., Kolberg, J., Urdea, M. S. (1993a). Classification of hepatitis C

virus into six major genotypes and a series of subtypes by phylogenetic analysis of the NS-5 region. *J Gen Virol*, **74 (Pt 11)**, 2391-2399.

Simmonds, P., McOmish, F., Yap, P. L., Chan, S. W., Lin, C. K., Dusheiko, G., Saeed, A. A., Holmes, E. C. (1993b). Sequence variability in the 5' non-coding region of hepatitis C virus: identification of a new virus type and restrictions on sequence diversity. *J Gen Virol*, **74 (Pt 4)**, 661-668.

Simonetti, A., Adamo, B., Tancredi, F., Triassi, M., Grandolfo, M. E. (2002). Evaluation of immunization practices in Naples-Italy. *Vaccine*, **20**, 1046-1049.

Simonsen, L., Kane, A., Lloyd, J., Zaffran, M., Kane, M. (1999). Unsafe injections in the developing world and transmission of bloodborne pathogens: a review. *Bull World Health Organ*, **77**, 789-800.

Singh, J., Bhatia, R., Gandhi, J. C., Kaswekar, A. P., Khare, S., Patel, S. B., Oza, V. B., Jain, D. C., Sokhey, J. (1998). Outbreak of viral hepatitis B in a rural community in India linked to inadequately sterilized needles and syringes. *Bull World Health Organ*, **76**, 93-98.

Singh, M., O'Hagan, D. (1999). Advances in vaccine adjuvants. *Nat Biotechnol*, **17**, 1075-1081.

Singh, N., Carrigan, D. R. (1996). Human herpesvirus-6 in transplantation: an emerging pathogen. *Ann Intern Med*, **124**, 1065-1071.

Skidmore, S. J., Pasi, K. J., Mawson, S. J., Williams, M. D., Hill, F. G. (1990). Serological evidence that dry heating of clotting factor concentrates prevents transmission of non-A, non-B hepatitis. *J Med Virol*, **30**, 50-52.

Smith, S. M., Markham, R. B., Jeang, K. T. (1996). Conditional reduction of human immunodeficiency virus type 1 replication by a gain-of-herpes simplex virus 1 thymidine kinase function. *Proc Natl Acad Sci U S A*, **93**, 7955-7960.

Sohn, Y. M., Rho, H. O., Park, M. S., Park, J. H., Choi, B. Y., Ki, M., Jang, W. I. (2000). The changing epidemiology of hepatitis A in children and the consideration of active immunization in Korea. *Yonsei Med J*, **41**, 34-39.

Soldan, K., Barbara, J. A., Heptonstall, J. (1998). Incidence of seroconversion to positivity for hepatitis C antibody in repeat blood donors in England, 1993-5. *Bmj*, **316**, 1413-1417.

Song, B. C., Kim, H., Kim, S. H., Cha, C. Y., Kook, Y. H., Kim, B. J. (2005). Comparison of full length sequences of hepatitis B virus isolates in hepatocellular carcinoma patients and asymptomatic carriers of Korea. *J Med Virol*, **75**, 13-19.

Sookoian, S. (2006). Liver disease during pregnancy: acute viral hepatitis. *Ann Hepatol*, **5**, 231-236.

Soza, A., Arrese, M., Gonzalez, R., Alvarez, M., Perez, R. M., Cortes, P., Patillo, A., Riquelme, A., Riquelme, A. (2004). Clinical and epidemiological features of 147 Chilean patients with chronic hepatitis C. *Ann Hepatol*, **3**, 146-151.

Spano, J. P., Busson, P., Atlan, D., Bourhis, J., Pignon, J. P., Esteban, C., Armand, J. P. (2003). Nasopharyngeal carcinomas: an update. *Eur J Cancer*, **39**, 2121-2135.

Speir, E., Yu, Z. X., Ferrans, V. J. (1998). Infectious agents in coronary artery disease: viral infection, aspirin, and gene expression in human coronary smooth muscle cells. *Rev Port Cardiol*, **17 Suppl 2**, II33-39.

Sprengers, D., Janssen, H. L., Kwekkeboom, J., Niesters, H. G., de Man, R. A., Schalm, S. W. (2003). In vivo immunization following virus suppression: a novel approach for inducing immune control in chronic hepatitis B. *J Viral Hepat*, **10**, 7-9.

Staras, S. A., Dollard, S. C., Radford, K. W., Flanders, W. D., Pass, R. F., Cannon, M. J. (2006). Seroprevalence of cytomegalovirus infection in the United States, 1988-1994. *Clin Infect Dis*, **43**, 1143-1151.

Steininger, C. (2007). Clinical relevance of cytomegalovirus infection in patients with disorders of the immune system. *Clin Microbiol Infect*, **13**, 953-963.

Stevens, S. J., Verschuuren, E. A., Pronk, I., van Der Bij, W., Harmsen, M. C., The, T. H., Meijer, C. J., van Den Brule, A. J., Middeldorp, J. M. (2001). Frequent monitoring of Epstein-Barr virus DNA load in unfractionated whole blood is

essential for early detection of posttransplant lymphoproliferative disease in high-risk patients. *Blood*, **97**, 1165-1171.

Stoszek, S. K., Abdel-Hamid, M., Narooz, S., El Daly, M., Saleh, D. A., Mikhail, N., Kassem, E., Hawash, Y., El Kafrawy, S., Said, A., El Batanony, M., Shebl, F. M., Sayed, M., Sharaf, S., Fix, A. D., Strickland, G. T. (2006a). Prevalence of and risk factors for hepatitis C in rural pregnant Egyptian women. *Trans R Soc Trop Med Hyg*, **100**, 102-107.

Stoszek, S. K., Abdel-Hamid, M., Saleh, D. A., El Kafrawy, S., Narooz, S., Hawash, Y., Shebl, F. M., El Daly, M., Said, A., Kassem, E., Mikhail, N., Engle, R. E., Sayed, M., Sharaf, S., Fix, A. D., Emerson, S. U., Purcell, R. H., Strickland, G. T. (2006b). High prevalence of hepatitis E antibodies in pregnant Egyptian women. *Trans R Soc Trop Med Hyg*, **100**, 95-101.

Stoszek, S. K., Engle, R. E., Abdel-Hamid, M., Mikhail, N., Abdel-Aziz, F., Medhat, A., Fix, A. D., Emerson, S. U., Purcell, R. H., Strickland, G. T. (2006c). Hepatitis E antibody seroconversion without disease in highly endemic rural Egyptian communities. *Trans R Soc Trop Med Hyg*, **100**, 89-94.

Strickland, G. T., Elhefni, H., Salman, T., Waked, I., Abdel-Hamid, M., Mikhail, N. N., Esmat, G., Fix, A. (2002). Role of hepatitis C infection in chronic liver disease in Egypt. *Am J Trop Med Hyg*, **67**, 436-442.

Stronks, K., van de Mheen, H., van den Bos, J., Mackenbach, J. P. (1997). The interrelationship between income, health and employment status. *Int J Epidemiol*, **26**, 592-600.

Stryker, W. S., Gunn, R. A., Francis, D. P. (1986). Outbreak of hepatitis B associated with acupuncture. *J Fam Pract*, **22**, 155-158.

Stuyver, L., Claeys, H., Wyseur, A., Van Arnhem, W., De Beenhouwer, H., Uytendaele, S., Beckers, J., Matthijs, D., Leroux-Roels, G., Maertens, G., De Paepe, M. (1996). Hepatitis C virus in a hemodialysis unit: molecular evidence for nosocomial transmission. *Kidney Int*, **49**, 889-895.

Stuyver, L., De Gendt, S., Van Geyt, C., Zoulim, F., Fried, M., Schinazi, R. F., Rossau, R. (2000). A new genotype of hepatitis B virus: complete genome and phylogenetic relatedness. *J Gen Virol*, **81**, 67-74.

Su, H. X., Xu, D. Z., Li, D., Zhang, J. X., Lu, J., Choi, B. C., Yan, Y. P. (2005). Heterogeneity analysis of the hepatitis B virus genome in intrauterine infection. *J Med Virol*, **77**, 180-187.

Suga, S., Suzuki, K., Ihira, M., Yoshikawa, T., Kajita, Y., Ozaki, T., Iida, K., Saito, Y., Asano, Y. (2000). Clinical characteristics of febrile convulsions during primary HHV-6 infection. *Arch Dis Child*, **82**, 62-66.

Sugauchi, F., Orito, E., Ichida, T., Kato, H., Sakugawa, H., Kakumu, S., Ishida, T., Chutaputti, A., Lai, C. L., Gish, R. G., Ueda, R., Miyakawa, Y., Mizokami, M. (2003). Epidemiologic and virologic characteristics of hepatitis B virus genotype B having the recombination with genotype C. *Gastroenterology*, **124**, 925-932.

Sugauchi, F., Orito, E., Ichida, T., Kato, H., Sakugawa, H., Kakumu, S., Ishida, T., Chutaputti, A., Lai, C. L., Ueda, R., Miyakawa, Y., Mizokami, M. (2002). Hepatitis B virus of genotype B with or without recombination with genotype C over the precore region plus the core gene. *J Virol*, **76**, 5985-5992.

Sumi, H., Yokosuka, O., Seki, N., Arai, M., Imazeki, F., Kurihara, T., Kanda, T., Fukai, K., Kato, M., Saisho, H. (2003). Influence of hepatitis B virus genotypes on the progression of chronic type B liver disease. *Hepatology*, **37**, 19-26.

Sun, C. A., Chen, H. C., Lu, C. F., You, S. L., Mau, Y. C., Ho, M. S., Lin, S. H., Chen, C. J. (1999). Transmission of hepatitis C virus in Taiwan: prevalence and risk factors based on a nationwide survey. *J Med Virol*, **59**, 290-296.

Sutanto, A., Suarnawa, I. M., Nelson, C. M., Stewart, T., Soewarso, T. I. (1999). Home delivery of heat-stable vaccines in Indonesia: outreach immunization with a prefilled, single-use injection device. *Bull World Health Organ*, **77**, 119-126.

Suzuki, K., Aikawa, T., Okamoto, H. (2002). Fulminant hepatitis E in Japan. *N Engl J Med*, **347**, 1456.

Sy, T., Jamal, M. M. (2006). Epidemiology of hepatitis C virus (HCV) infection. *Int J Med Sci*, **3**, 41-46.

Szmuness, W., Stevens, C. E., Harley, E. J., Zang, E. A., Oleszko, W. R., William, D. C., Sadovsky, R., Morrison, J. M., Kellner, A. (1980). Hepatitis B vaccine: demonstration of efficacy in a controlled clinical trial in a high-risk population in the United States. *N Engl J Med*, **303**, 833-841.

Tacke, F., Trautwein, C. (2007). Efficient recombinant hepatitis E virus vaccine: mission accomplished? *Hepatology*, **46**, 941-943.

Tagny, C. T., Mbanya, D., Tapko, J. B., Lefrere, J. J. (2008). Blood safety in Sub-Saharan Africa: a multi-factorial problem. *Transfusion*, **48**, 1256-1261.

Takahashi, K., Kang, J. H., Ohnishi, S., Hino, K., Mishiro, S. (2002a). Genetic heterogeneity of hepatitis E virus recovered from Japanese patients with acute sporadic hepatitis. *J Infect Dis*, **185**, 1342-1345.

Takahashi, K., Toyota, J., Karino, Y., Kang, J. H., Maekubo, H., Abe, N., Mishiro, S. (2004). Estimation of the mutation rate of hepatitis E virus based on a set of closely related 7.5-year-apart isolates from Sapporo, Japan. *Hepatol Res*, **29**, 212-215.

Takahashi, M., Kusakai, S., Mizuo, H., Suzuki, K., Fujimura, K., Masuko, K., Sugai, Y., Aikawa, T., Nishizawa, T., Okamoto, H. (2005). Simultaneous detection of immunoglobulin A (IgA) and IgM antibodies against hepatitis E virus (HEV) Is highly specific for diagnosis of acute HEV infection. *J Clin Microbiol*, **43**, 49-56.

Takahashi, M., Nishizawa, T., Yoshikawa, A., Sato, S., Isoda, N., Ido, K., Sugano, K., Okamoto, H. (2002b). Identification of two distinct genotypes of hepatitis E virus in a Japanese patient with acute hepatitis who had not travelled abroad. *J Gen Virol*, **83**, 1931-1940.

Takeuchi, K., Tanaka-Taya, K., Kazuyama, Y., Ito, Y. M., Hashimoto, S., Fukayama, M., Mori, S. (2006). Prevalence of Epstein-Barr virus in Japan: trends and future prediction. *Pathol Int*, **56**, 112-116.

Talaat, M., Kandeel, A., El-Shoubary, W., Bodenschatz, C., Khairy, I., Oun, S., Mahoney, F. J. (2003). Occupational exposure to needlestick injuries and hepatitis B vaccination coverage among health care workers in Egypt. *Am J Infect Control*, **31**, 469-474.

Tamada, Y., Yano, K., Yatsuhashi, H., Inoue, O., Mawatari, F., Ishibashi, H. (2004). Consumption of wild boar linked to cases of hepatitis E. *J Hepatol*, **40**, 869-870.

Tameda, Y., Kosaka, Y., Tagawa, S., Takase, K., Sawada, N., Nakao, H., Tsuda, F., Tanaka, T., Okamoto, H., Miyakawa, Y., Mayumi, M. (1996). Infection with GB virus C (GBV-C) in patients with fulminant hepatitis. *J Hepatol*, **25**, 842-847.

Tanaka, E., Matsumoto, A., Takeda, N., Li, T. C., Umemura, T., Yoshizawa, K., Miyakawa, Y., Miyamura, T., Kiyosawa, K. (2005). Age-specific antibody to hepatitis E virus has remained constant during the past 20 years in Japan. *J Viral Hepat*, **12**, 439-442.

Tanaka, H., Nishimura, T., Hakui, M., Sugimoto, H., Tanaka-Taya, K., Yamanishi, K. (2002). Human herpesvirus 6-associated hemophagocytic syndrome in a healthy adult. *Emerg Infect Dis*, **8**, 87-88.

Tarhan, N. C., Firat, A., Otken, A., Agildere, A. M., Demirceken, F. (2003). Central pontine myelinolysis secondary to cytomegalovirus hepatitis in a 10-month-old child. *Pediatr Radiol*, **33**, 44-46.

Tei, S., Kitajima, N., Takahashi, K., Mishiro, S. (2003). Zoonotic transmission of hepatitis E virus from deer to human beings. *Lancet*, **362**, 371-373.

Ter Borg, M. J., Leemans, W. F., de Man, R. A., Janssen, H. L. (2008). Exacerbation of chronic hepatitis B infection after delivery. *J Viral Hepat*, **15**, 37-41.

Thakur, V., Sarin, S. K., Rehman, S., Guptan, R. C., Kazim, S. N., Kumar, S. (2005). Role of HBV genotype in predicting response to lamivudine therapy in patients with chronic hepatitis B. *Indian J Gastroenterol*, **24**, 12-15.

Thoelen, S., Van Damme, P., Leentvaar-Kuypers, A., Leroux-Roels, G., Bruguera, M., Frei, P. C., Bakasenas, V., Safary, A. (1999). The first combined vaccine against hepatitis A and B: an overview. *Vaccine*, **17**, 1657-1662.

Thomas, D. L., Mahley, R. W., Badur, S., Palaoglu, K. E., Quinn, T. C. (1993). Epidemiology of hepatitis E virus infection in Turkey. *Lancet*, **341**, 1561-1562.

Thomson, S. A., Sherritt, M. A., Medveczky, J., Elliott, S. L., Moss, D. J., Fernando, G. J., Brown, L. E., Suhrbier, A. (1998). Delivery of multiple CD8 cytotoxic T cell epitopes by DNA vaccination. *J Immunol*, **160**, 1717-1723.

Thorgeirsson, S. S., Lee, J. S., Grisham, J. W. (2006). Functional genomics of hepatocellular carcinoma. *Hepatology*, **43**, S145-150.

Thorpe, L. E., Ouellet, L. J., Hershow, R., Bailey, S. L., Williams, I. T., Williamson, J., Monterroso, E. R., Garfein, R. S. (2002). Risk of hepatitis C virus infection among young adult injection drug users who share injection equipment. *Am J Epidemiol*, **155**, 645-653.

Tibbs, C. J. (1997). Tropical aspects of viral hepatitis. Hepatitis C. *Trans R Soc Trop Med Hyg*, **91**, 121-124.

Tiwari, S. C., Kumar, A. (2005). Development & standardization of a scale to measure socio-economic status in urban & rural communities in India. *Indian J Med Res*, **122**, 309-314.

Tokita, H., Harada, H., Gotanda, Y., Takahashi, M., Nishizawa, T., Okamoto, H. (2003). Molecular and serological characterization of sporadic acute hepatitis E in a Japanese patient infected with a genotype III hepatitis E virus in 1993. *J Gen Virol*, **84**, 421-427.

Tong, C. Y., Bakran, A., Williams, H., Cuevas, L. E., Peiris, J. S., Hart, C. A. (2001). Association of tumour necrosis factor alpha and interleukin 6 levels with cytomegalovirus DNA detection and disease after renal transplantation. *J Med Virol*, **64**, 29-34.

Tong, C. Y., Cuevas, L., Williams, H., Bakran, A. (1998). Use of laboratory assays to predict cytomegalovirus disease in renal transplant recipients. *J Clin Microbiol*, **36**, 2681-2685.

Toosi, M. N., Larti, F., Seifei, S., Abdollahi, A. (2008). Prevalence of viral hepatitis in hemodialysis patients in Tehran, Iran. *J Gastrointestin Liver Dis*, **17**, 233-234.

Topuzoglu, A., Ozaydin, G. A. N., Cali, S., Cebeci, D., Kalaca, S., Harmanci, H. (2005). Assessment of sociodemographic factors and socio-economic status affecting the coverage of compulsory and private immunization services in Istanbul, Turkey. *Public Health*, **119**, 862-869.

Toukan, A. U. (1996). Hepatitis B in the Middle East: aspects of epidemiology and liver disease after infection. *Gut*, **38 Suppl 2**, S2-4.

Toukan, A. U., Sharaiha, Z. K., Abu-el-Rub, O. A., Hmoud, M. K., Dahbour, S. S., Abu-Hassan, H., Yacoub, S. M., Hadler, S. C., Margolis, H. S., Coleman, P. J., et al. (1990). The epidemiology of hepatitis B virus among family members in the Middle East. *Am J Epidemiol*, **132**, 220-232.

Trautwein, C., Tacke, F., Amini-Bavil-Olyaee, S. (2009). Hepatitis E Vaccine: Current Status and Future Prospects. *Future Virology*, **4**, 143-154.

Trepo, C., Pradat, P. (1999). Hepatitis C virus infection in Western Europe. *J Hepatol*, **31 Suppl 1**, 80-83.

Trevisani, F., D'Intino, P. E., Morselli-Labate, A. M., Mazzella, G., Accogli, E., Caraceni, P., Domenicali, M., De Notariis, S., Roda, E., Bernardi, M. (2001). Serum alpha-fetoprotein for diagnosis of hepatocellular carcinoma in patients with chronic liver disease: influence of HBsAg and anti-HCV status. *J Hepatol*, **34**, 570-575.

Tsaparas, Y. F., Brigden, M. L., Mathias, R., Thomas, E., Raboud, J., Doyle, P. W. (2000). Proportion positive for Epstein-Barr virus, cytomegalovirus, human herpesvirus 6, Toxoplasma, and human immunodeficiency virus types 1 and 2 in heterophile-negative patients with an absolute lymphocytosis or an instrument-generated atypical lymphocyte flag. *Arch Pathol Lab Med*, **124**, 1324-1330.

Tsarev, S. A., Binn, L. N., Gomatos, P. J., Arthur, R. R., Monier, M. K., van Cuyck-Gandre, H., Longer, C. F., Innis, B. L. (1999). Phylogenetic analysis of hepatitis E virus isolates from Egypt. *J Med Virol*, **57**, 68-74.

Tsarev, S. A., Tsareva, T. S., Emerson, S. U., Govindarajan, S., Shapiro, M., Gerin, J. L., Purcell, R. H. (1994). Successful passive and active immunization of cynomolgus monkeys against hepatitis E. *Proc Natl Acad Sci U S A*, **91**, 10198-10202.

Tsarev, S. A., Tsareva, T. S., Emerson, S. U., Govindarajan, S., Shapiro, M., Gerin, J. L., Purcell, R. H. (1997). Recombinant vaccine against hepatitis E: dose response and protection against heterologous challenge. *Vaccine*, **15**, 1834-1838.

Tsega, E., Nordenfelt, E., Hansson, B. G. (1995). Hepatitis C virus infection and chronic liver disease in Ethiopia where hepatitis B infection is hyperendemic. *Trans R Soc Trop Med Hyg*, **89**, 171-174.

Tufenkeji, H. (2000). Hepatitis A shifting epidemiology in the Middle East and Africa. *Vaccine*, **18 Suppl 1**, S65-67.

Tzonou, A., Trichopoulos, D., Kaklamani, E., Zavitsanos, X., Koumantaki, Y., Hsieh, C. C. (1991). Epidemiologic assessment of interactions of hepatitis-C virus with seromarkers of hepatitis-B and -D viruses, cirrhosis and tobacco smoking in hepatocellular carcinoma. *Int J Cancer*, **49**, 377-380.

van Cuyck, H., Fan, J., Robertson, D. L., Roques, P. (2005). Evidence of recombination between divergent hepatitis E viruses. *J Virol*, **79**, 9306-9314.

Van Damme, P., Cramm, M., Safary, A., Vandepapeliere, P., Meheus, A. (1992). Heat stability of a recombinant DNA hepatitis B vaccine. *Vaccine*, **10**, 366-367.

Van der Bij, A. K., Coutinho, R. A., Van der Poel, C. L. (2006). Surveillance of risk profiles among new and repeat blood donors with transfusion-transmissible infections from 1995 through 2003 in the Netherlands. *Transfusion*, **46**, 1729-1736.

van der Sande, M. A., Waight, P., Mendy, M., Rayco-Solon, P., Hutt, P., Fulford, T., Doherty, C., McConkey, S. J., Jeffries, D., Hall, A. J., Whittle, H. C. (2006). Long-term protection against carriage of hepatitis B virus after infant vaccination. *J Infect Dis*, **193**, 1528-1535.

Varghese, A. M., Sayala, H., Evans, P. A., O'Connor, S. J., Patmore, R., Hillmen, P., Owen, R. G. (2008). Development of EBV-associated diffuse large B-cell lymphoma in Waldenstrom macroglobulinemia and mantle cell lymphoma. *Leuk Lymphoma*, **49**, 1618-1619.

Velati, C., Romano, L., Fomiatti, L., Baruffi, L., Zanetti, A. R. (2008). Impact of nucleic acid testing for hepatitis B virus, hepatitis C virus, and human immunodeficiency virus on the safety of blood supply in Italy: a 6-year survey. *Transfusion*.

Vellinga, A., Depoorter, A. M., Van Damme, P. (2002). Vaccination coverage estimates by EPI cluster sampling survey of children (18-24 months) in Flanders, Belgium. *Acta Paediatr*, **91**, 599-603.

Verkade, M. A., van Druningen, C. J., Op de Hoek, C. T., Weimar, W., Betjes, M. G. (2007). Decreased antigen-specific T-cell proliferation by moDC among hepatitis B vaccine non-responders on haemodialysis. *Clin Exp Med*, **7**, 65-71.

Vogt, T. M., Goldstein, S. T., Kuartei, S. (2006). Endemic hepatitis B virus infection and chronic liver disease mortality in the Republic of Palau, 1990-2002. *Trans R Soc Trop Med Hyg*, **100**, 1130-1134.

Waar, K., Herremans, M. M., Vennema, H., Koopmans, M. P., Benne, C. A. (2005). Hepatitis E is a cause of unexplained hepatitis in The Netherlands. *J Clin Virol*, **33**, 145-149.

Wagner, H. J., Fischer, L., Jabs, W. J., Holbe, M., Pethig, K., Bucsky, P. (2002). Longitudinal analysis of Epstein-Barr viral load in plasma and peripheral blood mononuclear cells of transplanted patients by real-time polymerase chain reaction. *Transplantation*, **74**, 656-664.

Wai, C. T., Chu, C. J., Hussain, M., Lok, A. S. (2002). HBV genotype B is associated with better response to interferon therapy in HBeAg(+) chronic hepatitis than genotype C. *Hepatology*, **36**, 1425-1430.

Waldhoer, T., Haidinger, G., Vutuc, C., Haschke, F., Plank, R. (1997). The impact of sociodemographic variables on immunization coverage of children. *Eur J Epidemiol*, **13**, 145-149.

Wang, J., Loveland, A. N., Kattenhorn, L. M., Ploegh, H. L., Gibson, W. (2006). High-molecular-weight protein (pUL48) of human cytomegalovirus is a competent deubiquitinating protease: mutant viruses altered in its active-site cysteine or histidine are viable. *J Virol*, **80**, 6003-6012.

Wang, L., Li, J., Chen, H., Li, F., Armstrong, G. L., Nelson, C., Ze, W., Shapiro, C. N. (2007). Hepatitis B vaccination of newborn infants in rural China: evaluation of a village-based, out-of-cold-chain delivery strategy. *Bull World Health Organ*, **85**, 688-694.

Wang, L., Zhuang, H. (2004). Hepatitis E: an overview and recent advances in vaccine research. *World J Gastroenterol*, **10**, 2157-2162.

Wang, Q., Chen, H., Cong, X., Fei, R., Gao, Y., Sun, J., Wei, L., Wang, Y. (2002). Transfusion of activated immune cells induced fluctuations of HCV titer without mutations in hepatitis C virus core and E2 region CTL epitopes. *Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi*, **16**, 329-332.

Wang, Y., Ling, R., Erker, J. C., Zhang, H., Li, H., Desai, S., Mushahwar, I. K., Harrison, T. J. (1999). A divergent genotype of hepatitis E virus in Chinese patients with acute hepatitis. *J Gen Virol*, **80 (Pt 1)**, 169-177.

Wansbrough-Jones, M. H., Frimpong, E., Cant, B., Harris, K., Evans, M. R., Teo, C. G. (1998). Prevalence and genotype of hepatitis C virus infection in pregnant women and blood donors in Ghana. *Trans R Soc Trop Med Hyg*, **92**, 496-499.

Ward, K. N. (1998). Infections due to the herpesvirus group in immunocompromised patients. *Curr Opin Infect Dis*, **11**, 425-430.

Ward, K. N. (2009). Unique route for human herpesvirus transmission: HHV-6 and chromosomal integration. *Future Virol.*, **4**, 221-224.

Ward, K. N., Thiruchelvam, A. D., Couto-Parada, X. (2005). Unexpected occasional persistence of high levels of HHV-6 DNA in sera: detection of variants A and B. *J Med Virol*, **76**, 563-570.

Wasley, A., Fiore, A., Bell, B. P. (2006). Hepatitis A in the era of vaccination. *Epidemiol Rev*, **28**, 101-111.

Webster, G., Barnes, E., Dusheiko, G., Franklin, I. (2001). Protecting travellers from hepatitis A. *Bmj*, **322**, 1194-1195.

Weild, A. R., Gill, O. N., Bennett, D., Livingstone, S. J., Parry, J. V., Curran, L. (2000). Prevalence of HIV, hepatitis B, and hepatitis C antibodies in prisoners in England and Wales: a national survey. *Commun Dis Public Health*, **3**, 121-126.

Weinbaum, C. M., Sabin, K. M., Santibanez, S. S. (2005). Hepatitis B, hepatitis C, and HIV in correctional populations: a review of epidemiology and prevention. *Aids*, **19 Suppl 3**, S41-46.

Weinreb, M., Day, P. J., Niggli, F., Powell, J. E., Raafat, F., Hesseling, P. B., Schneider, J. W., Hartley, P. S., Tzortzatou-Stathopoulou, F., Khalek, E. R., Mangoud, A., El-Safy, U. R., Madanat, F., Al Sheyyab, M., Mpofu, C., Revesz, T., Rafii, R., Tiedemann, K., Waters, K. D., Barrantes, J. C., Nyongo, A., Riyat, M. S., Mann, J. R. (1996). The role of Epstein-Barr virus in Hodgkin's disease from different geographical areas. *Arch Dis Child*, **74**, 27-31.

Weiss, L. M. (2000). Epstein-Barr virus and Hodgkin's disease. *Curr Oncol Rep*, **2**, 199-204.

West, D. J., Calandra, G. B. (1996). Vaccine induced immunologic memory for hepatitis B surface antigen: implications for policy on booster vaccination. *Vaccine*, **14**, 1019-1027.

Westall, G. P., Michaelides, A., Williams, T. J., Snell, G. I., Kotsimbos, T. C. (2003). Bronchiolitis obliterans syndrome and early human cytomegalovirus DNAaemia dynamics after lung transplantation. *Transplantation*, **75**, 2064-2068.

Westland, C., Delaney, W. t., Yang, H., Chen, S. S., Marcellin, P., Hadziyannis, S., Gish, R., Fry, J., Brosgart, C., Gibbs, C., Miller, M., Xiong, S. (2003). Hepatitis B virus genotypes and virologic response in 694 patients in phase III studies of adefovir dipivoxil1. *Gastroenterology*, **125**, 107-116.

White, P. D., Thomas, J. M., Kangro, H. O., Bruce-Jones, W. D., Amess, J., Crawford, D. H., Grover, S. A., Clare, A. W. (2001). Predictions and associations of fatigue syndromes and mood disorders that occur after infectious mononucleosis. *Lancet*, **358**, 1946-1954.

Whitley, R. J. (1994). Herpes simplex virus infections of women and their offspring: implications for a developed society. *Proc Natl Acad Sci U S A*, **91**, 2441-2447.

Whittle, H., Inskip, H., Bradley, A. K., McLaughlan, K., Shenton, F., Lamb, W., Eccles, J., Baker, B. A., Hall, A. J. (1990). The pattern of childhood hepatitis B infection in two Gambian villages. *J Infect Dis*, **161**, 1112-1115.

Whittle, H. C., Maine, N., Pilkington, J., Mendy, M., Fortuin, M., Bunn, J., Allison, L., Howard, C., Hall, A. (1995). Long-term efficacy of continuing hepatitis B vaccination in infancy in two Gambian villages. *Lancet*, **345**, 1089-1092.

WHO-UNICEF-UNFPA. (1999). *Joint statement on the use of auto-disable syringes in immunization services. In: Vaccine and Biological. 1-4. World health Organization, Geneva.*

WHO. (1993). Expanded Programme on Immunisation; Programme report for the year 1992., p. 85. World Health Organization Geneva.

WHO. (1999a). Global surveillance and control of hepatitis C. Report of a WHO Consultation organized in collaboration with the Viral Hepatitis Prevention Board, Antwerp, Belgium. *J Viral Hepat*, **6**, 35-47.

WHO. (1999b). Reduction of maternal mortality: a joint WHO/UNFPA/UNICEF/Worl Bank Statement., Geneva, Switzerland.

WHO. (2000a). Hepatitis A In: WHO/CDS/CSR/EDC/2000.7, p. 41.

WHO. (2000b). Hepatitis A vaccine: WHO position paper. *Weekly Epidemiological Record*, **5**, 38-44.

WHO. (2001a). Hepatitis E. 1, p. 30. WHO/CDS/CSR/EDC/2001.12.

WHO. (2001b). Introducing hepatitis B vaccine into national immunization services: Hepatitis B immunization. In: World Health Publications, WHO/V&B/01.31.

WHO. (2001c). Introduction of hepatitis B vaccine into childhood immunization services: Management guidelines, including information for health workers and parents. Department of Vaccines and Biologicals, Geneva

WHO. (2002a). Biennial report 2001. p. 201. World Health Organization, Department of Vaccines and Biologicals, Geneva,Switzerland.

WHO. (2002b). Hepatitis B. In: WHO/CDS/CSR/LYO/2002.2:Hepatitis B.

WHO. (2003a). Global Progress towards universal childhood hepatitis B vaccination, 2003. *Weekly Epidemiological Record*, **42**, 366-370.

WHO. (2003b). Hepatitis C Vius. In: Who.int/csr/disease/hepatitis/whocds.

WHO. (2004a). Global Database on Blood Safety Report 2001–2002. p. 30. WHO Blood Transfusion Safety unit, Geneva 27, Switzerland.

WHO. (2004b). Hepatitis B surface Antigen Assays: Operational Charectaristics (Phase I) Report 2. *Report*, 32.

WHO. (2004c). Hepatitis B vaccines: WHO position paper. *Weekly Epidemiological Record*, **28**, 255-263.

WHO. (2004d). Hepatitis E fact sheet *Wkly Epidemiol Rec*, **79**, 314-316.

WHO. (2005). International Classification of Diseases. In: WHO / Classification of Diseases, p. 63.

WHO. (2008a). Immunization Profile - the Republic of Yemen. In: http://www.who.int/vaccines/globalsummary/immunization/countryprofileresult.cfm?C='yem'. World health Organization.

WHO. (2008b). Universal Access to Safe Blood Transfusion. In: /08.03 (ed. by WHO/EHT), p. 28. WHO. Blood Transfusion Safety Unit, Geneva, Switzerland.

WHO/UNICEF. (2006). Review of National Immunization Coverage, Yemen.. 1-10.

Williams, H., Crawford, D. H. (2006). Epstein-Barr virus: the impact of scientific advances on clinical practice. *Blood*, **107**, 862-869.

Williams, S. L., Hartline, C. B., Kushner, N. L., Harden, E. A., Bidanset, D. J., Drach, J. C., Townsend, L. B., Underwood, M. R., Biron, K. K., Kern, E. R. (2003). In vitro activities of benzimidazole D- and L-ribonucleosides against herpesviruses. *Antimicrob Agents Chemother*, **47**, 2186-2192.

Wiwanitkit, V. (2005). Hepatitis G virus RNA positivity among the voluntary blood donors: a summary. *Ann Hepatol*, **4**, 43-46.

WorldBank. (2006). Yemen, Rep. Data Profile (ed. by http://devdata.worldbank.org/external/CPProfile.asp?SelectedCountry=YEM&CCODE=YEM&CNAME=Yemen%2C+Rep.&PTYPE=CP).

Worldbank. (2007). Yemen Economic Update. In: FALL 2007. 1-35. The world bank.

Wu, G. C., Zhou, W. P., Zhao, Y. R., Guo, S. H., Wang, Z. Y., Zou, S. B., Zhang, Q. H., Ren, H., Huang, A. L., Zhang, D. F. (2003). The natural history of chronic hepatitis B: a retrospective study. *Hepatobiliary Pancreat Dis Int*, **2**, 566-570.

Wu, T., Zhang, J., Su, Z. J., Liu, J. J., Wu, X. L., Lin, C. X., Ou, S. H., Yan, Q., Shih, J. W., Xia, N. S. (2008). Specific cellular immune response in hepatitis E patients. *Intervirology*, **51**, 322-327.

Xiang, J., Wunschmann, S., Schmidt, W., Shao, J., Stapleton, J. T. (2000). Full-length GB virus C (Hepatitis G virus) RNA transcripts are infectious in primary CD4-positive T cells. *J Virol*, **74**, 9125-9133.

Yaghi, C., Sharara, A. I., Rassam, P., Moucari, R., Honein, K., BouJaoude, J., Slim, R., Noun, R., Abdul-Baki, H., Khalifeh, M., Ramia, S., Sayegh, R. (2006). Hepatocellular carcinoma in Lebanon: Etiology and prognostic factors associated with short-term survival. *World J Gastroenterol*, **12**, 3575-3580.

Yang, H. I., Lu, S. N., Liaw, Y. F., You, S. L., Sun, C. A., Wang, L. Y., Hsiao, C. K., Chen, P. J., Chen, D. S., Chen, C. J. (2002). Hepatitis B e antigen and the risk of hepatocellular carcinoma. *N Engl J Med*, **347**, 168-174.

Yim, H. J., Lok, A. S. (2006). Natural history of chronic hepatitis B virus infection: what we knew in 1981 and what we know in 2005. *Hepatology*, **43**, S173-181.

Yoshikawa, T., Asano, Y. (2000). Central nervous system complications in human herpesvirus-6 infection. *Brain Dev*, **22**, 307-314.

Yoshizaki, T., Endo, K., Ren, Q., Wakisaka, N., Murono, S., Kondo, S., Sato, H., Furukawa, M. (2007). Oncogenic role of Epstein-Barr virus-encoded small RNAs (EBERs) in nasopharyngeal carcinoma. *Auris Nasus Larynx*, **34**, 73-78.

Yotsuyanagi, H., Yasuda, K., Iino, S., Moriya, K., Shintani, Y., Fujie, H., Tsutsumi, T., Kimura, S., Koike, K. (1998). Persistent viremia after recovery from self-limited acute hepatitis B. *Hepatology*, **27**, 1377-1382.

Young, K. H., Zhang, D., Malik, J. T., Williams, E. C. (2008). Fulminant EBV-driven CD8 T-cell Lymphoproliferative Disorder Following Primary Acute EBV Infection: A Unique Spectrum of T-Cell Malignancy. *Int J Clin Exp Pathol*, **1**, 185-197.

Young, L. S., Rickinson, A. B. (2004). Epstein-Barr virus: 40 years on. *Nat Rev Cancer*, **4**, 757-768.

Yousef Khalidah, A. M. (2003). Prevalence of hepatitis B among women in the perinatal period in Aden Governorate (2000-2002). *Yemeni Journal for Medical and Health Research*, **2**, 159-169.

Yu, M. L., Chuang, W. L., Dai, C. Y., Lu, S. N., Wang, J. H., Huang, J. F., Chen, S. C., Lin, Z. Y., Hsieh, M. Y., Tsai, J. F., Wang, L. Y., Chang, W. Y. (2001). The serological and molecular epidemiology of GB virus C/hepatitis G virus infection in a hepatitis C and B endemic area. *J Infect*, **42**, 61-66.

Yu, M. W., Chang, H. C., Chen, P. J., Liu, C. J., Liaw, Y. F., Lin, S. M., Lee, S. D., Lin, S. C., Lin, C. L., Chen, C. J. (2002). Increased risk for hepatitis B-related liver cirrhosis in relatives of patients with hepatocellular carcinoma in northern Taiwan. *Int J Epidemiol*, **31**, 1008-1015.

Yu, M. W., Chang, H. C., Liaw, Y. F., Lin, S. M., Lee, S. D., Liu, C. J., Chen, P. J., Hsiao, T. J., Lee, P. H., Chen, C. J. (2000). Familial risk of hepatocellular

carcinoma among chronic hepatitis B carriers and their relatives. *J Natl Cancer Inst*, **92**, 1159-1164.

Yuen, M. F., Fung, S. K., Tanaka, Y., Kato, T., Mizokami, M., Yuen, J. C., Wong, D. K., Yuan, H. J., Sum, S. M., Chan, A. O., Wong, B. C., Lai, C. L. (2004). Longitudinal study of hepatitis activity and viral replication before and after HBeAg seroconversion in chronic hepatitis B patients infected with genotypes B and C. *J Clin Microbiol*, **42**, 5036-5040.

Yuen, M. F., Sablon, E., Yuan, H. J., Wong, D. K., Hui, C. K., Wong, B. C., Chan, A. O., Lai, C. L. (2003). Significance of hepatitis B genotype in acute exacerbation, HBeAg seroconversion, cirrhosis-related complications, and hepatocellular carcinoma. *Hepatology*, **37**, 562-567.

Yuge, A., Kinoshita, E., Moriuchi, M., Ohno, Y., Haga, H., Moriuchi, H. (2004). Persistent hepatitis associated with chronic active Epstein-Barr virus infection. *Pediatr Infect Dis J*, **23**, 74-76.

Yun, H., Kim, S., Lee, H., Byun, K. S., Kwon, S. Y., Yim, H. J., Lim, Y. S., Jeong, S. H., Jee, Y. (2008). Genetic analysis of HAV strains isolated from patients with acute hepatitis in Korea, 2005-2006. *J Med Virol*, **80**, 777-784.

Yung, L., Linch, D. (2003). Hodgkin's lymphoma. *Lancet*, **361**, 943-951.

Yusuf, H., Daniels, D., Mast, E. E., Coronado, V. (2001). Hepatitis B vaccination coverage among United States children. *Pediatr Infect Dis J*, **20**, S30-33.

Zadeh, Z. R., Makhdumi, K., Lak, S. S. (2006). Epstein-Barr viral infection in renal allograft recipients: a single center experience. *Saudi J Kidney Dis Transpl*, **17**, 351-354.

Zago, A. M., Machado, T. F., Cazarim, F. L., Miranda, A. E. (2007). Prevalence and risk factors for chronic hepatitis B in HIV patients attended at a sexually-transmitted disease clinic in Vitoria, Brazil. *Braz J Infect Dis*, **11**, 475-478.

Zanetti, A. R. (2001). Update on hepatitis B vaccination in Italy 10 years after its implementation. *Vaccine*, **19**, 2380-2383.

Zanetti, A. R., Schlauder, G. G., Romano, L., Tanzi, E., Fabris, P., Dawson, G. J., Mushahwar, I. K. (1999). Identification of a novel variant of hepatitis E virus in Italy. *J Med Virol*, **57**, 356-360.

Zarski, J. P. (2006). [Epidemiology of chronic hepatitis B]. *Presse Med*, **35**, 304-307.

Zarski, J. P., Marcellin, P., Leroy, V., Trepo, C., Samuel, D., Ganne-Carrie, N., Barange, K., Canva, V., Doffoel, M., Cales, P. (2006). Characteristics of patients with chronic hepatitis B in France: predominant frequency of HBe antigen negative cases. *J Hepatol*, **45**, 355-360.

Zein, N. N. (2000). Clinical significance of hepatitis C virus genotypes. *Clin Microbiol Rev*, **13**, 223-235.

Zeng, G., Wang, Z., Wen, S., Jiang, J., Wang, L., Cheng, J., Tan, D., Xiao, F., Ma, S., Li, W., Luo, K., Naoumov, N. V., Hou, J. (2005). Geographic distribution, virologic and clinical characteristics of hepatitis B virus genotypes in China. *J Viral Hepat*, **12**, 609-617.

Zeng, Z., Guan, L., An, P., Sun, S., O'Brien, S. J., Winkler, C. A. (2008). A population-based study to investigate host genetic factors associated with hepatitis B infection and pathogenesis in the Chinese population. *BMC Infect Dis*, **8**, 1.

Zerr, D. M., Meier, A. S., Selke, S. S., Frenkel, L. M., Huang, M. L., Wald, A., Rhoads, M. P., Nguy, L., Bornemann, R., Morrow, R. A., Corey, L. (2005). A population-based study of primary human herpesvirus 6 infection. *N Engl J Med*, **352**, 768-776.

Zewdu, W. (1994). Acute renal failure in Addis Abeba, Ethiopia: a prospective study of 136 patients. *Ethiop Med J*, **32**, 79-87.

Zhang, J., Liu, C. B., Li, R. C., Li, Y. M., Zheng, Y. J., Li, Y. P., Luo, D., Pan, B. B., Nong, Y., Ge, S. X., Xiong, J. H., Shih, J. W., Ng, M. H., Xia, N. S. (2009). Randomized-controlled phase II clinical trial of a bacterially expressed recombinant hepatitis E vaccine. *Vaccine*, **27**, 1869-1874.

Zhang, J. Y., Wang, X., Han, S. G., Zhuang, H. (1998). A case-control study of risk factors for hepatocellular carcinoma in Henan, China. *Am J Trop Med Hyg*, **59**, 947-951.

Zhang, M., Emerson, S. U., Nguyen, H., Engle, R., Govindarajan, S., Blackwelder, W. C., Gerin, J., Purcell, R. H. (2002). Recombinant vaccine against hepatitis E: duration of protective immunity in rhesus macaques. *Vaccine*, **20**, 3285-3291.

Zhao, H., Kurbanov, F., Wan, M. B., Yin, Y. K., Niu, J. Q., Hou, J. L., Wei, L., Wang, G. Q., Tanaka, Y., Mizokami, M., Si, C. W. (2007). Genotype B and younger patient age associated with better response to low-dose therapy: a trial with pegylated/nonpegylated interferon-alpha-2b for hepatitis B e antigen-positive patients with chronic hepatitis B in China. *Clin Infect Dis*, **44**, 541-548.

Zhou, Y. H., Purcell, R. H., Emerson, S. U. (2004). An ELISA for putative neutralizing antibodies to hepatitis E virus detects antibodies to genotypes 1, 2, 3, and 4. *Vaccine*, **22**, 2578-2585.

Zou, P., Isegawa, Y., Nakano, K., Haque, M., Horiguchi, Y., Yamanishi, K. (1999). Human herpesvirus 6 open reading frame U83 encodes a functional chemokine. *J Virol*, **73**, 5926-5933.

Zuckerman, A. J. (1969). Viral hepatitis and the Australia-SH antigen. *Nature*, **223**, 569-572.

Zuckerman, A. J. (1982). Primary hepatocellular carcinoma and hepatitis B virus. *Trans R Soc Trop Med Hyg*, **76**, 711-718.

Zuckerman, A. J. (1996). Alphabet of hepatitis viruses. *Lancet*, **347**, 558-559.

Zuckerman, A. J. (1999). More than third of world's population has been infected with hepatitis B virus. *Bmj*, **318**, 1213.

Zuckerman, J. N. (2006). Vaccination against hepatitis A and B: developments, deployment and delusions. *Curr Opin Infect Dis*, **19**, 456-459.

Zuckerman, J. N., Zuckerman, A. J. (2000). Current topics in hepatitis B. *J Infect*, **41**, 130-136.

11 APPENDICES

Appendix (A): QUESTIONNAIRE (I)

Epidemiological Features and Risk Factors of Viral Hepatitis. Aden Governorate, Yemen.

Q. No	Question	Answer		Key Code
1.	**Clinic**	Al-Tawahi	1	1-8
		Al-Muala'a	2	
		Al-Meidan	3	
		Khormaksar	4	
		Al- Mansura	5	
		Al-Sheikh	6	
		Al-Buraika	7	
		Al-Dar	8	
2.	**Gender**	Female	1	1-2
		Male	2	
3.	**Age:**	Year Months		
4.	**Educational Level:**	Minor (<7 years)	0	0-6
		Illiterate	1	
		Read and write only	2	
		Basic education (years)	3	
		Secondary school (years)....	4	
		Higher diploma	5	
		University (years) ...	6	
5.	**Family Size:**	Total		
		No. of Children (< 5 years)		
		No. of Children (5- < 18 yrs)		
		No. of Adults (> 18 yrs)		
	If not a Child go to Q No. 8)			
6.	Where was she/he born?	Public Hospital	1	1-3
		Private clinic	2	
		Home	3	
7.	By whom was she/he assisted for the delivery?	Doctor	1	1-4
		Medical assistant	2	
		Nurse	3	
		Non medical	4	
8.	**Occupation:** Do you work:	Minor (<15 years)	0	0-2
		Yes	1	
		No	2	
9.	If he/she works, what is the nature of his work?	Teacher	1	1-10
		Marketing	2	
		Soldier	3	
		Manual work	4	
		Driver	5	
		Clerk	6	
		Professional	7	
		Retired	8	
		Fisherman	9	
		Other (specify).....	10	
	Infection Related Factors			
10.	Have you had hepatitis or jaundice?	yes	1	1-2
		no	2	
11.	If Yes, please specify when:	.....years ago.....month ago		
12.	Do you have any member of your family had previous history of hepatitis or jaundice?	yes	1	1-2
		no	2	
13.	If Yes, please specify the time when:	.....years ago.....month ago		
14.	Which one of the following do you believe as a cause of hepatitis?	virus	1	1-5

		bacteria	2	
		stress	3	
		fear	4	
		I didn't know	5	
15.	Have you been vaccinated against hepatitis B?	yes	1	1-3
		no	2	
		I didn't know	3	
16.	Have you ever been transfused with blood?	yes	1	1-3
		no	2	
		I didn't know	3	
17.	If yes: when were you transfused last time?	Month ..... years		
	Transfusion Product			
18.	Whole blood	yes	1	1-2
		no	2	
19.	Plasma	yes	1	1-2
		no	2	
20.	Red blood cells	yes	1	1-2
		no	2	
21.	Platelets	yes	1	1-2
		no	2	
22.	What old were you when you received the first transfusion?	..... years		
23.	Can you give the approximate number of transfusions you have had during your lifetime?	.....		
24.	In how many facilities have you received transfusions?	.....		
25.	How many transfusions did you received last year?	.....		
26.	Have you ever been hemodialysed?	yes	1	1-2
		no	2	
27.	If "yes", How many times?	..... times		
28.	How many years have you been hemodialysed?	..... Years ..... months		
29.	In how many different facilities have you been hemodialysed?	..... facilities		
30.	Have you ever been hospitalized?	yes	1	1-3
		no	2	
		I did not remember	3	
31.	If yes when was this?	..... years ..... Month		
32.	Number of hospitalizations during lifetime	..... times		
33.	Have you ever had surgery?	yes	1	1-3
		no	2	
		I don't remember	3	
34.	If yes. how many times:	..... times		
35.	When was the first surgery performed?	..... years ago ..... Month ago		
36.	Did you receive blood any of these times?	yes	1	1-2
		no	2	
37.	How many injections did you receive last year?	..... injection (s)		
38.	Have you ever received an intravenous medication?	yes	1	1-3
		no	2	
		I didn't know	3	
39.	Have you ever been to the dentist?	yes	1	1-3
		no	2	
		I did not remember	3	
40.	If "yes", how many times?	..... time(s)		

41.	Have you had dental extractions?	yes	1	**1-2**
		No	2	
42.	If yes, how many?	..... Extractions		
43.	Have you ever received acupuncture?	yes	1	**1-2**
		no	2	
44.	Have you ever received traditional care by a Hajjam?	yes	1	**1-2**
		no	2	
45.	If "yes", had you received injections from this person?	yes	1	**1-2**
		no	2	
46.	Did you undergo circumcision?	yes	1	**1-2**
		no	2	
47.	If Yes, by whom?	Medical		
		Non-medical		
		I didn't know		
48.	Do you shave your hair?	yes	1	**1-2**
		no	2	
49.	Do you ever share your razor blade?	yes	1	**1-2**
		no	2	
50.	Have you ever received scarification or tattoos?	yes	1	**1-2**
		no	2	
51.	How many sexual partners have you ever had?	..... time(s)		
52.	Have you ever used recreational intravenous drugs?	yes	1	**1-2**
		no	2	
53.	**Do you have past Medical history of:**	yes	1	**1-2**
	Malaria	no	2	
54.	Schistosomiasis	yes	1	**1-2**
		no	2	
55.	Tuberculosis	yes	1	**1-2**
		no	2	
56.	Cytotoxic therapy or immunosuppressive drugs	yes	1	**1-2**
		no	2	
57.	Do you ever been drinking Alcohol?	yes	1	**1-2**
☞		no	2	
58.	If "Yes" for how long?	..... Years ..... Months		
59.	Frequency of drinking:	Daily	1	**1-4**
		3-5 times/week	2	
		1-2 times/week	3	
		Occasionally	4	
60.	State of drinking:	current	1	**1-3**
		Stopped	2	
		decreased	3	
61.	Do you ever been smoked?	yes	1	**1-2**
☞		no **(if no, go to question No. 65)**	2	
62.	If yes, for how long?	..... Years .....months		
63.	Quantity of cigarette per day:	..... cigarette per day		
64.	State of smoking:	current	1	**1-3**
		Stopped	2	
		Decreased	3	

65.	Do you ever chew Qat?	yes	1	1-2
☞		no **(if no, go to question No. 69)**	2	
66.	If yes, since how long?	Months ..... Years		
67.	Frequency of chewing sessions:	.....times /week	1	1-4
68.	Do you used to wash Qat before chewing?	yes	1	1-2
		no	2	
	Environmental and socioeconomic Factors			
69.	Do you have piped water at home?	yes	1	1-2
		no	2	
70.	If not, what is the main source of drinking water?	.....		
71.	Do you have toilet	Yes	1	1-2
		no	2	
72.	If no, specify the currently used one	.....		
73.	Do you live in:	your own house	1	1-3
		rented one	2	
		family house	3	
74.	Type of house:	.....	1	1-6
75.	How many sleeping rooms do you have?	..... (room/s)		
76.	Do you use electricity in your house?	yes	1	1-2
		no	2	
77.	Do you attend public health clinics?	yes	1	1-2
		no	2	
78.	No. of times attended last year?	..... times		
79.	How long dose it takes you to reach the nearest health centre?	..... Minutes		
80.	Do you attend private clinics?	yes	1	1-2
		no	2	
81.	How many times did you attend in the last year?	..... Times		
82.	In your house do you have the following? Radio	yes	1	1-2
		no	2	
83.	• Television	yes	1	1-2
		no	2	
84.	• Satellite	yes	1	1-2
		no	2	
85.	• Fridge	yes	1	1-2
		no	2	
86.	• Mobile phone	yes	1	1-2
		no	2	
87.	• Land phone	yes	1	1-2
		no	2	
88.	• Computer	yes	1	1-2
		no	2	
89.	• Internet	yes	1	1-2
		no	2	
90.	• Cycle	yes	1	1-2
		no	2	
91.	• Car	yes	1	1-2
		no	2	

Interviewer: ..... Sign: Date: / /200

Blood sample taken: Yes ☐ No ☐: (name) .....

Appendix (B): Sample size and distribution in different health Facilities

Health institution	No. of attendants	Percentage %	Sample size
Al-Tawahi polyclinic	10929	4.8	26
Al-Maidan Polyclinic	25532	11.2	60
Al-Maala Polyclinic	32840	14.4	77
Khor-makser Polyclinic	3449	1.5	8
Dar-Saad Polyclinic	23329	10.2	55
Al-mansora Polyclinic	64724	28.3	152
Al-Sheik Othman Polyclinic	54447	23.8	128
Little Aden Polyclinic	13213	5.8	31
Total	228463	100.0	538

Appendix (C): Participants' identification registry form

S.N	Identification Code						Name "1st ,2nd & family"	Gender	Age Y:M	Health Facility	Reason for consultation	Date DD/MM/YY
	Letters		Numbers									

Appendix (D): QUESTIONNAIRE (II)

Epidemiological features and risk factors of HBV and HCV in cases with chronic hepatitis, cirrhosis and hepatocellular carcinoma. Aden Governorate, Yemen.

Q. No.	Question	Answer		Key Code
1.	**DIAGNOSIS**	Hepatocellular carcinoma	1	1-3
		Cirrhosis	2	
		Chronic liver disease	3	
		Others (specify)	4	
2.	Since when have you known?	..... years ..... months		
3.	Basis of diagnosis	.....		
	Baseline Data			
4.	**Hospital**	Al-Gamhoria	1	1-3
		Aden General	2	
		Al-Wahdah	3	
5.	**Gender**	Female	1	1-2
		Male	2	
6.	**Age:**	.....Year ..... Months		
7.	**Educational Level:**	Minor (<7 years)	0	0-6
		Illiterate	1	
		Read and write only	2	
		Basic education (years)	3	
		Secondary school (years)....	4	
		Higher diploma	5	
		University (years) .	6	
8.	**Family Size:**	Total		
		Children (< 5 yrs)		
		Children (5- < 18 yrs)		
		Adults (> 18 yrs)		
☞	**If not a Child go to Q No. 11)**			
9.	Where was she/he born?	Public Hospital	1	1-3
		Private clinic	2	
		Home	3	
10.	By whom was she/he assisted for the delivery?	Doctor	1	1-4
		Medical assistant	2	
		Nurse	3	
		Non medical	4	
11.	**Occupation:** Do you work:	Minor (<15 years)	0	0-2
		Yes	1	
		No	2	
12.	If he/she works, what is the nature of his work?	Teacher	1	1-10
		Marketing	2	
		Soldier	3	
		Manual work	4	
		Driver	5	
		Clerk	6	
		Professional	7	
		Retired	8	
		Fisherman	9	

		Other (specify).....	10	
	Infection Related Factors			
13.	Have you had hepatitis or jaundice?	yes	1	1-2
		no	2	
14.	If Yes, please specify when:	.....years .....month		
15.	Do you have any member of your family had previous history of hepatitis or jaundice?	yes	1	1-2
		no	2	
16.	If Yes, please specify the time when:	.....years ...month		
17.	Which one of the following do you believe as a cause of hepatitis?	virus	1	1-5
		bacteria	2	
		stress	3	
		fear	4	
		I didn't know	5	
18.	Have you been vaccinated against hepatitis B?	yes	1	1-3
		no	2	
		I didn't know	3	
19.	Have you ever been transfused with blood?	yes	1	1-3
☞		No	2	
		I didn't know	3	
20.	If yes: when were you transfused last time?	.....years .... Month		
	Transfusion Product			
21.	Whole blood	yes	1	1-2
		no	2	
22.	Plasma	yes	1	1-2
		no	2	
23.	Red blood cells	yes	1	1-2
		no	2	
24.	Platelets	yes	1	1-2
		no	2	
25.	What old were you when you received the first transfusion?	..... years		
26.	Can you give the approximate number of transfusions you have had during your lifetime?	.....		
27.	In how many facilities have received transfusions?	.....		
28.	How many transfusions did you received last year?	.....		
29.	Have you ever been haemodialysis?	yes	1	1-2
☞		no	2	
30.	If "yes", How many times?	.....times		
31.	How many years have you been hemodialysed?	.....Years ..... months		
	In how many different facilities have you been hemodialysed?	..... facilities		
33.	If yes when was this?	... years .... Month		
34.	Have you ever been hospitalized?	yes	1	1-3
☞		No	2	
		I did not remember	3	
35.	Number of hospitalizations during lifetime	..... times		
36.	Have you ever had surgery?	yes	1	1-3
☞		No	2	
		I don't remember	3	

37.	If yes. how many times:	..... times		
38.	When was the first surgery performed?	..... years ..... Month		
39.	Did you receive blood any of these times?	yes	1	1-2
		No	2	
40.	How many injections did you receive last year?	..... injection (s)		
41.	Have you ever received an intravenous medication?	yes	1	1-3
		no	2	
		I didn't know	3	
42.	Did you receive intravenous therapy for Schistosomiasis?	yes	1	1-2
☞		No	2	
43.	If yes, frequency:	.....Times		
44.	for how long was that	..... Years .....months		
45.	Have you ever been to the dentist?	yes	1	1-3
☞		No	2	
		I did not remember	3	
46.	If "yes", how many times?	..... time(s)		
47.	Have you had dental extractions?	yes	1	1-2
		No	2	
48.	If yes, how many?	..... Extractions		
49.	Have you ever received acupuncture?	yes	1	1-2
		No	2	
50.	Have you ever received traditional care by a Hajjam?	yes	1	1-2
		No	2	
51.	If "yes", had received injections from this person?	yes	1	1-2
		No	2	
52.	Did you undergo circumcision?	yes	1	1-2
		No	2	
53.	If Yes, by whom?	Medical		
		Non-medical		
		I didn't know		
54.	Do you shave your hair?	yes	1	1-2
		No	2	
55.	Do you ever share your razor blade?	yes	1	1-2
		No	2	
56.	Have you ever received scarifications or tattoos?	yes	1	1-2
		No	2	
57.	How many sexual partners have you ever had?	..... time(s)		
58.	Have you ever used recreational intravenous drugs?	yes	1	1-2
		No	2	
59.	Do you have past Medical history of:	yes	1	1-2
	Malaria	No	2	
60.	Schistosomiasis	yes	1	1-2
		No	2	
61.	Tuberculosis	yes	1	1-2
		No	2	
62.	Cytotoxic therapy or immunosuppressive drugs	yes	1	1-2
		No	2	

63.	Do you ever been drinking Alcohol?	yes	1	1-2
☞		No	2	
64.	If "Yes" for how long?	..... Years ... Months		
65.	Frequency of drinking:	Daily	1	1-4
		3-5 times/week	2	
		1-2 times/week	3	
		Occasionally	4	
66.	State of drinking:	current	1	1-3
		Stopped	2	
		decreased	3	
67.	Do you ever been smoked?	yes	1	1-2
☞		No	2	
68.	If yes, for how long?	..... Years .....months		
69.	Quantity of cigarette per day:	..... cigarette per day		
70.	State of smoking:	current	1	1-3
		Stopped	2	
		Decreased	3	
71.	Do you ever chew khat?	yes	1	1-2
☞		No	2	
72.	If yes, since how long?	..... Years .....months		
73.	Frequency of chewing sessions:	..... times /week	1	1-4
74.	Do you used to wash khat before chewing?	yes	1	1-2
		No	2	
	Environmental and socioeconomic Factors			
75.	Do you have piped water at home?	yes	1	1-2
		no	2	
76.	If not, what is the source of drinking water?	.....		
77.	Do you have toilet Yes No	Yes	1	1-2
		No	2	
78.	If no, specify the currently used one	.....		
79.	Do you live in:	your own house	1	1-3
		rented one	2	
		family house	3	
80.	Type of house:	flat	1	1-6
		break made house	2	
		Woody/ bush made house	4	
81.	How many sleeping rooms do you have?	..... (room/s)		
82.	Do you use electricity in your house?	Yes	1	1-2
		No	2	
83.	Do you attend public health clinics?	Yes	1	1-2
		No	2	
84.	No. of times attended last year?	..... times		
85.	How long dose it takes you to reach the nearest health centre?	..... Minutes		
86.	Do you attend private clinics?	Yes	1	1-2
		No	2	
87.	How many times did you attend in last year?	..... Times		

88.	In your house do you have the following? Radio	Yes	1	1-2
		No	2	
89.	Television	Yes	1	1-2
		No	2	
90.	Satellite	Yes	1	1-2
		No	2	
91.	Fridge	Yes	1	1-2
		No	2	
92.	Mobile phone	Yes	1	1-2
		No	2	
93.	Land phone	Yes	1	1-2
		No	2	
94.	Computer	Yes	1	1-2
		No	2	
95.	Internet	Yes	1	1-2
		No	2	
96.	Cycle	Yes	1	1-2
		No	2	
97.	Car	Yes	1	1-2
		No	2	

Interviewer: .....Sign: ..... Date: / /200

Blood sample taken: Yes ☐ No ☐

Appendix (E): QUESTIONNAIRE (III)

Epidemiological features and risk factors of HBV and HCV in cases with polytransfusion or hemodialysed. Aden Governorate, Yemen.

Q. No	Question	Answer		Key	Code
1.	**DIAGNOSIS**	Hemophilia A	1	1-8	
		Hemophilia B	2		
		Von Willebrand's disease	3		
		homozygous for thalassemia	4		
		homozygous for sickle cell	5		
		congenital methemoglobinemia	6		
		haemodialysis	7		
		Others (specify)	8		
2.	Since when was diagnosed?	..... years ..... months			
3.	Basis of *diagnosis*	.....			
4.	**Hospital:** Al-Gamhoria/ Aden General/ Al-Wahdah		1	1-3	
5.	**Gender**	Female	1	1-2	
		Male	2		
6.	**Age**:	.....Year ..... Months			
7.	**Educational Level:**	Minor (<7 years)	0	0-6	
		Illiterate	1		
		Read and write only	2		
		Basic education (years)	3		
		Secondary school (years)....	4		
		Higher diploma	5		
		University (years)	6		
8.	**Family Size:**	Total			
		Children (< 5 years)			
		Children (5- < 18 years)			
		Adults (> 18 years)			
☞	**If not a Child go to Q No. 11)**				
9.	Where was she/he born?	Public Hospital	1	1-3	
		Private clinic	2		
		Home	3		
10.	By who was she/he assisted for delivery?	Doctor	1	1-4	
		Medical assistant	2		
		Nurse	3		
		Non medical	4		
11.	**Occupation:** Do you work:	Minor (<15 years)	0	0-2	
		Yes	1		
		No	2		
12.	If he/she works, what type of work?	Teacher	1	1-5	
		Professional	2		
		Clerk	3		
		Non-professional	4		
		Other (specify).....	5		
	Infection Related Factors				
13.	Have you had hepatitis or jaundice?	yes	1	1-2	
		no	2		
14.	If Yes, please specify when:	.....years .....month			

15.	Do you have any member of your family had previous history of hepatitis?	yes no	**1** **2**	**1-2**
16.	If Yes, please specify the time when:	.....years .....month		
17.	Which one of the following do you believe as a cause of hepatitis?	virus bacteria stress fear I didn't know	**1** **2** **3** **4** **5**	**1-5**
18.	Have you been vaccinated against hepatitis B?	yes no I didn't know	**1** **2** **3**	**1-3**
19.	Have you ever been transfused with blood?	yes No I didn't know	**1** **2** **3**	**1-3**
20.	If yes: when were you transfused last time?	..... years ..... Month		
	Transfusion Product			
21.	Whole blood	yes no	**1** **2**	**1-2**
22.	Plasma	yes no	**1** **2**	**1-2**
23.	Red blood cells	yes no	**1** **2**	**1-2**
24.	Platelets	yes no	**1** **2**	**1-2**
25.	What old of the first transfusion?	..... years		
26.	number of transfusions during your lifetime?			
27.	Number of facilities received transfusions?	.....		
28.	How many transfusions did you received last year?	.....		
29.	Have you ever been hemodialysed?	yes no	**1** **2**	**1-2**
30.	If "yes", How many times?	..... times		
31.	How many years have you been hemodialysed?	..... Years ..... months		
32.	Number of facilities have you been hemodialysed?	..... facilities		
33.	Have you ever been hospitalized?	yes no I did not remember	**1** **2** **3**	**1-3**
34.	If yes when was this?	..... years ..... Month		
35.	Number of hospitalizations during lifetime	..... times		
36.	Have you ever had surgery?	yes no I don't remember	**1** **2** **3**	**1-3**
37.	If yes. how many times:	..... times		
38.	When was the first surgery performed?	..... years ..... Month		
39.	Did you receive blood any of these times?	yes No	**1** **2**	**1-2**
40.	How many injections did you receive last year?	..... injection (s)		
41.	Have you ever received an intravenous medication?	yes no	**1** **2**	**1-3**

		I didn't know	3	
42.	Have you ever been to the dentist?	yes	1	1-3
☞		no	2	
		I did not remember	3	
43.	If "yes", how many times?	..... time(s)		
44.	Have you had dental extractions?	yes	1	1-2
		No	2	
45.	If yes, how many?	..... Extractions		
46.	Have you ever received acupuncture?	yes	1	1-2
		No	2	
47.	Have you ever received traditional care by a	yes	1	1-2
	Hajjam?	No	2	
48.	If "yes", had you received injections from this	yes	1	1-2
	person?	No	2	
49.	Did you undergo circumcision?	yes	1	1-2
		No	2	
50.	If Yes, by whom?	Medical		
		Non-medical		
		I didn't know		
51.	Do you shave your hair?	yes	1	1-2
		No	2	
52.	Do you ever share your razor blade?	yes	1	1-2
		No	2	
53.	Have you ever received scarification or	yes	1	1-2
	Tattoos?	No	2	
54.	How many sexual partners have you ever had?	..... time(s)		
55.	Have you ever used recreational intravenous	yes	1	1-2
	drugs?	No	2	
56.	**Do you have past Medical history of:**	yes	1	1-2
	Malaria	No	2	
57.	Schistosomiasis	yes	1	1-2
		No	2	
58.	Tuberculosis	yes	1	1-2
		No	2	
59.	Cytotoxic therapy or immunosuppressive	yes	1	1-2
	drugs	No	2	
60.	Do you ever been drinking Alcohol?	yes	1	1-2
☞		no	2	
61.	If "Yes" for how long?	..... Years ... Months		
62.	Frequency of drinking:	.....times/week	1	
63.	State of drinking: current / Stopped/ decreased		1	1-3
64.	Do you ever been smoked?	yes	1	1-2
☞		no	2	
65.	If yes, for how long?	..... Years .....months		
66.	Quantity of cigarette per day:	..... cigarette per day		
67.	State of smoking: current / Stopped/ decreased		1	1-3
68.	Do you ever chew khat?	yes	1	1-2
☞		no	2	

69.	If yes, since how long?	..... Years .....months		
70.	Frequency of chewing sessions:	.....times/week	**1**	
71.	Do you used to wash khat before chewing?	yes	**1**	**1-2**
		No	**2**	
	Environmental and socioeconomic Factors			
72.	Do you have piped water at home?	yes	**1**	**1-2**
		no	**2**	
74.	Do you have toilet	Yes	**1**	**1-2**
		No	**2**	
76.	Do you live in:	your own house	**1**	**1-3**
		rented one	**2**	
		family house	**3**	
77.	Type of house:	flat	**1**	**1-6**
		break made house	**2**	
		Woody/ bush made house	**4**	
78.	How many sleeping rooms do you have?	..... (room/s)		
79.	Do you use electricity in your house?	Yes	**1**	**1-2**
		No	**2**	
80.	Do you attend public health clinics?	Yes	**1**	**1-2**
		No	**2**	
81.	No. of times attended last year?	..... times		
82.	How long dose it takes you to reach the nearest health centre?	..... Minutes		
83.	Do you attend private clinics?	Yes	**1**	**1-2**
		No	**2**	
84.	How many times did you attend in last year?	..... Times		
85.	In your house do you have the following? Radio	Yes	**1**	**1-2**
		No	**2**	
86.	Television	Yes	**1**	**1-2**
		No	**2**	
87.	Satellite	Yes	**1**	**1-2**
		No	**2**	
88.	Fridge	Yes	**1**	**1-2**
		No	**2**	
90.	Mobile phone	Yes	**1**	**1-2**
		No	**2**	
91.	Land phone	Yes	**1**	**1-2**
		No	**2**	
92.	Computer	Yes	**1**	**1-2**
		No	**2**	
93.	Internet	Yes	**1**	**1-2**
		No	**2**	
94.	Cycle	Yes	**1**	**1-2**
		No	**2**	
95.	Car	Yes	**1**	**1-2**
		No	**2**	

Interviewer: ..... Sign:..... Date: / /200

Blood sample taken: Yes No By: (name) .....

Appendix (F): QUESTIONNAIRE (IV).

Rate of coverage by hepatitis B vaccine among children under five in Aden Governorate, Yemen

Q. No.	Question	Answer		Key	Code
1.	**District**	Al-Tawahi	1	1-8	
		Al-Muala'a	2		
		Al-Meidan	3		
		Khormaksar	4		
		Al- Mansura	5		
		Al-Sheikh	6		
		Al-Buraika	7		
		Al-Dar	8		
2.	**Gender**	Female	1	1-2	
		Male	2		
3.	**Age:**	Year Months			
4.	**3- Vaccination History**				
4.1	Have you been vaccinated against hepatitis B?	yes	1	1-3	
		no	2		
		I didn't know	3		
4.2	If (**Yes**) please ask the parent to show you the card and inspect its data	valid	1	1-3	
		not valid	2		
		Not found	3		
5.	When the child got his first dose of the vaccine?	< 2 months	1	1-3	
		< 4 months	2		
		< 12 months	3		
6.	How many doses of the vaccine the child had already received?	one	1	1-4	
		two	2		
		three	3		
		more than three	4		
7.	Who take the child to be vaccinated?	his Father	1	1-3	
		his Mother	2		
		other person (specify)	3		
8.	Where the child got his vaccination?	polyclinic	1	1-4	
		MCH clinic	2		
		hospital	3		
		private clinic	4		
9.	Dose the vaccine against hepatitis B was accompanied with other vaccination?	yes	1	1-3	
		no	2		
		I didn't know	3		
10.	If the child was not vaccinated, what are the main reasons for that?				
11.	Level of attention received during vaccination of your child?	yes always	1	1-3	
		some times	2		
		never	3		
12.	Are you satisfied with the provided vaccination services?	Yes	1	1-2	
		no	2		
13.	Where was she/he born?	Public Hospital	1	1-3	
		Private clinic	2		
		Home	3		
14.	By whom was she/he assisted for the delivery?	Doctor	1	1-4	
		Medical assistant	2		
		Nurse	3		
		Non medical	4		

15.	**Father's educational**	Illiterate	**1**	**1-6**
		Read and write only	**2**	
		Basic education (years)	**3**	
		Secondary school	**4**	
		Higher diploma	**5**	
		University .	**6**	
16.	**Mother's educational**	Illiterate	**1**	**1-6**
		Read and write only	**2**	
		Basic education (years)	**3**	
		Secondary school	**4**	
		Higher diploma	**5**	
		University (years)	**6**	
17.	**Father's occupation**			
	Dose the father work?	yes	**1**	**1-2**
		No	**2**	
18.	What type of work?	teacher	**1**	**1-7**
		farmer	**2**	
		manual work	**3**	
		clerk	**4**	
		professional	**5**	
		fishermen	**6**	
		Others (specify)	**7**	
19.	**Mother's occupation**			
	Dose the mother work?	yes	**1**	**1-2**
		No	**2**	
20.	What type of work?	teacher	**1**	**1-7**
		farmer	**2**	
		manual work	**3**	
		clerk	**4**	
		professional	**5**	
		Home keeper	**6**	
		Others (specify)	**7**	
21.	**Family Size:**	Total		
		Children (< 5 years)		
		Children (5- < 18 years)		
		Adults (> 18 years)		
	Infection Related Factors			
10.	Dose has the child a history of hepatitis or jaundice?	yes	**1**	**1-2**
		no	**2**	
11.	If Yes, please specify when:	.....years .....month		
12.	Do you have any member of your family had previous history of hepatitis or jaundice?	yes	**1**	**1-2**
		no	**2**	
13.	If Yes, please specify the time when:	...years .....month		
14.	Which one of the following do you believe as a cause of hepatitis?	virus	**1**	**1-5**
		bacteria	**2**	
		stress	**3**	
		fear	**4**	
		I didn't know	**5**	
15.	Dose you believe on the importance of HBV vaccine?	yes	**1**	**1-3**
		no	**2**	
		I didn't know	**3**	

16.	Have you ever been transfused with blood?	yes	**1**	**1-3**
		No	**2**	
		I didn't know	**3**	
17.	If yes: when were you transfused last time?	Month … years …..		
	Transfusion Product			
18.	Whole blood	yes	**1**	**1-2**
		no	**2**	
19.	Plasma	yes	**1**	**1-2**
		no	**2**	
20.	Red blood cells	yes	**1**	**1-2**
		no	**2**	
21.	Platelets	yes	**1**	**1-2**
		no	**2**	
22.	What old were you when you received the first transfusion?	…….. years		
23.	Can you give the approximate number of transfusions you have had during your lifetime?	……		
24.	In how many facilities have you received transfusions?	……...		
25.	How many transfusions did you received last year?	……...		
26.	Have you ever been haemodialysis?	yes	**1**	**1-2**
		no	**2**	
27.	If "yes", How many times?	…… times		
28.	How many years have you been haemodialysis?	…. Years ... months		
29.	In how many different facilities have you been haemodialysed?	…… facilities		
30.	Have you ever been hospitalized?	yes	**1**	**1-3**
		No	**2**	
		I did not remember	**3**	
31.	If yes when was this?	… years … Month		
32.	Number of hospitalizations during lifetime	…….. times		
33.	Have you ever had surgery?	yes	**1**	**1-3**
		No	**2**	
		I don't remember	**3**	
34.	If yes. how many times:	…… times		
35.	When was the first surgery performed?	… years … Month		
36.	Did you receive blood any of these times?	yes	**1**	**1-2**
		No	**2**	
37.	How many injections did you receive last year?	…… injection (s)		
38.	Have you ever received an intravenous medication?	yes	**1**	**1-3**
		no	**2**	
		I didn't know	**3**	
39.	Have you ever been to the dentist?	yes	**1**	**1-3**
		No	**2**	
		I did not remember	**3**	
40.	If "yes", how many times?	…… time(s)		
41.	Have you had dental extractions?	yes	**1**	**1-2**
		No	**2**	
42.	If yes, how many?	……. extractions		
43.	Have you ever received acupuncture?	yes	**1**	**1-2**
		No	**2**	
44.	Have you ever received traditional care by a Hajjam?	yes	**1**	**1-2**
		No	**2**	
45.	If "yes", had you received injections from this person?	yes	**1**	**1-2**
		No	**2**	

46.	Did you undergo circumcision?	yes	**1**	**1-2**
		No	**2**	
47.	If Yes, by whom?	Medical		
		Non-medical		
		I didn't know		
48.	Do you shave your hair?	yes	**1**	**1-2**
		No	**2**	
49.	Do you ever share your razor blade?	yes	**1**	**1-2**
		No	**2**	
50.	Have you ever received tattoos?	yes	**1**	**1-2**
		No	**2**	
51.	How many sexual partners have you ever had?	..... time(s)		
52.	Have you ever used recreational intravenous drugs?	yes	**1**	**1-2**
		No	**2**	
53.	**Do you have past Medical history of:**	yes	**1**	**1-2**
	Malaria	No	**2**	
54.	Schistosomiasis	yes	**1**	**1-2**
		No	**2**	
55.	Tuberculosis	yes	**1**	**1-2**
		No	**2**	
56.	Cytotoxic therapy or immunosuppressive drugs	yes	**1**	**1-2**
		No	**2**	
57.	Do you ever been drinking Alcohol?	yes	**1**	**1-2**
☞		No	**2**	
58.	If "Yes" for how long?	..... Years ... Months		
59.	Frequency of drinking:	Daily	**1**	**1-4**
		3-5 times/week	**2**	
		1-2 times/week	**3**	
		Occasionally	**4**	
60.	State of drinking: current / Stopped/ decreased		**1**	**1-3**
61.	Do you ever been smoked?	yes	**1**	**1-2**
☞		No	**2**	
62.	If yes, for how long?	.... Years ...months		
63.	Quantity of cigarette per day:	..... cigarette per day		
64.	State of smoking: current / Stopped/ decreased		**1**	**1-3**
65.	Do you ever chew Qat?	yes	**1**	**1-2**
☞		No	**2**	
66.	If yes, since how long?	Months ..... Years		
67.	Frequency of chewing sessions:	Daily	**1**	**1-4**
		3-4 times /week	**2**	
		1-2 times /week	**3**	
		Occasionally	**4**	
68.	Do you used to wash Qat before chewing?	yes	**1**	**1-2**
		No	**2**	
	Environmental and socioeconomic Factors			
69.	Do you have piped water at home?	yes	**1**	**1-2**
		no	**2**	
70.	If not, what is the main source of drinking water?	.....		
71.	Do you have toilet	Yes	**1**	**1-2**
		No	**2**	
72.	If no, specify the currently used one	.....		

73.	Do you live in:	your own house	**1**	**1-3**
		rented one	**2**	
		family house	**3**	
74.	Type of house:	flat	**1**	**1-6**
		break made house	**2**	
		Villa	**3**	
		woody made house	**4**	
		bush made house	**5**	
		steel made house	**6**	
75.	How many sleeping rooms do you have?	..... (room/s)		
76.	Do you use electricity in your house?	Yes	**1**	**1-2**
		No	**2**	
77.	Do you attend public health clinics?	Yes	**1**	**1-2**
		No	**2**	
78.	No. of times attended last year?	..... times		
79.	How long does it take you to reach the nearest health centre?	..... Minutes		
80.	Do you attend private clinics?	Yes	**1**	**1-2**
		No	**2**	
81.	How many times did you attend in the last year?	..... Times		
82.	In your house do you have the following? Radio	Yes	**1**	**1-2**
		No	**2**	
83.	Television	Yes	**1**	**1-2**
		No	**2**	
84.	Satellite	Yes	**1**	**1-2**
		No	**2**	
85.	Fridge	Yes	**1**	**1-2**
		No	**2**	
86.	Mobile phone	Yes	**1**	**1-2**
		No	**2**	
87.	Land phone	Yes	**1**	**1-2**
		No	**2**	
88.	Computer	Yes	**1**	**1-2**
		No	**2**	
89.	Internet	Yes	**1**	**1-2**
		No	**2**	
90.	Cycle	Yes	**1**	**1-2**
		No	**2**	
91.	Car	Yes	**1**	**1-2**
		No	**2**	

Interviewer: .....Sign:..... Date: / / 200

Appendix (G): Guideline instruction for blood sample collection and management

(For laboratory technicians)

Collection, transport and processing of blood samples

Each blood sample should be attached with a form, which includes the following information:

of the participant, technical steps of collecting, transporting, and storing the blood sample.

the place of collection

the methodology of collection:

type of tubes (dry or anticoagulant filled tubes)

volume tube collected

number of aliquots to be prepared

identification of recruited individual

the methodology of transport

the methodology of processing

the methodology of storage: one of the aliquot shall be frozen at -80°C temperature, and be kept until transported (abroad) to the genetic laboratory for further molecular analysis.

Appendix (H): Blood Sample Management Form

CODE OF THE PATIENT / Serial No.

Gender	male	female
Age	year	months .....
Blood Sample:	(Venopuncture)	
Collected by		
Quantity of blood: ..... (ml)		
Blood clotted	yes	no .....
Transportation of blood sample to the lab.	After	(mints/hours)
Degree of temperature:	.....°C	
Centrifuged:	yes	no .....
Centrifuged of centrifugation:		
Devided blood sample in to A and B portion	Yes	no
Sera "B" is stored :	yes	no .....
If yes degree of stored temperature	.....°C	
Location:.....		

Reported by (name): ..... Signature: .....

Appendix (I): Informed consent

Dear participant:

This study is aiming to determine the seroprevalence of hepatitis viral infection and identify risk factors of acquiring the infection among the population. The result obtained will contribute in the application of appropriate control and preventive measures of the spread of this disease in the community. The gathered data will be used confidentially only for the research purpose.

We expect no health complication for you as a result of the use of the procedures in this study. If you agree to participate in the study, a blood sample of 10 ml (from 3-10 ml depend on the age-group) will be collected from you, also you required answering a questionnaire, which will take from you approximately 10 minutes. The questionnaire will ask about the socio-demographic information and others related to past history of acquiring the infection and its risk factors.

Appendix (J): Formal abstract of attended meetings

Sero-epidemiology of Viral Hepatitis in Aden City, Yemen

Amin A. Bawazir*, Luis Cuevas* and Prof. C Anthony Hart**
**Liverpool School of Tropical Medicine, ** Department of Medical Microbiology, University of Liverpool*

To determine the prevalence of viral hepatitis (A, B, C and E) and describe their prevalence by age among a population attending primary health care facilities in Aden Governorate.
Also to identify if HBV and HCV are significant risk factors among liver diseased patients as well as in polytransfused and haemodialysed patients. A cross-sectional study in eight polyclinics in Aden Governorate, from April to July 2005. 538 Individuals attending these polyclinics were enrolled independently of age and sex and were distributed proportionally across age groups (infants up to 78 years old) and polyclinic. For liver diseased patients, polytransfused and haemodialysed were enrolled from the different hospitals in Aden. A questionnaire was filled and serological assays were undertaken using ELISA.
258 (48%) were females and the rest were males. Prevalence of HAV was 86.6% and was followed by HBV, HEV, and HCV (16.2%, 10.7% and 0.4%, respectively). Children <15 had a HAV prevalence of 81.2% increasing to 95.7% in 15 to 44 years old. Similarly, HBV increased from 8.8% before 15 years of age to 32.6% after 45 years. HEV prevalence was 2.6% in <15 years old and increased to 21.3% after 45 years. HBV and HCV among liver diseased patients were (26.8% and 2.1% respectively) and it was (66.7% and 43.6%) among polytransfused patients.