HEPATITIS B VIRUS INFECTION IN THE REPUBLIC OF YEMEN

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Thesis submitted for the degree of Doctor of Philosophy

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June 2003

Abstract

A community-based cross-sectional household seroepdemiological survey was conducted in the Republic of Yemen to estimate the prevalence of HBV infection as well as hepatitis B vaccine coverage and effectiveness amongst children vaccinated in the country's Expanded Programme on Immunisation (EPI). Study subjects were randomly selected from five provinces in the country based on probability proportional to size.

The prevalence of HBV chronic infection amongst women of childbearing age participating in the survey was 5.08% and places Yemen amongst the group of countries with a high endemicity of HBV infection (> 5%). This is much lower than earlier estimates by hospital-based studies in Yemen estimating the prevalence of HBV chronic infection to be 12.5-16.6% amongst women of childbearing age. This prevalence estimate is more consistent with research findings from other neighbouring Middle East countries.

There was evidence that perinatal transmission is not a major mode of HBV infection in Yemen. This is mainly the result of the low prevalence of HBV chronic infection amongst women of childbearing age (5.08%), the low prevalence of hepatitis B e antigenaemia (HBeAg) (12.84%) amongst HBV chronic carrier women, and the low infectivity (21.14%) of HBeAg positive chronic carrier mothers.

The prevalence of children completely vaccinated with hepatitis B vaccination was 8.63%. This estimate is lower than hepatitis B vaccination coverage estimates presented by the EPI in Yemen. The prevalence of incompletely vaccinated children was 12.47%. If these children completed their vaccination schedule, this would have increased the proportion of completely vaccinated children to more double than its current level. There were differences in hepatitis B vaccine coverage by area/province of residence indicating inequitable distribution, availability or accessibility to health services, with a bias towards better provision, or at least uptake of immunisation services in urban areas. Hepatitis B vaccine was found to be highly immunogenic and effective in preventing HBV infection amongst children aged 1-3 participating in the survey.

In conclusion, this research demonstrates that HBV infection is not as major a public health problem in Yemen as originally expected, and this misconception needs to be corrected. Hepatitis B vaccine coverage, on the other hand, is low and must be increased. There is no need to amend the current hepatitis B vaccine schedule. Nevertheless, vaccine coverage should be increased with an emphasis on a more equitable distribution, access, and availability of hepatitis B vaccines.

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List of Abbreviations

Anti-HBc	Hepatitis B Core Antibody
Anti-HBe	Hepatitis B e Antibody
Anti-HBs	Hepatitis B Surface Antibody
AVH	Acute Viral Hepatitis
BCG	Bacillus Calmette-Guerin
CDC	Centre for Disease Control
CLD	Chronic Liver Disease
CMV	Cytomegalovirus
CSO	Central Statistical Organisation
DPT	Diphtheria Pertussis Tetanus
EBV	Epstein-Barr Virus
ELISA	Enzyme Linked Immunosorbent Assay
EPI	Expanded Programme on Immunisation
GAVI	Global Alliance for Vaccine and Immunisation
GCC	Gulf Council for Cooperation
GMC	Geometric Mean Concentration
GNP	Gross National Product
GZR	Gray Zone Reactive
HAV	Hepatitis A Virus
HBeAg	Hepatitis B e Antigen
HBsAg	Hepatitis B Surface Antigen
HBIG	Hepatitis B Immunoglobulin
HBV	Hepatitis B Virus
HBV DNA	Hepatitis B Virus Deoxyribonucleic Acid
HCC	Hepatocellular Carcinoma
HCV	Hepatitis C Virus
HDV	Hepatitis Delta Virus
HEV	Hepatitis E Virus
IDNO	Identification Number
IEC	Information, Education and Communication
IgM anti-HBc	Immunoglobulin M Hepatitis B Core Antibody
IgG anti-HBc	Immunoglobulin G Hepatitis B Core Antibody
MCA	Multiple Correspondence Analysis
MEIA	Microparticle Enzyme Immunoassay
MOE	Ministry of Education
MOPHP/MOPH	Ministry of Public Health and Population
MMR	Measles Mumps Rubella
OR	Odds Ratio
PHC	Primary Health Care
PPS	Probability Proportional to Size

RIA	Radioimmunoassay
RCF	Relative Centrifugal Force
RPHA	Reverse Passive Haemoagglutination
RR	Relative Risk
SE	Standard Error
STD	Sexually Transmitted Disease
UAE	United Arab Emirates
UNICEF	United Nations International Children's Education Fund
WHA	World Health Assembly
WHO	World Health Organisation
95% CI	95% Confidence Interval

Acknowledgements

Firstly, I would like to thank my supervisor Professor Andrew Hall. Not only did he supervise this research but he was also thoroughly involved in every step of it. I learnt from him a great deal, not only epidemiology but also communication skills, kindness, and understanding. I hope Yemen continues to benefit from his experience and expertise and that I will have the honour of being the link between him and Yemen.

I express my greatest gratitude to my father for giving me the opportunity to carryout this research. Had it not been for him and his support, this research would have never been initiated and would have never come to life. Throughout the past few years, as always, he has been the source of my motivation and reassurance.

Throughout my research my mother was continuously encouraging, caring, and provided excellent advice. Having been with her and seen what she went through when doing her Ph.D. enabled me to understand that I was not the only researcher encountering difficult times and frustrating moments.

A special thanks to my wife for her patience, kindness, supportive love, and so many other things I conveniently fail to remember. She truly deserves this Ph.D. as much as I do.

I am very much thankful and grateful to Sheikh Ahmed for generously providing funding for the fieldwork of this research, as well as Mr. Abdul Rahman for providing funding for purchasing additional supplies for the survey. When I requested the government of Yemen and other sources of funding they did not have the funds to support this survey. Had it not been for the generous funding provided by these two gentlemen, this survey would have never seen the light, and all the results and information obtained from this research would have not been known. I am also thankful to Dr. Hussein Al-Jezairy, Dr. Mohammed Haitham Al Khayat, Dr. Hashem Al Zain, Mrs. Fanous Violette, and Dr. Mona Al Midwahi at the WHO – EMRO.

I would also like to thank Dr. Neal Alexander for providing me with the statistical advice needed throughout my research, especially when designing the survey and in solving the many statistical problems that occurred during the analysis.

I would like to express special thanks and words of appreciation to Professor Abdulla Abdul Wali Nasher, Professor Abdul Nasser Al Munbari, Professor Yahia Al Shuaibi, Dr. Iain Murray-Lyon, Dr. Ali Al Midwahi, Dr. Fouad Al Khilli, Dr. Qassim Bereh, Dr.Abdulla Al Shaibah, Dr. Aimen Al Shami, Dr. Khaled Taj Aldeen, Dr. Ahmed Bourji, Dr. Yaslem Habtoor and Abdulla Al Aswadi. What they did was extremely important and significantly contributed to this research.

Chapter 1

Hepatitis B Virus Infection
A Global Health Problem

1.1 Introduction:

Worldwide approximately 2 billion individuals, a third of the world's population, have been infected with the hepatitis B virus (HBV) (Viral Hepatitis, 2000; Vaccine and Immunisation News, 1996). It is estimated that there are over 350 million chronic carriers infected with the virus across the globe (WHO, 2000; Zuckerman, 1996). The World Health Organisation estimates that 1 – 2 million deaths occur annually as a result of HBV infection (WHO, 2000; Zuckerman, 1996).

Approximately 25% of HBV chronic carriers develop chronic hepatitis, liver cirrhosis and hepatocellular carcinoma (HCC), the three main complications of HBV chronic infection. HCC is the fourth most common cause of mortality from cancer and the eighth most common incident cancer worldwide (Parkin et al., 1999). It is estimated that 54% - 80% of liver cancers can be attributed to HBV chronic infection (Parkin et al., 1999; Zuckerman, 1996). The HBV is described as the world's most common carcinogen, second only to tobacco (Maynard et al., 1989; Vaccine and Immunisation News, 1996). The course of infection depends on the age at exposure to HBV, with age at infection being the major determinant of whether a person becomes a chronic carrier. The risk of becoming a chronic carrier and consequently developing complications of HBV chronic infection is inversely proportional to age (Edmunds et al., 1993).

The distribution of HBV chronic carriers is not uniform across the globe and the majority of HBV chronic carriers are found in developing countries. Liver cancer is also mainly a problem of developing countries, which contribute 81% of all deaths resulting from liver cancer (Parkin et al., 1999). The global distribution of HCC follows a pattern similar to that of HBV chronic carriers and occurs predominantly in regions where HBV infection is highly endemic, particularly China and Sub-Saharan Africa.

In 1982, a vaccine was licensed for active immunoprophylaxis against HBV infection. The first vaccines available were plasma derived but later recombinant vaccines were developed. Both types of vaccine have proven to be safe, highly effective, and, recently

cost-effective, available at a cost most countries can afford. As the success of treatment for HBV infection remains low and antiviral therapy expensive, prevention of infection by vaccinating with hepatitis B vaccine remains the best option for health ministries and policy makers.

In 1991 the World Health Assembly (WHA), recommended the integration of hepatitis B vaccines to immunisation campaigns of countries with an intermediate to high endemicity by 1995, and to all countries by 1997. Although many countries have adopted this recommendation whilst struggling to keep their health systems afloat the main challenge facing developing countries is reaching highly deprived populations through a primary health care infrastructure that is in many cases exhausted and short of funds.

Hepatitis B Viral Epidemiology In The Middle East

1.2 Background:

The Middle East is comprised of a group of countries that show considerable variation in their economy, population characteristics and growth rate, literacy and educational levels, geography, climate, development, political system and level of health care. The Arabian Peninsula covers well over 3 million square kilometres of which Saudi Arabia alone comprises 2.3 million square kilometres. Countries of the Arabian Peninsula (Gulf States) include Saudi Arabia, Kuwait, Qatar, Bahrain, United Arab Emirates (UAE), Sultanate of Oman and the Republic of Yemen (Map 1). Middle East countries directly neighbouring the Arabian Peninsula include Egypt, Jordan, Iran, Iraq, Lebanon, Sudan and Syria.

The Gulf States of the Arabian Peninsula excluding Yemen form an economic, geographic and political alliance called the Gulf Council for Cooperation (GCC). These countries have been experiencing unprecedented growth since the discovery of oil. Health care has been a high priority in these countries, especially in Saudi Arabia and UAE, which provide free hospital care for all their citizens at a standard competing with the best in the world.

HBV infection is a major public health problem in the Middle East. The majority of the countries in the region fall into the group of countries with an intermediate and high endemicity of HBV infection.

In this chapter, the epidemiology of HBV infection in the Arabian Peninsula and its neighbouring Middle Eastern countries will be reviewed. Firstly, the incidence and prevalence of HBV infection in the region and the different modes and ages of transmission of infection in these countries will be discussed. This will be followed by a description of the risk factors associated with HBV infection in these countries. Then the preventive measures against HBV infection in the countries of the region will be presented. Finally, the epidemiology of HBV infection in The Republic of Yemen will be reviewed. Throughout the text the terms HBV chronic infection, HBV chronic carrier, HBsAg chronic carrier will be used interchangeably.

Map 1
Arabian Peninsula and Neighbouring Middle East Countries

1.3 Literature review search strategy:

Information and data in this review was obtained from four sources. The main source of information was literature published in international journals on the epidemiology of HBV infection and chronic carriage, hepatitis B vaccines, hepatitis B vaccine effectiveness, and control of HBV infection. This provided general information on the global situation of infection with HBV and regional (Middle East) information (with an emphasis on Yemen) on HBV infection, carrier status, mode of transmission, risk factors, and vaccination. Keywords used in the Thesaurus search of Medline and Popline, and the search of Pubmed were [hepatitis, hepatitis B virus, Middle East, hepatitis B vaccines, vaccine effectiveness, Bahrain, Egypt, Iran, Iraq, Jordan, Kuwait, Lebanon, Libya, Middle East, Morocco, Oman, Palestine, Qatar, Saudi Arabia, Sudan, Syria, Tunisia, United Arab Emirates and Yemen]. These terms were combined in the search limiting results to articles relevant to the review. A further search was conducted for articles cited in the primary articles, which expanded information obtained from primary reading material. On some occasions cited articles from journals not available at libraries in the UK or the internet were requested from overseas. The second source of information was Middle East country reports and workshops on viral hepatitis. The World Health Organisation (WHO) library at the Ministry of Public Health and Population (MOPHP) in Sanaa, Republic of Yemen was searched for all reports and publications on viral hepatitis in the region and the country. Reports on hepatitis B viral epidemiology in Yemen were obtained from the MOPHP. Information on vaccine types and coverage were kindly provided by Mr. Mohammed Kulaise, the Director of the Expanded Programme on Immunisation (EPI) at the MOPHP in Sanaa, Yemen. The third source of information was books and editorials on viral infections and HBV that were purchased, borrowed from the library of the London School of Hygiene and Tropical Medicine, or obtained from Professor Andrew Hall and Dr. Iain Murray-Lyon. Finally, the MOPHP kindly provided copies of its Expanded Programme on Immunisation (EPI) five-year plan, its plan to introduce hepatitis B vaccine to the EPI, its request for funding submitted to the Global Alliance for Vaccine and Immunisation (GAVI), as well as other useful and related reports and documents.

1.4 Epidemiology:

Based on the prevalence of HBV chronic carriers (individuals positive for hepatitis B surface antigen (HBsAg) for longer than six months) amongst adults in the general population, countries are divided into three main groups. Countries with a prevalence of HBV chronic carriers less than 2% are classified as countries with a low endemicity of infection; those with a prevalence of HBV chronic carriers between 2 – 5 % are classified as having an intermediate endemicity of infection; and countries with a prevalence of HBV chronic carriers exceeding 5% are classified as having a high endemicity of infection (Hall, 1994).

Middle East countries provide a good reflection of the global epidemiology of HBV infection, with the prevalence of HBV chronic carriers varying considerably between and within these countries. Countries of the region fall into all three groups of low, intermediate and high endemicity of HBV infection. However, the majority of countries in the region have an intermediate and high endemicity of HBV chronic carriers (Andre, 2000; Hall, 1994; WHO, 1995).

1.4.1 HBV chronic infection:

National HBV chronic carrier rates in the Middle East range from as low as 1.5% in Kuwait to as high as 18.5% in Yemen (Ashraf et al., 1986; El-Guneid et al., 1993).

Studies conducted in the Middle East show that the prevalence of chronic carriers in these countries ranges from 3 – 11% in Egypt, 1.1 – 2.5% in Iran, 3.4 – 5 % in Iraq, 2.6 – 10% in Jordan, 1.5 - 2.9% in Kuwait, 2.9% in Lebanon, 2 – 6% in Libya, 2.3 – 10% in Oman, 1.8 – 6% in Palestine, 2.8 – 16.7% in Saudi Arabia, 16 – 26% in Sudan, 3.3 - 6.5% in Tunisia, 2 – 6 % in the United Arab Emirates and 7.4 – 18.5% in Yemen (Al-Faleh et al., 1992; Al-Nakib et al., 1986; Al-Owais et al., 2000; Arya et al., 1985; Ashraf et al., 1986; Awidi et al., 1984; Basalamah et al., 1984; Coursaget et al., 1994; El-Gouli, 1984; El-Guneid et al., 1993; El-Hamzi, 1989; El-Shafie, 1992; Ghavanini et al., 2000; McCarthy et al., 1994; Nabulski et al., 1997; Nashef et al., 1992; Parande et al., 1986; Ragaa et al., 2001; Ramia et al., 1986; Scott et al., 1992; Scrimgeour et al., 1999; Soliman et al., 1995; Talkuder et al., 1982; Toukan et al., 1988; Toukan et al., 1990; WHO, 1995; Yassin et al.,

2002). These studies have been conducted on various populations with the intention of giving an accurate estimate of the prevalence of HBV chronic carriers in a representative sample of the general population (table 1.4.1 A). These groups included apparently healthy adults with no evidence of previous or present HBV infection, school children, infants, pregnant women, healthy blood donors and health care staff. The HBV carrier rates differed from one study to another, even when conducted amongst the same population and within the same country. This may be due to different study designs or sample populations, where, for example, higher rates of infection have been observed in community-based studies than in studies conducted amongst blood donors (Toukan, 1990). Blood donors do not necessarily represent the general population. In some settings, blood donors tend to be healthier than individuals in the general population and as a result are more willing and capable of donating blood. In one study in Saudi Arabia, blood donors appeared to have a lower prevalence of HBsAg (13.9%) when compared to the general population (16.7%) (p=0.4) (El-Hamzi, 1989). On the other hand, in countries where donors are paid for their blood donation, these blood donors may have higher rates of infection than the general population. Moreover, the blood donor relatives of patients suffering chronic liver disease (CLD) requiring blood transfusions may themselves have higher rates of viral hepatitis and liver disease than the general population. In Yemen, Scott et al found blood donors had a significantly higher prevalence of HBsAg chronic infection (20.6%) than apparently healthy non-donor individuals (12.1%) (p=0.02) (Scott et al., 1990). It is unclear whether these blood donors were paid or volunteer blood donors. As a result, one must not generalise findings based on studies conducted on blood donors. Another possible explanation for the differences in the HBV carrier rates may be due to the use of different measurement techniques used from one study to another. There are many kits and reagents available that differ in generation, specificity and sensitivity. Most of the serological assays performed were done using commercial Enzyme Linked Immunosorbent Assay (ELISA) or Microparticle Enzyme Immunoassay (MEIA), Radio-Immunoassay (RIA) and Reverse Passive Haemagglutination (RPHA). Tests done using RPHA were usually confirmed by ELISA or RIA. The majority of these studies did not clarify if samples positive by ELISA or MEIA were re-tested or tested by neutralisation to confirm the serological findings, which may be one explanation why the results were

so different. In Yemen El-Guneid et al, found a much higher hepatitis B e antigenaemia (HBeAg) (59.3%) amongst healthy individuals than Scott et al (9.7%). They attributed this to the more sensitive microparticle enzyme immunoassay technique (Imx HBe, Abbott) used for the detection of HBeAg in their study (El-Guneid et al., 1993). Still, a common finding for all these studies, irrespective of the study population or measurement technique, is that they all confirm high HBV carrier rates in the majority of the countries in the Middle East (see table 1.4.1 A).

Table 1.4.1 A
Studies investigating the prevalence of HBV chronic infection (HBsAg positive) in Middle East countries

No	Country	Region	Population	Type	Number	HBsAg Prevalence	Methods	Year	Reference
1.	**Egypt**	Kalyeoubeya (rural)	Pregnant women	Hospital based	150	8%	ELISA	1992-1993	El-Nawawy et al. 1996
2.	**Egypt**	Menofia Prov. (semi-rural)	Pregnant women	Hospital based	360	11.1%	ELISA	1983-1985	Ghaffar et al. 1989
3.	**Egypt**	El Menia & Alexandria Prov.	Apparently healthy individuals		1866	10.1%	RIA		Sherif et al. 1985
4.	**Iran**	Hamadan (rural + urban)	Apparently healthy individuals	Community based	4930	2.5%	ELISA	1989	Amini et al. 1993
5.	**Iran**	Shiraz	Blood donors	Laboratory/ blood bank based	7897	1.1%	ELISA	1988	Ghavanini et al. 2000
6.	**Iraq**		Blood donors	Hospital based	505	3.6%	CIEP	1973	Al-Kassab et al. 1973
7.	**Iraq**	Baghdad (urban)	Apparently healthy individuals		950	3.4%	CIEP		Omer et al. 1976
8.	**Jordan**	Amman (urban)	Volunteer blood donors	Hospital based	2500	4.4%	RPHA and RIA	1981	Awidi et al. 1984
9.	**Jordan**	3 rural villages and hospital in Amman (rural + urban)	Apparently healthy individuals	Community based in rural area and hospital based in urban	1,115 (of whom 241 are urban)	9.9%	RIA	1985	Toukan et al. 1988
10.	**Kuwait**	Kuwait (urban)	Pregnant women of various nationalities	Hospital based	1554	2.9%	RPHA and ELISA	1986	Al-Nakib et al. 1986
11.	**Lebanon**	Beirut (urban)	Pregnant women	Hospital based	558	2.9%	ELISA	1993-1995	Nabulski et al. 1997
12.	**Libya**	Tripoli	Hospital employees	Hospital based	459	4%	ELISA	1999	Daw et al. 2000

13.	**Oman**	Muscat	Blood donors	Hospital based	564	5.1%	ELISA		Al-Dhahry et al. 1994
14.	**Palestine**	East Jerusalem, Ramallah and Jordan valley	Pregnant women, blood donors, children and general population	Hospital + Community based	778	1.8%	ELISA	1991-1992	Nashef et al. 1992
15.	**Palestine**	Gaza	General population	Community based	810	3.5%	ELISA		Yassin et al. 2002
16.	**Saudi Arabia**	Riyadh	Blood donors, outpatients	Hospital based		4.6%	ELISA	1990	Al-Tuwaijri et al. 1990
17.	**Saudi Arabia**	Different provinces	Blood donors, school children, outpatients	Hospital based	2702	16.7%	RIA	1989	El-Hamzi. 1989
18.	**Saudi Arabia**	Different provinces	Children aged 1-10 years (pre-vaccination)	Community based	4575	6.7%	ELISA	1989-1990	Al-Faleh et al. 1992
19.	**Saudi Arabia**	Jaizan	Students, volunteer blood donors, pregnant women, newborns	Hospital based	724	12.7%	ELISA	1984	Arya et al. 1985
20.	**Saudi Arabia**	Jaizan	Infants and children	Hospital based	325	11.1%	ELISA	1984-1985	Parande et al. 1986
21.	**Saudi Arabia**	Riyadh	Blood donors, pregnant women, medical students	Hospital based	5467	4.3%	RPHA and RIA or ELISA	1986	Ramia et al. 1986
22.	**Saudi Arabia**	Eastern province	Healthy ambulatory men		3588	8.8%	RPHA	1982	Talkuder et al. 1982
23.	**Saudi Arabia**		Pregnant women	Hospital based	5000	2.8%	RPHA	1979-1982	Basalamah et al. 1984
24.	**Saudi Arabia**	Riyadh	Pregnant women	Hospital based	3020	3.9%	RPHA/ ELISA	1982-1984	Ramia et al. 1984
25.	**Saudi Arabia**	Buraidah	Blood donors	Hospital based	8003	4%	ELISA	1995 -1997	Mehdi et al. 2000
26.	**Saudi Arabia**	13 health regions	Children aged 1-12 years (post-vaccination)	Community based	4791	0.31%	ELISA		Al-Faleh et al. 1999
27.	**Sudan**	Juba	Outpatients	Hospital based	651	26%	ELISA	1989	McCarthy et al. 1994

28.	**Tunisia**	Tunis	Pregnant women	Hospital based	7162	3.3%	ELISA		Coursaget et al. 1994
29.	**Yemen**	Sanaa, Taiz	Pregnant women, blood donors	Hospital based	534	18.5%	ELISA	1993	El-Guneid et al. 1993
30.	**Yemen**	Sanaa, Taiz, Hajja, and Hodeidah	Pregnant women, blood donors, hospital patients, schools	Hospital + School based	879	12.7%	ELISA	1988	Scott et al. 1990
31.	**Yemen**	Sanaa, Taiz, Hajja, and Hodeidah	Pregnant women, blood donors, hospital patients, schools	Hospital + School based	348	13.5%	ELISA	1988	Scott et al. 1992
32.	**Yemen**	Sanaa	Respondents to a campaign to control HBV infection	Laboratory/blood bank based	2652	7.4%	RPHA and EIA	2000	Ragaa et al. 2001
33.	**Yemen**	Sanaa, Aden	Blood donors	Laboratory/blood bank based	999	10.7%	EIA and neutralisation	2002	Sallam et al. 2002
34.	**Yemen**	Hajjah	Blood donors	Hospital based	7868	9.8%	MEIA	1997- 1999	Haidar NA. 2002

In addition to the marked geographic variation of HBV carrier rates between Middle East countries, there is marked geographic variation of rates within these countries. In Saudi Arabia, for example, the prevalence of chronic carrier females was 25% in the Jaizan region (the Southwest province bordering Yemen) and this was significantly higher ($p<0.00003$) than the prevalence of chronic carrier females which was 7.3% in the Najran region (El-Hamzi, 1989). Jordan also shows significant differences ($p < 0.02$) in carrier rates between villages ranging from 5.7% in one village to 12.8% in another (Toukan et al., 1990).

While some studies conducted in the region found a significantly higher rate of anti-HBc positivity and HBV chronic carriers amongst males (Awidi et al., 1984; Ramia et al., 1986), another group did not find significant evidence of higher anti-HBc positivity or HBV chronic carrier rates amongst males (Al-Faleh., 1992; El-Hamzi, 1989; Parande et al., 1986). A WHO collaborative study on HBV in which 20 countries participated did not find a statistically significant difference in the prevalence of HBsAg between males and females (Sobeslavsky, 1980).

Table 1.4.1 B presents HBV chronic carrier rates and hepatitis B vaccination status and coverage in Middle East countries devised by Toukan (Toukan, 1997). Since its publication in 1997, there have been some changes and the table has been modified accordingly. In Yemen, for example, hepatitis B vaccination was introduced to the Expanded programme on Immunisation (EPI) in 1998. In Tunisia, hepatitis B vaccination has also been introduced to the EPI. In Iraq, coverage of the EPI has been markedly affected by the economic sanctions imposed on the country following the second Gulf War. In addition, Qatar is currently conducting selective vaccination of certain high-risk groups such as contacts of HBsAg positive pregnant women, in an attempt to improve the effectiveness of its hepatitis B control programme.

Table 1.4.1 B
HBV chronic carrier and hepatitis B vaccination status in Middle East countries
Adapted from Toukan A.U. 1997 - Control of Hepatitis B in the Middle East

Endemicity and country	HBsAg Carrier State	HBV Vaccine in EPI	Selective HBV Vaccination	Vaccination Coverage
Low endemicity				
Bahrain	0.9-1.25%	Yes	Yes	89%
Iran	1%	Yes		81%
Israel*	1-1.8%	Yes	Yes	92%
Kuwait	1.5%	Yes	Yes	94%
Intermediate Endemicity				
Cyprus	2-2.5%	Yes	Yes	68%
Iraq	4-5%	Yes		59%
Libya	2-6%	Yes		
UAE	2-5%	Yes	Yes	90%
High Endemicity				
Egypt	3-11%	Yes	Unclear	82%
Jordan	3-10%	Yes	No	90%
Oman	2-10%	Yes	Yes	99%
Palestine*	2-6%	Yes	Yes	
Saudi Arabia	7-17%	Yes	Yes	95%
Tunisia*	6.5%	Yes		
Yemen*	12-18.5%	Yes	No	12%
Unclear				
Lebanon	2.9%	No	No	
Syria		Yes	Yes	76%
Qatar*		Yes	Yes	90%

* countries with modified data.

1.4.2 Age-specific prevalence:

A few studies conducted in Saudi Arabia provide information on age-specific prevalence of HBV chronic infection. A community-based seroepidemiological survey in all provinces of Saudi Arabia conducted by Al-Faleh et al amongst 1575 Saudi Arabian children aged 1 – 10 years found the highest prevalence of HBsAg positivity (9.7%) among 1-year-old children (Al-Faleh et al., 1992). In a hospital-based serosurvey in the Riyadh province, Ramia et al found the prevalence of HBsAg positivity amongst children less than 3 years to be 4.8% and 4.4%, respectively, in males and females. Ramia et al found that the prevalence of HBV chronic infection peaked in 10-14 year-old children reaching 7.8% and 5.3%, respectively, in males and females (Ramia et al., 1986). Prevalence of HBV chronic infection decreased with advancing age in a similar pattern in

both sexes. A study among Saudi Arabian children living in the Jaizan province revealed HBsAg, anti-HBs and anti-HBc to be 11.1%, 9.5% and 7.4%, respectively (Arya et al., 1986). A study among 3588 healthy ambulatory men in the Eastern Province of Saudi Arabia found the highest prevalence of HBsAg to be 13.6% and 9.8%, respectively, among 35-45 year-old and 46-55 year-old men (Talkuder et al., 1982). These men were tested using RPHA and were not confirmed by ELISA or RIA.

In Jordan the prevalence of HBV chronic carriers was approximately 10% (95% C.I. 8.2 to 11.7) throughout all age groups, with evidence of HBV infection (any marker of HBV infection) increasing directly with age, reaching 92% in those over 60 years (Toukan et al., 1990). Amongst individuals positive for HBsAg, 32% were HBeAg positive (95% C.I. 23 to 40.7%). Most HBeAg positive individuals were children under 10 years of age, while anti-HBe was found mainly in adults (Toukan et al., 1990). A study in Palestine found the age-specific prevalence appeared to increase with age and HBsAg was highest amongst individuals aged 20-29 years (4.3%) and 30-39 years (5.3%), after-which the prevalence of HBsAg declined and remained approximately 2.8% (Yassin et al., 2002).

In Yemen, Scott et al found age to be a highly significant risk factor for HBV seropositivity and chronic infection. The estimated odds ratio associated with increasing 10-year age intervals was 1.37 and 1.51, respectively, for HBV chronic infection and total markers (Scott et al., 1990).

1.4.3 Incidence of HBV infection:

Information on the incidence of viral hepatitis in Middle East countries is limited. Available information shows that in 1994 the incidence of acute viral hepatitis (AVH) in the Middle East ranged from 22/100000 in Syria to 126/100000 in the United Arab Emirates (WHO, 1995). These incidence rates are for the general population and were presented at the WHO regional workshop on the prevention and control of viral hepatitis in 1995. The figures were obtained from regular reporting but it is unknown how reliable and accurate these figures are because few methodological details were described in the report. Consequently, estimating the role of HBV infection in the incidence of AVH in these countries poses a problem as does the comparison of incidence rates between them. Nevertheless, available data estimates that HBV is responsible for 61% of cases of AVH

in Egypt, 38% in Iraq, 40% in Algeria, 39 - 49% in Jordan, 12 – 36% in Kuwait, 10 – 20% in Libya, 5% in Qatar, 66% in Tunisia and 27% in Yemen (Al-Kandari et al., 1987; El-Gouli et al., 1984; El-Guneid et al., 1997; Toukan, 1990; Toukan et al., 1990; WHO, 1995).

There are a number of difficulties and limitations when estimating incidence rates of viral hepatitis attributable to HBV infection in the Middle East. First of all, the majority of HBV infections occur asymptomatically in childhood, resulting in a high carrier rate that passes undetected during the early years of life. Amongst adults and older children only 30 - 40% of cases of AVH become symptomatic and are diagnosed. These rates are only the tip of the iceberg, with the remaining majority of cases with sub-clinical infection and elevated biochemical markers undetected. As a result, whatever incidence rate is calculated will be an underestimate of the true incidence of HBV infection. Secondly, this estimate will have to be corrected for underreporting. In Middle Eastern countries this will largely be due to a lack of efficient surveillance programmes within these countries and deficiencies in diagnostic measures and techniques especially in rural areas. Thirdly, in many of these countries AVH is reported as one entity without the classification of cases of AVH according to etiologic agent. Fourthly, these incidence rates and reported cases are significantly affected by the health seeking behaviour of individuals in these countries. It is unknown what proportion of cases of AVH seek traditional therapists, pass unnoticed or seek professional medical treatment (and are thus consequently reported). It is also suspected that there is under-reporting of incident cases in some countries such as Jordan and Lebanon due to the large number of patients treated in the private sector. Table 1.4.3 shows the incidence rates of acute viral hepatitis in some countries in the Middle East.

Table 1.4.3
Incidence of acute viral hepatitis in some Middle East countries - 1994

Country	Incidence AVH	% due to HBV	% due to other viruses	Reference
Egypt		61%		WHO, 1995
Iran	26/100 000	Not available		WHO, 1995
Iraq	86/ 100 000	38%		WHO, 1995
Jordan	37/100 000	39 – 49%	48% HAV	WHO, 1995; Toukan et al., 1990
Kuwait	95/100 000	11.5 – 36.5%	25-77% HAV 18.7% HCV 3% HEV	WHO, 1995; Al-Kandari et al., 1987
Libya	30/ 100 000	Not available		WHO, 1995
Morocco	15.7/ 100 000	Not available		WHO, 1995
Oman	80/100 000	Not available		WHO, 1995
Qatar		5%		WHO, 1995
Syria	22/100 000	Not available		WHO, 1995
UAE	126/100 000	Not available		WHO, 1995
Yemen		27%	5.1% HAV 6.4% HCV 14% HEV 2.6% HCV 51.3% Unknown	El-Guneid et al., 1997

1.5 Mode of transmission:

HBV infection can be transmitted at 3 stages in life; around the time of birth, during childhood, and in adult life (Hall, 1994). The main modes of transmission are mother-to-child (perinatal), child-to-child (horizontal), sexual and parenteral. The role of each of these modes varies across the globe. In developed countries (also countries with low endemicity of HBV infection) sexual transmission and intravenous drug abuse in adolescence and adult life account for the majority of cases of HBV transmission. In developing countries (countries with intermediate and high endemicity of HBV infection), mother-to-child and child-to-child transmission during the early years of life are the major modes of transmission of HBV infection. Placental breakdown and leakage of maternal blood during delivery, in utero infection, infection postnatally through breastmilk, babies ingestion of blood, and small scratches to the baby during birth are postulated mechanisms of perinatal transmission (Zuckerman et al., 1996). There is no significant evidence supporting the role of the latter three mechanisms of perinatal

transmission. In about 10 – 50 % of cases of HBV infection the mode of transmission is unknown (Al-sowmely, 1998; Stevens et al., 1992).

The main factors determining perinatal transmission are the mothers HBsAg and HBeAg status (Hwang et al., 1985). The presence of HBeAg in the mother's serum correlates with HBV DNA load and is considered a marker of infectivity. If a HBV chronic carrier mother is HBeAg positive this indicates there is a high risk of the baby acquiring HBV infection and consequently becoming a HBV chronic carrier. Studies have found that 70 - 90% of infants born to mothers who were HBeAg positive became HBV chronic carriers compared to 10 - 31% of infants born to mothers who were HBeAg negative or anti-HBe positive (CDC, 1991; Gilbert, 1981; Hall, 1994; Hwang et al., 1985; Papaevangelou et al., 1979). Chotard et al found that children with a HBeAg positive mother had a higher risk of becoming HBsAg positive compared to children of HBeAg negative mothers (Relative Risk [RR] 124:1 95% C.I. 12 to 1277) (Chotard et al., 1991). In Southeast Asia, for example, the high rate of mother-to-child transmission of HBV infection has been attributed to the high rates of HBeAg amongst chronic carrier women of childbearing age in these countries. Nevertheless, one must be cautious when predicting the potential risk of perinatal transmission of HBV infection based on the prevalence of HBeAg amongst women of childbearing age. Hall clarifies that although HBeAg positivity in carrier women of childbearing age is a good reflector of the potential risk of perinatal transmission, HBeAg positive African women had a lower risk of transmitting HBV to their offspring because they had lower HBV DNA concentrations compared to their Southeast Asian counterparts (Hall, 1994; Wong et al., 1984). In a study in Senegal, out of 12 children born to HBeAg positive mothers, only two of the children became HBV chronic carriers (Chotard et al., 1992).

In the Middle East, the majority of HBV infections occur through childhood and perinatal transmission. Sexual and parenteral transmission are not common modes of infection in the Middle East. Sexual transmission is uncommon on a large scale probably due to the conservative culture of Middle Eastern societies. Nevertheless, wife-to-husband transmission was suggested to be important in one study in Tunisia (Coursaget et al., 1994). HBV chronic infection was found in 18% (6/33) of husbands of HBsAg carrier mothers compared to only 4-6% in the general population, and 75% (3/4) of husbands of

HBeAg positive mothers were HBsAg positive (Coursaget et al., 1994). Other studies supported evidence for sexual transmission of HBV infection in the Middle East based on finding a higher prevalence of HBV chronic infection amongst married individuals compared to single individuals but did not adjust for other factors such as age (Ragaa et al., 2001; Yassin et al., 2002). The role of parenteral transmission of HBV infection in health institutions should also be currently limited due to the routine screening of blood and blood products. However, it has been reported that in some countries of the Middle East hospital or health centre waste products are not treated in the proper manner and that a lot of these waste products lie on streets and are accessible to children which poses a serious health risk and hazard (Yassin et al., 2002). The number of intravenous drug abusers in the Middle East appears to remain low when compared to other regions.

Studies investigating the role of perinatal compared to childhood transmission in the Middle East have produced variable results. While one group of studies proposes that childhood transmission is a major mode of transmission of HBV infection with perinatal transmission being relatively unimportant, another group proposes that perinatal transmission plays an important and significant role in contributing to the pool of HBV chronic carriers. Considering the heterogeneity of Middle Eastern populations and the differences in the prevalence of chronic carriers and HBeAg positivity amongst women of childbearing age within them, inter-country differences in the mode of transmission probably exist.

Supporting the role of childhood transmission in the Middle East, Toukan et al suggested that person-to-person, non-sexual, non-parenteral and intrafamilial contact is the major mode of transmission between asymptomatic HBV carriers and susceptible individuals (Toukan et al., 1990). A combined study in four Middle-Eastern countries (Egypt, Jordan, Kuwait, Saudi Arabia) (see table 1.5.1) found that only 21% (n=25/120) of children born to HBsAg positive mothers became chronic carriers (Toukan , 1996). This was attributed to the low prevalence (13%) of HBeAg amongst HBsAg positive mothers, although 94% (n=15/16) of HBeAg positive mothers transmitted HBV to their infants.

A study in Kuwait found the prevalence of HBsAg among pregnant women to be 2.9% (n=45/1554) of whom 7.3% were positive for HBeAg, 65.8% were anti-HBe positive, and 26.8% had neither the HBeAg nor anti-HBe. Based on the low prevalence of HBeAg

amongst pregnant mothers they predicted a low transmission rate of virus to offspring. 30% of the infants of HBeAg positive mothers developed HBV infection (based on just one case) (Al-Nakib et al., 1986). In Lebanon, there is a similar prevalence of HBsAg (2.9%) (n=16/558) and HBeAg (6.3%) amongst pregnant women (Nabulski et al., 1997) and therefore one would expect a low mother-to-child transmission of HBV infection as in Kuwait.

In a study in Jaizan province in Saudi Arabia, it was in light of the low prevalence of HBsAg amongst women of childbearing age (4.9%) as well as the low HBeAg positivity of 9% (n=6/67) in males and females alike that it was suggested that perinatal transmission is unlikely to play a numerically important role in HBV hyperendemicity (Arya et al., 1985).

A study investigating materno-foetal transmission of HBV in Saudi Arabia found 2.8% (n=140/5000) of pregnant women positive for HBsAg. A two year follow up of fifty HBsAg positive women of whom 12% (n=6/50) were positive for HBeAg and their newborn children showed that none became HBsAg positive (Basalamah et al., 1984). A similar study conducted on 3020 women showed an overall prevalence of 3.9% for HBsAg amongst pregnant women in Saudi Arabia of which 11% (n=13/119) were positive for HBeAg (Ramia et al., 1984). A study by the same authors looking into transplacental transmission of HBV infection by HBsAg carrier mothers suggested a lack of evidence for perinatal transmission (Ramia et al., 1988). In another study in Saudi Arabia, based on finding high interfamilial clustering of HBV infection in families with HBsAg positive fathers but HBsAg negative mothers, Ramia suggested that person-to-person (horizontal) transmission rather than perinatal transmission was responsible for the spread of HBV infection within these families (Ramia, 1990).

Table 1.5.1
Maternal-neonatal transmission of HBV infection by mothers HBsAg/HBeAg status
Adapted from Toukan et al. 1990

	HBsAg Positive Mother	HBsAg Positive Baby		HBeAg Positive Mother		HBsAg Positive Baby		HBeAg Negative Mother		HBsAg Positive Baby		HBsAg Negative Mother	HBsAg Positive Baby	
Country	**Total No**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**No.**	**%**
Saudi Arabia	49	8	16	7	14	6	86	42	86	2	5	-	-	-
Egypt	35	13	37	6	16	6	100	29	83	7	24	30	0	0
Jordan	23	3	13	2	9	2	100	21	91	1	5	28	0	0
Kuwait	13	1	8	1	8	1	100	12	92	0	0	-	-	-
Total	120	25	21	16	13	15	94	104	87	10	10	58	0	0

On the other hand, there are studies supporting the role of perinatal transmission of HBV infection in the Middle East. Al-Faleh et al, in Saudi Arabia found a high rate of HBsAg positivity (9.7%) among children aged one year and accordingly suggested the possibility of significant HBV transmission during the perinatal period or soon after it (Al-Faleh et al., 1992). It is unlikely that these cases resulted from childhood transmission considering that this is uncommon in the first year of life.

In a study in Yemen, approximately 17% (n=40/243) of pregnant women were positive for HBsAg. Thirty-two percent (95% C.I. 3.8 to 67.8) of these HBV chronic carrier women were positive for HBeAg (n=9/28) (El-Guneid et al., 1993). The high prevalence of HBeAg amongst pregnant Yemeni women points to the potential importance of perinatal transmission of HBV infection from mother to child. Another study in Yemen suggested the rate of mother-to-child transmission of HBV infection from HBsAg positive mothers to their newborns to be 50% (n=10/20) based on cord blood specimens positive for HBsAg (Abdul Raheem et al., 1991). The prevalence of HBeAg was not measured in the 20 HBsAg positive mothers.

Studies in Egypt also indicate that perinatal transmission is relatively high. El-Nawawy et al detected HBsAg in 8% (n=12/150) of pregnant mothers and 17% (n=2/12) of their infants (El-Nawawy et al., 1996). None of the HBsAg positive mothers or their infants

was HBeAg positive. However, because the number of HBV chronic carriers is small, one cannot draw a definitive conclusion. Similarly, in another study conducted in Egypt, 17% (n=6/35) of infants born to HBsAg positive mothers were HBeAg positive (Ghaffar et al., 1989). HBsAg was positive in 25%, 22% and 37% of cord, 3 month and 6 month blood specimens, respectively. Out of six babies who became infected from HBeAg positive mothers, five (83%) showed infection at three months and only one showed infection at six months. Table 1.5.2 shows hepatitis B e antigen and antibody prevalence among HBsAg positive women found in some of the studies conducted in the region.

Table 1.5.2
HBeAg/anti-HBe status amongst HBV chronic carrier women in the Middle East

Country	HBsAg Positive Pregnant Women	HBeAg Positive Carrier Women	Anti-HBe Positive Carrier Women	Transmission Rate	Reference
Lebanon	2.9 %	6.3 %			Nabulski et al., 1997
Kuwait	2.9 %	7.3 %	65.8 %		Al-Nakib et al., 1986
Yemen	15%			50%	Abdul Raheem et al., 1991
Yemen	16.6 %	31.1 %			ElGuneid et al., 1993
Egypt	8 %	0		17%	El-Nawawy et al., 1996
Saudi Arabia	2.8 %	12 %	88%		Basalamah et al., 1984
Saudi Arabia	3.9 %	11 %	75 %	16%	Ramia et al., 1984

1.6 Factors associated with HBV infection:

The most common factors found to be associated with HBV infection and carrier status in the Middle East are family size, socioeconomic status, age, educational status and a history of previous blood transfusion, surgery or contact with a jaundiced person.

Evidence supporting the role of large family size in increasing the risk of HBV infection was observed in Jordan and Saudi Arabia (Ramia, 1990; Toukan, 1990). There was pronounced familial clustering of HBV infection and a significant correlation was found between family size and the proportion of HBsAg positive family members. In families where the mother was a HBsAg carrier there was higher intrafamilial clustering of HBV infection than in families where the father was a HBsAg carrier (Ramia, 1990). In Jordan,

34% of the siblings of HBsAg positive individuals were also found to be carriers of the virus (Toukan, 1990; Toukan, 1996). Toukan further emphasised that evidence for childhood transmission early in life existed because of a similar pattern of infection among adult HBV carriers. 39% of adult siblings of adult carriers were also found to carry the virus compared to 10% of their spouses.

The effect of socioeconomic status on HBV transmission in Jordan was examined using a specially developed socioeconomic index. This showed significantly greater HBsAg prevalence in lower (14.4%) than in upper (2.4%) socioeconomic groups (Toukan et al., 1990). Another study supporting this evidence showed the prevalence of HBsAg to be 11% and 4%, respectively, among low and high socioeconomic strata (Toukan, 1996). Earlier studies have also shown the prevalence of HBsAg to be 6.9% and 0.7%, respectively, in low and upper socioeconomic groups (Awidi et al., 1984). Similar findings have been reported from other Middle Eastern countries such as Syria, where the risk of infection was found to be higher in children of low socioeconomic status when compared to children of high socioeconomic status (WHO, 1995). Ghaffar et al, also found higher rates of HBV infection among children of low socioeconomic status in Egypt (Ghaffar et al., 1989). Members of poor families share bedding, eating and household utensils, which may be sources of infected saliva or serum. Poor families tend to live in small dwellings and crowding brings these individuals into close contact, which may facilitate childhood transmission (Toukan, 1990).

The association of a history of jaundice, previous blood transfusions, and surgery with HBV infection and carrier status has been reported in Jordan, Egypt, Libya and Yemen.

In Yemen, a multivariable analysis found age, a history of jaundice, and a combined history of blood transfusion and surgery, to be associated with HBV infection (Scott et al., 1990). The odds ratio associated with increasing 10-year age intervals was 1.37 and 1.51 for the carrier status and total markers, respectively. The adjusted odds ratio for a combined history of surgery and blood transfusion was 3.76 (95% C.I. 1.11 to 6.82) and the adjusted odds ratio for a history of jaundice was 1.42 (95% C.I. 1.01 to 2.01). Toukan et al reported similar findings in Jordan but additionally found past or present HBV infection associated with rural background, injections, tattooing, sexual exposure and surgical procedures (Toukan et al., 1990). It would be useful to know the nature of these

injections and by whom they were administered. Involvement of non-health personnel may be an important explanatory factor for infections resulting from these injections. In Egypt, for example, involvement of non-health personnel in parenteral and surgical procedures was found to be associated with a higher risk of HBV infection (WHO, 1995). A large number of surgical procedures such as tattooing and circumcision are also carried out by unqualified individuals in Egypt, which is the case in many other Middle Eastern countries and it is important to investigate the role ear piercing and circumcision may have in the transmission of HBV infection in these countries.

In Egypt, Ghaffar et al examined risk factors for perinatal transmission. Apart from the proven importance of HBeAg/ anti HBe status in perinatal transmission, they found that maternal history of schistosomal infection was significantly associated with perinatal transmission (Ghaffar et al., 1989). A possible explanation for this association was that schistosomal infection resulted in impaired cell-mediated immunity, which might contribute to the presence of a higher titre of HBsAg, and hence increased viraemia and infectivity. It was also suggested that due to a cross-reaction between schistosomal and HBV antigens, schistosomal antigens transmitted to the newborn from the mother can depress the newborns immune reaction against HBV by immunological tolerance or by an antigen specific suppressor mechanism. They also found higher rates of perinatal HBV transmission in rural areas, families of low socioeconomic status, as well as from mothers with low educational status. A negative correlation between prevalence of HBV infection and educational levels has also been reported from Iran (Amini et al., 1993; WHO, 1995). Families of low educational and socioeconomic status are more likely to be associated with poor personal hygiene and low standards of living which probably increase their risk of exposure to HBV infection.

1.7 Control:

The most common methods employed for the control of HBV infection in the Middle East are active immunisation (universal and targeted), routine blood screening for HBsAg, and ensuring safe injection practices. Due to belief that childhood transmission accounts for the majority of cases of HBV infection, its high cost and the large proportion of household deliveries in many Middle East countries, passive immunisation using

Hepatitis B Immunoglobulin (HBIG) is uncommon in the Middle East. Due to cultural sensitivity and expectations that sexual transmission is uncommon, safe sex is not officially promoted in most countries of the region.

1.7.1 Active vaccination:

In 1987, the WHO Hepatitis Technical Advisory Group recommended integration of hepatitis B vaccine into the Expanded Programme on Immunisation (EPI). In 1991, the Yaounde Conference witnessed the recommendation of global infant hepatitis B immunisation as part of the EPI by the EPI Global Advisory Group (EPI-GAG) Committee. In 1992, the World Health Assembly (WHA) endorsed a recommendation that hepatitis B vaccine should be integrated into national immunisation programmes in countries with high endemicity by 1995 and in all countries by 1997. Considering that the majority of HBV infections in the Middle East result from childhood and perinatal transmission, universal infant immunisation appears to be an attractive option for most countries of the region.

Hepatitis B vaccine is the first vaccine effective in preventing cancer or a sexually transmitted disease (STD). Two types of vaccine are currently available, plasma derived and recombinant hepatitis B vaccine, although the latter is superseding the former in national vaccination programmes. The efficacy of both vaccines is very similar. The effectiveness of hepatitis B vaccines has been demonstrated in numerous studies (Chotard et al., 1992; Coursaget et al., 1986; Hall et al., 1993; Lee et al., 1997; Maupas et al., 1981; Whittle et al., 1991). In the Gambia, for example, the vaccine given in infancy has been shown to be 84% effective in preventing HBV infection and 94% effective in preventing HBV chronic infection (Hall et al., 1993). Currently recommended and implemented schedules of hepatitis B vaccination vary, but generally follow a pattern of 2 –3 injections at monthly intervals followed by a booster dose 6 – 12 months after the initial dose (Stevens et al., 1992). Immunisation is considered to have occurred if the concentration of antibody (anti-HBs) is greater than 10mIU/L (Hall, 1993). Most individuals should remain protected for 5 – 10 years following vaccination even if anti-HBs is no longer detectable by immunoassay (Banatvala et al., 2000; CDC, 1991; Chotard et al., 1992; Hall, 1993; Stevens et al., 1992). This has been attributed to the

development of a rapid anamnestic response upon exposure to infection or booster doses, indicating persistence of immunologic memory (Stevens et al., 1992).

In order to prevent perinatal transmission, infants born to HBV chronic carrier mothers should receive HBIG immediately following delivery or vaccine as early as possible within the first 48 hours following delivery. Due to high cost, unavailability and logistical considerations of delivery, HBIG is not administered in most developing countries. On the other hand, vaccination at delivery appears to be an economically attractive option, particularly in developing countries. The most difficult challenge facing this option is how to identify children at risk and how to vaccinate children within 48 hours following delivery in developing countries with a high rate of perinatal transmission where the majority of deliveries occur at home.

Studies assessing the effectiveness of hepatitis B vaccine alone compared to hepatitis B vaccine with HBIG found that hepatitis B vaccine given alone within 48 hours of birth is nearly as effective as the combined regimen in preventing perinatal transmission of HBV (Mahoney et al., 1993; Poomerawan et al., 1990; Wong et al., 1984; Xu et al., 1985). Poomerawan et al found that only 3.4% (n=2/58) of newborns of HBeAg positive carrier mothers receiving vaccine alone within 12 hours became chronic carriers as opposed to an expected 70 – 90% (Poomerawan et al., 1990). Mahoney et al found that 3-4 year-old children whom were vaccinated at birth were protected from both perinatal and childhood transmission (Mahoney et al., 1993).

The earliest Middle Eastern countries to introduce hepatitis B vaccine into their EPI were Saudi Arabia and Qatar in 1989. These were followed by Oman in 1990, Bahrain, Iraq, Syria, UAE in 1991, Egypt in 1992, Palestine in 1993, and Jordan in 1995. In 1998, Yemen introduced Hepatitis B vaccine into its EPI providing free vaccine to all children under one-year of age. The vaccine was initially introduced to one district per governorate and later on expanded to all districts in the country starting with the main urban health facilities of each district. The Yemeni Ministry of Public Health and Population (MOPHP) also introduced a cost recovery programme, in which children aged more than one-year and adults could obtain hepatitis B vaccine after paying a nominal fee for the vaccine. The MOPHP claims that the cost recovery programme was highly successful in the first year and that the MOPHP collected considerable revenue but the

cost recovery programme was ended by the government mid 2001 because of financial constraints and legal implications (MOPHP(A), 2001). It is reported that hepatitis B vaccine was well accepted by the population and health workers in Yemen, however, at the beginning of 2000 some rumours about adverse effects were spread but the impact of these rumours was low on existing vaccine uptake (MOPHP(A), 2001).

Vaccination coverage in Middle East EPI vaccination programmes has been good overall. Gulf States in particular, are reported to have excellent coverage reaching 99%, 95%, 94% 90%, 90%, 90% and 89% respectively, in Oman, Saudi Arabia, Kuwait, UAE, Qatar, Jordan and Bahrain, for all recommended doses by one year of age (Toukan, 1997; WHO, 1995). It was estimated that by 1996, 90% of children under 12-years in Saudi Arabia were fully immunised (table 1.4.1 B).

A few studies have examined the effectiveness of EPI hepatitis B vaccination in preventing HBV infection and chronic infection in Middle Eastern countries. A study in Saudi Arabia demonstrated that the hepatitis B vaccination programme was highly effective and had a tremendous impact on the prevalence of HBV infection amongst children in Saudi Arabia. In 1999, ten years after introducing hepatitis B vaccination to the EPI, the prevalence of HBV chronic infection had declined to 0.31% compared to 6.7% which was the prevalence of HBV chronic infection just prior to introduction of hepatitis B vaccine to the EPI in October 1989 (Al-Faleh et al., 1999). In Palestine, there has also been a notable decline in the incidence rate of HBV infection since 1994, which was attributed to the universal hepatitis B vaccination programme started one year earlier. The incidence of acute HBV infection in 1999/2000 was one sixth the incidence of acute HBV infection in 1993, which was presumed to be largely due to the introduction of universal hepatitis B vaccination in 1993 (Yassin et al., 2002).

Many Middle Eastern countries such as Bahrain, Kuwait, Oman, Palestine, Qatar, Saudi Arabia, and UAE are reported to have also introduced vaccination targeted at high-risk groups (WHO, 1995). In Bahrain and Oman, for example, two high-risk groups are vaccinated; health care workers and susceptible contacts (e.g. household members) of HBsAg carriers. Most Middle East countries do not have mandatory vaccination for their health care employees. A study in Libya explained that health care workers are exposed to an occupational risk and argued the importance of introducing mandatory hepatitis B

vaccination of health care workers who come into direct contact with blood or blood products, and the need to provide advice and counselling to those who become infected (Daw et al., 2000). The study also emphasised the importance that hospitals and health service providers minimise such risks among their employees by implementing strict guidelines on sharp instruments, blood or body fluids, and decontamination. In general, it has been argued that targeted approaches are less effective due to the difficulty in reaching at-risk populations, at-risk people becoming infected before being reached, and the fact that up to 50% of reported hepatitis B cases have no identifiable risk or exposure (Stevens et al., 1992; Zuckerman., 1996).

Qatar is reported to have introduced good hepatitis B vaccine monitoring and surveillance activities (WHO, 1995). All contacts of carriers are traced and vaccinated. As part of their routine antenatal care, pregnant women are screened for HBsAg, and those positive are screened for HBeAg. All newborns in the country receive their first dose of hepatitis B vaccine in the delivery room. Babies born to HBeAg positive mothers also receive HBIG (WHO, 1995). In the UAE, in 1995, there were ongoing efforts to develop a national strategy for the prevention and control of viral hepatitis. There is no information on the effectiveness of such targeted programmes in these countries.

The major obstacles facing the introduction of hepatitis B vaccine to the EPI are economic and political. Sudan up until 1995, had not introduced hepatitis B vaccine to any groups due to a lack of financial resources (WHO, 1995). Iraq, during and shortly after the second Gulf War could not sustain its vaccination programme, but resumed its vaccination programme in 1994, which has most probably been disrupted during the third Gulf War. In 1995, Lebanon had not introduced hepatitis B vaccine to its EPI. It is believed that there are large numbers of children being vaccinated in the private sector in Lebanon but their number is unknown.

Although Yemen, the poorest country in the Arabian Peninsula is reported to have one of the highest HBV carrier rates in the Middle East, it was the last of these countries to introduce hepatitis B vaccine to its EPI. According to routine data the EPI vaccination coverage in 1991 for BCG, DPT (3 doses), Polio (3 doses) and Measles, respectively, was 99%, 89%, 89% and 74% by one year of age (Al-sowmely, 1998). More recently and realisticly, according to a proposal submitted by the Yemeni MOPHP to the Global

Alliance for Vaccines and Immunisation (GAVI), vaccine coverage in 2000 for BCG, DPT (3 doses), Polio (3 doses), and Measles, respectively, was 78.2%, 72.8%, 72.8%, and 71.4% by one year of age (MOPHP(B), 2001). In November 1998, the MOPHP introduced plasma-derived hepatitis B vaccine then changed to recombinant hepatitis B vaccine in December 1999, both purchased from Cheil-Jedang Corporation in South Korea. Both vaccines are low dose vaccines (1.5 micrograms) and their purchase in large quantities through UNICEF makes it an economically competitive and attractive option. There have been some studies showing that very low dose vaccines, as an economic option, produce acceptable immunogenic responses amongst infants of non-carrier mothers, similar to that of standard doses (Moyes et al., 1987). These studies have not investigated vaccine efficacy amongst infants of carrier mothers. The vaccine manufacturer Cheil-Jedang recommends giving double the dose to infants of carrier mothers (Cheil-Jedang, 1996). However, considering the reported high HBV chronic carrier rates amongst women of childbearing age in Yemen and the lack of routine screening for HBsAg amongst pregnant women allowing the identification of children at risk, it is highly unlikely that the vaccine manufacturer's recommendations are or will be implemented. This leaves infants born to HBV chronic carrier mothers at potential risk of acquiring HBV infection through perinatal transmission.

The first, second and third doses of hepatitis B vaccine, in Yemen, are administered at 2, 3 and 9 months respectively. The first and second doses of hepatitis B vaccine coincide with the first and second doses of DPT/Oral Polio vaccines and the third dose with Measles vaccine. As the MOPHP introduces the new pentavalent vaccine, consequently, the hepatitis B vaccine schedule will be modified and the first, second and third doses of hepatitis B vaccine will be given at 6 weeks, 10 weeks and 14 weeks, coinciding with the existing DPT/Oral Polio vaccine schedule (MOPHP(A), 2001). The BCG birth dose and Measles dose at 9 months will remain unchanged.

Data available from Yemen suggests that hepatitis B vaccine coverage was 9% in 1999 and increased to 14.8% in 2000 for all three doses of hepatitis B vaccine by one-year of age (MOPHP(B), 2001; MOPHP(C), 2001). In its five-year plan the EPI acknowledged that there was no independent assessment of hepatitis B vaccine coverage in Yemen performed in the last three years and expressed the importance that surveys must be

performed to assess hepatitis B vaccine coverage. According to the figures available it is clear that the MOPHP did not achieve its target of completely vaccinating 40% of children under one-year of age with hepatitis B vaccine. What the MOPHP claimed to achieve was 42% hepatitis B vaccine coverage with at least one dose of hepatitis B vaccine. This may be due to the later administration of the third dose of hepatitis B vaccine given with Measles at nine months of age. Nevertheless, according to MOPHP reports, uptake for Measles vaccine (71%) remained much higher than uptake for the third dose of HBV vaccine (9%), which was administered at the same time (MOPHP(C), 2001). A similar picture has been observed in South Africa where hepatitis B vaccine coverage dropped sharply from 99% to 53% to 34% for the first, second and third doses, respectively. In contrast polio vaccine coverage was maintained at 97 – 99% (Schoub et al., 1991). On the other hand, Gust et al found that integrating hepatitis B vaccine to the EPI increased children's contact with the EPI from 50% to 95%, and increased children fully immunised from 20% to 88% (Gust et al., 1991). Accordingly, it is probably incorrect to assume that uptake for a newly introduced vaccine will be good just because uptake for an existing vaccination programme is. However, one would expect good primary health care (PHC) services, a sufficient and sustainable supply of vaccine, adequate training of health care staff, and appropriate health education programmes to improve vaccine uptake and coverage. It remains to be seen if introduction of the pentavalent vaccine and the consequential reduction in the number of injections given to each child per visit, less time spent in health centre per visit, and better record keeping all contribute towards increasing hepatitis B vaccine coverage and the proportion of completely vaccinated children in Yemen.

1.7.2 Blood screening and safe injection:

All Middle Eastern countries routinely screen blood for HBsAg. Jordan and Egypt started screening as early as 1967 and 1970, respectively. In Yemen, it has been reported that occasional shortages of screening supplies occur (WHO, 1995). Some countries, such as Oman in 1993, have also started screening for hepatitis C virus (HCV).

The majority of Middle Eastern countries use disposable needles and syringes. It is also reported that they adequately sterilise surgical equipment and utensils (WHO, 1995).

1.8 Hepatitis B virus infection in Yemen

Yemen lies in the South-West corner of the Arabian Peninsula covering an area of 555,000 square kilometres. The Republic of Yemen was formed following the unification of former North and South Yemen in 1990. In 1999, the population of Yemen was approximately 18.5 million and had one of the highest population growth rates in the world (3.7%) with an estimated population doubling time of 20 years. The country is divided into 20 administrative regions called governorates (provinces) with each governorate consisting of a number of districts. The population of Yemen is widely dispersed throughout an irregular terrain of mountainous regions, coastal plain, desert, plateau, and to a much lesser extent islands. Yemen has a Gross National Product (GNP) per capita of 350 US$ and is categorised among the poorest countries in the world with approximately 50% of the population living below the poverty line and 30% of the population living in absolute poverty.

The overall public expenditure on the health sector is low in Yemen which accounts for only 1.4% of the Gross Domestic Product (GDP) and approximately 5% of total government expenditure. The MOPHP is responsible for all public health services in Yemen and is organised into three sectors: medical services and primary health care; health development and planning; and pharmaceutical and medical supplies; with an undersecretary chairing each sector. The EPI is part of the medical services and primary health care sector. Yemen adopted the primary health care approach to health services in 1978 and has utilised a traditional facility-based, three-tier health delivery system of health units, health centres and hospitals, which provide health care at three levels: primary health care supported by secondary and tertiary referral care. In Yemen, there are 711 temporary health units in small villages, 1149 fixed health units in medium size villages, 469 health centres in large villages, 79 district hospitals with 2968 beds, 25 governorate hospitals with 3517 beds and 14 specialised hospitals with 3814 beds (MOPHP(C), 2001). In 1999, there were 2.16 physicians and 5.9 hospital beds per 10,000 individuals in the general population. The widely dispersed population throughout Yemen, especially in rural areas where the population is spread over 65,000 rural villages and hamlets presents a significant challenge to the provision of health services. In 1995,

less than 50% of the total population and 30% of the rural population had access to health care (MOPHP(C), 2001).

Only a few studies have investigated the epidemiology of HBV infection in Yemen. These studies focused on the prevalence of HBV infection, risk factors associated with HBV infection, HBV genotyping, and on the etiologic agents of acute viral hepatitis in Yemen. These studies were mainly hospital-based seroepidemiological surveys carried out in the cities of Sanaa, Taiz, Hodeidah and Hajja. Study subjects were predominantly blood donors, pregnant women or outpatients. There were attempts to include rural populations into some of the studies in order to have a more representative sample of the general population. All the cities named above are located in the Northern Governorates (provinces) of Yemen (formerly North Yemen). No information has been found in the literature on the epidemiology of HBV infection in the Southern and Eastern regions of the country. Whether geographical differences between these regions and the Northern Governorates exist remains unknown. In a study by Scott et al, no statistically significant difference in the prevalence of hepatitis B carriers or seropositives was detected between the four different collection sites in North Yemen after controlling for age (Scott et al., 1990).

1.8.1 Acute viral hepatitis:

In a study investigating the etiologic agents of acute viral hepatitis (AVH) serum samples were collected from 78 patients in the acute phase of viral hepatitis. HBV was the most frequent causative agent being responsible for 26.9% (n=21/78) of cases of AVH. This was followed by hepatitis E virus (HEV) responsible for 14% (n=11/78) of cases. Hepatitis A virus (HAV), hepatitis C virus (HCV) and hepatitis D virus (HDV) were each responsible for 5.1% (n=4/78), 6.4% (n=5/78) and 2.6% (n=2/78), respectively, of cases of AVH (El-Guneid et al., 1997). In 51.3% (n=40/78) of cases of AVH no virological marker could be identified. These cases were not tested for Cytomegalovirus (CMV) and Epstein-Barr virus (EBV), however, the investigators suggested that these were unlikely to be the etiologic agents of AVH. As HBV mutants are becoming increasingly recognised and may not be detected with standard assays (Chen, 2000), a possibility

exists that HBV mutants may be responsible for these unclassified cases of AVH in Yemen.

These cases of AVH due to HBV infection are likely to be only the tip of the iceberg of HBV infection. In Yemen, the majority of infections occur during childhood and only 10% of infected children develop symptoms of acute viral hepatitis. Approximately 90% of infected children will have sub-clinical infections and pass undetected. Additionally, only 30 – 40% of infected adults develop symptoms with the remainder also passing unnoticed.

1.8.2 HBV chronic infection:

Studies in Yemen have shown the prevalence of HBV chronic infection to range from 7.4% to 18.5% amongst adults in the general population (Abdul Raheem et al., 1991; El-Guneid et al., 1993; Haidar NA, 2002; Ragaa et al., 2001; Sallam et al., 2002; Scott et al., 1990). The two studies carried out in the early nineties by El-Guneid et al and Scott et al found the prevalence of HBV chronic infection to be 12.7% and 18.5%, respectively (El-Guneid et al., 1993; Scott et al., 1990). El-Guneid et al suggested that the higher prevalence (18.5%) found in their study was probably due to differences in the prevalence of HBV markers in different geographic regions, age structure of the populations studied, and the use of more sensitive confirmatory tests for doubtful results. Such results would be confirmed by an enhanced enzyme immunoassay or using a third generation micro-particle enzyme immunoassay (El-Gunied et al., 1993). The total number of subjects with evidence of prior HBV infection was 399 (45.4%) and 321 (59.8%), respectively, in the studies conducted by Scott et al and El-Guneid et al. Scott et al and El-Guneid et al found the prevalence of HBeAg amongst HBV chronic carriers to be 9.7% (n=9/93) and 69.8% (n=50/86), respectively.

Scott et al and El-Guneid et al, respectively, showed the prevalence of HBsAg amongst pregnant women to be 12.5% (n=9/72) and 16.6% (n=40/241) ($p>0.46$). El-Guneid et al further found that out of 28 pregnant women positive for HBsAg, 31.1% were positive for HBeAg (95% C.I. 13.6% to 48.6%). Abdul Raheem et al found 15% of pregnant women positive for HBsAg and suggested that perinatal transmission occurred in 50% of cases (n=10/20) although they did not examine HBeAg amongst these pregnant women

(Abdul Raheem et al. 1991). Nevertheless, the high transmission rate presented in their study strongly suggests that a large proportion of these HBV chronic carrier women were HBeAg positive. This suggests a potentially high rate of perinatal transmission from HBsAg positive mothers contributing to the pool of HBV carriers in Yemen. Ideally this needed to be confirmed on a larger sample of women of childbearing age ascertaining the HBeAg and HBV DNA status of these women. It has been shown that the risk of perinatal transmission of HBV infection in countries with high HBV chronic carrier rates may reach 40 – 50% (Sobeslavsky, 1980).

A more recent study conducted on individuals attending at the central health laboratory in Sanaa in response to a campaign to control HBV infection found the prevalence of HBV chronic infection to be 7.4% (221/2321), and based on this the study investigators surprisingly proposed that Sanaa could be classified as an area of intermediate rather than high endemicity of HBV (Ragaa et al., 2001). There is no information on the nature of this campaign or how representative the respondents to the campaign were of the general population in Sanaa. The study by Ragaa et al provided the only existing information on the prevalence of HBV chronic infection amongst children less than five years which was 2.2%. The study investigators suggested that this was probably the result of perinatal transmission or early parenteral exposure through circumcision or ear piercing. Another study conducted from April 1997 to September 1999 investigating the prevalence of HBV infection amongst blood donors in a hospital in Hajjah, situated North of the capital Sanaa, found the prevalence of HBV chronic infection to be 9.8% amongst 7868 blood donors, and concluded that the endemicity of HBV infection was high in Hajjah governorate (Haidar NA, 2002).

Another recent study analysing the HBV core promoter sequences from blood donors at two blood banks in Yemen (Sanaa and Aden) found the prevalence of HBV chronic infection to be 10.7% (107/999) of whom 39% (42/107) had detectable DNA levels (Sallam et al., 2002). Surprisingly, only 3 (7.3%) out of 41 HBV DNA positive donors had detectable HBeAg when tested by commercial enzyme immunoassay. This raises doubts about the extent to which HBeAg positivity can be correlated with HBV DNA levels. As for genotyping, analysis performed on 18 surface gene sequences showed them to be of subtype ayw and with genotype D sequences.

Hepatitis Delta virus (HDV) super-infection was found among 2% of HBsAg positive sera in two studies (El-Guneid et al, 1993; Scott et al., 1990). This is lower than other Middle Eastern countries such as Kuwait and Saudi Arabia where super-infection with HDV occurred in 40% and 20%, respectively, of healthy HBV carriers (Toukan et al., 1990).

1.8.3 Chronic Liver Disease:

There is no national cancer registry in Yemen and it is important that one is developed in the near future, as in Saudi Arabia where a national cancer register was established in 1992 and all cancer cases in the country must be reported by law since 1994 (Al-Radi et al., 2000). Anecdotal reports by clinicians and hospital staff indicate that CLD is increasingly being recognised as a common health problem in Yemen. A study investigating the pattern and distribution of malignant neoplasms amongst Yemeni patients found gastrointestinal malignancies to be the most common cancers (22%) in Yemen (Al-thobhani et al., 2001). Within the gastrointestinal tract, the most commonly affected site was the liver (33%) and second most common type was hepatocellular carcinoma (Al-thobhani et al., 2001). This was consistent with earlier studies in the Southern-Eastern provinces in Yemen and studies in Saudi Arabia which also found gastrointestinal tract malignancies to be the most common cancers amongst patients in these populations (Bawazir et al., 1998; Al-Radi et al., 2000). In Saudi Arabia, HBV and HCV were suggested to be the main causative factor of hepatocellular carcinoma (Al-Radi et al., 2000). A model developed by the Vaccine Assessment and Monitoring (VAM) team at WHO headquarters estimates that over 2000 Yemeni adults die each year from hepatocellular carcinoma and cirrhosis resulting from HBV chronic infection (MOPHP(A), 2001). The evidence of an association between HBV and CLD is extensive across the globe (Arthur et al., 1984; Omer et al., 2001; Parkin et al., 1999; Toukan, 1996). In Yemen, HBV chronic carrier rates were significantly lower amongst healthy individuals (18.5%) than amongst patients with CLD (24.1%) ($p=0.03$) (El-Guneid et al., 1993). The presence of any HBV marker was 59.8% and 75.9% ($p=0.0016$) in healthy individuals and patients with CLD, respectively. Similarly, a study in Hajjah showed the prevalence of HBV chronic infection amongst patients suspected of having liver disease

(14.9%) was higher than healthy blood donors (9.8%) (Haidar NA, 2002). All this evidence strongly suggests that HBV infection plays a significant role in the etiology of CLD in Yemen and deserves further investigation.

1.8.4 Factors associated with HBV infection:

A number of different factors were associated with HBV chronic infection in Yemen by univariable analysis but only age, a history of jaundice, and a combined history of transfusion and surgery remained statistically significantly associated with HBV infection by multivariable analysis after adjusting for the remaining variables in the analysis (Scott et al., 1990). Multivariable analysis revealed age to be a highly significant risk factor for both HBV infection and HBV chronic infection. The adjusted odds ratio associated with increasing 10-year age intervals was 1.37 and 1.51 for HBV chronic infection and HBV infection, respectively (Scott et al., 1990). A history of surgery and blood transfusion were associated with HBV infection. However, these associations remained significant only when a combined history of blood transfusion and surgery was given [adjusted OR 2.76 (95% C.I. 1.11 to 6.82)] (Scott et al. 1990). A history of jaundice was also a significant risk factor for HBV infection [adjusted OR 1.42 (95% C.I. 1.01 to 2.01)]. When adjusted for age, neither sex nor qat chewing (a stimulant plant commonly chewed in Yemen) were significant risk factors associated with HBV infection (Scott et al., 1990). Similarly, a study in Hajjah found age to be significantly associated with HBV chronic infection by multivariable analysis but also found sex to be significantly associated with HBV chronic infection (Haidar NA, 2002).

No significant association was found between the presence of hepatitis B markers and parenteral treatment, visits to dentists and doctors, tattoos, and socioeconomic factors such as sewage disposal, water supply, electricity supply and household crowding (Scott et al., 1990).

1.9 Conclusion:

Upon reviewing the literature on the epidemiology of HBV infection and carrier status in the Middle East, HBV infection is clearly a major public health problem in many countries in the region. Toukan estimates that HBV infection might account for up to 2% of all eventual deaths in a Middle Eastern birth cohort (Toukan, 1996). In Jaizan province of Saudi Arabia the high HBsAg carrier rate of 19.9% amongst males was consistent with the provinces high male dominant prevalence of hepatocellular carcinoma (Arya et al., 1985). In Jordan there was a significantly higher prevalence of HBsAg in patients with CLD (54%) than in asymptomatic carriers (10%) (p=0.001) (Toukan et al., 1988). Similarly in Yemen, the prevalence of HBsAg amongst patients with CLD (24.1%) was significantly higher than asymptomatic carriers (18.5%) (p=0.03) (El-Guneid et al., 1993). The high prevalence of HBsAg amongst patients with CLD indicates that HBV infection most probably plays a significant role in the pathogenesis of CLD.

The majority of the countries in the region have an intermediate and high endemicity of HBV infection. In countries reporting low endemicity of HBV infection, it may well be that these figures are only the tip of the iceberg with many cases undetected due to the large number of sub-clinical infections, inadequate surveillance, diagnosis and underreporting.

There is marked variation in carrier rates between and within Middle Eastern countries. HBV chronic carrier rates range from as low as 1.5% in countries such as Kuwait to as high as 18.5% in countries such as Yemen. In Saudi Arabia, carrier rates witness a fourfold increase from one region to another. Why such differences exist within the same country remains unexplained. In Jordan, intra-country differences have been attributed to socioeconomic status. In the Saudi Arabian population, which is considered a relatively homogeneous population, socioeconomic status was not found to be an explanatory factor for these differences in prevalence of chronic carriers. Even when conducted on the same population, studies have produced different results. Many studies in the region have not examined age-specific prevalence of HBsAg carrier status, which makes examination of the pattern of infection in the region a difficult process. However, most of the studies in the region indicate that most transmission of HBV infection in the region occurs during the early years of life, with adult transmission being relatively uncommon.

Transmission of HBV infection occurring during the early years of life in the region, appears to be a combination of perinatal and childhood transmission. On one hand, a group of studies suggests that transmission predominantly occurs during childhood. On the other hand observing a high prevalence of infection amongst infants and a high rate of HBeAg amongst chronic carrier mothers in some settings suggests that perinatal transmission cannot be disregarded as an important mode of transmission contributing to the pool of HBV chronic carriers. Most probably the role played by either of these modes of transmission differs from one country to another. Obviously, determining the role either mode of transmission has will have important policy implications. If perinatal transmission is found to be a common mode of transmitting HBV infection, this will indicate that vaccinating children against HBV at 6-8 weeks of age will be too late to prevent mother-to-child HBV infection. Under such circumstances, one must aim to vaccinate newborns at birth or within 48 hours of delivery at the latest. In some settings, such as Yemen, where the majority of deliveries occur at home, introducing such a procedure will encounter numerous obstacles. One of the major concerns will be the difficulty of delivering vaccines to home deliveries occurring in remote areas considering that 75% of the Yemeni population lives in rural areas and is dispersed over approximately 65,000 rural hamlets.

Hepatitis B vaccination strategies may vary depending on the prevalence of chronic carriers, mode and age of infection, and health sector resources. Control measures vary from one Middle Eastern country to another. Some countries, such as Qatar have excellent control programmes. Not only do they vaccinate all newborns in the delivery room but they also screen and vaccinate eligible contacts of carriers. The effectiveness of these measures in reducing infection and carrier rates has not been examined. Another group of countries, such as Sudan, due to economic constraints, has not yet introduced hepatitis B vaccination, despite having one of the highest rates of chronic carriers in the region.

Yemen, the poorest country in the Arabian Peninsula has the highest prevalence of chronic carriers. There is limited information available on the pattern of transmission and age-specific prevalence of HBV in the population. The high prevalence of HBeAg amongst pregnant women found in one study and the high rate of materno-foetal

transmission suggested in another indicate that a high rate of perinatal transmission of HBV infection may exist. However, these findings require further confirmation by a larger and, most importantly, a more representative sample from the Yemeni population.

The studies conducted in the Middle Eastern region have collectively found that HBV infection is a serious public health problem in the region. Still many related questions specific to the region remain unanswered. Effectiveness of hepatitis B vaccine in preventing HBV infection and chronic carrier status, risk factors for infection and carrier status, the proportion of CLD attributable to HBV infection, the extent of perinatal transmission of HBV infection, and the pattern of HBV infection amongst different age-groups are only examples of the many questions that deserve further investigation.

Chapter 2: Study Methods:

2.1 Study objectives:

This study was conducted with the intention of satisfying the following objectives:

2.1.1 Primary objectives:

1- Measure the prevalence of HBV infection (hepatitis B core antibody positive) and HBV chronic infection (hepatitis B surface antigen positive) amongst children aged 1-3 years in Yemen and estimate the proportion of these cases resulting from perinatal transmission (i.e. the extent of perinatal transmission of HBV infection and chronic infection in Yemen).

2- Measure the prevalence of HBV infection, HBV chronic infection and hepatitis B e antigenaemia (HBeAg) amongst women of childbearing age in Yemen and estimate the potential risk of perinatal transmission of HBV from these women.

3- Investigate whether the prevalence of HBV infection and chronic infection amongst children and mothers varies between geographic regions and provinces in Yemen.

2.1.2 Secondary objectives:

1- Evaluate the effectiveness of the hepatitis B vaccination programme in preventing HBV infection and HBV chronic infection amongst children vaccinated in the country's Expanded Programme on Immunisation (EPI).

2- Measure hepatitis B vaccination coverage amongst children aged 1-3 years old participating in the study.

3- Examine the pattern of HBV infection and HBV chronic infection amongst children aged 4-9 years in Yemen prior to the introduction of HBV vaccination to these age-groups and its influence on the prevalence of HBV infection amongst these cohorts of children. This would serve as a baseline measure of the prevalence of HBV infection amongst children aged 4-9 years against which the effectiveness of hepatitis B vaccination in reducing the prevalence of HBV infection can be compared in the future.

To achieve these objectives a survey was conducted consisting of two main parts. Firstly, a population-based cross-sectional household sero-epidemiological survey of a representative sample of children aged 1-3 and their mothers (women of childbearing age). This was followed by a nested case-control analysis of children aged 1-3 and mothers participating in the survey. Secondly, a population-based cross-sectional sero-epidemiological survey of children aged 4-9 years, sampled from within the same households of children aged 1-3.

2.2 Cross-sectional survey amongst children aged 1-3 and their mothers:

2.2.1 Type of study:

A community-based cross-sectional household sero-epidemiological survey was conducted amongst Yemeni children aged 1-3 years and their mothers. This measured the prevalence of anti-HBc positivity and HBV chronic infection amongst children aged 1 – 3 and women of childbearing age, perinatal transmission of HBV, hepatitis B vaccine coverage, and geographic variation in the prevalence of anti-HBc positivity and HBV chronic infection.

2.2.2 Population:

This study was conducted on a representative sample of Yemeni children aged 1-3 and the mothers of these children participating in the survey.

2.2.3 Sample size:

Sample size was calculated separately for each of the study objectives. This was mainly due to the different population groups under investigation (mothers and children) and the different prevalence rates for the investigated variables expected amongst them. Expected prevalence rates were estimated based on the prevalence of the variable under investigation reported by previous studies amongst the relevant age-groups in Yemen or neighbouring Middle-Eastern countries, as well as the expected or known mode of transmission of HBV infection in the country or region.

All sample sizes were calculated using the WHO Practical Manual for Sample Size Determination (Lwanga SK, 1991) and EPI-INFO Version 6 using the STATCALC utility.

2.2.3.1 Prevalence of HBV chronic infection amongst children aged 1-3 years:

The sample size was calculated based on an expected HBV chronic infection prevalence of 10% amongst Yemeni children aged 1-3 and a desired precision of 3% (7-13%) with 95% confidence. The expected prevalence of 10% was based on a prevalence of approximately 18% for HBV chronic infection amongst adults in the general population in Yemen and evidence suggesting mother-to-child and horizontal transmission as being the major modes of transmission of HBV infection in the region. This sample size was adequate if the prevalence of HBV chronic infection was found to be 5%. In order to adjust for the clustering of the sampling process, it was estimated that it was sufficient to multiply the calculated sample size by a factor of 2 to adjust for the design effect of the survey and obtain the same desired precision. This was also done to ensure a sufficient number of study participants required for the case-control analysis. This was further extended to the remaining sample size calculations of children aged 1-3 and mothers participating in the survey.

Expected prevalence	10%
Level of Confidence	95%
Precision (7% - 13%)	3%

Table (2.2.3.1) shows sample size needed to measure the prevalence of HBV chronic infection amongst children aged 1-3 with a 2% and 3% precision at the 90 and 95% levels of confidence, adjusted for design effect.

Table 2.2.3.1
Sample size for measuring the prevalence of HBV chronic infection amongst children aged 1-3 years - adjusted for design effect (x2)

Expected Prevalence	Absolute Precision	Level of Confidence	Sample Size
10%	2%	95%	1728
10%	3%	95%	**768**
10%	2%	90%	1218
10%	3%	90%	542

2.2.3.2 Prevalence of HBV chronic infection amongst mothers:

The prevalence of HBV chronic infection amongst Yemeni women of childbearing age was expected to be approximately 16% with a desired precision of 3% (13-19%) at the 95% confidence level. The expected prevalence of 16% was based on a reported prevalence of 15% to 18% for HBV chronic infection amongst pregnant women in Yemen.

Expected prevalence	16%
Level of Confidence	95%
Precision (13% - 19%)	3%

Table (2.2.3.2) shows the sample size adjusted for design effect (x2) needed to measure the prevalence of HBV chronic infection amongst women of childbearing age with a 2% and 3% precision at the 90 and 95% levels of confidence.

Table 2.2.3.2
Sample size for measuring the prevalence of HBV chronic infection amongst mothers - adjusted for design effect (x2)

Expected prevalence	Absolute Precision	Level of Confidence	Sample Size
16%	2%	95%	2450
16%	3%	95%	**1088**
16%	2%	90%	1726
16%	3%	90%	766

The sample size required to achieve a 3% precision at the 95% level of confidence was 1088 women. This was large enough to cover the sample size required for measuring the prevalence of HBV chronic carriers amongst Yemeni children aged 1-3 (768 children).

The final sample size of children aged 1-3 and their mothers was rounded up to 1100 children aged 1-3 and their mothers to be included in the cross-sectional study, which was expected to be sufficient to fulfil the primary objectives of the survey with the desired power.

2.2.3.3 Hepatitis B vaccine coverage:

Measuring hepatitis B vaccine coverage was one of the secondary objectives of the study. The sample size of 1100 children was expected to be sufficiently large enough to estimate vaccine coverage to within 3 percentage points of the true value at the 95% level of confidence based on a vaccine coverage of 42% for all three doses by one year of age, as has been proposed in anecdotal evidence.

2.2.4 Sampling methods:

This survey followed a complex sampling scheme. This scheme was adopted to accommodate a number of factors. Firstly, there was no epidemiological data on HBV infection from the Southern and Eastern regions of the country (formerly South Yemen) and it was suspected that differences existed in the pattern and prevalence of HBV infection between these regions and the more mountainous regions in the North. Therefore, the study was designed in a way to ensure the inclusion of these regions in the study population. Secondly, the recently introduced hepatitis B vaccination programme was gradually introduced starting with one health centre in each governorate and is being further introduced to all health centres in provinces. Thirdly, it was intended to draw a sample representative of the prevalence of HBV infection in the country overall, which would provide generalisable information essential for public health policy-making.

The sample population of the survey was drawn from five main survey domains with each governorate (province) included in the study forming one survey domain. It was planned to draw an equal number of study participants from each survey domain. The governorates included in the survey were Sanaa City municipality, Aden, Taiz, Shabwa, and Hodeidah (excluding Taiz and Hodeidah cities). The population of these five governorates constitutes approximately 24 % of the country's overall population.

These governorates were selected because they are considered representative of Yemen's geographic regions, tribes and socioeconomic classes; include governorates from the Southern and Eastern regions of the country (former South Yemen); and because of the feasibility of doing a household survey in these governorates. The total population of these five governorates combined together is 68% rural and 32% urban. The sample selected from Sanaa City municipality (political capital) and Aden (economic capital)

was considered representative of urban populations, and the sample selected from Taiz, Hodeidah and Shabwa (which are mainly rural areas except for Taiz and Hodeidah cities, which were excluded from Taiz and Hodeidah governorates sample population) predominantly represented rural populations. This was adopted because of the difficulty in clearly dividing the population living in these governorates into urban and rural populations based on characteristics other than population size. The Central Statistical Organisation (CSO) in Yemen classifies any population exceeding 5000 individuals as urban. However, there are many populations in Yemen exceeding 5000 individuals that cannot be correctly considered urban populations due to the lack of essential services and infrastructure that an urban population would normally have. Additionally, population size projected for 2001 by the CSO was only done at the district level with no projections done at the village and town level. To have this done at the village level for each and every population in the five governorates of the survey would be time consuming and expensive. Moreover, the changes that took place in geographic and administrative divisions in 1999 significantly changed governorate population size and require new projections to be done at the village level.

Households were selected from within these five provinces and any child aged 1-3 years, regardless of sex, fulfilling the inclusion criteria whose parents witnessed verbal consent was obtained participated in the survey. The sampling methodology performed was similar to that developed by the EPI of the WHO, which is a type of cluster sampling survey, used to estimate vaccination status among young children (Bennett et al., 1991). Within each province, five districts/enumeration areas were selected as primary sampling units based on probability proportional to size (PPS). This was carried out by creating a cumulative population list for all the districts/enumeration areas in each of the five provinces and selecting a systematic sample starting from a random point. The sampling interval was calculated by dividing the total population size of the province by the number of districts/enumeration areas to be selected (in this survey five). A number lying within the range of this sampling interval was randomly chosen, using Epi-info, and was fitted into position in the cumulative population list and whatever district this number was part of was taken as the first district/enumeration area to be sampled in the province. The sampling interval was then added to the random number to select the second

district/enumeration area and this process was repeated until five districts/ enumeration areas were selected from each province. Data on population size was based on population size and distribution projections for 2001 prepared by the Yemeni CSO.

The minimum number of individuals selected from each district/enumeration area was 44 children aged 1-3 and their mothers equally sampled from two different starting points in the district/enumeration area (minimum 22 children aged 1-3 and mothers from each of the two starting points in the district/enumeration area). This gave every individual living in the province an equal probability of being selected (self-weighting sampling procedure). The daily minimum target number of children aged 1-3 and mothers sampled was 22 with no more than two days spent in each district/enumeration area.

2.2.4.1 Selection of households:

Three teams of study investigators participated in the fieldwork throughout the survey and these would either split up in the streets of towns and cities or in the compounds and aggregations of households of villages. The teams would visit one household after another until the daily target number of children aged 1-3 and mothers was achieved.

Different procedures were used for the selection of individuals from rural governorates (Taiz, Shabwa, and Hodeidah) than urban governorates (Sanaa City municipality and Aden).

Once the five districts from Taiz, Hodeidah and Shabwa were selected based on PPS sampling using the CSO projected district population size and distribution data for 2001, villages and towns were selected from within these districts based on PPS (as described in the selection of districts within provinces) using these district's village and town cumulative population list projected for 2001 specifically prepared for the study. Within villages and towns households were randomly selected. The first household to be sampled in a town/village was selected based on selecting a central point or street in the town/village, numbering the households on that street or vicinity and randomly selecting one of the households using numbered cards. If the household was not inhabited by a child aged 1-3, a coin was tossed (to determine whether to go right or left) and when appropriate every third household was visited until an eligible household was identified.

However, if this was an aggregation of households in a village and there were not many households then all the households were visited. Once identifying and gaining access to an eligible household and after obtaining consent an interviewer administered questionnaire was completed and children aged 1 – 3 years and their mothers were bled to test for serological markers of HBV infection. Only one child aged 1 – 3 per mother was included in the study sample. If more than one child aged 1 –3 per mother was present in the household, one of these children was randomly selected, by tossing a coin, for inclusion to the study sample. If the study investigators failed to obtain a blood sample from the randomly selected child aged 1-3 then the study investigator would obtain a sample from the remaining child aged 1-3. If there was more than one mother with a child aged 1 –3 in the household, all consenting mothers with a child aged 1 –3 present in the household were included in the study sample. Within a household each mother and child pair that were eligible were considered to be a family unit. Once the first consenting household had been surveyed all the neighbouring households (starting with the nearest) in the village or town were approached until the target number of 22 children and their mothers from this starting point of the primary sampling unit had been attained. It was initially planned to use village/town maps when available to mark surveyed households and streets in villages/towns, however, most commonly none of these were available nor were these needed.

In villages usually all the households in the village had to be visited to obtain the target number of children aged 1-3. If a village was completely surveyed and the target sample size from this starting point of the primary sampling unit had not been achieved, the team would move on to the nearest village and the same process for selecting the first and subsequent households for sampling was repeated in the next village until the target number of children aged 1-3 and mothers was attained. The same process was repeated in all five districts of the three rural provinces (Taiz, Shabwa and Hodeidah). The process implemented at the second starting point was identical to what was done at the first starting point.

In Sanaa City municipality and Aden, which are urban areas and for which maps were available, five enumeration areas were selected as primary sampling units based on PPS.

Population size for enumeration areas was projected for 2001 with a fixed number of 44 individuals selected from each enumeration area. Within these enumeration areas, sampling was initiated from a street blindly pointed out from a map of this enumeration area. The first household to be sampled on the street was selected based on numbering the households on the street and randomly selecting a household from the street using numbered cards. If the household was not inhabited by a child aged 1-3, a coin was tossed (to determine whether to go right of left) and every third household was visited until an eligible household was identified. Once identifying and gaining access to an eligible household and after obtaining consent an interviewer administered questionnaire was completed and both child aged 1 – 3 years and the mother were bled and tested for serological markers of HBV infection. Only one child aged 1 – 3 per mother was included in the study sample. If more than one child aged 1 –3 per mother was present in the household, one of these children was randomly selected, by tossing a coin, for inclusion to the study sample. If the study investigators failed to obtain a blood sample from the randomly selected child aged 1-3 then the study investigator would obtain a sample from the other child aged 1-3. If there was more than one mother with a child aged 1 –3 in the household, all consenting mothers with a child aged 1 –3 present in the household were included in the study sample. Within a household each mother and child pair that were eligible were considered to be a family unit.Upon the completion of surveying the first household all the households that roughly lied within a 50 metre radius of the first household were surveyed including to the study population all eligible consenting households inhabited by a 1 –3 year old child until the target number of 22 children aged 1-3 and their mothers from this starting point of the primary sampling unit had been attained. If no more eligible households could be identified within the 50-metre radius the team would move on to the nearest street and the same process for selecting the first and subsequent households for sampling was repeated until the target number of children aged 1-3 and mothers was attained. The process implemented at the second starting point was the same as what was done at the first starting point. The same process was repeated in all five enumeration areas of both urban provinces (Sanaa City municipality and Aden).

2.2.4.2 Community participation:

In order to maximise community co-operation mainly in rural areas attempts were made to promote community acceptance. Prior to surveying a village or town, community leaders, tribal leaders, village elders, and relevant local authorities were informed about the nature of the visit of the study investigators. Appropriate meetings and discussions were held focusing on the objectives and benefits of the survey. These community leaders in turn were requested to encourage the village population to participate in the survey and co-operate with study investigators. This proved to be an effective mechanism of leading study participants to feel part of the survey and perceive it to be for their benefit, thereby improving their cooperation. Moreover, following the selection of a village or town at least one local facilitator was identified in advance, or contacted on the day of the fieldwork, prior to visiting households, and recruited to maximise the co-operation of the village inhabitants and to assist in the days activities in the area.

Inclusion criteria:

All children aged 1-3 years and their mothers willing to participate in the survey from whom witnessed verbal consent was obtained.

Exclusion criteria:

1- Children and mothers refusing to participate in the study (children's exclusion was based on their guardian's refusal to allow them to participate in the study).
2- Seriously ill children or children and mothers with a history of bleeding disorders would have been excluded although such children were not encountered.

2.2.4.3 Obtaining consent:

Upon identifying and gaining access to a household occupied by a child aged 1-3 years the team of study investigators (consisting of two individuals) would introduce themselves or be introduced by a facilitator to the head of the household and/or the mother of the child. In order to ensure that the same correct and complete information was given to each study participant, study investigators used an information sheet to give

the head of the household and/or the mother of the child a brief explanation of the research objectives. They were also informed that the visit to their household would involve two main procedures. The first was the completion of an interviewer administered questionnaire. The second was obtaining a blood sample from both mother and child to test for markers of HBV infection. Potential participants in the study were provided with sufficient information on potential risks and benefits to enable them to make an informed decision on whether or not to participate in the study. Study subjects were reassured on confidentiality and safety issues involving the questionnaire and blood sampling and were then asked if they had any questions. These were answered by the study investigators. Literate parents were offered an Arabic language information sheet prepared specifically for the household survey describing the research objectives, procedures to be carried-out in the household, and the benefits from the study (refer to information sheet, Annex III).

Following the above, study participants were asked for their witnessed verbal consent for the mothers and child's inclusion in the study sample of the cross-sectional survey. The reason verbal consent was requested rather than written consent is because approximately 75% of the Yemeni population is illiterate and this is more pronounced in rural areas. Requesting study participants to provide written consent would have been difficult and impractical. It was attempted to obtain the consent of both mother and father of the child. If the father was not present the mother was asked if the father might object to their inclusion in the study sample (which only happened on a few occasions). In these cases an attempt was made to obtain the fathers consent. When either parent refused the inclusion of the child and mother in the study population, efforts were made to address their concerns and alleviate their fears and they were encouraged to participate in the survey. If however, following this either parent refused to participate in the survey, the household members were not included in the study sample, with subjects refusing to participate being free to do so at their own wish. Out of the 1577 surveyed eligible households that were inhabited by a child aged 1-3 years old, 16% (260) refused to allow their child to participate in the survey for various reasons such as fear of injections, side effects, complications, or suspicions about the intentions of the study investigators.

2.2.4.4 Questionnaire:

Individuals participating in the survey were subjected to an interviewer administered questionnaire. The questionnaire focusing on the child aged 1-3 and the mother asked about the child's personal and vaccination history, mother, father, the household's characteristics and socioeconomic status (refer to questionnaire I, Annex I). The questionnaire did not include any sensitive or embarrassing questions.

The mother or father of the child was the target person to be interviewed. If this was not possible the aunt, uncle, grandfather or grandmother of the child was interviewed.

Interviews were conducted in study participant's households and the questionnaire was administered and filled by one of the study investigators participating in the survey specifically trained for this purpose. The study investigator would systematically go over the questions reading them out as written and fill the form according to the response of the person interviewed. This was expected to minimise inter-observer and responder bias, especially because both respondent and investigator did not know the child's serological status at the time the interview was conducted. Chapter 3 presents a full description and discussion of the serological tests performed on study participants.

2.2.4.5 Quality control:

Everyday after completing the household survey the primary study investigator would review every questionnaire completed by the other study investigators as well as questionnaires completed by himself. During this process the primary study investigator would check questionnaires for errors, missing information and inconsistencies, and filled the coding column on the questionnaire preparing data from the questionnaire to be entered into the database. If errors, inconsistencies, or missing information was detected the study investigator completing the questionnaire was asked to clarify these issues. It was expected that having conducted the interview on the same day the interviewer would have a better recollection and memory of the issues s/he might be asked about compared to if s/he had been asked about this at a later date. If however, there was missing information the study investigator could not confidently clarify it was left blank and was coded as missing data. On a few occasions, when it was practical and reasonable,

information on hepatitis B vaccination status recorded on vaccination cards was verified by checking children's vaccination records retained at vaccinating health centres. Quality control checks and procedures were also implemented throughout the process of data entry and analysis of data (refer to Chapter 4 Data Description and Methods of Analysis).

2.3 Case-control analysis:

Following the cross-sectional survey and analysis a case-control analysis was performed on children aged 1-3 years and their mothers, and children aged 4-9 participating in the cross-sectional survey, drawing cases and controls according to the variables or the outcome being investigated.

The case-control study analysed:

1- The effectiveness of the EPI hepatitis B vaccination programme in preventing HBV infection (anti-HBc positivity) amongst children by comparing HBV infection amongst vaccinated and unvaccinated children.
2- The effectiveness of the hepatitis B vaccination programme in preventing HBV chronic infection amongst children by comparing HBV chronic infection amongst vaccinated and unvaccinated children.
3- Variables associated with hepatitis B vaccine coverage amongst children aged 1-3 years.
4- Variables associated with anti-HBc positivity and HBV chronic infection amongst children aged 1-3 and mothers, as well as children aged 4-9 years.

2.3.1 The effectiveness of hepatitis B vaccination in preventing HBV chronic infection:

Cases:

HBV chronic carrier children aged 1-3 years (children repeatedly positive for HBsAg and negative for IgM anti-HBc).

Controls:

Children negative for hepatitis B surface antigen.

Exposure:

Hepatitis B vaccination. Children receiving all three doses of vaccine in whatever schedule were considered completely vaccinated. Children receiving one or two doses were considered incompletely vaccinated children.

This allowed calculation of the odds of exposure which estimated the relative odds of HBV chronic infection amongst completely vaccinated compared to unvaccinated children. Sample size was calculated based on an expected 42% vaccination coverage (based on anecdotal evidence) amongst children negative for HBsAg (controls) and an expected relative risk of 0.3 with an 80% power at the 0.05 level of significance. Table 2.3.1 shows that 38 cases were needed to carryout the analysis with an 80% power. All sample sizes for the case-control analysis were calculated using Power Epicentre Software version 1.3 by Dr. Inskip and MRC Laboratories.

Table 2.3.1
Number of cases required in analysis for estimating the effectiveness of hepatitis B vaccination in preventing HBV chronic infection amongst vaccinated children. Control case ratio 9:1, power = 80%, level of significance = 0.05

	Proportion vaccinated amongst controls						
Relative Risk	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**
0.2	105	51	34	25	20	17	16
0.3	146	73	49	**38**	32	28	27
0.4	210	107	74	58	50	46	47
0.5	317	165	116	94	83	79	82

It was predicted that if the prevalence of HBV chronic carriers generated from the sero-epidemiological survey of children aged 1-3 years was 5 – 10%, a sufficient number of cases would be available to allow the case-control analysis with an 80% power at the 0.05 level of significance.

2.3.2 The effectiveness of hepatitis B vaccination in preventing anti-HBc positivity:

Cases:

HBV infected children aged 1-3 years (children repeatedly positive for anti-HBc).

Controls:

Children negative for hepatitis B core antibody.

Exposure:

Hepatitis B vaccination. Children receiving all three doses of vaccine in whatever schedule were considered completely vaccinated. Children receiving one or two doses were considered incompletely vaccinated children. This allowed calculation of the odds of exposure which estimated the relative odds of HBV infection amongst completely vaccinated compared to unvaccinated children.

Sample size was calculated based on an expected 42% vaccination coverage amongst children negative for anti-HBc and an expected relative risk of 0.3 with an 80% power at the 0.05 level of significance. Table 2.3.2 shows that 42 cases were needed to carryout the analysis with an 80% power. The cross-sectional survey was expected to generate a sufficient number of cases for estimating the effectiveness of HBV vaccination in preventing HBV infection with an 80% power.

Table 2.3.2
Number of cases required in analysis for estimating the effectiveness of hepatitis B vaccination in preventing anti-HBc positivity amongst vaccinated children. Control case ratio 4:1, power = 80%, level of significance = 0.05

	Proportion vaccinated amongst controls						
Relative Risk	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**
0.2	116	57	38	28	23	20	19
0.3	161	81	55	**42**	36	32	32
0.4	232	119	82	65	56	53	53
0.5	352	184	130	105	93	89	93

Inclusion and exclusion criteria in case-control analysis for estimating the effectiveness of hepatitis B vaccination in preventing anti-HBc positivity and HBV chronic infection:

Inclusion criteria:

Children aged 1-3 years who had a full and accurate vaccination history whether by vaccination cards or vaccination records from a vaccinating health centre.

Exclusion criteria:

Children aged 1-3 years from who an accurate vaccination history could not be obtained.

2.4 Cross-sectional survey amongst children aged 4-9 years:

2.4.1 Type of study:

A population-based cross-sectional household sero-epidemiological survey was conducted amongst Yemeni children aged 4-9 years in order to examine the pattern and age-specific prevalence of HBV infection and chronic infection amongst unvaccinated Yemeni children. This would provide a baseline measure of HBV infection and chronic infection amongst a cohort of unvaccinated children upon which the effectiveness of the hepatitis B vaccination programme in reducing HBV infection and chronic infection could be evaluated in the future. It would also give a picture of the pattern of childhood transmission of HBV infection in Yemen.

2.4.2 Population:

This study was conducted on a representative sample of Yemeni children aged 4-9 years.

2.4.3 Sample size:

Sample size was calculated based on an expected prevalence for HBV chronic infection of approximately 10%, based on a prevalence of approximately 18% for HBV chronic infection amongst adults in the general population in Yemen and evidence suggesting mother-to-child and horizontal transmission as being the major modes of transmission of HBV infection in the region with a desired precision of 3% (7%-13%) at the 90% level of confidence. The sample size was multiplied by 1.5 to adjust for the design effect in the study. Sample size was calculated using the WHO Practical Manual for Sample Size Determination (Lwanga SK, 1991) and EPI-INFO Version 6 using the STATCALC utility. Table 2.4.3 shows that this can be achieved with a sample size of 407 children. This was rounded up to a target number of 420 children aged 4-9.

Table 2.4.3
Sample size for measuring the prevalence of HBV chronic infection amongst Yemeni children aged 4-9 years - adjusted for design effect (x 1.5)

Expected Prevalence	Absolute Precision	Level of Confidence	Sample Size
10%	3%	95%	576
10%	3%	90%	**407**

It was planned to equally divide the sample size over the six years of age (age-groups) extending from 4-9 years, with 70 children sampled from each year of age. It was also initially intended to sample an equal number of children from each year of age (age-group) from each province. In other words approximately fourteen children would have been randomly selected for each of the six age-groups extending from 4-9 years from each survey domain giving a total of eighty-four children aged 4-9 from each of the five survey domains.

2.4.4 Sampling methods:

At the same time as the household survey amongst children aged 1-3 years was being carried out, any child present in the household aged 4-9 years was considered eligible to participate in the cross-sectional survey of children aged 4-9 years.

From approximately 50% of the households of children aged 1-3, children aged 4-9 years were randomly selected for inclusion to the survey of children aged 4-9. Prior to filling in the interviewer administered questionnaire and blood sampling of these 4-9 year old children, parents or guardians were also requested for their witnessed verbal consent for the inclusion of these children to the study sample of children aged 4-9 (as was done for children aged 1-3). Parents or guardians refusing were free to do so at their own wish.

Upon obtaining witnessed verbal consent from the child's parent/guardian, children participating in the survey of children aged 4-9 were subjected to the completion of an interviewer administered questionnaire and the withdrawal of a blood sample for serological testing, similar to what occurred with children aged 1-3.

The questionnaire focusing on the child aged 4-9 was similar to the questionnaire of children aged 1-3 and asked about the child's personal and vaccination history, mother, father, the household's characteristics and socio-economic status (refer to questionnaire

II, Annex II). The sample quality control procedures described in 2.2.4.5 were applied to the questionnaires of these children as well.

2.5 Staff recruitment and training:

The group of study investigators conducting the fieldwork consisted of a minimum of six members divided into three teams with two study investigators in each team. Each team consisted of a qualified medical doctor or psychologist and a qualified nurse or laboratory technician. One of the qualified medical doctors was the primary study investigator. The qualified medical doctors/psychologists were recruited based on having a minimum of three years experience in their profession or a reasonable relation and background with the governorates or villages surveyed. Nurses/laboratory technicians were recruited based on having a minimum of three years experience in a paediatric ward and active involvement in drawing blood samples from children and infants. Depending on the circumstances different study investigators had to be recruited in some of the governorates surveyed.

Study investigators were trained on all aspects of the survey such as filling questionnaires and blood sampling during a two-week workshop prior to initiating the seroepidemiological survey. During this workshop study investigators learnt about the types of hepatitis virus infections, the objectives of this survey, survey methodology, and the types of bias and how to avoid them. They were trained on selecting and approaching households, procedures within households, inclusion and exclusion criteria of study participants, obtaining consent, blood sampling and handling procedures, how to conduct the interviewer administered questionnaire, specific responsibilities during the survey, and finally health and safety guidelines. Every study investigator was given a survey manual specifically prepared for this survey which contained all the necessary information and guidelines of any procedures which were implemented throughout the survey (refer to fieldworker manual, Annex IV). Study investigators were advised to keep the fieldworkers manual with them throughout the survey as a reference. Additionally, throughout the fieldwork, each team of study investigators had continuous 2-way radio contact with the primary study investigator in case they needed his advice, instructions, or feedback.

2.6 Ethical issues:

The seroepidemiological survey was designed taking into account the confidentiality, freedom, anonymity, benefits, and safety of study participants.

Ethical approval for this study was obtained from the Ministry of Public Health and Population in Yemen and from the Ethics Committee of the London School of Hygiene and Tropical Medicine.

2.6.1 Confidentiality:

During the interview and prior to filling the questionnaire respondents were asked about their preferences on the presence or absence of subjects in the room. Every effort was made to meet the interviewee's preferences. No more than two study investigators were present in the room at the same time when the questionnaire was being filled. Whilst the interview was being carried out, individuals present from the study subjects side were kept to a minimum, unless this was contrary to the respondents wishes. After filling the questionnaire strict confidentiality was ensured for the respondents personal and household characteristics and details. Completed questionnaires were labelled with a unique identification number for both mother and child separately, and kept in a safe and inaccessible place where they were kept under lock and key. No one other than the primary study investigator had access to individuals completed questionnaires. No information on study participants was discussed or disclosed to anybody else.

2.6.2 Anonymity:

Blood samples sent for laboratory analysis were labelled with the unique identification number of the study participant. Consequently, the primary study investigator as well as laboratory staff handling and testing samples were unable to identify study participants. Laboratory results were recorded for identification numbers and not named individuals (i.e. maintaining anonymity of study participants).

At the stage of data analysis the only individuals gaining access to the study subject's details were the primary investigator, his supervisor, and his statistical advisor.

2.6.3 Freedom:

Only individuals from whom witnessed verbal consent was obtained were included in the study population. Subjects refusing to participate in the survey were free to do so at their own wish and were not included in the study sample.

2.6.4 Safety:

A basic principle of health interventions and epidemiological survey's is first to do no harm to study participants, whether this harm may be mental or physical.

It was anticipated before the study was carried out that discomfort or distress amongst study participants may result from the interviewer administered questionnaire or venesection of mother and more importantly the child. Therefore, this was taken into consideration when the questionnaire was designed. First of all the questionnaire did not investigate sensitive or culturally unacceptable issues. Secondly, there were no embarrassing questions. Thirdly, the focus of the questionnaire was on the child and to a lesser extent the mother, which was less likely to embarrass the mother. Finally, the questionnaire was kept as short as possible.

Venesection was the main procedure that may have caused discomfort to subjects participating in the study. This was more common amongst children than adults mainly because children perceive venesection as a more frightening experience than adults and because children were more likely to perceive the study investigators in their household as strangers and dislike contact with them. This would reach its peak when the child was held to obtain the blood sample. Therefore, attempts were made to minimise this occurring especially whilst the questionnaire was being completed. During the interview the study team attempted to be as friendly, nice and playful with the child as possible. Before the blood sample was withdrawn the mother was requested to hold the child giving him/her a sense of security. Following this one study investigator calmly and firmly held the child in place and distracted his/her attention from the other study investigator performing venepuncture.

As far as household safety was concerned, the medical doctor/psychologist in the team of study investigators visiting households was responsible for supervising the

implementation of health and safety guidelines and would have dealt with health related events if they occurred.

It was anticipated that the main potential health and safety hazards in this study would result from the sampling, transportation and storage of blood and the disposal of used needles. In order to ensure the safety of study subjects, study investigators and the environment the following measures and guidelines were strictly implemented:

1- All blood samples were collected aseptically using alcohol swabs and sterilised disposable needles.
2- The study investigator withdrawing blood ensured that there was no bleeding from the site of venepuncture and no contamination of the household with drops of blood. The study investigator applied a bandage to the site of venepuncture when needed.
3- Children sampled for blood were held in their mother's lap in order to reassure the child. When withdrawing blood samples from children both study investigators with the co-operation and permission of the mother held the child in a secure and fixed position. This was to prevent injury that may result from sudden movements or attempts by the child to remove the needle that may occur at the time of blood sampling or due to the sting of the needle.
4- All products involved in blood sampling such as used needles, cotton wool and bandages were disposed of in a safe needle disposable box, which was taken with the team back to base and disposed of in the local health centre at the end of each working day. Under no circumstances were used or unused items involved in blood sampling procedures left in households of study participants.
5- For transportation, blood samples were collected in sealed vaccutainers. This prevented any spillage or leaking of blood. Sealed vaccutainers were held in racks and stored in mobile refrigerated blood transportation systems.
6- Upon arrival to base, blood samples were centrifuged and serum was separated from the residual blood clot. The serum from each study participant was divided into two serum samples, one original and one backup, which were stored in sealed cryovials in a freezer. Residual products were disposed of safely.

7- Throughout the process of handling blood or its derivatives study investigators were wearing disposable gloves.

8- Study investigators participating in the study were screened for hepatitis B surface antigen to exclude any of them being a HBV chronic carrier.

9- Study investigators, particularly those handling blood, were asked if they were vaccinated against HBV. Those who were not already vaccinated were advised to get vaccinated as early as possible before the fieldwork was initiated and were offered free hepatitis B vaccination.

Chapter 3: Laboratory Methods:

In this chapter the methods of handling and storage of samples will be described, serological tests and assays performed will be presented, and the interpretation of these serological tests according to the manufacturer's recommendations will be explained. Finally, internal and external quality control measures and procedures to ensure reliable and valid results will be discussed.

3.1 Serum sample handling and storage:

The primary study investigator ensured that serum samples were adequately stored and handled throughout the survey. The primary study investigator also supervised the laboratory processing and handling of samples and attended as an observer approximately 25% of the laboratory assay shifts and discussed relevant issues with laboratory technicians performing the runs or the laboratory manager. The serum samples were processed at Sanaa Medical Laboratory, Hadda Street, Sanaa, Republic of Yemen. This laboratory was selected after assessing all the laboratories in Yemen capable of performing the assays and was visited by Professor Andrew Hall and the primary study investigator on 10th July 2001 to discuss with the manager assay procedures, quality control and related issues. Serum samples were stored, handled and processed as follows:

1- Serum was separated from blood after it was allowed to clot at a temperature between 10 to 37 degrees centigrade, as this prevents the aggregation of immunoglobulin in the clot.
2- Laboratory technicians and study investigators handling or processing serum specimens were all vaccinated with hepatitis B vaccine.
3- The serum sampled from every subject participating in the survey was placed in two separate cryovials. One was referred to as the original sample and the second was referred to as the backup sample. This was done to avoid repeated freezing/thawing cycles (which were kept to a minimum) or an insufficient quantity of serum. The second sample was used as a backup sample in case anything went wrong with the original one or its quantity was insufficient.
4- All samples, original and backup, were placed in cryovials with a plastic screw cap.

5- Cryovials were labelled using pre-printed labels capable of withstanding wetting and freezing with the corresponding study subjects identification number (IDNO) (children aged 1-3 IDNO 1001 to 2286, children aged 4-9 IDNO 7001 to 7469, and mother IDNO 4001 – 5286).

6- Serum samples were stored at -10 to -20 degrees centigrade in chest freezers with lid locks and temperature warning lights.

7- Original and backup samples were stored in different freezers placed in two different locations that both had a backup electricity supply in case there were any power failures and were checked everyday, once in the morning and once at night.

8- Once a serum sample was frozen it remained frozen until the time of serological testing in order to avoid bacterial contamination. This is also why sterile vaccutainers were used for the collection of blood and the skin puncture site was cleansed with an antiseptic prior to blood collection.

9- Twenty-four hours prior to performing the first serological assay, samples were taken out of the freezer and placed in a refrigerator overnight.

10- After samples were allowed to thaw in a refrigerator overnight these were removed from the refrigerator early in the morning of performing the serological assay.

11- Serum samples and all human sourced materials (e.g. positive controls) were handled on a potentially infectious basis and using disposable gloves.

12- Before pipetting serum samples the cryovials were mixed by gently inverting the cryovials a few times ensuring the mixture of any precipitate settled on the base of the cryovials during their storage, in order to ensure the consistency of the results.

13- Haemolysed samples or samples showing particulate matter were centrifuged prior to testing in order to avoid inconsistent results.

14- When it was recommended by the manufacturer's assay procedures, serum samples were centrifuged prior to pipetting and performing the analysis (IMx HBsAgV2, IMx AUSAB) or prior to repeat testing (IMx HBe2, IMx HBsAgV2).

15- The plastic screw caps of cryovials were removed and 200 micrograms of serum was pipetted from each cryovial and placed in the reaction cell (sample well) of the micro-particle enzyme immunoassay (MEIA).

16- The plastic screw caps were replaced onto the cryovials, which were put back in the refrigerator until the next serological assay was performed according to the order of serological tests described below.

17- A new sterile disposable pipette tip was used for each serum sample in the survey and caution was exercised in handling patient specimens to prevent cross contamination of specimens or the contamination of the laboratory with drops of serum. No serum samples were pipetted by mouth.

18- The sample IDNO on the cryovial was cross-checked with the sample IDNO entered into the IMx system in order to ensure that there were no numbering errors of samples.

19- According to manufacturer's recommendations all specimens requiring repeat testing or that were Gray Zone Reactive (GZR) were centrifuged at 8000 Relative Centrifugal Force (RCF) for 10 minutes prior to any repeat or confirmatory testing. GZR specimens are specimens with S/N (Sample rate/Calibrator rate) between 1.6 and 2.0. The German Federal Public Authority (Paul Ehrlich Institute, Langen, Germany) recommends that GZR specimens be retested (Abbott, 2000).

3.2 Serological tests:

3.2.1 Children:

Serological tests on the serum of children aged 1-3 and 4-9 were carried out according to the following order:

1- All children were tested for HBsAg.
2- Children initially positive for HBsAg or GZR were retested for HBsAg.
3- All children initially positive for HBsAg were tested for Immunoglobulin-M hepatitis B core antibody (IgM anti-HBc) with the intention of retesting IgM anti-HBc positive samples. However, none of the samples was initially IgM anti-HBc positive so no retesting had to be done for this serological marker.
4- All children were tested for total hepatitis B core antibody (anti-HBc).
5- Children initially positive for anti-HBc were retested for anti-HBc.

6- Children aged 1-3 with evidence of hepatitis B vaccination were tested for hepatitis B surface antibody (anti-HBs), which was measured in milli-international units per millilitre (mIU/mL).

3.2.2 Mothers:

Serological tests on mother's sera were carried out according to the following order:

1- All mothers were tested for HBsAg.
2- Mothers initially positive for HBsAg or GZR were retested for HBsAg.
3- All mothers initially positive for HBsAg were tested for hepatitis B e antigen (HBeAg).
4- Mothers initially positive for HBeAg were retested for HBeAg.
5- All mothers were tested for total anti-HBc.
6- Mothers initially positive for anti-HBc were retested for anti-HBc.

Serum samples were tested for all of these serological markers (HBsAg, IgM anti-HBc, total anti-HBc, HBeAg, and anti-HBs) using Microparticle Enzyme Immunoassay (MEIA) referred to as MEIA from here onwards.

3.3 Serological assays:

Serological assays were performed on the IMx System (Abbott Laboratories, USA) using the IMx System Software Module version 6.0 minimum and the IMx Hepatitis B Assay Module version 11.0 minimum. All the laboratory tests were performed in Sanaa starting on the 17th October 2001 and were completed by the end of January 2002. The laboratory equipment was accurately calibrated and maintained in accordance with the manufacturer's recommendations. Reagent kits, calibrators, controls, and diluent buffers were stored and used according to manufacturer's instructions and recommendations. Prior to performing serological assays all reagent kits, calibrators, controls, and diluent buffers were checked to ensure that none of these was beyond or reaching the expiry date. What follows is a presentation of the serological assays performed on the serum samples of study participants.

3.3.1 Abbott/IMx HBsAg(V2) kit (No. 2228-22):

The IMx HBsAg(V2) assay is a qualitative third generation MEIA for the detection of HBsAg in human serum or plasma. This assay was designed for use with human serum or plasma from individual patient and donor specimens (Abbott, 2000).

The HBsAg(V2) assay kit consists of IMx HBsAg(V2) Reagent Pack, 100 reaction cells, and IMx HBsAg(V2) MODE 1 Calibrator developed by Abbott Laboratories Diagnostics Division, USA, for use on the IMx System (Abbott, 2000).

In addition to the HBsAg(V2) Kit, performing the HBsAg assay requires the IMx HBsAg Controls, IMx Probe Cleaning Solution, and IMx MEIA Diluent Buffer.

The principles of the procedure are as follows (Abbott, 2000):

1- Initially, a probe removes the serum sample from the sample well in the carousel of the IMx System and delivers the sample and anti-HBs coated microparticles to the incubation well of the reaction cell.
2- If HBsAg is present in the serum sample it will bind to the anti-HBs coated microparticles forming an antibody-antigen complex.
3- A biotinylated anti-HBs solution is then added to the reaction mixture forming an antibody-antigen-antibody complex.
4- A portion of the mixture containing the antibody-antigen-antibody complex bound to the microparticles is transferred to a glass fibre matrix, onto which the microparticles irreversibly bind.
5- An anti-biotin: alkaline phosphatase conjugate is dispensed onto the matrix and binds to the antibody-antigen-antibody complex.
6- The matrix is then washed to remove unbound material.
7- A substrate, 4-methylumbelliferyl phosphate is added to the matrix and the fluorescent product is measured by the MEIA optical assembly.
8- The presence or absence of HBsAg is determined by comparing the rate of formation of fluorescent product to the Cutoff, which is calculated from the IMx HBsAg MODE 1 Calibrator rate (the noise signal of a completely negative sample). The IMx HBsAg MODE 1 Calibrator is recalcified human plasma nonreactive for HBsAg, HIV-1 Ag, anti-HBs, anti-HCV, and anti-HIV-1/HIV-2.

Interpretation of HBsAg results:

The IMx HBsAg(V2) assay calculates a ratio of the sample rate to the MODE 1 Calibrator rate where, S/N = Sample Rate/MODE 1 Calibrator Rate. This was interpreted according to the manufacturer's recommendations as follows:

1- Specimens and controls with S/N < 2.000 were classified as nonreactive by the IMx HBsAg(V2) assay and did not need further testing (HBsAg negative).
2- Specimens and controls with S/N > 2.000 were classified as initially reactive, and all specimens that were initially reactive were retested after centrifugation. Specimens were centrifuged according to the manufacturer's recommendations at 8000 RCF for 10 minutes.
3- If repeat testing showed the specimen to be less than the cutoff, the original assay was classified as non-repeatedly reactive (HBsAg negative). The main causes of non-repeatedly reactive samples are contamination or the presence of particulate matter in the serum sample such as fibrin clots or cellular material (Abbott, 2000).
4- If repeat testing showed the specimen to be greater than or equal to the cutoff, the specimen was classified as repeatedly reactive and was presumed positive for HBsAg (HBsAg positive).
5- Grey Zone Reactive (GZR) samples were samples with 1.600 < S/N < 1.999. These samples were retested and their results were interpreted as described in 3 and 4 above.

3.3.2 Abbott/IMx anti-HBc Core kit (No. 2259-20):

The IMx Core assay is a MEIA for the qualitative detection of total antibody to hepatitis B core antigen in human serum or plasma and is indicated as an aid in the diagnosis of ongoing or previous hepatitis B virus infection (Abbott, 1992).

The hepatitis Core kit consists of IMx Core Reagent Pack and IMx Core MODE 1 Calibrator developed by Abbott Laboratories Diagnostics Division, USA, for use on the IMx System. In addition to the IMx Core Kit, performing the assay requires the IMx Core Controls, IMx Probe Cleaning Solution, and IMx MEIA Diluent Buffer.

The principles of the procedure are as follows (Abbott, 1992):

1- Initially, a probe removes the serum sample from the sample well in the carousel of the IMx System and delivers the sample, specimen diluent, and recombinant DNA derived hepatitis B core antigen (HBcAg) coated microparticles to the incubation well of the reaction cell.
2- If anti-HBc is present in the serum sample it binds to the HBcAg coated microparticles forming an antibody-antigen complex.
3- A portion of the mixture containing the antibody-antigen complex bound to the microparticles is transferred to a glass fibre matrix, onto which the microparticles irreversibly bind.
4- Antibody to hepatitis B core antigen: alkaline phosphatase conjugate is dispensed onto the matrix and binds with the antigenic sites of HBcAg which are not bound with sample anti-HBc.
5- The matrix is then washed to remove unbound material.
6- A substrate, 4-methylumbelliferyl phosphate is added to the matrix and the fluorescent product is measured by the MEIA optical assembly.
7- The presence or absence of anti-HBc in the serum sample is determined by comparing the rate of formation of fluorescent product to the Cutoff, which is calculated from the IMx Core MODE 1 Calibrator rate. The IMx Core MODE 1 Calibrator consists of recalcified human plasma nonreactive for HBsAg, HIV-1 Ag, anti-HCV, anti-HIV-1/HIV-2, anti-HBc, and anti-HBs.

Interpretation of anti-HBc results:

The IMx Core assay calculates a ratio of the sample rate to the cutoff rate where, S/CO = Sample Rate/Cutoff Rate. The Cutoff rate is determined by dividing the MODE 1 Calibrator Rate by 2. The S/CO rate was interpreted according to the manufacturer's recommendations as follows:

1- Specimens and controls with S/CO rate in the range of 1 to 3 were classified as nonreactive by the criteria of the IMx Core assay and did not require further testing (anti-HBc negative).

2- Specimens and controls with S/CO in the range of 0.002 to 1 were classified as initially reactive by the criteria of the IMx Core assay. All specimens that were initially reactive were retested.
3- Specimens which were found to be repeatedly reactive were considered anti-HBc positive by the criteria of the IMx Core assay (anti-HBc positive).

3.3.3 Abbott/IMx IgM anti-HBc Core -M kit (No. 2260-20):

The IMx Core-M assay is a MEIA for the qualitative determination of IgM antibody to hepatitis B core antigen (HBcAg) in human serum or plasma and is indicated for use as an aid in the diagnosis of acute or recent (usually six months or less) hepatitis B virus infection (Abbott (A), 1997).

The hepatitis Core-M kit consists of IMx Core-M Reagent Pack, IMx Core-M MODE 1 Calibrator, and MEIA reaction cells developed by Abbott Laboratories Diagnostics Division, USA, for use on the IMx system. In addition to the IMx Core-M Kit, performing the assay requires the IMx Core-M Controls, IMx Probe Cleaning Solution, and IMx MEIA Diluent Buffer.

The principles of the procedure are as follows (Abbott (A), 1997):

1- Initially, a probe removes the serum sample from the sample well in the carousel of the IMx System and dilutes it.
2- Microparticles coated with goat anti-human antibody, specific for human IgM, are used to immunologically bind the IgM in the sample.
3- A recombinant DNA HBcAg solution is added. If IgM anti-HBc is present in the sample, the HBcAg binds to the microparticle-antibody complex.
4- Antibody to hepatitis B core antigen: alkaline phosphatase conjugate is dispensed and binds to the microparticle-antibody-antigen complex.
5- After washing to remove unbound material, a substrate, 4-methylumbelliferyl phosphate is added to the matrix and the fluorescent product is measured by MEIA optical assembly.
6- IMx Core-M sample rate values are proportional to the quantity of IgM anti-HBc in the sample. Sample rate values are compared to the IMx Core-M MODE 1

Calibrator rate to give an index value, which is used to determine whether a sample is positive or negative.

Interpretation of IgM anti-HBc results:

The presence or absence of IgM anti-HBc is determined by the index value comparing the rate of formation of fluorescent product in the sample to the MODE 1 Calibrator rate. Index values were interpreted according to the manufacturer's recommendations as follows:

1- Specimens with indexes less than 0.8 were considered nonreactive for IgM anti-HBc by the criteria of the IMx Core-M assay (IgM anti-HBc negative).
2- Specimens with indexes greater than 1.2 were considered reactive for IgM anti-HBc by the criteria of the IMx Core-M assay (IgM anti-HBc positive).
3- There were no specimens with indexes between 0.8 and 1.2, which would have been considered grey zone reactive (GZR). It is recommended that individuals with GZR results be monitored (one week intervals) to distinguish rising from falling IgM anti-HBc levels.

3.3.4 Abbott/IMx HBe2 kit (No. 4B14-20):

The IMx HBe2 assay is a MEIA for the qualitative determination of HBeAg in human serum or plasma screened positive for HBsAg and is indicated as an aid in the diagnosis of hepatitis B viral infection (Abbott (B), 1997).

The hepatitis HBe2 kit consists of IMx HBe2 Reagent Pack, IMx HBe2 MODE 1 Calibrator, and reaction cells developed by Abbott Laboratories Diagnostics Division, USA, for use on the IMx system. In addition to the IMx HBe2 Kit, performing the assay requires IMx HBe2 Controls, IMx MEIA Diluent Buffer, and IMx Probe Cleaning Solution.

The principles of the procedure are as follows (Abbott (B), 1997):

1- Initially, a probe delivers the serum sample and microparticles coated with anti-HBe to the incubation well of the reaction cell.

2- If present, the HBeAg in the sample binds to the anti-HBe coated microparticles forming an antibody-antigen complex.
3- A portion of the antigen-antibody complex bound to the microparticles is transferred to a glass fibre matrix.
4- The microparticles bind irreversibly to the glass fibre matrix.
5- The matrix is washed to remove unbound materials.
6- An anti-HBe: alkaline phosphatase conjugate is dispensed onto the matrix and binds to the antibody-antigen complex.
7- After washing to remove unbound material, a substrate, 4-methylumbelliferyl phosphate is added to the matrix and the fluorescent product is measured by MEIA optical assembly.
8- The presence or absence of HBeAg is determined by comparing the rate of formation of fluorescent product to the cutoff which is calculated from the IMx HBe2 MODE 1 Calibrator rate. The IMx HBe2 MODE 1 Calibrator consists of recalcified human plasma nonreactive for HBsAg, anti-HBs, anti-HBc, anti-HCV, anti-HIV-1/HIV-2, antiHBe, HBeAg and HIV Ag.

Interpretation of HBe2 results:

The IMx HBe2 assay calculates a ratio of the sample rate to the MODE 1 Calibrator rate S/N = sample rate/MODE1 calibrator rate. S/N rates were interpreted according to the manufacturer's recommendations as follows:

1- Samples with rates less than the cutoff (<2.1) are nonreactive by the criteria of the IMx HBe2 assay.
2- Samples with rates equal to or greater than the cutoff rate (>2.1) are considered initially reactive by the criteria of the IMx HBe2 assay. Initially reactive samples must be centrifuged at 8000 RCF for 10 minutes and retested in duplicate.
3- If repeat testing shows both assay values to be less than the cutoff, the sample was classified as nonreactive.
4- If either of the repeat assay values was greater than or equal to the cutoff the sample was classified repeatedly reactive.

5- Samples found to be repeatedly reactive are positive for HBeAg by the criteria of the IMx HBe 2 assay.
6- Samples with S/N values less than 0.000 are invalid and must be repeated.

3.3.5 Abbott/IMx AUSAB kit (No. 2262-21):

The IMx AUSAB assay is a MEIA for the qualitative and quantitative determination of anti-HBs. It is intended for the measurement of anti-HBs following HBV infection or vaccination (Abbott, 1998).

The AUSAB assay kit consists of IMx AUSAB Reagent Pack, IMx AUSAB MODE 1 Calibrator, and 100 reaction cells developed by Abbott Laboratories Diagnostics Division, USA, for use on the IMx System (Abbott, 1998).

In addition to the AUSAB Kit, performing the AUSAB assay requires the IMx AUSAB Calibrators, IMx AUSAB Controls, IMx AUSAB Specimen Diluent, and IMx Probe Cleaning Solution.

The principles of the procedure are as follows (Abbott, 1998):

1- Initially, a probe delivers recombinant hepatitis B surface antigen (rDNA HBsAg) coated microparticles to the reaction cell, followed by the serum sample.
2- The anti-HBs in the sample binds to the microparticles forming an antibody-antigen complex.
3- An aliquot of the reaction mixture is transferred onto a glass fibre matrix, onto which microparticles bind irreversibly to the matrix.
4- Biotinylated rDNA HBsAg is then added to the matrix forming an antigen-antibody-antigen complex.
5- An anti-biotin: alkaline phosphate conjugate is dispensed onto the matrix and binds to the antigen-antibody-antigen complex.
6- After washing unbound materials, a substrate, 4-methylumbelliferyl phosphate is added to the matrix and the fluorescent product is measured by the MEIA optical assembly.
7- The presence or absence of anti-HBs in the specimen is determined by comparing the rate of formation of fluorescent product to the cutoff rate, which is calculated

from the IMx AUSAB Calibrator A or MODE 1 adjusted calibrator A rate. The IMx AUSAB MODE 1 calibrator consists of recalcified human plasma nonreactive for anti-HIV-1/HIV-2, anti-HCV, HBsAg and HIV-1 Ag, containing anti-HBs at 100 mIU/ml. The IMx AUSAB calibrator A is nonreactive for anti-HBs, while calibrators B, C, D, E and F have an anti-HBs concentration of 10, 50, 100, 500 and 100 mIU/mL, respectively.

Interpretation of AUSAB results:

Specimens and controls in the IMx AUSAB assay are determined to be reactive or nonreactive based only on the cutoff rate. The IMx AUSAB assay calculates a ratio of the sample to the MODE 1 adjusted cutoff rate. This was interpreted according to the manufacturer's recommendations as follows:

1- Samples with rates less than the cutoff (<1.000) rate are nonreactive by the IMx AUSAB assay and need not be further tested.
2- Specimens whose rates are greater than or equal to the MODE 1 adjusted Calibrator A rate (>1.000) are reactive.
3- Specimens with anti-HBs < 10 mIU/mL are not immune to HBV following vaccination.
4- Specimens with anti-HBs > 10 mIU/mL are considered immune to HBV following vaccination.

3.3.6 Serological kits:

The codes, Lot numbers and expiry dates of the MEIA kits used to perform serological analysis of HBV infection markers amongst the participants in this survey are presented in table 3.3.6.

Table 3.3.6
MEIA reagents used in the analysis of HBV infection amongst participants in the survey

Serological Kit	Kit Code	Manufacturer	Lot. Number/s	Expiry Date/s
IMx HBsAg (V2)	2228-22	Abbott Laboratories	78155LU02 80279LU01 81277LU00 81277LU01 82182LU01	07/12/2001 08/02/2002 16/01/2002 16/01/2002 04/03/2002
IMx Core	2259-20	Abbott Laboratories	81237LU00 81237LU03	20/03/2002 20/03/2002
IMx Core-M	2260-66	Abbott Laboratories	78547M200	07/05/2002
IMx HBe2	4B14-66	Abbott Laboratories	80232M300	23/03/2002
IMx AUSAB	2262-21	Abbott Laboratories	81207LU00	07/05/2002
IMx HBsAg Control	2228-12	Abbott Laboratories	82271LU00	02/03/2002
IMx Core Control	2259-10	Abbott Laboratories	78621M00	03/04/2002
IMx Core-M Control	2260-10	Abbott Laboratories		
IMx HBe 2 Control	4B14-10	Abbott Laboratories		
IMx AUSAB Control	2262-10	Abbott Laboratories	82603HP00	17/07/2002
IMx AUSAB Calibrator	2262-01	Abbott Laboratories	82601HP00	08/07/2002

3.4 Laboratory quality control procedures:

There were three main quality control procedures that were implemented throughout the laboratory analysis of serum specimens collected during the survey in order to ensure valid, accurate and reliable laboratory results. Two of these procedures, referred to below as internal and external quality control, were procedures required and applied specifically for the laboratory analysis of serum specimens collected during this survey and were under the supervision of the primary study investigator. The third, referred to below as routine laboratory quality control procedures, were standard and routine quality control

procedures implemented by the laboratories performing the MEIA assays and were not procedures specifically introduced for the analysis of the specimens of this survey.

3.4.1 Internal quality control:

1- In accordance with manufacturer's recommendations a calibration was performed each time a reagent pack with a new lot number was used and if the IMx HBsAg/ Core/Core-M/HBe2/AUSAB control or IMx HBsAg/Core/HBe2/AUSAB MODE 1 Calibrator value was out of its specified range.

2- In accordance with every IMx HBsAg/Core/Core-M/HBe2/AUSAB calibration, the corresponding positive and negative IMx HBsAg/Core/Core-M/HBe2/AUSAB control was processed as a means of evaluating the calibration.

3- A minimum of one positive and negative IMx HBsAg/Core/Core-M/HBe2/AUSAB control was run with every 50 serum samples processed.

4- In accordance with manufacturer's recommendations at least one IMx HBsAg/Core/Core-M/HBe2 positive control was run per 8 hour shift.

5- In accordance with manufacturer's recommendations at least one IMx AUSAB positive control was run per IMx AUSAB MODE 1 assay.

6- The characteristics, values, and normal range of the IMx HBsAg/Core/Core-M/HBe2/AUSAB positive and negative controls and AUSAB calibrators are summarised in table 3.4.1. All the results for the positive and negative controls in the kits were within the specified control range.

Table 3.4.1
Characteristics of IMx HBsAg/Core/Core-M/HBe2/AUSAB
Abbott MEIA control kits

Control Kit	Kit Number	Serology	Colour	Concentration of Marker/ Minimum Titre	Control Range
IMx HBsAg	2228-12	HBsAg Positive HBsAg Negative	Blue Natural	4-15 ng/mL* 0 ng/mL	25 – 140 S/N 0.5 – 1.5 S/N
IMx Core	2259-10	anti-HBc Positive anti-HBc Negative	Blue Natural	0.5– 2 PEI unit/mL** 0 PEI unit/mL	0.05 – 0.8 S/CO 1.7 – 2.3 S/CO
IMx Core-M	2260- 10	IgM anti-HBc +ve IgM anti-HBc –ve	Blue Natural	1:1.5 0	> 1.5 < 0.4
IMx HBe 2	4B14-10	HBeAg Positive HBeAg Negative	Blue Natural	1:2 0	15 – 100 S/N 0.5 – 1.5 S/N
IMx AUSAB	2262-10	anti-HBs Positive anti-HBs Negative	Blue Natural	80 mIU/mL*** 0	60 – 100 0
IMx AUSAB Calibrator (6 bottles A-F)	2262-01	A B C D E F		0 mIU/mL 10 mIU/mL 50 mIU/mL 100 mIU/mL 500 mIU/mL 1000 mIU/mL	

* nanogram per millilitre.
** Concentration of anti-HBc standardised against the Reference Standard of the Paul-Ehrlich-Institut, Langen, Germany.
*** Concentration of anti-HBs standardised against the World Health Organisation Reference Standard.

3.4.2 External quality control:

All serum samples that were initially reactive or repeatedly reactive for HBsAg and HBeAg in the laboratory carrying out the initial serological testing in the survey were retested at a confirmatory laboratory by MEIA using the same laboratory methods and assays described above and implementing at minimum the same internal quality control measures described above. The results of these confirmatory laboratory tests were consistent with the results of the initial laboratory.

3.4.3 Routine laboratory quality control procedures:

In addition to the internal and external quality control procedures described above that were implemented throughout the analysis the laboratory performing the serological assays routinely carried out the following quality control procedures:

1- A positive and negative control was included with every new kit or lot number of MEIA.
2- A positive and negative control was included with every MEIA calibration performed.
3- As external quality control known positive and negative samples sent from a reference laboratory were tested in the laboratory once every three months.
4- The laboratory had its own known positive and negative HBsAg/anti-HBc samples that were included with every 100 MEIA tests.

3.5 Definitions of HBV infection and chronic carrier status:

The definition of the different HBV infection status according to the laboratory results for HBV serological markers was as follows:

3.5.1 Perinatally infected HBV chronic carrier child:

A child 1-3 years old was considered a perinatally infected HBV chronic carrier if repeatedly HBsAg positive, IgM anti-HBc negative, anti-HBc positive, and was born from a repeatedly HBsAg positive mother.

3.5.2 Childhood infected HBV chronic carrier child:

A child 1-3 years old was considered a HBV chronic carrier infected by childhood transmission if repeatedly HBsAg positive, IgM anti-HBc negative, anti-HBc positive, and was born from a HBsAg negative mother.

3.5.3 Child with ongoing or previous HBV infection:

A child was considered HBV infected (anti-HBc positive), whether ongoing or previous, if repeatedly positive for anti-HBc.

3.5.4 Child seronegative for HBV infection:

A child was considered negative for HBV infection if negative for HBsAg and anti-HBc (total).

3.5.5 HBV chronic carrier mother:

A mother was considered a HBV chronic carrier if repeatedly HBsAg positive.

3.5.6 HBeAg positive HBV chronic carrier mother:

A HBV chronic carrier mother was considered a HBeAg positive chronic carrier mother if repeatedly HBeAg positive.

3.5.7 Mother with ongoing or previous HBV infection:

A mother was considered HBV infected (anti-HBc positive), whether ongoing or previous, if repeatedly positive for anti-HBc.

3.5.8 Mother seronegative for HBV infection:

A mother was considered negative for HBV infection if negative for HBsAg and anti-HBc (total).

3.5.9 Child successfully vaccinated with hepatitis B vaccine:

A child was considered successfully vaccinated (immune) with hepatitis B vaccine if the child had an anti-HBs concentration greater than 10 mIU/mL after receiving all three doses of hepatitis B vaccine.

Chapter 4: Data Management, Methods of Analysis and Description of the Data

This chapter consists of four main sections. In the first section (4.1) the databases created in the survey are described and data management is presented. The second section (4.2) describes the statistical techniques and methods applied in the analysis. The third section (4.3) defines the dependent and independent variables in the survey. Finally, the fourth section (4.4), describes the data contained in the databases (mainly of children aged 1-3) and focuses on describing, examining, analysing and reducing the independent variables included in the analysis of HBV infection and hepatitis B vaccine coverage amongst study participants.

4.1 Database description and data management:

There were a total of seven Epi-info database files created in the survey. These database files were designed, constructed, and managed by the primary study investigator, who also functioned as one of the data-entry clerks who entered data from the questionnaires into their corresponding databases. The database files were as follows:

1- Database file 1 containing data from questionnaire of children aged 1-3 and their mothers (questionnaire 1, annex 1).
2- Database file 2 containing data from questionnaire of children aged 4-9 (questionnaire 2, annex 2).
3- Database file 3 containing laboratory results of children aged 1-3.
4- Database file 4 containing laboratory results of mothers of children aged 1-3.
5- Database file 5 containing laboratory results of children aged 4-9.

Data entered into database files 1 to 5 was double-entered, checked, validated, and corrected as necessary. After the databases above were completed two more databases were created by the primary study investigator as follows:

6- Database file 6 containing data from questionnaire of children aged 1-3 and their mothers, together with their corresponding laboratory results.
7- Database file 7 containing data from questionnaire of children aged 4-9 and their corresponding laboratory results.

There were 1285 completed questionnaires of children aged 1-3 and their mothers with their corresponding children's and mother's blood sample results, and 467 completed questionnaires of children aged 4-9 with their corresponding blood sample results.

Database file 1 had 74 fields, and database file 2 had 66 fields, with each field corresponding to a field in questionnaires 1 and 2, respectively. Database files 1 and 2 had a similar format and structure, except for 8 fields focusing on birth history of the child that were not present in database 2 because these were not originally part of questionnaire 2.

Data from these questionnaires was entered into their corresponding databases in Sanaa during the months of September, October, and November 2001. The data was entered by two different data entry clerks, into two different database files. The database files were compared and differences were rectified by referring to the original questionnaires and the data was adjusted in the database accordingly. This was repeated until the data contained in both databases was identical, resulting in final checked and validated database files 1 and 2.

In both database files 1 and 2 the fields were set where appropriate, with checks, legal values, must enter format, minimum and maximum values, unique value, and skips. For example, field (or question) two in database files 1 and 2, which is the child's identification number, was a must enter field, with a unique value and within a specific range. In other words, this field could not be left empty, the value entered must lie within the range of 1001 to 2286, and only one record could be given this identification number. Another example is field 27 in database 1, which is "Have you breastfed this child?" This was a must enter field, with legal values of 1 or 2, corresponding to yes or no, and

had a skip option to skip the following field 28, which was "For how long have you breastfed this child?" if the answer to this question was no, which was not applicable in this case.

Three database files were constructed for the laboratory results. One of these was for children aged 1-3 (database file 3), one for mothers of the children aged 1-3 (database file 4), and one for children aged 4-9 (database file 5). Laboratory results were entered twice on different occasions, for each of the three database files, over a period extending from November 2001 until February 2002. The two database files were compared and although no differences were found, these would have been rectified by referring to the original laboratory results provided by the laboratory and adjusting the database accordingly.
The laboratory database files were also set with the appropriate checks and legal values.

Finally, database file 1 was merged with database files 3 and 4, producing database file 6 containing data from the questionnaire of children aged 1-3 and their mothers together with their corresponding laboratory results. Similarly, database file 2 was merged with database file 5, producing database file 7 containing data from the questionnaire of children aged 4-9 with their corresponding laboratory results.

Database files 6 and 7 were transformed from Epi-info files to Stata files using the programme Stat-Transfer version 6.
These Stata files were set using the appropriate commands in the statistical software Stata 7 to adjust for the stratification, clustering, and sample weights resulting from the survey design. This avoids biased results when computing point estimates, standard error, and 95% confidence intervals (explained in methods of analysis).
These database files were used for subsequent analysis and were further developed by creating new variables as and when needed.

4.2 Statistical techniques and methods of analysis:

All the statistical analysis was performed using the statistical software Stata 7.

Because the survey had a multistage sampling design and the sampling was not carried out by simple random sampling, the stratification, clustering, and probability of being selected (probability/sampling weights) resulting from the survey design had to be accounted for in order to avoid biased estimates. In other words, adjustments had to be made in the data analysis to account for the stratification, clustering, and probability weights involved in the survey design. This is essential in order to obtain the correct point estimates, standard errors, 95% confidence intervals and test statistics (StataCorp (A), 2001). In the survey, there were a total of 5 strata with each province constituting one stratum and 25 clusters or primary sampling units (PSU) with each district constituting one cluster. The sampling (probability) weight is equal or proportional to the inverse of the probability of being selected (StataCorp (A), 2001). This was calculated for all the study participants of each district by dividing the total population size of the districts province by the number sampled from that district. Data on stratification, clustering and sampling weights was held in the database in the variables "stratum", "psuid", and "finalwg4", respectively.

The statistical software Stata 7 has a group of survey commands, specifically developed for the analysis of survey data that all start with the prefix "svy" (short for survey). These make the necessary adjustments in the analysis to account for clustering, probability weights and stratification in the design. Before using the survey commands (e.g. svylogit, svytab) the dataset in Stata 7 had to be survey set (setting the dataset so that the statistical survey commands make the necessary adjustments in the analysis), which basically requires the specification of variables containing data on sampling weights, strata, and cluster identifier variables. In both combined datasets 6 and 7, probability weight was specified to be "finalwg4", strata was specified to be "stratum", and cluster was specified to be "psuid". Once these were set, the survey commands automatically used these design specifications in all subsequent survey command estimations unless they were cleared or changed (StataCorp (B), 2001).

Once the data had been survey set, the survey estimation commands (e.g. svylogit) were used in the same manner as the non-survey version of the command (e.g. logit). All the analysis and computations mentioned were performed using survey estimation commands unless indicated otherwise. Similarly all the percentages, proportions, prevalence estimates, odds ratios (OR), 95% confidence intervals (95% CI) were weighted estimates that took into account the survey design unless indicated otherwise. It should be noted that the Stata user's guide explains that the "likelihood" in the output for weighted and cluster designs is not a true likelihood (pseudolikelihood), that is, the likelihood is not the distribution of the sample and that the point estimates are from a weighted "ordinary" maximum-likelihood estimator. The consequence of this pseudo-likelihood means that the standard likelihood tests are no longer valid and cannot be performed (StataCorp (A), 2001).

A socioeconomic index was constructed for this survey by performing a multiple correspondence analysis (MCA) (Van Kerm, 1998). Correspondence analysis is a method for deriving a set of coordinate values representing the row and column categories of a contingency table, allowing the associations in the table to be displayed graphically. MCA actually conducts an adjusted simple correspondence analysis on the Burt matrix constructed with a list of variables, which is a matrix of frequency counts resulting from all two-way cross-tabulations of the variables in the list including cross-tabulations of each variable with itself (Van Kerm, 1998).

The MCA was done in Stata using a Stata command developed by Philippe Van Kerm at the Boston College Department of Economics. The command MCA produces numerical results and graphical representations for MCA (Van Kerm, 1998). This allowed identification of all the variables that corresponded well with the remaining variables and with itself. In other words, variables with subcomponents or categories that were clearly demarcated from each other and that were closely linked with the subcomponents or categories of the remaining variables. These variables were then combined together forming the socioeconomic index. Variables that did not correspond well were excluded from the index. All of the variables included in the MCA were and must be categorical variables for the MCA to be performed. The socioeconomic index allowed the

classification of study participants into low, middle and upper socioeconomic classes based on a group of variables identified in the MCA effectively representing household characteristics, household possessions, as well as educational and social status of the mother and father. The variables identified in the MCA representing household characteristics were household ownership, type of household, what the household was built of, availability of an electricity supply, source of water supply, and the crowding index of the household. Variables representing household possessions were ownership of a fridge/freezer, television, home telephone, mobile telephone, motorbike, radio and car. Variables representing educational and social status were educational status of the mother, educational status of the father and employment status of the mother. Different cut-off points for the index and the significance of the difference between the cut-off points of the index was examined. Finally, study participants that had a socioeconomic index of 1-9 (39.4%) were classified as low socioeconomic status, those with an index of 10-13 (39.6%) were classified as middle socioeconomic status, and those with an index of 14-19 (21%) were classified as upper socioeconomic status.

Following data entry and after the data was checked, verified and cleaned a data analysis was performed using survey command cross-tabulations. These tables have the same cell frequencies as standard tables, but the proportions, percentages, and confidence intervals are adjusted according to the study design. They were produced for area of residence for all variables such as baseline information on the child's age, birth order, personal and vaccination history, mother's characteristics, father's characteristics, and the household's characteristics and possessions. Where appropriate, variables were examined for percentage and cumulative distribution, minimum and maximum values, mean, median, and were reduced to categorical variables.

The first objectives of the survey to be measured were the prevalence objectives. These were measuring the prevalence of anti-HBc positivity and HBV chronic carrier status amongst children aged 1-3, mother's of children aged 1-3 (women of childbearing age), children aged 4-9, and hepatitis B vaccine coverage amongst children aged 1-3 participating in the survey. This was performed using survey cross-tabulations for each

outcome of interest (dependent variable) in the dataset by the relevant independent variables. These computed the weighted prevalence of each outcome of interest by the relevant independent variables, 95% CI of the prevalence estimates, number of observations, adjusted design-based Pearson chi square test of significance with Rao and Scott second order correction, and p-values. The adjusted design-based Pearson chi square test of significance with Rao and Scott second order correction is the recommended test of significance for cross-tabulations using survey commands (StataCorp (A), 2001). Examining the output from the survey cross-tabulations and their design-based adjusted Pearson chi square values allowed the identification of variables potentially associated with the outcomes of interest that may be significant in the univariable and multivariable analysis.

Secondly, a univariable analysis was done to estimate the association of each dependent variable in the analysis with the explanatory variables under investigation in the survey. This was performed using survey logistic regression which computed crude odds ratios (OR), 95% confidence intervals (CI), standard errors (SE), adjusted Wald tests and p-values. These were computed as log OR, 95% CI, and SE and by using an option in the regression model these estimates were anti-logged and transformed back to the normal scale. In the case of binary explanatory variables the univariable analysis was performed directly. In the case of categorical explanatory variables, where appropriate (such as in the case of ordered categorical variables), the linear OR (OR per one unit increase in the variable), its 95% CI, SE, adjusted Wald tests were computed, and the p-value of the linear OR was indicative of the significance of the association with the dependent variable. When it was not appropriate to use a linear OR, such as type of household where the categories of the variable cannot be ordered, an adjusted Wald test (survey test) was performed to compute the significance of association of joint effect of the categories of this variable with the dependent variable.

After completing the univariable analysis the explanatory variables were ordered according to the p-value, which was considered indicative of the significance of their association with the dependent variable. A p-value less than or equal to 0.05 was considered indicative of evidence of a significant association between the explanatory

variable and the outcome of interest. Nevertheless, variables with a p-value less than 0.2 were taken forward to be included in the multivariable model in order to avoid missing any statistically significant associations that may have been confounded by other explanatory variables.

A few explanatory variables were treated as *a priori* variables and it was decided to take these variables forward to the multivariable analysis and include them in the final model regardless of the significance of their association with the outcome of interest. This was done either because of evidence suggesting an association of these variables with the dependent variable under investigation or the importance of obtaining information about these variables. These *a priori* variables were age-group, sex, area of residence, and mothers educational status..

Thirdly, a multivariable analysis was done using multiple logistic regression to investigate the association of the dependent variables with independent variables adjusting for the remaining independent variables in the model. Explanatory variables associated with the dependent variable identified in the univariable analysis with a p-value less than 0.2 were added to the multivariable model, one at a time, starting with the variable with the most significant association (smallest p-value) with the dependent variable in the univariable analysis. This process was repeated until all explanatory variables with p-values less than 0.2 were added to the model (forward fitting). The *a priori* variables were also fitted to the model according to the significance of their association with the dependent variable. All the independent variables in the multivariable model were then tested for their significance in the model and variables with a p-value greater than 0.05 were removed from the model one at a time, starting with the least significantly associated independent variable (largest p-value) with the dependent variable until only significantly associated independent variables remained in the model. If *a priori* variables were removed during the process of removing variables according to their p-value, these were added back to the final model at the end of the process.

After completing the forward fitting multivariable modelling a backward fitting multivariable model was done to compare results obtained by the two methods. This was

done by adding the explanatory variables identified in the univariable analysis with a p-value less than 0.2 all in one go. Independent variables were then removed from the model, one at a time, starting with the variable with the least significant association (largest p-value) until only significantly associated variables remained in the model. If *a priori* variables were removed during the process, these were added back to the final model. If an independent variable was found to be statistically significantly associated with anti-HBc positivity or HBV chronic infection in the final multivariable model of either of these two dependent variables, this independent variable was added to the final multivariable model of the other dependent variable, regardless of the strength of its association, resulting in two models, one for anti-HBc positivity and one for HBV chronic infection, with identical independent variables in them.

In order to examine the association of the socioeconomic status variable with anti-HBc positivity and HBV chronic infection the exact process of the multivariable analysis described above conducted on all the variables was repeated using the socioeconomic status variable alone with the remaining variables but excluding all the socioeconomic indicator variables that contributed to and were included in the socioeconomic status variable (household characteristics, household possessions, and mothers and fathers educational status).

The multiple logistic regression computed adjusted OR, 95% CI, SE, adjusted Wald tests and p-values. These were computed as log OR, 95% CI, and SE and were anti-logged and transformed back to the normal scale.

Finally, the effectiveness of hepatitis B vaccination in preventing anti-HBc positivity and HBV chronic infection amongst children aged 1-3 was measured using the formula, vaccine effectiveness = 1 – relative risk. Since anti-HBc positivity was found to be a rare disease in Yemen, and in rare diseases the risk ratio approximates the odds ratio and rate ratio, the odds ratio was used to estimate vaccine effectiveness. Vaccine effectiveness was estimated by comparing completely vaccinated to unvaccinated children.

The OR of anti-HBc positivity by hepatitis B vaccination status was computed using logistic univariable analysis and this OR was used to estimate the effectiveness of hepatitis B vaccination in preventing anti-HBc positivity using the formula described

above. When it was attempted to estimate the effectiveness of hepatitis B vaccine in preventing HBV chronic infection it could not be estimated using logistic univariable analysis because no OR or 95% confidence intervals could be computed since there were no cases of HBV chronic infection amongst vaccinated children and this group predicted perfect failure. Therefore, the OR was computed using survey cross-tabulations of HBV chronic infection by vaccination status (comparing completely vaccinated with unvaccinated children) using the weighted percentages of the number of observations in the cells. The validity and reliability of this method was tested by comparing the effectiveness of hepatitis B vaccination in preventing anti-HBc positivity computed by logistic univariable analysis and survey cross-tabulations, and it was found that both methods produced the same results. The vaccine effectiveness computed by this technique is equivalent to the prevented fraction amongst exposed group computed by the epi-tab table for epidemiologists from cross-sectional or case-control studies, but in this case it was adjusted for design effect.

4.3 Definition of dependent and independent variables:

4.3.1 Dependent variables:

1- Hepatitis B surface antigenaemia amongst mothers (MsAg3).
2- Hepatitis B surface antigenaemia amongst children aged 1-3 (CsAg3).
3- Hepatitis B surface antigenaemia amongst children aged 4-9 (sAg3).
4- Hepatitis B core antibody amongst mothers (mcab1).
5- Hepatitis B core antibody amongst children aged 1-3 (ccab1).
6- Hepatitis B core antibody amongst children aged 4-9 (cab1).
7- Hepatitis B surface antibody amongst children aged 1-3 (csab1).
8- Hepatitis B e antigen (MeAg2) amongst chronic carrier mothers.
9- Immunoglobulin-M hepatitis B core antibody (IgM anti-HBc) amongst children.
10- Vaccination status of the children (vacstat1).

4.3.2 Independent (explanatory) variables:

The main independent variables in the survey were:

1- Age of mother, age of child 1-3, and age of child 4-9.
2- Sex of child aged 1-3 and sex of child aged 4-9.
3- Province and area of residence.
4- Qualifications of the person managing delivery of the child.
5- Setting in which the child was delivered.
6- Type of delivery.
7- Antenatal care during pregnancy.
8- Breastfeeding.
9- Birth order of mother, child aged 1-3 and child aged 4-9.
10- Knowing of hepatitis B vaccine.
11- Mothers education.
12- Mothers employment.
13- Fathers education.
14- Fathers employment.
15- Household ownership.
16- Type of household.
17- Availability of an electricity supply.
18- Household source of water supply.
19- Crowding index in the household.
20- Television, refrigerator/freezer, radio, telephone, mobile telephone, motorcycle, and bicycle ownership.
21- Schooling of child aged 4-9.
22- Number of doses of hepatitis B vaccine.

4.4 Data description:

4.4.1 Database of children aged 1-3 (Database 1):

The final dataset contained 1285 records with each record representing one child aged 1-3 and the child's mother participating in the survey. The dataset had 74 fields, representing the fields in questionnaire 1 (annex 1). The dataset consisted of data on the child and mother. It contained baseline information such as identification number, province, district, city or village, street, household and area of sampling. It also consisted of information on the child's personal and vaccination history, mother, father, and the household's characteristics and indicators of socioeconomic status.

Child and mother identification numbers:

Each record had two unique identification numbers one for the child aged 1-3 starting with 1001 and ending with 2286, and another for the mother starting with 4001 and ending with 5286. Mothers starting with 4xxx had the same last three digits as their children aged 1-3 starting with 1xxx, and mothers starting with 5xxx had the same last three digits as their children aged 1-3 starting with 2xxx.

One record had missing data on anti-HBc status of the child. Because this was one of the principal outcomes under investigation, record number 247 (child identification number 1247 and consequently mother identification number 4247) was dropped from the analysis.

Province, district, street, city or village, household and area:

These recorded the province, district, and city or village from which the study participant was sampled, as well as street name and household number where appropriate.

The variable "area" classified areas in the survey into urban or rural, and each study subject was recorded as either being sampled from an urban or rural area.

Interview time and date, interviewer, and person interviewed:

These recorded the date and time the interview was started. The name and identification code of the study investigator completing the interviewer administered questionnaire was recorded, as well as the name of the person interviewed and the relationship of this person to the child. However, the name of the person interviewed as well as study participant was not entered to the database to ensure confidentiality and anonymity of study participants. The mother or father of the child was the target person to be interviewed and this was fulfilled in 95% of the interviews with study participants. If this was not possible the aunt, uncle, grandfather or grandmother of the child was interviewed.

Age of child, date of birth and source of child age:

The first question attempting to determine the age of the child was the date of birth of the child. The date of birth of the child was known for only 45% of children aged 1-3 participating in the survey, in which case the source of the date of birth was determined by a birth certificate, vaccination card, or parents memory or records.

Parents were then directly asked for the age of their child in months (12 to 36 months). If the date of birth was known, this was used to compute the age of the child in months. If the date of birth was not known the age was recorded after asking the parents a series of questions in order to determine the age of their child as accurately as possible. These aimed to determine when during the year the child was born (e.g. summer, winter) or to link the birth of the child to a memorable date during the year (for example Eid Alfitr, Eid Al Adha, Ramadan).

The mean age of children participating in the survey was 23.7 months (95% CI 23.1 to 24.4) ranging from 12 to 36 months. After examining the percentage and cumulative distribution, and minimum and maximum values of age, this was reduced to the categorical variable age-group that was initially divided into 8 and finally 4 categories, ensuring that the baseline group was large enough to avoid generating wide confidence intervals (table 4.4.1 A).

Table 4.4.1 A
Age-group of children participating in survey of children aged 1-3

No	Age-group	Frequency	Percent	Cumulative Percentage
	Age-group in months (8 categories)			
	12 to 14	139	10.8	10.8
	15 to 17	130	10.1	20.9
	18 to 20	225	17.5	38.4
	21 to 23	159	12.4	50.8
	24 to 26	187	14.6	65.4
	27 to 29	88	6.9	72.2
	30 to 32	165	12.8	85.1
	33 to 36	192	14.9	100
	Total	1285	100	
	Age-group in months (4 categories)			
	12 to 17	269	20.9	20.9
	18 to 23	384	29.9	50.8
	24 to 29	275	21.4	72.2
	30 to 36	357	27.8	100
	Total	1285	100	

The source of the child's age was recorded as an indicator of the accuracy of the reported age of the child (table 4.4.1 B).

Table 4.4.1 B
Source of age for children participating in survey of children aged 1-3

No.	Source	Frequency	Percent
1	Approximate	132	13.4
2	Health records	422	28.5
3	Birth certificate	61	3.9
4	Parents memory	645	51.9
5	Parents records	22	1.9
6	ID card or passport	3	0.3
	Total	1285	100

When the source of the child's age was a birth certificate, health record (e.g. vaccination card), parents records, or identification card or passport, and evidence of the child's age was verified by one of the study investigators the age of the child was considered accurately determined. Parents memory was recorded when either one of the parents remembered the month of birth of the child, or linked the birth of their child to a memorable event but no evidence of the child's age was seen by the study investigator. Approximate was recorded when no evidence of age or date of birth was seen by the study investigator and neither of the parents could confidently specify when their child was born. This was estimated by linking the birth of the child to a memorable event, for example, asking when in the year the child was born (summer or winter), or relating the birth of the child to memorable events such as Eid celebrations or Ramadan.

Sex of the child:

This recorded whether the child was male or female. 52% of the children aged 1-3 participating in the survey were male and 48% were female.

Place of birth of child:

Approximately 19% of children participating in the survey were born in a health setting and 81% in a home setting. Table 4.4.1 C shows the place of birth of children aged 1-3 participating in the survey.

Table 4.4.1 C
Place of birth of child participating in survey of children aged 1-3

No.	Place of delivery of child	Urban % (no)	Rural % (no)	Total % (no)
1	General hospital	41.0 (235)	4.7 (57)	16.2 (292)
2	Private hospital	4.4 (19)	1.8 (13)	2.6 (32)
3	Health centre	0.6 (2)	0.3 (4)	0.4 (6)
4	Father/mother home	44.5 (200)	75.7 (565)	65.8 (765)
5	Grand Parents home	8.3 (46)	13.4 (92)	11.8 (138)
6	Other	1.3 (7)	4.1 (45)	3.2 (52)
	Total	100 (509)	100 (776)	100 (1285)

There were significant differences in place of delivery by area of residence. While 45% of urban deliveries took place in the home of the father/mother of the child, 76% of rural deliveries took place in the home of the father/mother of the child. On the other hand, while 41% of deliveries in urban areas took place in a general hospital, only 5% of rural deliveries took place in a general hospital ($p<0.0001$). Differences also existed in place of delivery between the two urban areas in the survey where 35% of deliveries in Sanaa took place in a health setting compared to 69% of deliveries in Aden ($p=0.003$).

Who delivered child and type of delivery:

Approximately 39% of deliveries of children aged 1-3 participating in the survey were attended by a medically qualified individual (doctor, nurse, or midwife), and the remaining 61% were attended by a relative or traditional birth attendant (table 4.4.1 D).

Table 4.4.1 D
Person delivering child participating in survey of children aged -3

No.	Person who delivered the child	Urban % (no)	Rural % (no)	Total % (no)
1	Doctor	49.3 (263)	9.4 (99)	21.9 (362)
2	Nurse	5.6 (30)	5.7 (44)	5.7 (74)
3	Midwife	8.3 (48)	11.2 (75)	10.3 (123)
4	Traditional birth attendant	8.5 (45)	20.5 (123)	16.7 (168)
5	Relative	22.7 (102)	49.5 (401)	41.0 (503)
6	Alone or with husband	3.5 (13)	1.8 (21)	2.4 (34)
7	Other	1.9 (8)	2.1 (13)	2.0 (21)
	Total	100 (509)	100 (776)	100 (1285)

The person who delivered the child varied by area. While 63% of urban deliveries were attended by a medically qualified individual, only 26% of rural deliveries were attended by a medically qualified individual ($p<0.0001$). Differences also existed between the two urban provinces in the survey. Whilst 54% of deliveries in Sanaa were attended by a medically qualified individual, 82% of deliveries in Aden were attended by a medically qualified individual ($p=0.005$).

Overall, 98% (1249/1285) of the children participating in the survey were delivered by normal vaginal delivery and the remaining 2% (36/1285) by caesarean section. 92% (34/36) of caesarean sections were carried out in urban areas, where health care facilities and qualified staff are available, compared to the remaining 8% of sections done in rural areas ($p<0.0001$). Only one woman in Taiz and one woman in Shabwa underwent caesarean sections. No women in Hodeidah underwent caesarean sections. It is unknown if women undergoing caesarean sections in Sanaa and Aden (urban areas) included women from other provinces whom were transported to urban areas due to complications during labour and were consequently delivered by caesarean section. Another possibility is that availability of facilities for caesarean section may have tempted health care providers to perform a caesarean section for a child who would have been delivered by normal vaginal delivery had they not intervened with the normal course of labour. There was no evidence suggesting that caesarean section was more common in private hospitals, which may be indicative of supplier induced demand.

Antenatal care during pregnancy:

A woman was considered to have received antenatal care if she came into contact with a qualified health care provider at any time during her pregnancy with her child aged 1-3 involved in this survey before the onset of labour. Overall, 45.2% of women participating in the survey received antenatal care during their pregnancy, 54.7% did not, and 0.1% could not recall if they received antenatal care (table 4.4.1 E).

Table 4.4.1 E
Antenatal care during pregnancy for mothers of children aged 1-3

No.	Received antenatal care	Urban % (no)	Rural % (no)	Total % (no)
1	Yes	65.0 (350)	36.1 (269)	45.2 (619)
2	No	34.7 (157)	63.9 (504)	54.7 (661)
3	Don't know	0.3 (2)	0.1 (1)	0.1 (3)
	Total	100 (509)	100 (774)	100 (1283)

There were marked differences in receiving antenatal care between provinces. 65% of women in urban areas reported having received antenatal care at anytime during their pregnancy compared to 36% of women in rural areas ($p<0.0001$). Differences also existed in receiving antenatal care between the two urban areas in the survey. 56% of women in Sanaa received antenatal care during pregnancy compared to 82% of women in Aden ($p=0.003$). Records 678 and 682 had missing data on whether they received antenatal care or not.

Breastfeeding and its duration:

98.6% (1262/1284) of mothers breastfed their child aged 1-3 participating in the survey and 1.4% (22/1284) of mothers did not breastfeed their child. Record number 711 had missing data on whether the child was breastfed or not. Out of the 1262 mothers whom breastfed their child aged 1-3 participating in the survey, there was missing data from three mothers (record numbers 357, 581 and 709), leaving 1259 mothers with data on duration they breastfed their child.

The mean duration of breast feeding was 16.2 months (95% CI 15.4 to 16.9) ranging from 1 to 36 months. After examining the percentage and cumulative distribution, and minimum and maximum values of duration of breastfeeding, this was reduced to a categorical variable and was divided into 3 categories, up to 1 year, 1 to 2 years, and 2 to 3 years breastfeeding (table 4.4.1 F).

Table 4.4.1 F
Duration breastfeeding of children aged 1-3 participating in the survey

No.	Duration breastfeeding	Urban % (no)	Rural % (no)	Total % (no)
1	Up to 1 year	41.5 (209)	31.6 (262)	34.7 (471)
2	1 to 2 years	51.6 (250)	58.0 (436)	56.0 (686)
3	2 to 3 years	6.8 (35)	10.5 (67)	9.3 (102)
	Total	100 (494)	100 (765)	100 (1259)

Approximately 91% of women participating in the survey breastfed their child aged 1-3 up to 2 years. A higher proportion of rural women breastfed their children for 1 to 2 years, and 2 to 3 years than urban women ($p=0.009$).

Child birth order:

The mean number of deliveries by mothers of the children aged 1-3 participating in the survey was 4.6 (95% CI 4.4 to 4.8) ranging from 1 to 18. The mean birth order of children aged 1-3 participating in the survey was 4.5 (95% CI 4.2 to 4.8) ranging from 1 to 18. After examining the distribution of child birth order, and its minimum and maximum values, child birth order was reduced to a categorical variable and was divided into 6 categories, with birth order's 6, 7 up to 18 combined into one category 6 to 18 (table 4.4.1 G).

Table 4.4.1 G
Birth order of children aged 1-3 participating in the survey

No.	Childs birth order	Urban % (no)	Rural % (no)	Total % (no)
1	1	25.4 (130)	14.2 (111)	17.7 (241)
2	2	18.1 (97)	16.5 (113)	17.0 (210)
3	3	15.2 (84)	13.5 (114)	14.0 (198)
4	4	13.4 (71)	8.0 (70)	9.7 (141)
5	5	8.7 (42)	9.5 (81)	9.3 (123)
6	6 to 18	19.2 (85)	38.3 (287)	32.2 (372)
	Total	100 (509)	100 (776)	100 (1285)

Approximately 50% of children aged 1-3 participating in the survey were one of the first three children in birth order. 32% of children aged 1-3 participating in the survey were between the 6^{th} to 18^{th} child in birth order. A higher proportion of the children aged 1-3 born in urban areas were the 1^{st}, 2^{nd}, 3^{rd}, or 4^{th} in birth order, whereas a higher proportion of children born in rural areas were the 5^{th} or 6^{th} to 18^{th} in birth order ($p<0.0001$). This meant that children aged 1-3 living in rural areas were more likely to have older siblings than children living in urban areas.

Vaccination card:

84% (1109/1285) of parents of children aged 1-3 participating in the survey said their child aged 1-3 had a vaccination card. There was no evidence of a significant difference in the proportion of parents saying their child had a vaccination card in urban areas (90%) compared to rural areas (81%) ($p=0.06$) (table 4.4.1 H).

Table 4.4.1 H
Having a vaccination card amongst children aged 1-3 participating in the survey

No.	Has vaccination card	Urban % (no)	Rural % (no)	Total % (no)
1	Yes	89.6 (467)	81.3 (642)	83.9 (1109)
2	No	10.4 (42)	18.7 (134)	16.1 (176)
	Total	100 (509)	100 (776)	100 (1285)

When study participants who said their child had a vaccination card were asked to show it to the study investigator only 54% (596/1108) had the vaccination card available and 46% (512/1108) did not. There was no significant difference in vaccination card availability by area (p=0.4). There was one record (record number 643) that had missing data on vaccine availability amongst those reporting having a vaccination card.

Knowledge of hepatitis B vaccine:

Overall, approximately 78% of study participants claimed to know of hepatitis B vaccine, 22% claimed they did not know of hepatitis B vaccine and less than 0.5% could not determine whether they knew of the vaccine or not (table 4.4.1 I)

Table 4.4.1 I
Knowledge of hepatitis B vaccine amongst parents of children aged 1-3 participating in the survey

No.	Know of hepatitis B vaccine	Urban % (no)	Rural % (no)	Total % (no)
1	Yes	92.1 (468)	71.3 (504)	77.8 (972)
2	No	7.5 (39)	28.4 (266)	21.8 (305)
3	Undetermined	0.4 (2)	0.3 (7)	0.4 (7)
	Total	100 (509)	100 (775)	100 (1284)

There were significant differences in knowledge of hepatitis B vaccine by area. 92% of urban participants claimed to know of hepatitis B vaccine compared to 71% of rural participants (p<0.0001). There was no significant difference in knowledge of hepatitis B vaccine between the urban provinces in the survey, Sanaa and Aden, where the proportion of respondents who claimed to know of hepatitis B vaccine were 93 and 91%, respectively (p=0.7). Similarly, there was no significant difference in knowledge of

hepatitis B vaccine between the rural provinces, Taiz, Shabwa, and Hodeidah, included in the survey (p=0.1).

Been vaccinated with hepatitis B vaccine and number of doses:

Although 78% of parents interviewed claimed to know of hepatitis B vaccine only 32% of parents interviewed said their child aged 1-3 was vaccinated with hepatitis B vaccine, 66% said their child did not receive the vaccine and 2% did not know whether their child aged 1-3 received the vaccine or not (table 4.4.1 J).

Table 4.4.1 J
Parent's response whether their child aged 1-3 participating in the survey received hepatitis B vaccine

No.	Child was vaccinated with hepatitis B vaccine	Urban % (no)	Rural % (no)	Total % (no)
1	Yes	43.9 (239)	26.4 (174)	31.9 (413)
2	No	52.9 (251)	72.2 (589)	66.1 (840)
3	Don't know	3.2 (19)	1.5 (12)	2.0 (31)
	Total	100 (509)	100 (775)	100 (1284)

44% of urban participants said that their child aged 1-3 received hepatitis B vaccine compared to 26% of rural participants. On the other hand, although 72% of rural participants said their child did not receive hepatitis B vaccine compared to 53% of urban participants, this difference was not statistically significant (p=0.06). Record number 643 had missing data on parent's response whether their child aged 1-3 was vaccinated. Differences existed between the two urban provinces in the survey. 37% of participants in Sanaa said their child received hepatitis B vaccine compared to 57% of participants in Aden (p=0.02). There was no evidence of a significant difference in parents response regarding their child's vaccination between rural provinces (p=0.6).

Out of the study participants who said that their child received hepatitis B vaccine, 31% (107/414) said their child received one dose, 29% (118/414) said their child received two doses, and 39% (187/414) said their child received three doses. The number of doses of hepatitis B vaccine received by the children who had a vaccination card was copied from

the card whatever the person interviewed reported (table 4.4.1 K). If the vaccination card had three doses recorded on it the child was classified as completely vaccinated. If the card had one or two doses recorded on it the child was recorded as incompletely vaccinated. Children were classified as unvaccinated if the child was reported to be unvaccinated by the parents or vaccinated by the parents but their vaccination card showed them to be unvaccinated. Children were classified as unknown vaccination status if they were reported to be vaccinated but did not have evidence of their vaccination to show the study investigator or if the parents did not know their child's vaccination status. The vaccination status of 1124/1285 children aged 1-3 was accurately determined, and 13% (161/1285) of children had an unknown vaccination status.

Table 4.4.1 K
Hepatitis B vaccination status amongst children aged 1-3 participating in the survey

No.	Number of doses	Frequency	Percent
1	Zero	840	65.9
2	One	66	6.3
3	Two	81	6.2
4	Three	137	8.6
5	Unknown	161	13.0
	Total	1285	100

Hepatitis B vaccination status was determined by study investigators by checking the source of the child's vaccination status. 44% of children's vaccination status was determined by a vaccination card and 56% of children's vaccination status was determined by parent's memory.

After verifying hepatitis B vaccination status by study investigators, 21% of children participating in the survey received at least one dose of hepatitis B vaccine, and 8.6% received all three doses of vaccine. There was a significant difference in vaccination status by area. 17.3% of children in urban areas were completely vaccinated compared to 4.6% of children in rural areas (p=0.05). Moreover, there was a significantly higher proportion of completely vaccinated children in Aden (34.27%) compared to Sanaa (8.5%) (p=0.002).

Mother's age, place of birth and birth order:

Three questions were asked before directly asking about the mother's age. These were the age of the mother at her first marriage, age of the mother's oldest child, and how long after marriage she delivered her first child. The years of these questions were added to give an approximate idea of the age of the mother. After this the mother was asked what her age was. This allowed evaluation of the reliability of the reported age of the mother. The mean age of mothers of children aged 1-3 participating in the survey was 28.5 years (95% CI 28.1 to 28.9) ranging from 15 to 49 years. Less than 5% of mothers were younger than 20 years and just over 5% of the mothers were older than 40 years. After examining the percentage and cumulative distribution, and minimum and maximum values of mothers age, this was reduced to the categorical variable mothers age-group that was initially divided into 7 and finally 5 categories, ensuring that the baseline group was large enough to avoid generating wide confidence intervals (table 4.4.1 L).

Table 4.4.1 L
Age-group of mothers participating in survey of children aged 1-3

No	Mothers age-group	Frequency	Percent	Cumulative Percentage
1	Age-group in years (7 categories)			
	15 to 19	46	3.6	3.6
	20 to 24	354	27.6	31.1
	25 to 29	349	27.2	58.3
	30 to 34	268	20.9	79.1
	35 to 39	177	13.8	92.9
	40 to 44	79	6.2	99.1
	45 to 49	12	0.9	100
	Total	1285	100	
2	Age-group in years (5 categories)			
	15 to 24	400	31.1	31.1
	25 to 29	349	27.2	58.3
	30 to 34	268	20.9	79.1
	35 to 39	177	13.8	92.9
	40 to 49	91	7.1	100
	Total	1285	100	

88% of mothers age was approximately estimated (i.e. the mother did not know the date, month, or year she was born), 8% was obtained from family records, 3% from an identification card (ID) or passport, and 1% from a birth certificate.
Having an ID card or passport as source of age does not necessarily mean that the age recorded is accurate. Mothers or their relatives may have guessed their age and recorded it in the passport or ID card. Nevertheless, 3% of mothers age was determined by ID card or passport whereas a lower proportion 0.3% of children's age was determined by passport or ID indicating that with increasing age individuals are more likely to have an ID card or passport particularly amongst males.

Out of the mothers participating in the survey 35% were born in Taiz, 28% in Hodeidah, 9% in Sanaa, 8% in Aden, 6% in Shabwa, and 14% in provinces other than the five targeted in the survey. Over 90% of women sampled from Taiz, Hodeidah, and Shabwa were born in these provinces. On the other hand, only 41% of women sampled from Sanaa were born there. 42% of women sampled in Sanaa were born in provinces other than those targeted in the survey, 11% in Taiz, 4% in Hodeidah, and 2% in Aden. Similarly, 70% of women sampled from Aden were born there, 21% were born in provinces other than those targeted in the survey, 7% were born in Taiz, 1% in Hodeidah and 1% were born in Shabwa.

The mean number of siblings per mothers was 7.2 (95% CI 7.0 to 7.4) ranging from 0 to 20. The mean birth order of mothers participating in the survey was 3.9 (95% CI 3.8 to 4.1) ranging from 1 to 19. After examining the distribution, minimum and maximum values of mothers birth order it was reduced to 6 categories with birth order 6 and above combined into one category 6 to 19 (table 4.4.1 M).

Table 4.4.1 M
Birth order of mothers participating in survey of children aged 1-3

No.	Mothers birth order	Urban % (no)	Rural % (no)	Total % (no)
1	1	22.1 (109)	22.5 (169)	22.4 (278)
2	2	15.9 (80)	15.3 (123)	15.5 (203)
3	3	16.0 (80)	16.7 (124)	16.5 (204)
4	4	13.6 (67)	12.1 (93)	12.6 (160)
5	5	12.2 (61)	9.0 (71)	10.0 (132)
6	6 to 19	20.2 (112)	24.5 (196)	23.1 (308)
	Total	100 (509)	100 (776)	100 (1285)

Over 50% of mothers participating in the survey were one of the first three of their siblings in birth order. 23% of mothers participating in the survey were between the 6^{th} to 19^{th} child in birth order. There was no evidence of a significant difference in mothers birth order by area (p=0.3).

Mothers educational and employment status:

Overall, 37% (95% CI 29.9 to 44.1) of mothers participating in the survey claimed to be educated compared to 63% (95% CI 55.91 to 70.07) who were uneducated. The proportion of mothers completing schooling and university was significantly higher in urban areas compared to rural areas (p<0.0001). 29%, 14%, and 5% of mothers participating in the survey claimed to have completed primary school, secondary school and university or college, respectively, in urban areas compared to 9%, 4% and 0.6% of mothers in rural areas (table 4.4.1 N).

Table 4.4.1 N
Educational status of mothers participating in survey of children aged 1-3

No.	Educational status	Urban % (no)	Rural % (no)	Total % (no)
1	Uneducated	37.0 (158)	75.4 (580)	63.3 (738)
2	Read and write (Basic)	15.0 (70)	10.7 (109)	12.1 (179)
3	Completed primary	28.7 (159)	9.4 (62)	15.5 (221)
4	Completed secondary	14.3 (91)	3.9 (22)	7.2 (113)
5	Completed university	4.9 (31)	0.6 (3)	1.9 (34)
	Total	100 (509)	100 (776)	100 (1285)

97% of mothers participating in the survey were not employed with only 3% (55/1285) of mothers participating in the survey in paid employment. Approximately 8% of urban women participating in the survey were in paid employment compared to 1.4% of rural women (p=0.003). Approximately 50% of women in paid employment worked as teachers, 25% as secretaries in the public and private sector, and the remaining 25% worked for example in factories, ministries, or as cleaners.

Household ownership, type and characteristics:

Overall, 85% of households in the survey were owned and 15% were rented. 63% of urban households were owned compared to 96% of rural households. In other words, only 4% of rural households were rented compared to 37% of urban households (p<0.0001). Apartments constituted 36% of urban residences and 1.4% of rural residences. 17% and 81% of rural households were shacks (ill defined households built of wood, corrugated steel sheets or non-specific mixed materials and with no concrete roof) and houses, respectively, compared to 3% and 60% of urban households (p<0.0001).

Rural households were more likely to be built of stone, mud or clay, and mixed materials compared to urban households, which were more likely to be built of cement (p=0.007).

The mean number of rooms per household was 2.8 (95% CI 2.5 to 3.2) ranging from 1 to 25. Approximately 90% of households had 1 to 7 rooms. The mean number of people per household was 8.9 (95% CI 8.4 to 9.3) ranging from 2 to 60.

The crowding index was calculated by dividing the number of people by the number of rooms per household, giving the average number of individuals per room. The mean crowding index overall, was 4.1 (95% CI 3.7 to 4.5) individuals per room, ranging from 1 to 18 individuals per room. After examining its percentage distribution, minimum and maximum values, crowding index was reduced to a categorical variable of six categories with indexes 6 to 18 combined into one category (table 4.4.1 O).

Table 4.4.1 O
Crowding index in households of children aged 1-3 participating in the survey

No	Crowding index	Urban % (no)	Rural % (no)	Total % (no)
1	One	6.7 (35)	4.9 (65)	5.5 (100)
2	Two	33.6 (165)	14.1 (158)	20.2 (323)
3	Three	31.0 (155)	18.3 (157)	22.3 (312)
4	Four	15.9 (78)	20.8 (143)	19.2 (221)
5	Five	6.9 (39)	14.5 (87)	12.1 (126)
6	Six to eighteen	6.0 (37)	27.6 (166)	20.7 (203)
	Total	100 (509)	100 (776)	100 (1285)

Crowding indexes 1, 2 and 3 were more common in urban areas, and crowding indexes 4, 5, and 6 to 18 were more common in rural areas ($p<0.0001$), indicating that rural residents were living in more crowded households compared to their urban counterparts. The mean number of individuals sleeping in the same room as the child aged 1-3 participating in the survey was 5.3 (95% CI 5.1 to 5.6) ranging from 2 to 17.

Out of all the households participating in the survey 56% had an electricity supply. 98% of urban households had an electricity supply compared to 37% of rural households ($p<0.0001$). Out of the households that had an electricity supply, 99.7% of urban households had an electricity supply all the time (excluding power failures), whereas only 61% of the rural households that had an electricity supply had it all the time and the remaining 35% of rural households that had an electricity supply had it after sunset ($p=0.0008$). 28% of households in the survey had a governmental water supply, 29% a local project, 9% were supplied by car trucks, and 34% collected water using buckets (table 4.4.1 P).

Table 4.4.1 P
Source of water in households of children aged 1-3 participating in the survey

No.	Source of water supply	Urban % (no)	Rural % (no)	Total % (no)
1	Governmental	68.8 (384)	8.6 (52)	27.6 (436)
2	Local project	2.3 (9)	41.2 (434)	28.9 (443)
3	Car truck	23.9 (96)	2.5 (26)	9.2 (122)
4	Buckets	4.9 (19)	47.5 (263)	34.1 (282)
5	Other	0.2 (1)	0.2 (1)	0.2 (2)
	Total	100 (509)	100 (776)	100 (1285)

There was a significant difference in source of water according to area of residence ($p<0.0001$). Governmental water supply was more characteristic of urban areas, whereas local projects, established by local inhabitants and supported by the government and external donors, were more common in rural areas. Both these methods delivered tapped water directly to the household. 69% of urban households had a governmental water supply compared to 9% of rural households. On the other hand, 41% of rural households had a local project water supply compared to 2% of urban households. In urban areas, the main alternative source of water was car truck where 24% of urban households obtained water this way. In rural areas, the main alternative source of water was using buckets to collect water from a water source where 48% of rural households obtained water this way.

Fathers level of education and employment:

27% of fathers in the survey were uneducated and 73% of fathers claimed they could read and write, regardless of their level of education (table 4.4.1 Q).

Table 4.4.1 Q
Educational status of fathers of children participating in survey of children aged 1-3

No.	Fathers level of education	Urban % (no)	Rural % (no)	Total % (no)
1	Uneducated	13.1 (51)	33.8 (219)	27.3 (270)
2	Read and write	14.3 (63)	18.5 (152)	17.2 (215)
3	Completed primary school	27.6 (147)	20.2 (193)	22.5 (340)
4	Completed secondary school	23.1 (136)	17.0 (148)	19.0 (284)
5	Completed university/college	21.9 (112)	10.5 (64)	14.1 (176)
	Total	100 (509)	100 (776)	100 (1285)

28%, 23%, and 22% of urban men claimed they had completed primary school, secondary school and university or college, respectively, compared to 20%, 17% and 11% of their rural counterparts (p=0.0009).

The fathers of the children aged 1-3 in the survey had the following occupations: 30% government employees, 39% manual labourers, 7% farmers, 5% private sector employees, 3.8% shop owners, 3.6% worked overseas, 3% drivers, 1% professionals (doctor, lawyer, engineers, academic staff at universities), 0.5% businessmen, 1% students, 4.5% were unemployed and 1% were dead. Manual labourers worked for example as builders, painters, or as carpenters. Individuals working overseas were mainly in Saudi Arabia and the United Arab Emirates. Individuals in these countries worked as manual labourers, cooks or waiters in restaurants, textile shops, sales assistants, and to a lesser extent businessmen.

Household possessions:

Overall, approximately 30% of households of study participants had a fridge/freezer, 59% had a television, 65% a radio, 16% a home telephone, 4% a mobile telephone, 19% a car, 5% a motorcycle, and 9% a bicycle (table 4.4.1 R). A significantly higher proportion of households in urban areas had the household possessions listed in table 4.4.1 R except for motorcycle, which was more common in rural areas, and particularly in Hodeidah province.

Table 4.4.1 R
Household possessions of participants in the survey of children aged 1-3

No.		Urban % (no)	Rural % (no)	Total % (no)	P value
1	Freezer				
	Yes	64.0 (361)	14.8 (229)	30.3 (590)	
	No	36.1 (148)	85.2 (547)	69.7 (695)	<0.0001
		100 (509)	100 (776)	100 (1285)	
2	Television				
	Yes	89.9 (468)	44.5 (404)	58.8 (872)	
	No	10.2 (41)	55.5 (372)	41.2 (413)	<0.0001
		100 (509)	100 (776)	100 (1285)	
3	Radio				
	Yes	76.5 (407)	60.1 (914)	65.3 (914)	
	No	23.5 (102)	39.9 (269)	34.7 (371)	0.03
		100 (509)	100 (776)	100 (1285)	
4	Home telephone				
	Yes	41.9 (240)	3.4 (25)	15.6 (265)	
	No	58.1 (269)	96.6 (751)	84.5 (1020)	<0.0001
		100 (509)	100 (776)	100 (1285)	
5	Mobile phone				
	Yes	9.6 (51)	1.5 (9)	4.0 (60)	
	No	90.4 (458)	98.6 (767)	96.0 (1225)	0.0008
		100 (509)	100 (776)	100 (1285)	
6	Car				
	Yes	26.0 (135)	15.1 (182)	18.6 (317)	
	No	74.0 (594)	84.9 (594)	81.4 (968)	0.0006
		100 (509)	100 (776)	100 (1285)	
7	Motor Cycle				
	Yes	2.7 (11)	6.5 (42)	5.3 (53)	
	No	97.3 (498)	93.5 (734)	94.7 (1232)	0.019
		100 (509)	100 (776)	100 (1285)	
8	Bicycle				
	Yes	13.2 (55)	6.7 (80)	8.8 (135)	
	No	86.8 (454)	93.3 (696)	91.2 (1150)	0.03
		100 (509)	100 (776)	100 (1285)	

4.4.2 Database of children aged 4-9 (Database 2):

The final dataset contained 467 records with each record representing one child aged 4-9 participating in the survey. The dataset had 68 fields representing the fields in questionnaire 2 (annex 2). The fields in database 2 were identical to the fields in database 1 of children aged 1-3 except for not having a few questions in database 1 that investigated the setting in which the child was delivered (health versus home), the qualifications of the person managing delivery of the child, the type of delivery, receiving antenatal care during pregnancy, as well as breastfeeding and its duration.
Otherwise database 2 consisted of data on the child aged 4-9 and the child's mother, and similar to database 1 it contained baseline information such as identification number, province, district, city or village, street, household and area of sampling. It also consisted of information on the child's personal and vaccination history, mother, father, and the household's characteristics and indicators of socioeconomic status.

76% (356/467) of children aged 4-9 participating in the survey were the older siblings of participants in the survey of children aged 1-3 and the data and results produced by this dataset were similar to that produced by dataset 1. Therefore the results produced by the variables and questions in this dataset will not be repeated except for variables that were either unique to dataset 2 or that were noticeably different from those resulting from dataset 1.

Child aged 4-9, mothers and sibling aged 1-3 identification numbers:

Each record had a unique identification number for the child aged 4-9 starting with 7001 and ending with 7469. If the child was the older sibling of a child aged 1-3 included in database 1, two fields recorded the mothers and child aged 1-3 identification numbers, which allowed linking children in database 1 with children in database 2.
Two records had missing data on anti-HBc status of the child and because this was one of the principal outcomes under investigation, record numbers 98 and 113 (child identification numbers 7098 and 7113) were dropped from the analysis.

Age of child, date of birth and source of child age:

The date of birth was known for 41% of children aged 4-9 participating in the survey. The mean age of children aged 4-9 participating in the survey was 6.5 years (95% CI 5.9 to 7.1) ranging from 4 to 9 years. The source of the child's age was recorded as an indicator of the accuracy of the reported age of the child (table 4.4.2 A). A higher proportion of children in this dataset had their age approximately determined and a lower proportion had their age determined by health records than children participating in the survey of children aged 1-3.

Table 4.4.2 A
Source of age for children participating in survey of children aged 4-9

No.	Source	Frequency	Percent
1	Approximate	101	26.2
2	Health records	96	14.9
3	Birth certificate	34	4.4
4	Parents memory	224	53.4
5	Parents records	10	1.0
6	ID card or passport	2	0.2
	Total	467	100

Sex of the child:

43% of the children aged 4-9 participating in the survey were male and 57% were female.

Child birth order:

The mean birth order of children aged 4-9 participating in the survey was 3.2 (95% CI 3.0 to 3.4) ranging from 1 to 6, which was lower than the mean birth order 4.5 (95% CI 4.2 to 4.8) of children aged 1-3 participating in the survey. Record number 212 (child identification number 7212) had missing data on the birth order of the child. After examining the distribution of child birth order, and its minimum and maximum values, child birth order was reduced to a categorical variable and was divided into 6 categories, with birth order's 6, 7 up to 15 combined into one category 6 to 15 (table 4.4.2 B).

Table 4.4.2 B
Birth order of children aged 4-9 participating in the survey

No.	Child birth order	Urban % (no)	Rural % (no)	Total % (no)
1	1	33.5 (76)	24.3 (53)	27.2 (129)
2	2	26.1 (61)	16.9 (40)	19.8 (101)
3	3	12.8 (35)	11.1 (30)	11.7 (65)
4	4	7.7 (21)	12.5 (25)	11.0 (46)
5	5	8.4 (15)	12.0 (25)	10.9 (40)
6	6 to 15	11.6 (33)	23.1 (52)	19.5 (85)
	Total	100 (241)	100 (225)	100 (466)

47% of children aged 4-9 participating in the survey were one of the first two children in birth order. 19% of children aged 1-3 participating in the survey were between the 6th to 15th child in birth order.

Vaccination card:

71% (346/467) of parents of children aged 4-9 participating in the survey said their children had a vaccination card compared to 84% of parents of children aged 1-3. There was no evidence of a significant difference in the number of parents saying their child had a vaccination card between urban (79%) and rural areas (67%) (p=0.08) (table 4.4.2 C).

Table 4.4.2 C
Having a vaccination card amongst children aged 4-9 participating in the survey

No.	Has vaccination card	Urban % (no)	Rural % (no)	Total % (no)
1	Yes	79.4 (194)	67.0 (152)	70.9 (346)
2	No	20.6 (47)	33.0 (74)	29.1 (121)
	Total	100 (241)	100 (226)	100 (467)

When study participants who said their child had a vaccination card were asked to present it only 33% (139/346) had the vaccination card available to show the study investigator. Amongst those who said their child had a vaccination card, there was evidence suggesting a significantly (p=0.03) higher availability of vaccination cards in urban areas (46%) compared to rural areas (26%).

Been vaccinated with hepatitis B vaccine and number of doses:

Although 78% of parents interviewed knew of hepatitis B vaccine only 10% of parents interviewed said their child aged 4-9 was vaccinated with hepatitis B vaccine, 88% said their child was not vaccinated and less than 2% did not know (table 4.4.2 D).

Table 4.4.2 D
Parent's response whether their child aged 4-9 participating in the survey received hepatitis B vaccine

No.	Vaccinated with hepatitis B vaccine	Urban % (no)	Rural % (no)	Total % (no)
1	Yes	16.5 (36)	7.2 (12)	10.1 (48)
2	No	81.3 (199)	91.6 (211)	88.3 (410)
3	Don't know	2.2 (6)	1.3 (3)	1.6 (9)
	Total	100 (241)	100 (226)	100 (467)

There was no evidence of a significant difference in parents response whether the child received hepatitis B vaccine between urban and rural residents (p=0.06). Nevertheless, differences did exist between the two urban provinces in the survey. 22% of participants in Sanaa said their child aged 4-9 participating in the survey received hepatitis B vaccine compared to 7% of participants in Aden (p=0.03). There was no evidence of a significant difference in parents response regarding their child's vaccination between rural provinces Taiz, Shabwa and Hodeidah, where 2.5%, 6.6% and 12.8%, respectively, of parents said their child was vaccinated with hepatitis B vaccine (p=0.2).

The number of doses received by children who had a vaccination card was taken from the card whatever the person interviewed reported (table 4.4.2 E). The vaccination status of 90% (419/467) of children aged 4-9 was determined and 10% (48/467) of children had an unknown vaccination status. This was determined by the study investigators checking the source of the child's vaccination status.

Table 4.4.2 E
Hepatitis B vaccination status amongst children aged 4-9 participating in the survey

No.	Number of doses	Frequency	Percent
1	Zero	410	88.3
2	One	2	0.2
3	Two	3	1.3
4	Three	4	0.5
5	Unknown	48	9.8
	Total	467	100

Twenty one percent of children's vaccination status was determined by a vaccination card and 79% of children's vaccination status was determined by the parent's memory.

Overall, 1.9% of children aged 4-9 participating in the survey received at least one dose of hepatitis B vaccine, and 0.5% received all three doses of vaccine. This low vaccine coverage amongst children aged 4-9 is because these children are not targeted for hepatitis B vaccination in the first place, and the low percentage of these children that were vaccinated were done so by their parents own initiative.

There was a significant difference in vaccination status by area. 1.5% of children aged 4-9 living in urban areas were completely vaccinated compared to 0% of children in rural areas. Similarly, 4.3% of children aged 4-9 in urban areas received one or two doses of hepatitis B vaccine compared to 0.1% of children in rural areas (p=0.004).

Schooling:

This recorded whether the child aged 4-9 participating in the survey attended school. Overall, 48% of children participating in the survey of the children aged 4-9 went to school. There was no evidence of a significant difference in school attendance between children in urban areas (54%) and rural areas (45%) (p=0.5). The odds ratio for attending school was 2.4 (95% CI 2.1 to 2.6) per one year increase in the age of the child.

4.4.3 Database of laboratory results of children aged 1-3 (Database 3):

The final dataset had 12 fields containing 1285 records with each record representing the laboratory results of one child aged 1-3 participating in the survey.

The dataset contained laboratory results of all children aged 1-3 for hepatitis B surface antigen (HBsAg) with S/N ratio, hepatitis B core antibody (anti-HBc) with S/N ratio, immunoglobulin M hepatitis B core antibody (IgM anti-HBc) with S/N ratio, and hepatitis B surface antibody (anti-HBs) with S/N ratio. On data entry results were coded as 1 if the test result was positive and 0 if the result was negative.

One record had missing data on anti-HBc status of the child and because this was one of the principal outcomes under investigation, record number 247 (child identification number 1247 and consequently mother identification number 4247) was dropped from the analysis.

4.4.4 Database of laboratory results of mothers (Database 4):

The final dataset had 12 fields containing 1285 records with one record representing the laboratory results of the mother of each child aged 1-3 participating in the survey.

The dataset contained laboratory results of mothers of all children aged 1-3 participating in the survey for hepatitis B surface antigen (HBsAg) with S/N ratio, hepatitis B core antibody (anti-HBc) with S/N ratio, and hepatitis B e antigen (HBeAg) with S/N ratio. On data entry results were coded as 1 if the test result was positive and 0 if the result was negative.

One record (mother identification number 4247) was dropped from the analysis because the child of this mother did not have anti-HBc results, and the objectives would not be fulfilled by looking at the mothers results alone.

4.4.5 Database of laboratory results of children aged 4-9 (Database 5):

The final dataset had 9 fields containing 467 records with each record representing the laboratory results of one child aged 4-9 participating in the survey.

The dataset contained the laboratory results of children aged 4-9 participating in the survey tested for the same serological markers of children aged 1-3, and the results were coded similarly.

Two records 98 and 113 were dropped (child identification number 7098 and 7113) because the child did not have anti-HBc results.

4.4.6 Combined database of children aged 1-3, their mothers, and laboratory results (Database 6):

This database contained data from the questionnaire of children aged 1-3 and their mothers (database 1), together with the corresponding laboratory results of children aged 1-3 (database 3) and laboratory results of mothers of children aged 1-3 (database 4).

This database was created by merging database files 1, 3 and 4 using unique identifier variables, which were the child's and mothers identification numbers which were unique to each study participant.

4.4.7 Combined database of children aged 4-9 and their laboratory results (Database 7):

This database contained data from questionnaire of children aged 4-9 (database 2) and their corresponding laboratory results (database 5).

This database was created by merging database files 2 and 5 using a unique identifier variable, which was the child's identification number that was unique to each study participant.

Chapter 5: Results Women of Childbearing Age:

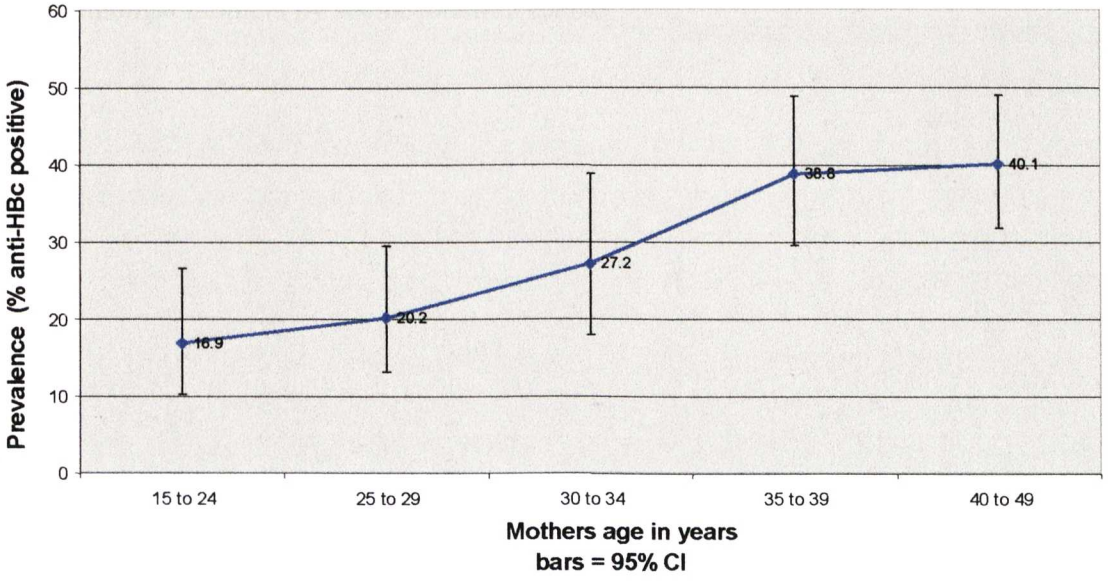
In this chapter the results of the prevalence, univariable analysis, and multivariable analysis of HBV infection and chronic carrier status amongst women of childbearing age (mothers of the children aged 1-3 participating in the survey) are presented. The terms mothers and women of childbearing age will be used interchangeably.

HBV Infection:

5.1 Prevalence of mothers hepatitis B core antibody (anti-HBc positive):

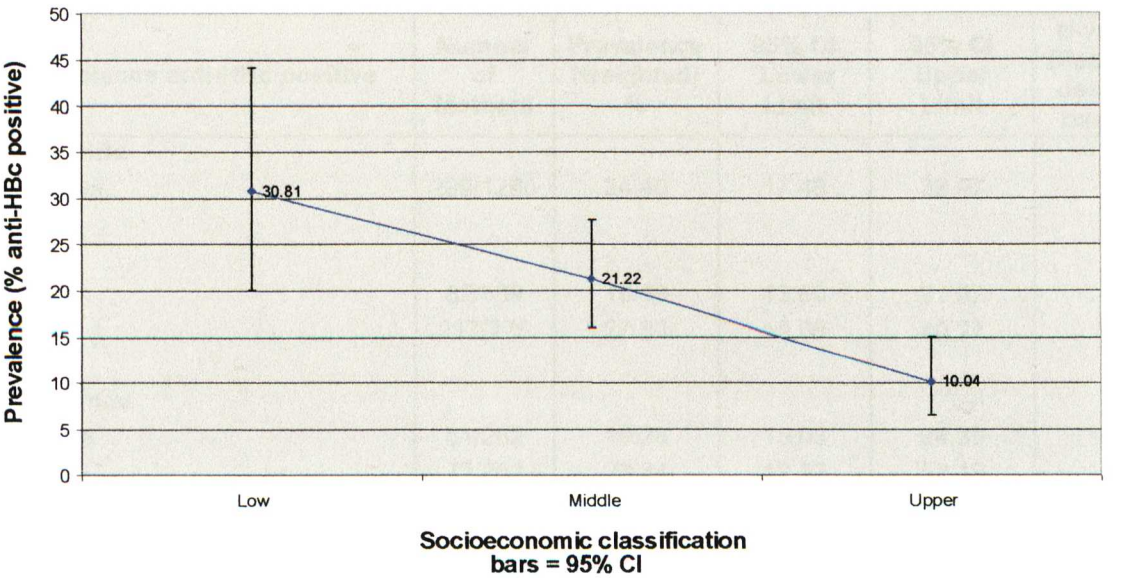
The overall prevalence of anti-HBc amongst women of childbearing age in Yemen was 24.4% (95% CI 17.48 to 32.97). A total of 299 out of the 1285 women participating in the survey were anti-HBc positive. There was a statistically significant difference in the prevalence of anti-HBc amongst mothers by age, area of residence, socioeconomic status, and educational status. There was a clear increase in the prevalence of anti-HBc amongst mothers with increasing age ($p<0.0001$). The prevalence of anti-HBc was lowest amongst mothers aged 15 to 24 (16.87%) and highest amongst mothers aged 40 to 49 (40.1%). Figure 5.1.1 shows the prevalence of anti-HBc amongst mothers by age.

Figure 5.1.1
Prevalence of anti-HBc amongst mothers by age



The prevalence of anti-HBc amongst mothers living in urban areas (16.99%) was lower than the prevalence of anti-HBc amongst mothers living in rural areas (27.83%) and this difference unadjusted for age was statistically significant (p=0.04). When the prevalence of anti-HBc amongst mothers was compared by urban versus rural area of birth of mother the results were similar to that by area of residence of the mother. The prevalence of anti-HBc was 15% (41/293) amongst mothers born in urban areas and 27% (215/793) amongst mothers born in rural areas, and this difference was also statistically significant (p=0.04). The prevalence of anti-HBc amongst uneducated women (29.41%) was nearly double the prevalence of anti-HBc amongst women claiming to have any level of education (15.77%) and this difference was statistically significant (p=0.0005). Despite this there was no evidence of a statistically significant difference in the prevalence of anti-HBc by mothers level of education (p=0.68). The prevalence of anti-HBc amongst mothers claiming to have attended University or College was 8.98%, which was lower than the prevalence of anti-HBc amongst mothers claiming that they could read and write, completed primary school, or completed secondary school, which was 15.87%, 16.47%, and 15.93%, respectively. The prevalence of anti-HBc was 30.81% amongst mothers of low socioeconomic status, 21.22% amongst women of middle socioeconomic status, and 10.04% amongst women of upper socioeconomic status unadjusted for age and this was statistically significant (p=0.002). Figure 5.1.2 shows the prevalence of anti-HBc amongst mothers by socioeconomic status.

Figure 5.1.2
Prevalence of anti-HBc amongst mothers by socioeconomic status



The prevalence of anti-HBc was lower amongst mothers in paid employment (18.19%) compared to mothers who were not in paid employment (24.62%), however there was no evidence of a statistically significant difference in the prevalence of anti-HBc according to the employment status of the mother (p=0.35). The prevalence of anti-HBc amongst mothers was 19.28% in Sanaa, 28.34% in Taiz, 12.57% in Aden, 28.64% in Shabwa, and 27.03% in Hodeidah. There was no evidence of a statistically significant difference in the prevalence of anti-HBc when comparing all five provinces (p=0.58), the three rural provinces (p=0.97), or the two urban provinces (p=0.13) in the survey. Table 5.1.1 shows the prevalence of anti-HBc amongst mothers by mothers demographic and educational characteristics.

Table 5.1.1
Prevalence of anti-HBc positive mothers by women's demographic and educational characteristics

Prevalence anti-HBc positive	Number of Mothers	Prevalence (weighted) %	95% CI Lower Limit	95% CI Upper Limit	p-value (Pearson design-based)
anti-HBc					
Overall	299/1285	24.40	17.48	32.97	
Area					
Urban	82/509	16.99	13.60	21.02	
Rural	217/776	27.83	18.06	40.27	0.04
Province					
Sanaa	51/262	19.28	15.03	24.39	
Taiz	72/252	28.34	12.53	52.19	
Aden	31/247	12.57	7.67	19.92	
Shabwa	76/268	28.64	12.87	52.16	
Hodeidah	69/256	27.03	17.36	39.53	0.41
Mother's Age					
15 to 24	60/400	16.87	10.23	26.55	
25 to 29	71/349	20.16	13.20	29.54	
30 to 34	73/268	27.15	17.94	38.85	
35 to 39	62/177	38.81	29.62	48.86	
40 to 49	33/91	40.10	31.82	48.98	<0.0001
Mother's Educational Status					
Educated	81/547	15.77	10.64	22.76	
Uneducated	218/733	29.41	20.67	39.99	0.0005
Mother's Level of Education					
Read and Write	28/179	15.87	8.02	28.99	
Completed Primary School	33/221	16.47	11.85	22.44	
Completed Secondary School	16/113	15.93	9.54	25.40	
Completed University or College	4/34	8.98	4.04	18.78	0.68
Mother's Employment					
Employed	11/55	18.19	9.77	31.36	
Not employed	288/1230	24.62	17.48	33.50	0.35
Socioeconomic Status					
Low	158/506	30.81	20.03	44.18	
Middle	110/508	21.22	15.95	27.66	
Upper	31/270	10.04	6.55	15.09	0.002

5.2 Mothers anti-HBc univariable analysis:

The univariable analysis of the association of anti-HBc positivity amongst women of childbearing age with exposure variables are here classified into three types of results. The first had an OR that was statistically significantly different from 1. The second type of result had notably increased or decreased OR by more or less than 30% but these were not statistically significant. The third type of result neither had notably increased nor decreased OR. These results are shown in tables 5.2.1 and 5.2.2.

Overall, the exposure variables that had a statistically significant association with anti-HBc positivity amongst mothers by univariable analysis were age of the mother, area of residence, mothers educational status, socioeconomic status, ownership of the household, source of water in the household, and the availability of electricity, television, fridge/freezer, and home telephone in the household.

The age of the mother in years was the exposure variable with the most significant association with mothers anti-HBc positivity. There was an increase in the odds of mothers anti-HBc positivity with increasing age of the mother (p=0.0001) with a significant difference in the odds of anti-HBc positivity across mothers age-groups. The OR of anti-HBc positivity was 2.22 amongst uneducated mothers and this was statistically significantly higher than anti-HBc positivity amongst mothers claiming to be educated (p=0.001). The OR of anti-HBc positivity (OR=1.88) amongst mothers living in rural areas was statistically significantly higher than mothers living in urban areas (p=0.04). The OR of being anti-HBc positive was statistically significantly lower amongst mothers of middle (OR=0.6) and upper (OR=0.25) socioeconomic status compared to mothers of low socioeconomic status (p=0.003). Fridge/freezer, television, and home telephone were the three household commodities that were statistically significantly associated with anti-HBc positivity amongst women of childbearing age. The OR of being anti-HBc positive was 2.35 amongst women living in households without a fridge/freezer (p=0.003), 1.99 amongst mothers living in households without a television (p=0.04) and 2.9 amongst mothers living in households without a home telephone (p=0.002). The OR of being anti-HBc positive was 1.7 amongst mothers living

in households without an electricity supply (p=0.05) and 0.58 amongst mothers living in rented households (p=0.03). There was an association between mothers anti-HBc positivity and the households source of water (p=0.008), with a higher odds of mothers being anti-HBc positive in households that obtained water through a local project or bucket. Table 5.2.1 shows the results of the univariable analysis of the association of mothers demographic characteristics with anti-HBc positivity amongst mothers in Yemen.

Table 5.2.1
Association of anti-HBc positivity amongst mothers with women's demographic characteristics

Variable		Number Controls	Number Cases	OR	p value	95% C.I.
Age of mother (in years)	15 to 24	340	60	1		
	25 to 29	278	71	1.24	0.26	0.84 – 1.84
	30 to 34	195	73	1.84	< 0.001	1.37 – 2.47
	35 to 39	115	62	3.13	< 0.001	2.10 – 4.64
	40 to 49	58	33	3.30	0.001	1.73 – 6.29
	Joint significance				**0.0001**	
	Linear trend OR			1.41	< 0.001	1.24 – 1.60
Province	Sanaa	211	51	1		
	Taiz	180	72	1.66	0.33	0.57 – 4.78
	Aden	216	31	0.60	0.11	0.32 – 1.12
	Shabwa	192	76	1.68	0.31	0.59 – 4.77
	Hodeidah	187	69	1.55	0.17	0.82 – 2.95
	Joint significance				0.18	
Area	Urban	427	82	1		
	Rural	559	217	1.88	**0.04**	1.02 – 3.49
Mothers Birth Order	1	205	73	1		
	2	159	44	0.65	0.26	0.30 – 1.40
	3	163	41	0.64	0.004	0.47 – 0.85
	4	113	47	1.06	0.86	0.53 – 2.14
	5	104	28	0.63	0.1	0.35 – 1.11
	6 to 18	242	66	0.66	0.24	0.33 – 1.35
	Joint significance				0.07	
	Linear Trend OR			0.95	0.36	0.83 – 1.07
Mothers Education	Educated	466	81	1		
	Uneducated	520	218	2.22	**0.001**	1.47 – 3.36
Mothers Employment	Unemployed	942	288	1		
	Employed	44	11	0.68	0.36	0.29 – 1.59
Husbands Education	Educated	799	216	1		
	Uneducated	187	83	1.45	0.11	0.91 – 2.32

Exposure variables that had notably increased or decreased OR by more or less than 30% but were not statistically significantly associated with anti-HBc positivity amongst mothers were province of sampling, employment status of the mother, husbands educational status, type of household, crowding index of the household, and mothers birth order.

The OR of mothers being anti-HBc positive was 1.66, 1.68, 1.55 and 0.60 in Taiz, Shabwa, Hodeidah, and Aden, respectively, compared to Sanaa (p=0.18). Women in paid employment appeared to have a lower odds of being anti-HBc positive (OR=0.68) compared to women who were not in paid employment (p=0.36). Women with an uneducated husband had a higher odds of being anti-HBc positive (OR=1.45) than women with an educated husband (p=0.11). The odds of anti-HBc positivity amongst women living in apartments or shacks appeared to be lower than women living in houses (p=0.15). The OR of mothers anti-HBc positivity associated with crowding index of the household did not follow a clear trend but households with higher crowding indexes appeared to have a higher odds of being anti-HBc positive (p=0.09). Likewise, the OR of anti-HBc positivity associated with mothers birth order was not significant and did not follow a clear trend (p=0.07). Table 5.2.2 shows the results of the univariable analysis of the association of household characteristics and possessions with anti-HBc positivity amongst mothers in Yemen.

Table 5.2.2
Association of anti-HBc positivity amongst mothers with household characteristics and possessions

Variable		Number Controls	Number Cases	OR	p value	95% C.I.
Household Ownership	Owned	829	269	1		
	Rented	157	30	0.58	**0.03**	0.36 – 0.93
Household Type	House	707	241	1		
	Apartment	188	33	0.54	0.05	0.29 – 0.99
	Shack	90	25	0.74	0.51	0.29 – 1.90
	Joint significance				0.15	
Household Construction	Stone	289	85	1		
	Block	457	127	0.99	0.99	0.57 – 1.75
	Mud or Clay	141	49	1.04	0.95	0.32 – 3.39
	Mixed	99	38	1.12	0.79	0.47 – 2.67
	Joint significance				0.99	
Crowding Index	1	77	23	1		
	2	270	53	0.91	0.81	0.42 – 1.97
	3	244	68	1.26	0.53	0.59 – 2.73
	4	155	66	2.12	0.05	0.99 – 4.55
	5	97	29	1.47	0.37	0.61 – 3.52
	6 to 18	143	60	2.39	0.02	1.21 – 4.74
	Joint significance				0.09	
	Linear Trend OR			1.22	0.003	1.08 – 1.39
Electricity Supply	Yes	724	180	1		
	No	262	119	1.7	**0.05**	0.99 – 2.94
Source of Water	Governmental	374	62	1		
	Local Project	321	122	2.43	0.002	1.46 – 4.03
	Water Truck	94	28	1.50	0.35	0.62 – 3.66
	Buckets	195	87	2.70	0.048	1.01 – 7.2
	Joint significance				**0.008**	
Freezer	Yes	498	92	1		
	No	488	207	2.35	**0.003**	1.38 – 4.01
Television	Yes	707	165	1		
	No	279	134	1.99	**0.04**	1.05 – 3.79
Radio	Yes	714	200	1		
	No	272	99	1.27	0.25	0.83 – 1.94
Home Phone	Yes	233	32	1		
	No	753	267	2.90	**0.002**	1.58 – 5.31
Mobile Phone	Yes	51	9	1		
	No	935	290	1.97	0.09	0.88 – 4.37
Car	Yes	244	73	1		
	No	742	226	1.07	0.74	0.70 – 1.63
Socioeconomic Status	Low	348	158	1		
	Middle	398	110	0.6	0.07	0.35 – 1.04
	Upper	239	31	0.25	0.001	0.12 – 0.51
	Joint significance				**0.003**	

Exposure variables that neither showed notable increased or decreased OR nor evidence of a statistically significant association with anti-HBc positivity amongst mothers were ownership of a radio (p=0.25) or car (p=0.74), and the material used in the construction of the household (p=0.99).

5.3 Mothers anti-HBc multivariable analysis:

The independent variables that had a statistically significant association with anti-HBc positivity amongst women of childbearing age in the multiple logistic regression analysis after adjusting for the remaining variables in the model were age of the mother, ownership of a fridge/freezer, and ownership of a home telephone.

There was an increase in the adjusted OR of anti-HBc positivity amongst mothers with increasing age of the mother (p=0.0001). The OR of being anti-HBc positive was 1.29, 1.83, 3.15, and 2.99 amongst women aged 25 to 29, 30 to 34, 35 to 39, and 40 to 49, respectively. The adjusted OR of anti-HBc positivity was 1.76 (95% CI 1.04 to 2.97) amongst mothers who did not have a fridge or freezer in their household and 1.59 (95% CI 1.03 – 2.46) amongst mothers living in households that did not have a home telephone. The adjusted OR of anti-HBc positivity associated with area of residence was 1.12 (95% CI 0.58 to 2.14) amongst mothers living in rural areas and this was not significant (p=0.72) after adjusting for the remaining variables in the model, although in the univariable analysis the odds of anti-HBc positivity amongst women living in rural areas was double the odds of anti-HBc positivity amongst women living in urban areas. Mothers birth order (p=0.11) and educational status (p=0.19) did not show evidence of a statistically significant association with anti-HBc positivity amongst mothers, however, they were found to be significantly associated with HBV chronic infection (HBsAg chronic carrier status) amongst mothers. Therefore, they were added to the final multivariable model of mothers anti-HBc positivity resulting in two models, one for mothers anti-HBc positivity and the other for mothers HBV chronic infection, both including identical independent variables. There was no evidence of interaction between the independent variables in the multivariable model. Table 5.3.1 shows the results of the

multivariable analysis of the association of independent variables with anti-HBc positivity amongst mothers in Yemen.

Table 5.3.1
Adjusted logistic regression of the association of anti-HBc positivity amongst mothers with independent exposure variables

Variable		OR*	p value**	95% C.I.***
Mothers Age (in years)	15 to 24	1		
	25 to 29	1.29	0.231	0.84 – 1.98
	30 to 34	1.83	<0.001	1.37 – 2.44
	35 to 39	3.15	<0.001	2.13 – 4.66
	40 to 49	2.99	0.002	1.55 – 5.75
	Joint significance	****	***0.0001***	
Mothers Birth Order	1	1		
	2	0.64	0.25	0.29 – 1.41
	3	0.65	0.02	0.46 – 0.91
	4	1.13	0.73	0.55 – 2.34
	5	0.73	0.26	0.41 – 1.29
	6 to 18	0.69	0.31	0.32 – 1.46
	Joint significance		*0.23*	
Area	Urban	1		
	Rural	1.12	0.72	0.58 – 2.14
Mothers Educational Status	Educated	1		
	Uneducated	1.37	0.19	0.84 – 2.22
Fridge/Freezer Ownership	Yes	1		
	No	1.76	**0.04**	1.04 – 2.97
Home Telephone Ownership	Yes	1		
	No	1.59	**0.04**	1.03 – 2.46

* adjusted OR computed by survey logistic regression multivariable analysis.
** p-value of the adjusted Wald test statistic.
*** 95% Confidence Intervals.
**** p-value of the adjusted survey Wald test statistic of the significance of the joint effect of the categories of the independent variable on the dependent variable.

The multivariable analysis of variables associated with anti-HBc positivity amongst mothers was repeated excluding the socioeconomic variables that contributed to the socioeconomic index constructed in the survey and substituting them with this single socioeconomic status variable. The OR and p-values of anti-HBc positivity associated with mothers age and birth order were extremely similar in this model as in table 5.3.1. The adjusted OR of anti-HBc positivity associated with residing in a rural area increased

from 1.12 to 1.41 (p=0.2). Socioeconomic status, on the other hand, was significantly associated with anti-HBc positivity, and there was a clear decrease in the OR of anti-HBc positivity associated with increasing socioeconomic status. Table 5.3.2 shows the results of the multivariable analysis of the association of independent variables and the socioeconomic status variable with anti-HBc positivity amongst mothers participating in the survey.

Table 5.3.2
Adjusted logistic regression of the association of anti-HBc positivity amongst mothers with independent exposure variables and socioeconomic status

Variable		OR*	p value**	95% C.I.***
Mothers Age (in years)	15 to 24	1		
	25 to 29	1.29	0.24	0.84 – 1.98
	30 to 34	1.79	0.001	1.29 – 2.48
	35 to 39	3.08	<0.001	2.09 – 4.54
	40 to 49	2.91	0.003	1.49 – 5.68
	Joint significance	****	***0.0005***	
Mothers Birth Order	1	1		
	2	0.62	0.20	0.29 – 1.32
	3	0.65	0.01	0.47 – 0.91
	4	1.07	0.83	0.54 – 2.14
	5	0.69	0.18	0.40 – 1.21
	6 to 18	0.64	0.23	0.31 – 1.35
	Joint significance		*0.23*	
Area	Urban	1		
	Rural	1.41	0.20	0.82 – 2.44
Socioeconomic Status	Low	1		
	Middle	0.73	0.21	0.44 – 1.22
	Upper	0.32	0.001	0.17 – 0.58
	Joint significance		***0.004***	

* adjusted OR computed by survey logistic regression multivariable analysis.
** p-value of the adjusted Wald test statistic.
*** 95% Confidence Intervals.
**** p-value of the adjusted survey Wald test statistic of the significance of the joint effect of the categories of the independent variable on the dependent variable.

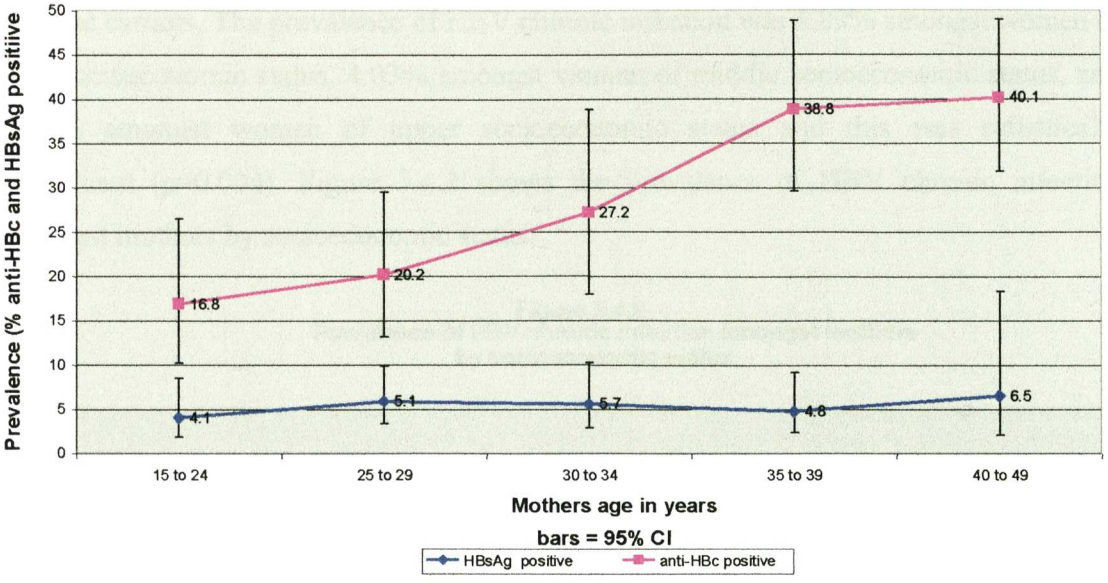
HBV Chronic Infection (HBsAg chronic carrier):

5.4 Prevalence of mothers HBV chronic infection:

The overall prevalence of HBV chronic infection amongst women of childbearing age in Yemen was 5.08% (95% CI 3.56 to 7.20). 59 out of the 1285 women participating in the survey were chronic carriers of HBV (HBsAg positive). The prevalence of hepatitis B e antigenaemia was 12.84% (95% CI 6.12 to 24.99) amongst these HBV chronically infected women with 8 out of 59 HBV chronic carrier women being hepatitis B e antigen positive. There was a statistically significant difference in the prevalence of HBV chronic infection amongst mothers by area of residence, socioeconomic status, and educational status.

The prevalence of HBV chronic infection was lowest amongst women aged 15 to 24 years (4.03%) and highest amongst women aged 40 to 49 years (6.49%). However, there was no evidence of a statistically significant difference in the prevalence of HBV chronic infection by mothers age (p=0.78) nor was there a clear pattern of increasing prevalence of HBV chronic infection with increasing mothers age. Figure 5.4.1 shows the prevalence of HBV infection amongst mothers by age.

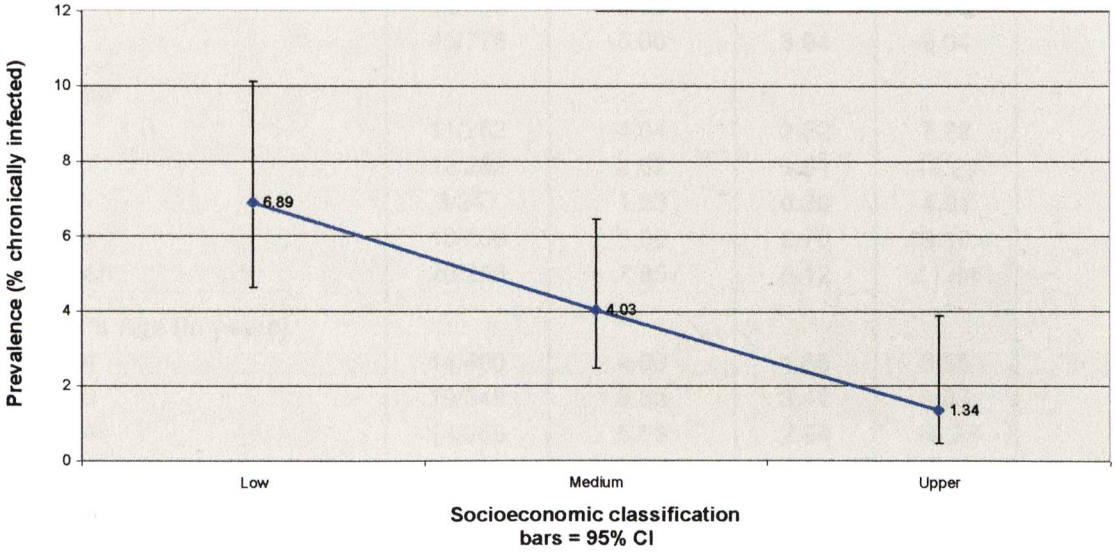
Figure 5.4.1
Prevalence of HBV infection amongst mothers by age



The prevalence of HBV chronic infection amongst mothers in urban areas (3.08%) was half the prevalence of HBV chronic infection amongst mothers in rural areas (6%) and this difference unadjusted for age was statistically significant (p=0.05). When the prevalence of HBV chronic infection amongst mothers was compared by urban versus rural area of birth of mother the results were similar to what was found by area of residence of the mother. The prevalence of HBV chronic infection was 2.9% (7/293) amongst mothers born in urban areas and 5.9% (45/793) amongst mothers born in rural areas (p=0.06). The prevalence of HBV chronic infection amongst uneducated women (7.2%) was higher than the prevalence of HBV chronic infection amongst women claiming to have any level of education (1.43%) and this difference was statistically significant (p=0.001). However, there was no evidence of a statistically significant difference in the prevalence of HBV chronic infection by mothers level of education (p=0.13).There were only 7 cases of HBV chronic infection amongst women claiming to have any level of education. There were no cases (0/34) of HBV chronic infection amongst women claiming to have completed University or College. 1 out of 113 (0.62%) women claiming to have completed secondary school was a HBV chronic carrier, 4 out

of 221 (2.78%) women claiming to have completed primary school were HBV chronic carriers, and 2 out of 179 (0.4%) women claiming they could read and write were HBV chronic carriers. The prevalence of HBV chronic infection was 6.89% amongst women of low socioeconomic status, 4.03% amongst women of middle socioeconomic status, and 1.34% amongst women of upper socioeconomic status and this was statistically significant (p=0.004). Figure 5.4.2 shows the prevalence of HBV chronic infection amongst mothers by socioeconomic status.

Figure 5.4.2
Prevalence of HBV chronic infection amongst mothers by socioeconomic status



The prevalence of HBV chronic infection was slightly lower amongst women in paid employment (4.62%) compared to women who were not in paid employment (5.1%) but this difference was not statistically significant (p=0.91). The prevalence of HBV chronic infection amongst mothers was 4.04% in Sanaa, 4.62% in Taiz, 1.23% in Aden, 5% in Shabwa, and 7.85% in Hodeidah. There was no evidence of a statistically significant difference in the prevalence of HBV chronic infection when comparing all five provinces (p=0.13), the three rural provinces (p=0.33), or the two urban provinces (p=0.12) in the survey. Table 5.4.1 shows the prevalence of HBV chronic infection amongst mothers by mothers demographic and educational characteristics.

Table 5.4.1
Prevalence of HBV chronic infection (HBsAg positive) amongst mothers by women's demographic and educational characteristics

HBV Chronic Infection (HBsAg positive) Prevalence	Number of Mothers	Prevalence (weighted) %	95% CI Lower Limit	95% CI Upper Limit	p-value (Pearson design-based)
HBsAg					
Overall	59/1285	5.08	3.56	7.20	
HBeAg/ HBsAg positive					
Positive	8/59	12.84	6.12	24.99	
Negative	51/59	87.16	75.01	93.88	
Area					
Urban	14/509	3.08	1.78	5.28	
Rural	45/776	6.00	3.94	9.04	0.05
Province					
Sanaa	11/262	4.04	2.22	7.22	
Taiz	12/252	4.62	1.81	11.29	
Aden	3/247	1.23	0.30	4.91	
Shabwa	13/268	5.00	2.70	9.10	
Hodeidah	20/256	7.85	5.12	11.84	0.13
Mother's Age (in years)					
15 to 24	14/400	4.03	1.85	8.55	
25 to 29	19/349	5.85	3.41	9.87	
30 to 34	14/268	5.56	2.94	10.25	
35 to 39	8/177	4.75	2.41	9.12	
40 to 49	4/91	6.49	2.11	18.30	0.78
Mother's Educational Status					
Educated	7/547	1.43	0.60	3.37	
Uneducated	52/738	7.20	5.01	10.24	0.001
Mother's Level of Education					
Read and Write	2/179	0.40	0.05	3.17	
Completed 1ry	4/221	2.78	1.04	7.23	
Completed 2ry	1/113	0.62	0.08	4.77	
Completed Univ.	0/34	0.00			0.13
Mother's Employment					
Employed	2/55	4.62	0.87	21.09	
Not Employed	57/1230	5.10	3.50	7.35	0.91
Socioeconomic Status					
Low	34/506	6.89	4.63	10.12	
Medium	21/508	4.03	2.48	6.47	
Upper	4/270	1.34	0.46	3.88	0.004

5.5 Mothers HBV chronic infection univariable analysis:

Similar to the univariable analysis of HBV infection, the univariable analysis of the association of HBV chronic infection amongst women of childbearing age with exposure variables are also classified into three types of results. The first had an OR that were statistically significantly different from 1. The second type of result had notably increased or decreased OR by more or less than 30% but these were not statistically significant. The third type of result neither had notable increased or decreased OR. These results are shown in tables 5.5.1 and 5.5.2.

Overall, the exposure variables statistically significantly associated with mothers HBV chronic infection by univariable analysis were educational status, area of residence, mothers birth order, type and crowding index of the household, socioeconomic status, and the availability of television and freezer in the household.

Mothers educational status was the exposure variable with the most significant association with mothers HBV chronic infection. The OR of HBV chronic infection was 5.36 amongst uneducated women and this was statistically significantly higher than HBV chronic infection amongst women claiming to be educated (p=0.002). The OR of HBV chronic infection (OR=2) amongst women living in rural areas was statistically significantly higher than women living in urban areas (p=0.05). The OR of HBV chronic infection was statistically significantly lower amongst women of middle (OR=0.57) and upper (OR=0.18) socioeconomic status compared to women of low socioeconomic status (p=0.01). Fridge/freezer and television were the two household commodities that were statistically significantly associated with HBV chronic infection amongst women of childbearing age. The OR of HBV chronic infection was 3.65 amongst women living in households without a fridge/freezer (p=0.001) and 2.22 amongst mothers living in households without a television (p=0.01). The OR of HBV chronic infection was 0.12 amongst mothers living in apartments compared to mothers living in houses (p=0.003). The odds of mothers HBV chronic infection appeared to be higher in households with a crowding index of 4 and above (p=0.05). HBV chronic infection was significantly associated with mothers birth order but did not follow a clear trend (p=0.04). Table 5.5.1

shows the results of the univariable analysis of the association of mothers demographic characteristics with HBV chronic infection amongst mothers in Yemen.

Table 5.5.1
Association of HBV chronic infection (HBsAg positive) amongst mothers with women's demographic characteristics

Variable		Number Controls	Number Cases	OR	p value	95% C.I.
Age of mother (in years)	15 to 24	386	14	1		
	25 to 29	330	19	1.48	0.38	0.60 – 3.65
	30 to 34	254	14	1.40	0.41	0.61 – 3.22
	35 to 39	169	8	1.19	0.75	0.39 – 3.61
	40 to 49	87	4	1.65	0.52	0.33 – 8.3
	Joint significance				*0.92*	
	Linear trend OR			1.09	0.51	0.84 – 1.41
Province	Sanaa	251	11	1		
	Taiz	240	12	1.15	0.8	0.37 – 3.62
	Aden	244	3	0.30	0.12	0.06 – 1.40
	Shabwa	255	13	1.25	0.6	0.51 – 3.05
	Hodeidah	236	20	2.02	0.07	0.94 – 4.35
	Joint significance				*0.13*	
Area	Urban	495	14	1		
	Rural	731	45	2.00	**0.05**	0.98 – 4.12
Mothers Birth Order	1	267	11	1		
	2	195	8	0.7	0.45	0.27 – 1.82
	3	197	7	0.83	0.6	0.40 – 1.73
	4	147	13	2.19	0.02	1.18 – 4.07
	5	126	6	1.14	0.72	0.53 – 2.49
	6 to 18	294	14	1.08	0.88	0.39 – 3.00
	Joint significance				***0.04***	
	Linear Trend OR			1.06	0.5	0.89 – 1.28
Mothers Education	Educated	540	7	1		
	Uneducated	686	52	5.36	**0.002**	2.01 – 14.3
Mothers Employment	Unemployed	1173	57	1		
	Employed	53	2	0.9	0.91	0.14 – 5.64
Husbands Education	Educated	974	41	1		
	Uneducated	252	18	1.55	0.29	0.67 – 3.61

Exposure variables that had notably increased or decreased OR by more or less than 30% but were not statistically significantly associated with HBV chronic infection amongst mothers were province of sampling, husbands educational status, household ownership and construction, household source of water, availability of an electricity supply, home telephone, and mobile telephone in the household.

The OR of HBV chronic infection amongst women was 1.15, 1.25, 2.02 and 0.3 in Taiz, Shabwa, Hodeidah and Aden, respectively, compared to Sanaa (p=0.13). Women with an uneducated husband had a higher odds of HBV chronic infection (OR=1.55) than women with an educated husband (p=0.29). The OR of HBV chronic infection was 0.36 amongst mothers living in rented households compared to mothers living in owned houses (p=0.15). The odds of HBV chronic infection was 1.33, 1.65, and 2.04 amongst women living in households built of block, mud/clay, or mixed material, respectively (p=0.69). There was a higher odds of mothers HBV chronic infection in households that obtained water through a local project, bucket, or truck when compared to households with a governmental water supply (p=0.2). The OR of HBV chronic infection was 2.36 amongst mothers living in households without a home telephone (p=0.11), 2.27 amongst mothers living in households without a mobile telephone (p=0.42), and 1.91 amongst mothers living in households without an electricity supply (p=0.08). Table 5.5.2 shows the results of the univariable analysis of the association of household characteristics and possessions with HBV chronic infection amongst mothers in Yemen.

Table 5.5.2
Association of HBV chronic infection amongst mothers with household characteristics and possessions

Variable		Number Controls	Number Cases	OR	p value	95% C.I.
Household Ownership	Owned	1043	55	1		
	Rented	183	4	0.36	0.15	0.08 – 1.51
Household Type	House	898	50	1		
	Apartment	219	2	0.12	0.003	0.03 – 0.43
	Shack	108	7	1.08	0.9	0.31 – 3.78
	Joint significance				***0.01***	
Household Construction	Stone	359	15	1		
	Block	561	23	1.33	0.39	0.67 – 2.63
	Mud or Clay	179	11	1.65	0.34	0.56 – 4.84
	Mixed	127	10	2.04	0.26	0.57 – 7.34
	Joint significance				*0.69*	
Crowding Index	1	96	4	1		
	2	312	11	0.91	1.13	0.30 – 4.28
	3	302	10	1.26	0.78	0.14 – 4.31
	4	208	13	2.12	1.91	0.45 – 8.07
	5	119	7	1.47	2.15	0.46 – 10.04
	6 to 18	189	14	2.39	2.37	0.53 – 10.55
	Joint significance				***0.05***	
	Linear Trend OR			1.25	0.03	1.03 – 1.53
Electricity Supply	Yes	872	32	1		
	No	354	27	1.91	0.08	0.92 – 3.95
Source of Water	Governmental	425	11	1		
	Local Project	418	25	2.48	0.03	1.10 – 5.61
	Water Truck	116	6	1.70	0.35	0.54 – 5.30
	Buckets	265	17	2.36	0.09	0.86 – 6.46
	Joint significance				*0.2*	
Freezer	Yes	576	14	1		
	No	650	45	3.65	**0.001**	1.81 – 7.36
Television	Yes	841	31	1		
	No	385	28	2.22	**0.01**	1.21 – 4.06
Radio	Yes	876	38	1		
	No	350	21	1.01	0.97	0.55 – 1.87
Home Phone	Yes	259	6	1		
	No	967	53	2.36	0.11	0.82 – 6.85
Mobile Phone	Yes	59	1	1		
	No	1167	58	2.27	0.42	0.29 – 17.92
Car	Yes	305	12	1		
	No	921	47	1.17	0.57	0.66 – 2.07
Socioeconomic Status	Low	472	34	1		
	Middle	487	21	0.57	0.07	0.31 – 1.04
	Upper	266	4	0.18	0.004	0.06 – 0.55
	Joint significance				***0.01***	

Exposure variables that neither showed notable increased or decreased OR nor evidence of a statistically significant association with HBV chronic infection amongst mothers were age of the mother (p=0.92), mothers employment (p=0.91), and the ownership of a radio (p=0.97) or car (p=0.57).

5.6 Mothers HBV chronic infection multivariable analysis:

The independent variables that had a statistically significant association with HBV chronic infection amongst women of childbearing age in the multiple logistic regression analysis after adjusting for the remaining variables in the model were mothers birth order, educational status of the mother and the ownership of a fridge/freezer.

The association between mothers birth order and mothers HBV chronic infection did not follow a clear pattern, however, mothers birth order was significant in the multivariable model (p=0.01). The OR of HBV chronic infection was 2.5 amongst mothers 4^{th} in birth order (p=0.004) and women of higher birth order appeared to have a higher odds of HBV chronic infection. The adjusted OR of HBV chronic infection amongst women claiming to be uneducated was 4.5 (95% CI 1.47 to 13.7). Similar to findings with anti-HBc positivity amongst mothers, the adjusted OR of HBV chronic infection was 2.5 (95% CI 1.20 to 5.15) amongst mothers living in households that did not have a fridge/freezer (p=0.02). The adjusted OR of HBV chronic infection associated with living in a rural area of residence was 1.04 (95% CI 0.52 to 2.08) amongst mothers and this was not significant (p=0.9) after adjusting for the remaining variables in the model, although in the univariable analysis the odds of HBV chronic infection amongst women living in rural areas was double the odds of HBV chronic infection amongst women living in urban areas. Age of the mother and ownership of a home telephone did not show evidence of a statistically significant association with mothers HBV chronic infection, however, they were associated with mothers being anti-HBc positive. Therefore, these were added to the final multivariable model of mothers HBV chronic infection resulting in two models one for anti-HBc positivity and the other for HBV chronic infection both including identical independent variables. There was no evidence of interaction between the independent variables in the multivariable model. Table 5.6.1 shows the results of the multivariable

analysis of the association of the independent variables with HBV chronic infection amongst mothers in Yemen.

Table 5.6.1
Adjusted logistic regression of the association of HBV chronic infection amongst mothers with independent exposure variables

Variable		OR*	p value**	95% C.I.***
Mothers Age (in years)	15 to 24	1		
	25 to 29	1.54	0.33	0.62 – 3.85
	30 to 34	1.30	0.54	0.54 – 3.11
	35 to 39	0.98	0.98	0.34 – 2.89
	40 to 49	1.27	0.76	0.26 – 6.12
	Joint significance	****	*0.68*	
Mothers Birth Order	1	1		
	2	0.79	0.61	0.31 – 2.05
	3	0.85	0.64	0.41 – 1.74
	4	2.53	0.004	1.38 – 4.64
	5	1.32	0.48	0.59 – 2.98
	6 to 18	1.10	0.85	0.41 – 2.94
	Joint significance		***0.01***	
Area	Urban	1		
	Rural	1.04	0.9	0.52 – 2.08
Mothers Educational Status	Educated	1		
	Uneducated	4.49	**0.01**	1.47 – 13.7
Fridge/Freezer Ownership	Yes	1		
	No	2.48	**0.02**	1.20 – 5.15
Home Telephone Ownership	Yes	1		
	No	0.75	0.65	0.20 – 2.78

* adjusted OR computed by survey logistic regression multivariable analysis.
** p-value of the adjusted wald test statistic.
*** 95% Confidence Intervals.
**** p-value of the adjusted survey wald test statistic of the significance of the joint effect of the categories of the independent variable on the dependent variable.

The multivariable analysis of the association of variables with HBV chronic infection amongst mothers was repeated excluding the socioeconomic variables that contributed to the socioeconomic index constructed in the survey and substituting these variables with the single socioeconomic status variable. The OR and p-values of HBV chronic infection associated with mothers age and birth order were extremely similar in this model as in table 5.6.1. The adjusted OR of HBV chronic infection associated with residing in a rural

area increased from 1.04 to 1.44 (p=0.31). Socioeconomic status, on the other hand, was significantly associated with HBV chronic infection, and there was a clear decline in the OR of HBV chronic infection with increasing socioeconomic status. Table 5.6.2 presents the results of the multivariable analysis of the association of independent variables and the socioeconomic status variable with HBV chronic infection amongst mothers participating in the survey.

Table 5.6.2
Adjusted logistic regression of the association of HBV chronic infection amongst mothers with independent exposure variables and socioeconomic status

Variable		OR*	p value**	95% C.I.***
Mothers Age (in years)	15 to 24	1		
	25 to 29	1.49	0.39	0.58 – 3.79
	30 to 34	1.31	0.52	0.56 – 3.09
	35 to 39	1.09	0.86	0.36 – 3.32
	40 to 49	1.42	0.65	0.29 – 6.96
	Joint significance	****	*0.88*	
Mothers Birth Order	1	1		
	2	0.74	0.51	0.29 – 1.87
	3	0.86	0.66	0.42 – 1.75
	4	2.26	0.01	1.20 – 4.27
	5	1.22	0.62	0.54 – 2.76
	6 to 18	1.05	0.92	0.39 – 2.83
	Joint significance		**0.03**	
Area	Urban	1		
	Rural	1.44	0.31	0.70 – 2.95
Socioeconomic Status	Low	1		
	Middle	0.60	0.14	0.31 – 1.19
	Upper	0.22	0.004	0.09 – 0.59
	Joint significance		***0.01***	

* adjusted OR computed by survey logistic regression multivariable analysis.
** p-value of the adjusted Wald test statistic.
*** 95% Confidence Intervals.
**** p-value of the adjusted survey Wald test statistic of the significance of the joint effect of the categories of the independent variable on the dependent variable.

5.7 Discussion:

There are no previous community-based seroepidemiological surveys investigating HBV infection in Yemen. This is the first study to focus on the prevalence of HBV infection amongst women of childbearing age as a specific age-group in the Republic of Yemen. The overall prevalence of anti-HBc positivity (24.4%) and HBsAg chronic carriers (5.1%) amongst women of childbearing age participating in this household seroepidemiological survey was lower than initially expected compared to earlier studies in Yemen and neighbouring Middle Eastern countries. Rather than obtaining an estimated 570 anti-HBc positive and 190 HBsAg positive chronic carrier mothers as was expected based on the prevalence of HBV infection reported from previous studies conducted in Yemen, the survey generated 299 anti-HBc positive and 59 HBsAg positive chronic carrier mothers. Earlier studies have shown the prevalence of anti-HBc positivity to range from 45% to 60% and HBV chronic infection to range from 13% to 19% amongst adults in hospital populations, and the prevalence of HBV chronic infection to range from 12.5% to 16.6% amongst women of childbearing age attending hospitals in Yemen (El-Guneid et al.,1993; Scott et al., 1990; Abdul Raheem et al., 1991). Unfortunately, there is no information on age-specific prevalence of anti-HBc positivity and HBV chronic infection amongst women participating in these earlier studies. Similarly, studies in neighbouring countries in the Middle-East have shown that these countries have an intermediate to high endemicity of HBV infection. Noteworthy to mention is that anti-HBc positivity is a crude marker of HBV infection as anti-HBc positive mothers may have been infected many years earlier or during childhood with anti-HBc positivity being the only indicator of previous or past exposure to HBV infection.

The prevalence of HBV chronic infection [5.08% (95% CI 3.56 to 7.2)] amongst mothers participating in this survey barely places Yemen amongst the group of countries highly endemic with HBV infection. Actually, this prevalence estimate of HBV chronic infection amongst mothers in Yemen makes the endemicity of HBV infection in Yemen more consistent with the endemicity of HBV infection in other Middle-Eastern countries in the region such as Saudi Arabia, Kuwait, Lebanon, Tunisia and Iran (Al-Nakib et al., 1986; Amini et al., 1993; Basalamah et al., 1984; Coursaget et al., 1994; Nabulski et al., 1997; Ramia et al., 1984). This is contrary to earlier studies conducted in Yemen, which

suggested that HBV infection is hyperendemic in Yemen and much higher than other countries in the region.

Why were the prevalence estimates of anti-HBc positivity and HBsAg chronic carriers amongst mothers participating in this survey much lower than the prevalence estimates from previous studies in Yemen? The difference between the prevalence of anti-HBc positivity and HBV chronic infection amongst mothers participating in this survey and previous studies is not expected to be the result of the effect of time (cohort effect) on the prevalence of anti-HBc positivity and HBV chronic infection amongst mothers. This is mainly because there is no significant decline in the prevalence of HBV chronic infection amongst the youngest mothers (15 to 24 years) compared to the oldest mothers (40 to 49 years) participating in the survey, similar to what was observed with anti-HBc positivity amongst mothers. The difference between the prevalence estimates is also not expected to be due to differences in measurement technique or serological assay which is another potential explanation for the difference between the prevalence estimates. The most likely explanation for the difference in the prevalence of HBV infection amongst mothers participating in this survey and earlier studies is due to the mothers in this survey and the mothers in previous studies being drawn from two totally different populations. The prevalence estimates of this survey are based on a community-based household seroepidemiological survey with mothers randomly selected from the general population across the country based on probability proportional to size, whereas the prevalence estimates of the previous studies were based on hospital-based serosurveys in major cities with study participants representing a specific subgroup of the population. Not everyone in the general population has an equal chance of being included in a hospital-based sample, and therefore, the sample can only be considered representative of the hospital population or the community that it serves. The prevalence estimates of community-based surveys, on the other hand, are more generalisable, accurate, reliable and representative of the general population than those of hospital-based serosurveys. In Yemen, the results of these earlier hospital-based surveys are commonly and incorrectly reported by unqualified individuals as the prevalence estimates of HBV chronic infection in the general population. This results in an exaggerated and overestimated prevalence of HBV chronic infection amongst adults in the general population in Yemen with the

widespread belief that 15% to 20% of the general population is chronically infected with HBV.

One of the most important findings of this survey was the low infectiousness of HBV chronic carrier mothers in Yemen. Infectiousness of HBV chronic carrier mothers is determined by their HBeAg status, which correlates with HBV DNA levels with HBeAg positive chronic carriers being highly infectious (Hall, 1994; Hwang et al., 1985). Out of the 59 HBV chronic carrier mothers identified in this survey, 12.8% (8/59) were HBeAg positive, which is lower than the prevalence of HBeAg (31%) amongst chronic carrier women reported by El-Guneid et al (El-Guneid et al., 1993), but similar to the prevalence of HBeAg amongst HBV chronic carriers reported by Scott et al in Yemen (Scott et al., 1990), and similar to the prevalence of HBeAg amongst HBV chronic carrier women of childbearing age reported by many other studies in Middle-Eastern countries (Al-Nakib et al., 1986; Amini et al., 1993; Basalamah et al., 1984; Coursaget et al., 1994; Nabulski et al., 1997; Ramia et al., 1984). This reduces the prevalence of HBeAg positive chronic carrier mothers in Yemen by approximately two-thirds, and consequently the prevalence of potentially highly infectious HBV chronic carrier women, and thereby the risk of perinatal transmission of HBV infection in Yemen. The number of HBeAg positive mothers was too small to allow examination of HBeAg status by age of mother.

Another important finding was the clear increase in the prevalence of anti-HBc positivity with increasing age of the mother ($p=0.0001$). As the age of the mother increases the more likely she is to be exposed to HBV regardless of means or setting. The adjusted OR of anti-HBc positivity was 1.29, 1.83, 3.15, and 2.99 amongst women aged 25 to 29, 30 to 34, 35 to 39, and 40 to 49, respectively ($p=0.0001$). This association was demonstrated in an earlier study in Yemen which showed that after adjusting for other variables age was highly significantly associated with being seropositive (adjusted OR 1.51), and is consistent with findings of numerous international studies as well as other studies in the region (Scott et al., 1990). On the other hand, the relatively stable prevalence of HBV chronic infection from young to older mothers, and the apparently low risk of perinatal transmission of HBV infection suggests that mothers with HBV chronic infection were most likely infected by child-to-child transmission during childhood, which has been

suggested to be the major mode of transmission of HBV infection in the Middle East (Toukan et al., 1990).

There was approximately a 50% decline in the prevalence of anti-HBc positivity ($p=0.04$) and HBV chronic infection ($p=0.05$) amongst mothers living in urban areas compared to mothers living in rural areas unadjusting for age. Nevertheless, this difference did not remain significant in the multivariable analysis after adjusting for the remaining variables in the model. It is suspected that the difference in the prevalence of HBV infection by area of residence may be attributed to socioeconomic factors including mother's educational status. It has already been shown in chapter 1 that numerous studies in the Middle-East have demonstrated the prevalence of anti-HBc positivity and HBV chronic infection to be inversely related to socioeconomic status. International research as well as studies in Middle-Eastern countries, most commonly in Jordan and Saudi Arabia, show the prevalence of anti-HBc positivity and HBV chronic infection amongst individuals of upper socioeconomic status to be lower than the prevalence of anti-HBc positivity and HBV chronic infection amongst individuals of middle and low socioeconomic status (Awidi et al., 1984; Ghaffar et al., 1989; Toukan et al., 1990; Toukan, 1996; WHO, 1995). Similarly, the socioeconomic classification in this survey demonstrated a clear and statistically significant decline in the prevalence and OR of anti-HBc positivity and HBV chronic infection with increasing socioeconomic status by univariable and multivariable analysis. According to the socioeconomic index of this survey study participants were divided into low, middle, and upper socioeconomic status. This was based on a number of household characteristics, household possessions, and levels of educational attainment of mother and father. Fridge/freezer ownership was one of the household possessions contributing to the socioeconomic index constructed in the survey that was statistically significantly associated with anti-HBc positivity and HBV chronic infection amongst mothers in Yemen in the multivariable analysis after adjusting for the remaining variables in the model. There was also a statistically significant increase in fridge/freezer ownership with increasing socioeconomic status ($p<0.0001$), mothers educational status ($p<0.0001$), and fathers educational status ($p<0.0001$), as well as in urban areas of residence ($p<0.0001$). Home telephone ownership was another household possession contributing to the socioeconomic index constructed in the survey that was statistically

significantly associated with anti-HBc positivity in the multivariable analysis [OR 1.59 (95% CI 1.03 to 2.46)]. There was a statistically significant increase in home telephone availability with increasing socioeconomic status ($p<0.0001$) as well as a statistically significant difference in home telephone availability by area of residence with only 3% of rural households having a home telephone compared to 42% of urban households ($p<0.0001$). However, area of residence was not statistically significantly associated with being anti-HBc positive or HBV chronic infection in the multivariable analysis in this survey, which is similar to findings observed in an earlier study in Yemen (Scott et al., 1990).

Mothers educational status, another variable contributing to the socioeconomic index constructed in the survey, was also statistically significantly associated with HBV chronic infection amongst mothers in Yemen. Being an uneducated mother had a highly significant association with HBV chronic infection in the multivariable analysis [adjusted OR 4.49 (95% CI 1.47 to 13.7)] ($p=0.01$). The adjusted OR of being anti-HBc positive amongst uneducated women was also increased in the multivariable analysis but did not reach statistical significance [OR 1.37 (95% CI 0.84 to 2.22)] ($p=0.19$). Female education is a well established determinant of health. It has been demonstrated that women who received even one to three years of schooling acquired greater knowledge and learnt better health practices which were enough to reduce child mortality by 15% (Abel-Smith, 1998).

Women of higher birth order had an increased adjusted OR of HBV chronic infection in the multivariable analysis than women of relatively lower birth order. This suggested that women of higher birth order had a greater risk of becoming infected with HBV by their older siblings during childhood and indicates that childhood transmission was occurring and contributed to the pool of HBV chronic carrier women in Yemen. Family size, on the other hand, which takes into account the number of younger siblings, as opposed to child birth order which only takes into account the number of older siblings, was not significantly associated with HBV chronic infection nor did it alter the OR of HBV chronic infection amongst mothers associated with mothers birth order indicating that mothers were not at risk of becoming infected with HBV from their younger siblings.

The prevalence estimates of anti-HBc positivity and chronic infection amongst mothers participating in this survey are the first estimates obtained from this age-group through a random population-based household seroepidemiological survey in Yemen. The reason for selecting this age-group was to measure the prevalence of HBV chronic infection amongst women of childbearing age and the proportion of these women HBeAg positive, which would allow assessing the infectiousness of these women and thereby the risk of perinatal transmission of HBV infection from these women to their children. It is believed that these results are reliable, accurate, and based on a representative sample taken for the general population. It is not suspected that these results are biased and a general discussion of the possible effect of bias or confounding on these results will be presented in chapter 9.

Chapter 6: Results Children Aged 1-3:

In this chapter the results of the prevalence, univariable analysis, and multivariable analysis of anti-HBc positivity and HBsAg chronic carrier status amongst children aged 1-3 participating in the survey will be presented.

HBV Infection:

6.1 Prevalence of hepatitis B core antibody (anti-HBc):

The overall prevalence of anti-HBc positive children aged 1-3 participating in the survey in Yemen was 2.38% (95% CI 1.25 to 4.49). The prevalence of anti-HBc positive children aged 1-3 participating in the survey was statistically significantly different according to mothers HBV infection status, sex of the child, qualifications of the person managing delivery of the child, and socioeconomic status.

The prevalence of anti-HBc positivity was 9.86% amongst children born from HBV chronic carrier mothers, 5.09% amongst children born from anti-HBc positive mothers, and 1.19% amongst children born from uninfected mothers (p=0.0001). When examining the risk of the child being anti-HBc positive by hepatitis B e antigen (HBeAg) status of HBV chronic carrier mothers, 2/8 (21%) of children born from HBeAg positive chronic carrier mothers were anti-HBc positive compared to 3/51 (8.2%) of children born from HBeAg negative chronic carrier mothers, however, this difference was not statistically significant probably due to the small number of HBeAg positive chronic carrier mothers (p=0.45).

The prevalence of anti-HBc positivity amongst males (3.16%) was twice as high as it was amongst females (1.54%) (p=0.05). The prevalence of anti-HBc positivity amongst children whose delivery was managed by a medically qualified individual (1.02%) was statistically significantly lower than the prevalence of anti-HBc positivity amongst children whose delivery was managed by an unqualified individual (3.32%) (p=0.02). Similarly, the prevalence of anti-HBc positivity amongst children delivered in a health setting (0.87%) was significantly lower than the prevalence of anti-HBc positivity amongst children delivered in a home setting (2.71%) (p=0.06). There was a significant difference in the prevalence of anti-HBc positivity between the three socioeconomic

classes in the survey (p=0.01). The prevalence of anti-HBc positivity was 3.61% amongst children of low socioeconomic status, 0.92% amongst children of middle socioeconomic status, and 1.43% amongst children of upper socioeconomic status. The higher prevalence of anti-HBc positivity amongst children of upper socioeconomic status compared to children of middle socioeconomic status was not statistically significant (p=0.52). Table 6.1.1 shows the prevalence of anti-HBc positivity amongst children aged 1-3 by personal and health care characteristics.

The prevalence of anti-HBc positivity was lower amongst completely vaccinated (0.5%) compared to unvaccinated (2.95%), and partially vaccinated (3.17%) children (p=0.34). When completely vaccinated children were compared to unvaccinated children alone the p-value became smaller (p=0.06). The prevalence of anti-HBc positivity amongst children aged 1-3 in urban areas (1.46%) was nearly half the prevalence of anti-HBc positivity in rural areas (2.79%) but this difference was not statistically significant (p=0.26). When anti-HBc positivity was compared by urban/rural area of birth of child the prevalence of anti-HBc positivity was 1.1% (5/454) amongst children born in urban areas and 2.8% (20/755) amongst children born in rural areas (p=0.07). There was no evidence of a statistically significant difference in the prevalence of anti-HBc positivity between all five provinces (p=0.58), urban provinces (p=0.31), or rural provinces (p=0.68) in the survey. Table 6.1.2 shows the prevalence of anti-HBc positivity amongst children aged 1-3 by characteristics of the mother.

Table 6.1.1
Prevalence of anti-HBc positive children aged 1-3 by demographic and health care characteristics

Prevalence anti-HBc positive	Number of Children	Prevalence (weighted) %	95% CI LL	95% CI UL	p-value (Pearson design-based)
Overall	27/1285	2.38	1.25	4.49	
Sex					
Male	19/652	3.16	1.71	5.79	
Female	8/633	1.54	0.62	3.79	0.05
Age (in months)					
12 to 17	5/269	2.02	0.48	8.18	
18 to 23	8/384	2.24	0.74	6.59	
24 to 29	3/275	1.07	0.29	3.82	
30 to 36	11/357	3.80	1.96	7.24	0.33
Province					
Sanaa	5/262	1.84	0.68	4.91	
Taiz	6/252	2.36	0.65	8.18	
Aden	2/247	0.81	0.22	2.87	
Shabwa	5/268	1.84	0.49	6.62	
Hodeidah	9/256	3.53	1.20	9.92	0.58
Area					
Urban	7/509	1.49	0.64	3.44	
Rural	20/776	2.79	1.29	5.97	0.26
Vaccination Status					
Not Vaccinated	22/840	2.95	1.44	5.92	
Completely Vaccinated	1/137	0.50	0.06	3.78	
Partially Vaccinated	4/147	3.17	1.02	9.47	0.34
Person Managing Delivery					
Medically Qualified Personnel	6/559	1.02	0.48	2.18	
Unqualified Personnel	21/705	3.32	1.57	6.87	0.02
Setting of Delivery					
Health Setting	4/330	0.87	0.31	2.43	
Home Setting	22/903	2.71	1.35	5.38	0.06
Antenatal Care					
Yes	9/619	1.91	0.85	4.24	
No	18/661	2.78	1.28	5.90	0.42
Socioeconomic Status					
Low	17/506	3.61	1.92	6.69	
Middle	6/508	0.92	0.29	2.87	
Upper	4/270	1.43	0.48	4.17	0.01

Table 6.1.2
Prevalence of anti-HBc positive children aged 1-3 by characteristics of the mother

Prevalence anti-HBc positive	Number of Children	Prevalence (weighted) %	95% CI LL	95% CI UL	p-value (Pearson design-based)
Mothers HBV Status					
Mother Chronic Carrier	5/59	9.86	5.35	17.47	
Mother anti-HBc positive	12/240	5.09	2.45	10.28	
Mother Uninfected	10/986	1.19	0.56	2.50	0.0001
Mothers HBeAg Status					
Positive	2/8	21.14	2.40	74.51	
Negative	3/51	8.20	2.97	20.68	0.45
Mothers Age (In years)					
15 to 24	12/400	3.17	1.60	6.16	
25 to 29	6/349	2.30	0.68	7.49	
30 to 34	5/268	1.65	0.56	4.76	
35 to 39	2/177	1.14	0.20	6.29	
40 to 49	2/91	3.44	0.74	14.58	0.61
Mother's Educational Status					
Educated	9/547	1.61	0.68	3.77	
Uneducated	18/738	2.83	1.49	5.32	0.12
Mother's Level of Education					
Read and Write	5/179	2.28	0.62	8.06	
Primary School	3/221	1.75	0.48	6.21	
Secondary School	0/113	0.00			
University or College	1/34	2.22	0.22	19.06	0.61
Mother's Employment					
Employed	1/55	1.27	0.13	11.28	
Not Employed	26/1230	2.42	1.26	4.60	0.56

6.2 Children's anti-HBc univariable analysis:

The variables statistically significantly associated with children aged 1-3 being anti-HBc positive by univariable analysis were HBV infection status of the mother, qualifications of the person managing delivery of the child, availability of electricity in the household, television ownership, fridge/freezer ownership, and socioeconomic status.

The HBV infection status of the mother was the independent variable with the most significant association with children being anti-HBc positive. The OR of being anti-HBc positive amongst children aged 1-3 associated with having an anti-HBc positive mother was 4.47 (95% CI 1.62 to 12.33) and the OR associated with having a HBV chronic carrier mother was 9.11 (95% CI 4.29 to 19.36) compared to children born from uninfected mothers ($p<0.001$). The OR of being anti-HBc positive was 3.32 (95% CI 1.19 to 9.22) amongst children whose delivery was managed by an unqualified individual which was statistically significantly higher than children whose delivery was managed by a medically qualified individual ($p=0.02$). The OR of being anti-HBc positive was significantly lower amongst children of middle (OR=0.25) and upper (OR=0.39) socioeconomic status compared to children of low socioeconomic status ($p=0.03$). Fridge/freezer and television were the two household commodities that were statistically significantly associated with children aged 1-3 being anti-HBc positive. The OR of being anti-HBc positive was 2.95 (95% CI 1.04 to 8.37) amongst children who did not have a fridge/freezer in their household ($p=0.04$), 4.17 (95% CI 1.35 to 12.84) amongst children who did not have a television in their household ($p=0.02$) and 3.55 (95% CI 1.04 to 12.12) amongst children living in households that did not have an electricity supply ($p=0.04$). Table 6.2.1 shows the results of the univariable analysis of the association of demographic and health care variables with anti-HBc positivity amongst children aged 1-3.

Table 6.2.1
Association of anti-HBc positivity amongst children aged 1-3 with demographic and health care characteristics

Variable		Number Controls	Number Cases	OR	p value	95% C.I.
Sex	Male	633	19	1		
	Female	625	8	0.48	0.06	0.23 – 1.02
Age of child (in months)	12 to 17	264	5	1		
	18 to 23	376	8	1.11	0.88	0.27 – 4.52
	24 to 29	272	3	0.52	0.52	0.07 – 4.01
	30 to 36	346	11	1.91	0.39	0.41 – 8.84
	Joint significance				*0.25*	
	Linear trend OR			1.24	0.42	0.72 – 2.11
Province	Sanaa	257	5	1		
	Taiz	246	6	1.29	0.75	0.25 – 6.72
	Aden	245	2	0.43	0.3	0.08 – 2.24
	Shabwa	263	5	0.99	1	0.19 – 5.32
	Hodeidah	247	9	1.95	0.36	0.44 - 8.72
	Joint significance				*0.57*	
Area	Urban	502	7	1		
	Rural	756	20	1.90	0.26	0.59 – 6.11
Child Birth Order	1	235	6	1		
	2	208	2	0.51	0.55	0.05 – 5.32
	3	195	3	0.99	0.99	0.33 – 3.02
	4	135	6	2.72	0.16	0.65 – 11.46
	5	120	3	1.73	0.57	0.24 – 12.43
	6 to 18	365	7	0.80	0.7	0.24 – 2.66
	Joint significance				*0.29*	
	Linear Trend OR			1.03	0.78	0.82 – 1.3
Duration Breastfed	Up to 1 year	462	9	1		
	1 to 2 years	670	16	2.34	0.12	0.78 – 7.04
	More than 2 yrs	100	2	1.68	0.49	0.37 – 7.64
	Joint significance				*0.31*	
Vaccination Status	Unvaccinated	818	22	1		
	Partially	143	4	1.08	0.9	0.29 – 4.01
	Completely	136	1	0.17	0.09	0.02 – 1.42
	Joint significance				*0.24*	
Person Managing Delivery	Qualified Medic	553	6	1		
	Unqualified	684	21	3.32	**0.02**	1.19 – 9.22
Setting of Delivery	Health Setting	326	4	1		
	Home Setting	881	22	3.12	0.08	0.88 – 11.43

Variables that had notably increased or decreased OR by more or less than 30% but not found to be statistically significantly associated with anti-HBc positivity amongst children aged 1-3 were age and sex of the child, area and province of residence, vaccination status of the child, breastfeeding, setting of delivery, mothers and fathers

educational status, mothers employment, household crowding index, ownership of a car or home telephone, and household ownership, type and construction. These are all presented in tables 6.2.1, 6.2.2 and 6.2.3. Some of the most notable findings were the OR of anti-HBc positivity was 0.17 amongst completely vaccinated children compared to unvaccinated children (p=0.09), the odds of anti-HBc positivity was 0.43 amongst children aged 1-3 in Aden compared to children in Sanaa (p=0.3), the odds of anti-HBc positivity amongst children aged 30 to 36 months was 1.91 compared to children aged 12 to 17 months (p=0.39), and children living in households with high crowding indexes appeared to have a higher odds of being anti-HBc positive (p=0.06). Table 6.2.2 shows the results of the univariable analysis of the association of mothers and fathers characteristics with anti-HBc positivity amongst children aged 1-3.

Table 6.2.2
Association of anti-HBc positivity amongst children aged 1-3 with mothers and fathers characteristics

Variable		Number Controls	Number Cases	OR	P value	95% C.I.
Mother HBV Status	Uninfected	976	10	1		
	anti-HBc positive	228	12	4.47	0.006	1.62 – 12.33
	Chronic carrier	54	5	9.11	<0.001	4.29 – 19.36
	Joint significance				***<0.001***	
Mothers Age (in years)	15 to 24	388	12	1		
	25 to 29	343	6	0.72	0.53	0.24 – 2.12
	30 to 34	263	5	0.51	0.35	0.12 – 2.18
	35 to 39	175	2	0.35	0.22	0.06 – 1.95
	40 to 49	89	2	1.09	0.9	0.27 – 4.35
	Joint significance				*0.48*	
	Linear Trend OR			0.86	0.44	0.58 – 1.27
Mothers Education	Educated	538	9	1		
	Uneducated	720	18	1.78	0.12	0.85 – 3.76
Mothers Birth Order	1	277	1	1		
	2	200	3	2.86	0.41	0.21 – 38.54
	3	198	6	9.85	0.07	0.82 – 118
	4	155	5	7.25	0.12	0.59 – 88.79
	5	129	3	9.14	0.07	0.79 – 105
	6 to 19	299	9	6.71	0.06	0.91 – 49.19
	Joint significance				*0.13*	
	Linear Trend OR			1.24	0.01	
Mothers Employment	Unemployed	1204	26	1		
	Employed	54	1	0.52	0.57	0.05 – 5.6
Fathers Education	Educated	998	17	1		
	Uneducated	260	10	2.02	0.2	0.66 – 6.15

Variables that neither showed notably increased or decreased OR nor evidence of a statistically significant association with anti-HBc positivity amongst children aged 1-3 participating in the survey were ownership of mobile telephone (p=0.99), radio (p=0.94), or household source of water (p=0.11). Likewise the OR of anti-HBc positivity amongst children aged 1-3 associated with child birth order (p=0.29), mothers birth order (p=0.13) and mothers age (p=0.12) was not significant and did not follow a clear trend (see tables 6.2.1, 6.2.2, 6.2.3). Table 6.2.3 shows the association of anti-HBc positivity amongst children aged 1-3 with household characteristics and possessions.

Table 6.2.3
Association of anti-HBc positivity amongst children aged 1-3 with household characteristics and possessions

Variable		Number Controls	Number Cases	OR	P value	95% C.I.
Household Ownership	Owned	1073	25	1		
	Rented	185	2	0.45	0.25	0.11 – 1.81
Household Type	House	928	20	1		
	Apartment	218	3	0.9	0.86	0.26 – 3.14
	Shack	111	4	1.7	0.33	0.56 – 5.01
	Joint significance				*0.57*	
Household Construction	Stone	368	6	1		
	Block	571	13	1.83	0.26	0.62 – 5.41
	Mud or Clay	187	3	0.85	0.88	0.08 – 8.6
	Mixed	132	5	2.61	0.22	0.54 – 12.67
	Joint significance				*0.59*	
Crowding Index	1	99	1	1		
	2	317	6	3.7	0.23	0.41 – 33.09
	3	310	2	1.03	0.98	0.08 – 14.1
	4	213	8	10.16	0.04	1.11 – 93.27
	5	124	2	3	0.38	0.23 – 38.69
	6 to 18	195	8	11.04	0.03	1.27 – 95.85
	Joint significance				*0.06*	
	Linear Trend OR			1.41	0.009	1.1 – 1.81
Electricity Supply	Yes	892	12	1		
	No	366	15	3.55	**0.04**	1.04 – 12.12
Source of Water	Governmental	431	5	1		
	Local Project	434	9	2.3	0.21	0.61 – 8.69
	Water Truck	117	5	3.16	0.03	1.13 – 8.86
	Buckets	274	8	2.56	0.14	0.71 – 9.22
	Joint significance				*0.11*	
Fridge/Freezer	Yes	583	7	1		
	No	675	20	2.95	**0.04**	1.04 – 8.37
Television	Yes	861	11	1		
	No	397	16	4.17	**0.02**	1.35 – 12.84
Radio	Yes	894	20	1		
	No	364	7	0.98	0.94	0.53 – 1.79
Home Phone	Yes	261	4	1		
	No	997	23	1.66	0.37	0.53 – 5.24
Mobile Phone	Yes	59	1	1		
	No	1199	26	0.99	0.99	0.13 – 7.48
Car	Yes	312	5	1		
	No	946	22	1.71	0.49	0.35 – 8.42
Socioeconomic Status	Low	489	17	1		
	Middle	502	6	0.25	0.01	0.08 – 0.73
	Upper	266	4	0.39	0.11	0.12 – 1.27
	Joint significance				***0.03***	

6.3 Children's anti-HBc multivariable analysis:

After adjusting for the remaining variables included in the multivariable analysis, the only variables found to have a statistically significant association with anti-HBc positivity amongst children aged 1-3 were HBV infection status of the mother and the ownership of a television.

The adjusted OR of anti-HBc positivity amongst children aged 1-3 born from anti-HBc positive mothers was 3.97 (95% CI 1.38 to 11.43) and increased to 7.84 (95% CI 3.78 to 16.25) amongst children aged 1-3 born from HBV chronic carrier mothers.

The adjusted OR of anti-HBc positivity amongst children aged 1-3 born from families that did not own a television in their household was 3.34 (95% CI 1.07 to 10.41) compared to families that owned a television (p=0.04). Adding television ownership to the multivariable model changed the adjusted OR of anti-HBc positivity amongst children aged 1-3 living in rural areas from 1.6 (p=0.5) in the model without television ownership to 0.78 (p=0.68) in the model including television ownership. Age, sex, and area of residence did not show evidence of a statistically significant association with anti-HBc positivity amongst children aged 1-3, but because these were *a priori* variables, they were added to the final model at the end of the multivariable modelling.

The adjusted OR of the qualifications of the person managing delivery of the child associated with anti-HBc positivity amongst children aged 1-3 was 2.5 (95% CI 0.8 to 7.82) but showed no evidence of a statistically significant association (p=0.11). Nevertheless, because qualifications of the person managing delivery of the child was significantly associated with HBV chronic infection it was added to the final multivariable model of anti-HBc positivity, regardless of the significance of its association with anti-HBc positivity, resulting in two models, one for anti-HBc positivity and the other for HBV chronic infection, both including identical independent variables. There was no evidence of interaction between the independent variables in the model. Table 6.3.1 shows the results of the multivariable analysis of the association of the independent variables with anti-HBc positivity amongst children aged 1-3.

Table 6.3.1
Adjusted logistic regression of the association of anti-HBc positivity amongst children aged 1-3 with independent exposure variables

Variable		OR*	p value**	95% C.I.***
Mother HBV Status	Uninfected	1		
	anti-HBc positive	3.97	0.01	1.38 – 11.43
	Chronic Carrier	7.84	<0.001	3.78 – 16.25
	Joint significance	****	***<0.0001***	
Sex	Male	1		
	Female	0.52	0.13	0.22 – 1.22
Age of Child (in months)	12 to 17	1		
	18 to 23	1.18	0.78	0.36 – 3.90
	24 to 29	0.45	0.43	0.06 – 3.57
	30 to 36	1.88	0.4	0.41 – 8.66
	Joint significance		*0.33*	
Area	Urban	1		
	Rural	0.61	0.46	0.16 – 2.38
Person Managing Delivery	Qualified Medic	1		
	Unqualified	2.5	0.11	0.80 – 7.82
Television Ownership	Yes	1		
	No	3.34	**0.04**	1.07 – 10.41

* adjusted OR computed by survey logistic regression multivariable analysis.
** p-value of the adjusted Wald test statistic.
*** 95% Confidence Intervals.
**** p-value of the adjusted survey Wald test statistic of the significance of the joint effect of the categories of the independent variable on the dependent variable.

The multivariable analysis of variables associated with anti-HBc positivity amongst children aged 1-3 was repeated excluding all the socioeconomic variables and after substituting them with the single socioeconomic status variable. The OR and p-values of anti-HBc positivity associated with age and sex of the child aged 1-3, as well as mothers HBV infection status, were similar in the model including the socioeconomic status variable as the model including all the socioeconomic variables. The adjusted OR of anti-HBc positivity associated with residing in a rural area in the model including the socioeconomic status variable changed from 0.61 to 0.92 (p=0.2). Socioeconomic status, on the other hand, was not significantly associated with anti-HBc positivity amongst children aged 1-3 (p=0.25). Table 6.3.2 shows the results of the multivariable analysis of the association of independent variables and the socioeconomic status variable with anti-HBc positivity amongst children aged 1-3 participating in the survey.

Table 6.3.2
Adjusted logistic regression of the association of anti-HBc positivity amongst children aged 1-3 with independent exposure variables and socioeconomic status

Variable		OR*	p value**	95% C.I.***
Mother HBV Status	Uninfected	1		
	anti-HBc positive	4.14	0.01	1.39 – 12.34
	Chronic Carrier	8.62	<0.001	3.91 – 19.01
	Joint significance	****	***<0.0001***	
Sex	Male	1		
	Female	0.51	0.12	0.22 – 1.17
Age of Child (in months)	12 to 17	1		
	18 to 23	1.21	0.74	0.36 – 4.07
	24 to 29	0.47	0.45	0.06 – 3.54
	30 to 36	1.72	0.46	0.39 – 7.55
	Joint significance		*0.38*	
Area	Urban	1		
	Rural	0.92	0.90	0.24 – 3.57
Person Managing Delivery	Qualified Medic	1		
	Unqualified	2.82	0.08	0.87 – 9.16
Socioeconomic Status	Low	1		
	Middle	0.37	0.12	0.11 – 1.28
	Upper	0.88	0.83	0.25 – 3.05
	Joint significance		*0.25*	

* adjusted OR computed by survey logistic regression multivariable analysis.
** p-value of the adjusted Wald test statistic.
*** 95% Confidence Intervals.
**** p-value of the adjusted survey Wald test statistic of the significance of the joint effect of the categories of the independent variable on the dependent variable.

As well as being one of the important factors under investigation in the survey, hepatitis B vaccination was an important factor determining the HBV infection status of the child. In the univariable analysis, the OR of being anti-HBc positive was 0.17 amongst completely vaccinated children compared to unvaccinated children (p=0.09). Therefore, even though vaccination status was dropped from the multiple logistic regression model, it was added to the final model in order to examine the effect of vaccination status of the child on the remaining variables in the model. Vaccination status was not statistically significant in the final model (p=0.65). Adding vaccination status to the multivariable model of anti-HBc positivity amongst children aged 1-3 had a minor effect on the OR and p-values. It reduced the OR of anti-HBc positivity associated with not owning a

television in the household from 3.34 to 2.9 and its statistical significance from 0.04 to 0.08. Table 6.3.3 shows the results of the multivariable analysis of the association of the independent variables including hepatitis B vaccination status with anti-HBc positivity amongst children aged 1-3.

Table 6.3.3
Adjusted logistic regression of the association of anti-HBc positivity amongst children aged 1-3 with independent exposure variables including vaccination status of the child

Variable		OR*	p value**	95% C.I.***
Mother HBV Status	Uninfected	1		
	anti-HBc positive	4.03	0.01	1.38 – 11.78
	Chronic Carrier	7.57	<0.001	3.83 – 14.94
	Joint significance	****	*<0.0001*	
Sex	Male	1		
	Female	0.54	0.12	0.24 – 1.20
Age of Child (in months)	12 to 17	1		
	18 to 23	1.17	0.79	0.35 – 3.92
	24 to 29	0.47	0.44	0.06 – 3.47
	30 to 36	1.97	0.37	0.43 – 9.12
	Joint significance		*0.29*	
Area	Urban	1		
	Rural	0.53	0.34	0.14 – 2.06
Person Managing Delivery	Qualified Medic	1		
	Unqualified	2.29	0.17	0.69 – 7.65
Television Ownership	Yes	1		
	No	2.86	0.08	0.86 – 9.47
Vaccination status	Unvaccinated	1		
	One Dose	1.82	0.46	0.34 – 9.73
	Two Doses	0.65	0.67	0.08 – 5.15
	Three Doses	0.34	0.32	0.04 – 3.08
	Joint significance		*0.65*	

* adjusted OR computed by survey logistic regression multivariable analysis.
** p-value of the adjusted Wald test statistic.
*** 95% Confidence Intervals.
**** p-value of the adjusted survey Wald test statistic of the significance of the joint effect of the categories of the independent variable on the dependent variable.

HBV Chronic Infection:

6.4 Prevalence of HBV chronic infection (HBsAg positive):

The prevalence of HBV chronic infection amongst children aged 1-3 participating in the survey in Yemen was 0.99% (95% CI 0.35 to 2.76). Only 11 out of 1285 children tested were positive for HBsAg, all of whom were negative for IgM anti-HBc, indicating that these children did not have acute HBV infection and were chronically infected with HBV (infected for longer than six months). All of these chronically infected children were a subset of children aged 1-3 positive for anti-HBc. There was evidence of a statistically significant difference in the prevalence of HBV chronic infection amongst children aged 1-3 by mothers HBV infection and HBeAg status, qualifications of the person managing delivery of the child, setting in which the child was delivered, and socioeconomic status.

The prevalence of HBV chronic infection was 2.72% amongst children born from HBV chronic carrier mothers, 3.85% amongst children born from anti-HBc positive mothers, and 0.14% amongst children born from uninfected mothers (p=0.0002). 89% of HBV chronic carrier children and 62% of anti-HBc positive children were born from HBV infected/chronic carrier mothers. When examining the risk of the child developing HBV chronic infection by HBeAg status of HBV chronic carrier mothers, 2/8 (21%) of children born from HBeAg positive chronic carrier mothers were chronically infected with HBV compared to 0/51 (0%) of children born from HBeAg negative chronic carrier mothers (p=0.02).

The prevalence of HBV chronic infection amongst children whose delivery was managed by a medically qualified individual (0.06%) was statistically significantly lower than the prevalence of HBV chronic infection amongst children whose delivery was managed by an unqualified individual (3.32%) (p=0.0003). Similarly, the prevalence of HBV chronic infection amongst children delivered in a health setting (0.12%) was statistically significantly lower than the prevalence of HBV chronic infection amongst children delivered in a home setting (1.25%) (p=0.02). The prevalence of HBV chronic infection was 1.6% amongst children of low socioeconomic status, 0.15% amongst children of middle socioeconomic status, and 0.78% amongst children of upper socioeconomic status

(p=0.04). Similar to findings with anti-HBc positivity amongst children aged 1-3 the higher prevalence of HBV chronic infection amongst children of upper socioeconomic status compared to children of middle socioeconomic status was not statistically significant (p=0.07). Table 6.4.1 shows the prevalence of HBV chronic infection amongst children aged 1-3 by demographic and health care characteristics.

All HBV chronic carrier children participating in the survey were unvaccinated and the prevalence of HBV chronic carriers amongst unvaccinated children was 1.5% (95% CI 0.53 to 4.16). There were no HBV chronic carriers amongst the 284 completely or partially vaccinated children. However, there was no evidence of a statistically significant difference in the prevalence of HBV chronic carriers between completely vaccinated, partially vaccinated and unvaccinated children (p=0.58). Likewise, there was no evidence of a statistically significant difference in the prevalence of HBV chronic carriers between vaccinated children (completely and partially combined) and unvaccinated children (p=0.28). Similarly, there was no evidence of a statistically significant difference in the prevalence of HBV chronic carriers between completely vaccinated and unvaccinated children (p=0.5). Similar to findings with anti-HBc positivity amongst children aged 1-3 the prevalence of HBV chronic infection amongst children aged 1-3 in urban areas (0.61%) was nearly half the prevalence of HBV chronic infection in rural areas (1.17%) but this difference was not statistically significant (p=0.58). When HBV chronic infection was compared by urban/rural area of birth of child the prevalence of HBV chronic infection was 0.3% (1/459) amongst children born in urban areas and 1.17% (9/775) amongst children born in rural areas (p=0.26). Table 6.4.2 shows the prevalence of HBV chronic infection amongst children aged 1-3 by characteristics of the mother.

Table 6.4.1
Prevalence of HBV chronically infected children aged 1-3 by demographic and health care characteristics

Prevalence HBsAg Positive	Number of Children	Prevalence (weighted) %	95% CI LL	95% CI UL	p-value (Pearson design-based)
Overall	11/1285	0.99	0.35	2.76	
Sex					
Male	6/652	1.00	0.31	3.20	
Female	5/633	0.99	0.37	2.60	0.98
Age (in months)					
12 to 17	1/269	0.63	0.08	4.93	
18 to 23	3/384	0.92	0.24	3.47	
24 to 29	2/275	0.56	0.10	3.01	
30 to 36	5/357	1.67	0.52	5.17	0.43
Province					
Sanaa	2/262	0.93	0.11	7.16	
Taiz	3/252	1.18	0.14	8.95	
Aden	0/247	0.00			
Shabwa	3/268	1.10	0.28	4.25	
Hodeidah	3/256	1.17	0.29	4.56	0.90
Area					
Urban	2/509	0.61	0.08	4.79	
Rural	9/776	1.17	0.36	3.75	0.58
Vaccination Status					
Not Vaccinated	11/840	1.50	0.53	4.16	
Completely Vaccinated	0/137	0.00			
Partially Vaccinated	0/147	0.00			0.58
Person Managing Delivery					
Medically Qualified Personnel	1/559	0.06	0.01	0.52	
Unqualified Personnel	10/705	1.61	0.55	4.65	0.0003
Setting of Delivery					
Health Setting	1/330	0.12	0.02	1.02	
Home Setting	10/903	1.25	0.42	3.60	0.02
Antenatal Care					
Yes	3/619	0.79	0.16	3.86	
No	8/661	1.16	0.46	2.90	0.55
Socioeconomic Status					
Low	7/506	1.60	0.53	4.73	
Middle	2/508	0.15	0.04	0.56	
Upper	2/270	0.78	0.14	4.34	0.04

Table 6.4.2
Prevalence of HBV chronically infected children aged 1-3 by characteristics of the mother

Prevalence HBsAg Positive	Number of Children	Prevalence (weighted) %	95% CI LL	95% CI UL	p-value (Pearson design-based)
Mothers HBV Status					
Mother Chronic Carrier	2/59	2.72	0.53	12.76	
Mother anti-HBc Positive	8/240	3.85	1.37	10.33	
Mother Uninfected	1/986	0.14	0.02	1.18	0.0002
Mothers HBeAg Status					
Positive	2/8	21.14	2.40	74.51	
Negative	0/51	0.00			0.02
Mothers Age (in years)					
15 to 24	6/400	1.71	0.63	4.59	
25 to 29	2/349	0.51	0.09	2.86	
30 to 34	1/268	0.13	0.02	1.03	
35 to 39	1/177	0.96	0.12	7.03	
40 to 49	1/91	1.83	0.21	14.38	0.21
Mother's Educational Status					
Educated	3/547	0.40	0.09	1.69	
Uneducated	8/738	1.34	0.43	4.04	0.11
Mother's Level of Education					
Read and Write	2/179	0.41	0.11	1.54	
Primary School	1/221	0.63	0.08	4.90	
Secondary School	0/113	0.00			
University or College	0/34	0.00			0.78
Mother's Employment					
Employed	0/55	0.00			
Not Employed	11/1230	1.03	0.36	2.86	0.68

6.5 Children's HBV chronic infection univariable analysis:

The variables that showed evidence of a statistically significant association with HBV chronic infection amongst children aged 1-3 by univariable analysis were HBV infection status of the mother, qualifications of the person managing delivery of the child, mother's age, birth order of the child, duration breastfeeding, car ownership and socioeconomic status.

The HBV infection status of the mother had the most significant association with children's HBV chronic infection. The OR of HBV chronic infection amongst children aged 1-3 associated with having an anti-HBc positive mother was 27.6 (95% CI 3.37 to 226) and the OR associated with having a HBV chronic carrier mother was 19.2 (95% CI 1 to 385) compared to children born from uninfected mothers (p=0.0001). Also similar to findings with anti-HBc positivity amongst children aged 1-3, children whose delivery was managed by an unqualified individual had an OR of 25.95 (95% CI 2.43 to 277) for HBV chronic infection and this was statistically significantly higher than children whose delivery was managed by a medically qualified individual (p=0.01). Children breastfed for more than 1 year (OR=2.45) or more than 2 years (OR=4.91) had a significantly higher odds of HBV chronic infection than children breastfed up to 1 year (p=0.05).

The OR of HBV chronic infection was significantly lower amongst children of middle (OR=0.09) and upper (OR=0.49) socioeconomic status compared to children of low socioeconomic status (p=0.03). The OR of HBV chronic infection was 9.32 amongst children living in households without a car (p=0.05). The birth order of the child was significantly associated with HBV chronic infection (p=0.01) but did not follow a clear pattern. Children fourth in birth order had the highest OR of HBV chronic infection (OR=30) (95% CI 3 to 292). Likewise, the association between children's HBV chronic infection and mothers age did not follow an obvious pattern but generally appeared to decline with increasing age of the mother (p=0.007). Table 6.5.1 shows the results of the univariable analysis of the association of demographic and health care variables with HBV chronic infection amongst children aged 1-3.

Table 6.5.1
Association of HBV chronic infection amongst children aged 1-3 with demographic and health care characteristics

Variable		Number Controls	Number Cases	OR	p value	95% C.I.
Sex	Male	646	6	1		
	Female	628	5	0.99	0.98	0.51 – 1.93
Age of child (in months)	12 to 17	268	1	1		
	18 to 23	381	3	1.45	0.55	0.4 – 5.27
	24 to 29	273	2	0.88	0.92	0.06 – 13.3
	30 to 36	352	5	2.66	0.28	0.43 – 16.55
	Joint significance				*0.3*	
	Linear trend OR			1.36	0.38	0.67 – 2.78
Province	Sanaa	260	2	1		
	Taiz	249	3	1.27	0.87	0.06 – 26.24
	Aden	247	0	-	-	-
	Shabwa	265	3	1.18	0.89	0.09 – 15.33
	Hodeidah	253	3	1.26	0.85	0.1 - 16.42
	Joint significance				*0.99*	
Area	Urban	507	2	1		
	Rural	767	9	1.91	0.58	0.17 – 21.4
Child Birth Order	1	240	1	1		
	2	208	2	8.52	0.15	0.44 – 164
	3	198	0	-	-	-
	4	137	4	29.97	0.01	3.08 – 292
	5	122	1	8.87	0.13	0.49 – 161
	6 to 18	369	3	6.64	0.19	0.36 – 121
	Joint significance				***0.01***	
	Linear Trend OR			1.14	0.45	0.79 – 1.68
Duration Breastfed	Up to 1 year	468	3	1		
	1 to 2 years	680	6	2.45	0.39	0.29 – 20.9
	More than 2 yrs	100	2	4.91	0.02	1.42 – 17
	Joint significance				***0.05***	
Vaccination Status	Unvaccinated	829	11	1		
	Partially	137	0	-	-	-
	Completely	147	0	-	-	-
Person Managing Delivery	Qualified med	558	1	1		
	Unqualified	695	10	25.95	**0.01**	2.43 – 277
Setting of Delivery	Health setting	329	1	1		
	Home setting	893	10	10.13	0.06	0.94 – 108

Variables that had an OR associated with HBV chronic infection amongst children aged 1-3 that was increased or decreased by more or less than 30% but failed to reach statistical significance were age of the child, area of residence, setting of delivery, mothers and fathers educational status, household crowding index, ownership of a home telephone, mobile telephone, fridge/freezer and television, household electricity and

water supply, as well as household ownership, type and construction. These are presented in tables 6.2.1, 6.2.2, and 6.2.3. Notable findings are that there were no cases of HBV chronic infection amongst completely or partially vaccinated children, the OR of HBV chronic infection amongst children born from an uneducated mother was 3.38 (p=0.12), and the odds of HBV chronic infection amongst children aged 30 to 36 months (OR=2.66) was more than double the odds of HBV chronic infection amongst children aged 12 to 17 months, however, there was no evidence of a statistically significant difference in the odds of HBV chronic infection amongst children aged 1-3 by age of the child (p=0.3). Table 6.5.2 shows the results of the univariable analysis of the association of mother and father characteristics with HBV chronic infection amongst children aged 1-3.

Table 6.5.2
Association of HBV chronic infection amongst children aged 1-3 with mothers and fathers characteristics

Variable		Number Controls	Number Cases	OR	p value	95% C.I.
Mother HBV Status	Uninfected	985	1	1		
	anti-HBc positive	232	8	27.58	0.004	3.37 – 226
	Chronic carrier	57	2	19.22	0.05	1 – 385
	Joint significance				***0.0001***	
Mothers age (in years)	15 to 24	394	6	1		
	25 to 29	347	2	0.3	0.15	0.24 – 2.12
	30 to 34	267	1	0.07	0.03	0.12 – 2.18
	35 to 39	176	1	0.56	0.52	0.06 – 1.95
	40 to 49	90	1	1.07	0.94	0.27 – 4.35
	Joint significance				***0.007***	
	Linear Trend OR			0.85	0.69	0.36 – 1.98
Mothers Education	Educated	544	3	1		
	Uneducated	730	8	3.38	0.12	0.66 – 17.3
Mothers Birth Order	1	277	1	1		
	2	202	1	0.36	0.48	0.21 – 38.54
	3	201	3	5.3	0.22	0.82 – 118
	4	158	2	2.5	0.5	0.59 – 88.79
	5	131	1	2.65	0.5	0.79 – 105
	6 to 19	305	3	2.53	0.29	0.91 – 49.19
	Joint significance				*0.59*	
	Linear Trend OR			1.15	0.02	
Mothers employment	Unemployed	1219	11			
	Employed	55	0	-	-	-
Fathers Education	Educated	1007	8	1		
	Uneducated	267	3	0.81	0.76	0.18 – 3.51

Variables that neither showed notable increased or decreased OR nor were they associated with HBV chronic infection amongst children aged 1-3 were sex of the child (p=0.98), province of sampling in the survey (p=0.99) and ownership of a radio (p=0.98). Likewise the OR of HBV chronic infection amongst children aged 1-3 associated with mothers birth order (p=0.59) was not significant and did not follow a clear trend. Table 6.5.3 shows the association of HBV chronic infection amongst children aged 1-3 with household characteristics and possessions.

Table 6.5.3
Association of HBV chronic infection amongst children aged 1-3 with household characteristics and possessions

Variable		Number Controls	Number Cases	OR	p value	95% C.I.
Household Ownership	Owned	1088	10	1		
	Rented	186	1	0.63	0.65	0.07 – 5.24
Household Type	House	941	7	1		
	Apartment	219	2	2.21	0.39	0.34 – 14.5
	Shack	113	2	2.32	0.4	0.3 – 17.9
	Joint significance				*0.67*	
Household Construction	Stone	371	3	1		
	Block	581	3	0.89	0.88	0.16 – 4.91
	Mud or Clay	188	2	1.03	0.98	0.06 – 17.23
	Mixed	134	3	2.68	0.41	0.23 – 31.31
	Joint significance				*0.75*	
Crowding Index	1	99	1	1		
	2	320	3	2.48	0.48	0.41 – 33.09
	3	312	0	-	-	-
	4	217	4	4.54	0.23	1.11 – 93.27
	5	125	1	2.17	0.58	0.23 – 38.69
	6 to 18	201	2	2.9	0.45	1.27 – 95.85
	Joint significance				*0.84*	
	Linear Trend OR			1.17	0.54	0.68 – 2.03
Electricity Supply	Yes	899	5	1		
	No	375	6	3.55	0.22	0.45 – 28.12
Source of Water	Governmental	435	1	1		
	Local Project	439	4	2.65	0.45	0.19 – 37.86
	Water Truck	120	2	3.82	0.01	1.41 – 10.35
	Buckets	278	4	4.28	0.27	0.3 – 61.76
	Joint significance				*0.08*	
Fridge/Freezer	Yes	587	3	1		
	No	687	8	2.57	0.26	0.47 – 14
Television	Yes	867	5	1		
	No	407	6	3.92	0.16	0.56 – 27.4
Radio	Yes	906	8	1		
	No	368	3	0.99	0.98	0.3 – 3.23
Home Phone	Yes	264	1	1		
	No	1010	10	1.71	0.61	0.2 – 14.51
Mobile Phone	Yes	59	1	1		
	No	1215	10	0.38	0.33	0.05 – 2.86
Car	Yes	316	1	1		
	No	958	10	9.32	**0.05**	0.96 – 90.7
Socioeconomic Status	Low	499	7	1		
	Middle	506	2	0.09	0.01	0.02 – 0.51
	Upper	268	2	0.49	0.42	0.08 – 3.06
	Joint significance				***0.03***	

6.6 Children's HBV chronic infection multivariable analysis:

Following the adjustment for the remaining variables, independent variables significantly associated with HBV chronic infection amongst children aged 1-3 in the multivariable analysis were mothers HBV infection status and qualifications of the person managing delivery of the child.

The adjusted OR of HBV chronic infection amongst children born from anti-HBc positive mothers was 27.3 (95% CI 3.15 to 237), and the OR of HBV chronic infection amongst children aged 1-3 born from mothers chronically infected with HBV was 15.6 (95% CI 0.65 to 378). The adjusted OR of HBV chronic infection amongst children aged 1-3 whose delivery was managed by an unqualified individual was 25.5 (95% CI 1.79 to 362), indicating that children whose delivery was attended by an unqualified individual had a 25 times higher odds of developing HBV chronic infection compared to children whose delivery was managed by a medically qualified individual after adjusting for the remaining independent variables in the model. Age, sex, and area of residence did not show evidence of a significant association with HBV chronic infection of children aged 1-3, however, being *a priori* variables these were added to the final model. The adjusted OR of HBV chronic infection amongst children aged 1-3 associated with not owning a television [2.8 (95% CI 0.85 to 9.17)] was not statistically significant (p=0.09), however, because it was associated with anti-HBc positivity it was added to the multivariable model of HBV chronic infection in order to have two models both including identical independent variables. There was no interaction between the independent variables in the model. Table 6.6.1 shows the results of the multivariable analysis of the association of the independent variables with HBV chronic infection amongst children aged 1-3.

Table 6.6.1
Adjusted logistic regression of the association of HBV chronic infection amongst children aged 1-3 with independent exposure variables

Variable		OR*	p value**	95% C.I.***
Mother HBV Status	Uninfected	1		
	anti-HBc positive	27.3	0.005	3.15 – 237
	Chronic Carrier	15.64	0.09	0.65 – 378
	Joint significance	****	***0.02***	
Sex	Male	1		
	Female	1.14	0.7	0.57 – 2.28
Age of child (in months)	12 to 17	1		
	18 to 23	1.4	0.4	0.62 – 3.18
	24 to 29	0.65	0.73	0.05 – 8.27
	30 to 36	2.29	0.38	0.34 – 15.49
	Joint significance		*0.35*	
Area	Urban	1		
	Rural	0.41	0.43	0.04 – 4.11
Person Managing Delivery	Qualified Medic	1		
	Unqualified	25.45	**0.02**	1.79 – 362
Television	Yes	1		
	No	2.79	0.09	0.85 – 9.17

* adjusted OR computed by survey logistic regression multivariable analysis.
** p-value of the adjusted Wald test statistic.
*** 95% Confidence Intervals.
**** p-value of the adjusted survey Wald test statistic of the significance of the joint effect of the categories of the independent variable on the dependent variable.

The multivariable analysis of variables associated with HBV chronic infection amongst children aged 1-3 was repeated excluding the socioeconomic variables and substituting these variables with the single socioeconomic status variable. The OR and p-values of HBV chronic infection associated with age and sex of the child aged 1-3, as well as mothers HBV infection status, were similar in the model including the socioeconomic status variable as the model including all the socioeconomic variables. The adjusted OR of HBV chronic infection associated with residing in a rural area in the model including the socioeconomic status variable changed from 0.4 to 0.6 (p=0.09). Socioeconomic status, on the other hand, was not significantly associated with HBV chronic infection amongst children aged 1-3 (p=0.07) as none of the socioeconomic variables were directly significantly associated with HBV chronic infection amongst children aged 1-3. Table 6.3.2 shows the results of the multivariable analysis of the association of independent

variables and the socioeconomic status variable with HBV chronic infection amongst children aged 1-3 participating in the survey.

Table 6.6.2
Adjusted logistic regression of the association of HBV chronic infection amongst children aged 1-3 with independent exposure variables and socioeconomic status

Variable		OR*	p value**	95% C.I.***
Mother HBV Status	Uninfected	1		
	anti-HBc positive	27.6	0.01	2.94 – 259
	Chronic Carrier	17.7	0.08	0.69 – 451
	Joint significance	****	***0.02***	
Sex	Male	1		
	Female	1.11	0.75	0.57 – 2.15
Age of Child (in months)	12 to 17	1		
	18 to 23	1.35	0.42	0.63 – 2.88
	24 to 29	0.62	0.70	0.05 – 7.54
	30 to 36	1.80	0.52	0.28 – 11.8
	Joint significance		*0.52*	
Area	Urban	1		
	Rural	0.60	0.69	0.04 – 8.48
Person Managing Delivery	Qualified Medic	1		
	Unqualified	26.6	**0.02**	1.88 - 378
Socioeconomic Status	Low	1		
	Middle	0.15	0.04	0.03 – 0.90
	Upper	1.64	0.45	0.43 – 6.32
	Joint significance		*0.07*	

* adjusted OR computed by survey logistic regression multivariable analysis.
** p-value of the adjusted Wald test statistic.
*** 95% Confidence Intervals.
**** p-value of the adjusted survey Wald test statistic of the significance of the joint effect of the categories of the independent variable on the dependent variable.

As well as being one of the important variables investigated in the survey, hepatitis B vaccination was an important factor determining the HBV infection status of the child. In the univariable analysis, no OR of HBV chronic infection by vaccination status was computed because there were no cases of HBV chronic infection amongst completely and partially vaccinated children. Therefore, vaccination status was not added to the final multiple logistic regression model to examine the effect of vaccination status on the remaining variables in the model.

6.7 Discussion:

There was no previous data or information on the prevalence of HBV infection amongst children in the Republic of Yemen before this survey was conducted. The overall prevalence of anti-HBc and HBsAg positivity amongst children aged 1-3 participating in this household seroepidemiological survey was 2.4% and 0.99%, respectively, which was lower than initially expected. A recent study published in 2002 conducted amongst individuals presenting at Sanaa central health laboratory in response to a campaign to control HBV infection, as described by its authors, found the prevalence of HBV chronic infection amongst children aged less than 5 years to be 2.2% (Ragaa et al., 2002). The prevalence of HBV chronic infection amongst children aged 1-3 participating in this survey was expected to be approximately 5% based on a number of factors. Firstly, the few studies conducted in Yemen reported a high prevalence of HBV chronic infection amongst adults in the general population as well as women of childbearing age and all concluded that HBV infection is either highly endemic or hyperendemic in Yemen. Secondly, the studies that included women of childbearing age found a high prevalence of HBeAg amongst HBV chronic carrier women of childbearing age which indicates a potentially high rate of perinatal transmission of HBV infection in Yemen. Finally, studies conducted in the Middle-East and more specifically neighbouring countries of Yemen, described in preceding chapters, showed a high prevalence of anti-HBc positivity and HBV chronic infection amongst children and suggested childhood and perinatal transmission as the major contributors to the pool of HBV chronic carriers in these countries. According to these factors it was expected that the majority of HBV chronic infections in Yemen would be the result of childhood and perinatal transmission, and consequently, there would be a large proportion of anti-HBc and HBsAg positive children. Nevertheless, rather than obtaining an estimated 120 anti-HBc positive and 60 HBsAg positive children based on the expected prevalence, the survey generated 27 anti-HBc positive and 11 HBsAg positive children. This had important consequences on the power of the study and one major concern resulting from the unexpected small number of anti-HBc positive and HBV chronic carrier children in the survey was the possibility of an insufficient power to detect a statistically significant difference or association even if this existed. This was despite the fact that there was overachievement in the sampling

process and the total actual sample size (1285 children aged 1-3) exceeded the total planned sample size (1100 children aged 1-3) by 17%. For example, hepatitis B vaccination is well known to be effective in preventing anti-HBc positivity and HBV chronic infection. The prevalence of anti-HBc positivity amongst unvaccinated children in the survey was 2.95% (22/840) and this was higher than the prevalence of anti-HBc positivity amongst completely vaccinated children which was 0.5% (1/137), however, this difference was not statistically significant (p=0.6). Similarly, there were no cases of HBV chronic infection amongst completely vaccinated children (0/137) with all cases of HBV chronic infection occurring amongst unvaccinated children (11/840), however, this difference did not reach statistical significance (p=0.5). Moreover, even though the prevalence of anti-HBc positivity and chronic infection amongst children in urban areas was half the prevalence in rural areas this did not reach statistical significance. Nevertheless, it would be incorrect to conclude that the results obtained from this survey did not reflect true differences or associations because these differences or associations failed to reach statistical significance when this may be the result of an insufficient power of the study to yield a statistically significant result. Systematically repeating such an error would bias the results of this study towards the null.

One of the major findings of the survey was the decline in the prevalence of anti-HBc positivity (p=0.0001) and HBV chronic infection (p=0.0002) amongst children aged 1-3 according to the HBV infection status of the mother. It is well established that children born from HBV chronic carrier mothers have the highest risk of becoming infected with HBV. The multivariable analysis showed a clear increase in the adjusted OR of anti-HBc positivity amongst children born from anti-HBc positive mothers [OR=3.97 (95% CI 1.38 to 11.43)] and mothers chronically infected with HBV [OR=7.84 (95% CI 3.78 to 16.25)], which provided strong evidence of the association between HBV infection amongst children aged 1-3 and the HBV infection status of mothers. Similarly, multivariable analysis showed a statistically significant association between HBV chronic infection amongst children aged 1-3 and HBV infection status of the mother.

The risk of children becoming infected with HBV from a HBV chronic carrier mother is highly dependent on the mothers HBeAg status, with HBeAg positive mothers being

more infectious than HBeAg negative mothers. In this survey, there was evidence that the prevalence of HBV chronic infection was statistically significantly higher amongst children born to HBeAg positive chronic carrier mothers (21%) compared to HBeAg negative chronic carrier mothers (0%) (p=0.02). The same rate of HBV transmission from HBsAg positive mothers (21%) was observed in Tunisia, which had the same rate of HBeAg (9.6%) amongst HBV chronic carrier mothers (Coursaget et al., 1994). The proportion of children born from HBeAg positive chronic carrier mothers developing HBV chronic infection (21%) in this survey was much lower than 70% - 90% of children reported from numerous international studies (Hwang et al., 1985; Hall AJ, 1994), or an earlier study in Yemen which suggested that 50% of HBsAg chronic carrier mothers transmitted HBV chronic infection to their children although they did not measure the HBeAg status of the mothers (Abdul Raheem at al., 1991). Such differences in the risk of transmission of HBV infection to children of HBeAg positive mothers have been previously attributed to differences in HBV DNA levels between women in Africa and South-East Asia (Hall AJ, 1994).

The low prevalence of HBV chronic infection amongst children aged 1-3, the low prevalence of HBeAg amongst chronic carrier mothers, and the low transmission of HBV infection from HBeAg positive chronic carrier mothers to their children in this survey provides evidence that Yemen follows the pattern of studies suggesting that perinatal transmission is unlikely to be an important mode of transmission of HBV infection in the Middle East (Basalamah et al., 1984; Coursaget et al., 1994; Toukan, 1996) or a major contributor to the pool of HBV chronic carriers in Yemen. Childhood transmission, on the other hand, cannot be ruled out, which is supported by the increasing prevalence of HBV chronic infection amongst children aged 4-9 included in this survey presented in chapter 7. The importance of childhood transmission has been supported by other studies in the Middle East (Amini et al., 1993; Arya et al., 1985; Ramia, 1990).

In this survey 21% (2/8) of children born from HBeAg positive chronic carrier mothers became anti-HBc positive compared to 8% (3/51) of children born from HBeAg negative chronic carrier mothers, however, this difference did not reach statistical significance (p=0.5). It should be noted that mothers lose their HBeAg positive status over time and

that a few of the HBV chronic carrier mothers who were HBeAg negative at the time of the survey may have been HBeAg positive 1 to 3 years earlier at the time of delivery of their child aged 1-3 participating in the survey. Therefore, these women may have been highly infectious and actually infected their child at the time of delivery. Such changes in the HBV serological status of the mother (HBsAg/HBeAg) over time make it difficult to interpret the exact relationship between the child and the mothers HBV infection status. This may result in underestimating the risk of HBV infection amongst children born to HBeAg positive chronic carrier mothers, and therefore, the importance of HBeAg positivity amongst mothers in determining perinatal transmission will be underestimated. Moreover, due to the cross-sectional nature of the survey a causal relationship between mothers HBV infection and the child's HBV infection cannot be firmly established based on the results of this survey alone, as both the child's and mothers serological markers were measured at the same point of time and, theoretically, it is not known for sure if the mothers HBV infection proceeded her child's infection or vice versa (temporal relationship). Nevertheless, the prevalence of anti-HBc positivity and chronic infection amongst mothers is much higher than children and many of the Bradford Hill criteria still hold such as plausibility, consistency, and the strength of relationship.

Another important finding was the lower prevalence of anti-HBc positivity ($p=0.02$) and HBV chronic infection ($p=0.00003$) amongst children aged 1-3 whose delivery was managed by medically qualified personnel compared to unqualified persons. In multivariable analysis, there was an increased OR of anti-HBc positivity (OR=2.5) and HBV chronic infection (OR=25) amongst children aged 1-3 delivered by an unqualified person but only the latter of the two reached statistical significance ($p=0.02$). This may be due to qualified medical personnel using sterilised equipment and conducting adequate procedures during management of labour in proper settings (which reduces the risk of infecting the child), or a better chance of children receiving hepatitis B vaccine, or more hazardous procedures carried-out by unqualified personnel. De La Hoz found a similar association between anti-HBc positivity and qualifications of the person delivering the child in Colombia, and suggested that practices at the time of birth carried-out by unqualified persons such as cutting the umbilical cord may increase the child's risk of

HBV infection (De La Hoz, 2002). This survey also found a significantly lower prevalence of HBV chronic infection (p=0.02) and anti-HBc positivity (p=0.06) amongst children aged 1-3 delivered in a health setting compared to a home setting in Yemen. Parents of children delivered by medically qualified personnel or in a health setting are likely to have better health awareness or access to health services, and whether on their own or according to medical personnel's recommendations are more likely to visit health centres to vaccinate their child thereby reducing the child's risk of HBV infection.

It has already been demonstrated in chapter 1 that many studies in the Middle East have shown the prevalence of HBV chronic infection to be inversely related to socioeconomic status. International research and studies from neighbouring countries show that the prevalence of HBV infection declines with improving socioeconomic status. The socioeconomic classification of this survey also showed a decline in the prevalence of anti-HBc positivity (p=0.01) and HBV chronic infection (p=0.04) amongst children with increasing socioeconomic status. However, unexpected was the higher prevalence of anti-HBc positivity and HBV chronic infection amongst children of upper socioeconomic status compared to children of middle socioeconomic status. It was suspected that the higher prevalence of anti-HBc positivity and HBV chronic infection amongst children of upper socioeconomic status compared to children of middle socioeconomic status may have resulted from design and the cut-off points in the construction of the socioeconomic status variable, however, this was not the case when anti-HBc positivity and HBV chronic infection amongst mothers was analysed. When investigating the difference in prevalence of anti-HBc positivity (p=0.52) and HBV chronic infection (p=0.07) between children of upper and middle socioeconomic status this was not statistically significant.

The independent variables that were statistically significantly associated with anti-HBc positivity amongst children aged 1-3 in the multivariable analysis after adjusting for the remaining variables in the model were mothers HBV infection status and television ownership. The nature of the association between anti-HBc positivity amongst children aged 1-3 and not owning a television in the household is unexplained [OR = 3.34 (1.07 to 10.41)]. The adjusted OR of the association between HBV chronic infection amongst children and not owning a television was also increased [OR=2.79 (95% CI 0.85 to 9.17)]

although it did not reach statistical significance. The most likely explanation is that television ownership is an indicator of financial wellbeing and socioeconomic status. When the socioeconomic status variable was included in the multivariable analysis it showed a decreased OR of anti-HBc positivity and HBV chronic infection amongst children of middle and upper socioeconomic status compared to children of low socioeconomic status but neither of these associations reached statistical significance. Television ownership was highly dependent on the availability of an electricity supply in the household. In this survey, 88% of households that had an electricity supply had a television whereas only 21% of households without an electricity supply had a television in the household ($p<0.0001$). The availability of electricity supply, on the other hand, is highly dependent on area of residence. 98% of urban households had an electricity supply compared to 37% of rural households ($p<0.0001$). Noteworthy to mention is that 30% of rural households participating in the survey that had a television did not have electricity but used alternative sources such as batteries to power their television sets. Interestingly, after television ownership was added to the multiple logistic regression model it reversed the odds of anti-HBc positivity amongst children aged 1-3 associated with living in a rural area from 1.6 to 0.78. On the other hand, adding the socioeconomic status variable to the multiple logistic regression model did not have such a profound effect on the odds of anti-HBc positivity associated with living in a rural area. Why this occurred is not fully understood but a possible explanation is the highly significant difference ($p<0.0001$) in the prevalence of television ownership by area of residence (table 6.7.1).While 90% of urban households owned televisions less than half (45%) of rural households owned a television. Moreover, 92% of households that did not own a television were in rural areas, whereas only 8% of households that did not own a television were in urban areas.

Table 6.7.1
Television Ownership by Area

	Area of Residence	
Television Ownership	Urban % (no)	Rural % (no)
Yes	89.9 (468)	44.5 (404)
No	10.2 (41)	55.52 (372)
Total	100 (509)	100 (776)

This suggests that there may have been positive correlation between television ownership and area of residence. It is already known that pooling dissimilar subsets (in this case with reference to television ownership) in an analysis can suggest a difference or association where one does not exist, or even suggest an association or difference the opposite way around to one which does exist (Kirkwood, 1997) . This might be a possible explanation for the reversal of the odds of anti-HBc positivity associated with living in a rural area after adding television ownership to the multivariable model.

The results of the prevalence of anti-HBc positivity and HBV chronic infection amongst children aged 1-3 participating in this survey are the first results obtained from this age-group in Yemen. The reason for selecting this age-group was in order to confidently determine cases of HBV chronic infection resulting from perinatal transmission based on serological markers of the child and mother. At this age it was expected that the parents or guardians of these children would have the children's vaccination records of the type and dates of vaccines received. It is believed that these results are reliable and true findings based on a representative sample taken from the general population. It is unlikely that these results are biased and a general discussion of the possible effect of bias or confounding on these results is presented in chapter 9.

Chapter 7: Results Children Aged 4-9:

This chapter presents the results of the prevalence, univariable analysis, and multivariable analysis of HBV infection amongst children aged 4-9 participating in the survey in Yemen. These children were surveyed to simply provide a baseline measure of the prevalence of HBV infection amongst children in Yemen prior to the effect of hepatitis B vaccination on the prevalence of HBV infection amongst these children. These children were not included to examine factors associated with HBV infection.

HBV Infection:

7.1 Prevalence of hepatitis B core antibody (anti-HBc):

The prevalence of anti-HBc positive children aged 4-9 participating in the survey in Yemen was 4.75% (95% CI 2.34 to 9.41). Out of the 467 children aged 4-9 participating in the survey, 19 were anti-HBc positive.

Amongst the children aged 4-9 participating in the survey there was only evidence of a statistically significant difference in the prevalence of anti-HBc positivity by socioeconomic status and educational status of the mother. The prevalence of anti-HBc positive children aged 4-9 participating in the survey born to uneducated women (6.59%) was higher than the prevalence of anti-HBc positive children born to women claiming to have any level of education (1.55%) and this difference was statistically significant (p=0.02). There were no anti-HBc positive children aged 4-9 participating in the survey born from women who completed secondary school, attended college/university, or who were in paid employment. There was a significant difference in the prevalence of anti-HBc positivity between the three socioeconomic classes in the survey (p=0.002). The prevalence of anti-HBc positivity was 7.82% amongst children of low socioeconomic status, 0.83% amongst children of middle socioeconomic status, and 1.49% amongst children of upper socioeconomic status. The higher prevalence of anti-HBc positivity amongst children of upper socioeconomic status compared to children of middle socioeconomic status was not statistically significant (p=0.55). There was an increase in the prevalence of anti-HBc positive children with increasing age across the six age-

groups of children participating in the survey of children aged 4-9 but this did not reach statistical significance (p=0.72). Figure 7.1.1 shows the distribution of anti-HBc positivity amongst children aged 4-9 participating in the survey by age of the child.

Figure 7.1.1
Prevalence of anti-HBc positivity amongst children aged 4-9

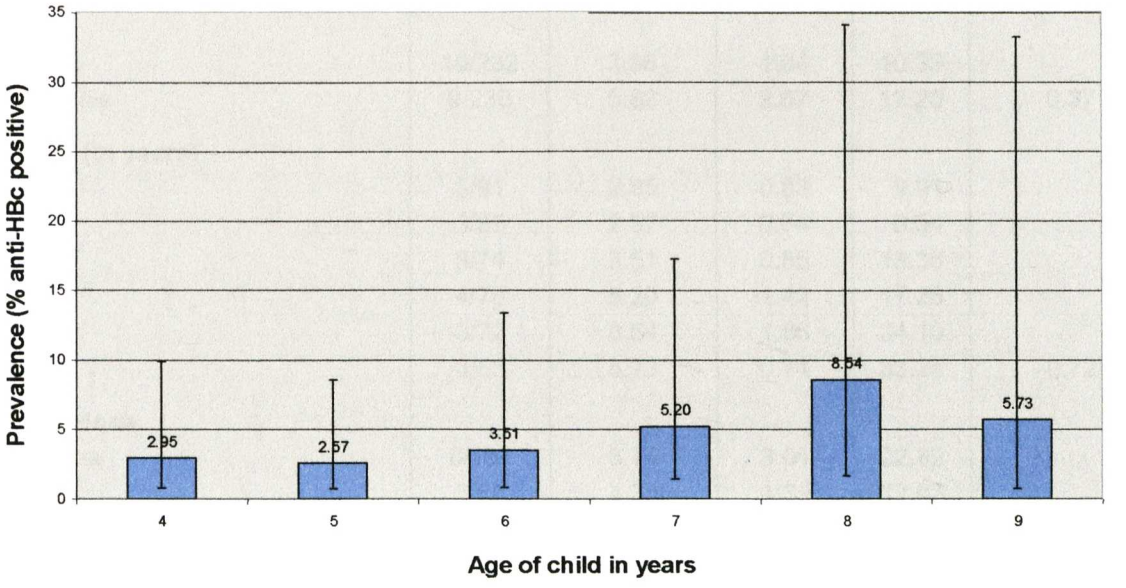


Table 7.1.1 shows the prevalence of anti-HBc positivity amongst children aged 4-9 participating in the survey by demographic characteristics.

Table 7.1.1
Prevalence of anti-HBc positive children aged 4-9 by children's demographic characteristics

Prevalence anti-HBc positive	Number of Children	Prevalence (weighted) %	95% CI LL	95% CI UL	p-value (Pearson design-based)
Overall	19/467	4.75	2.34	9.41	
Sex					
Male	10/232	3.36	1.04	10.33	
Female	9/235	5.82	2.67	12.20	0.37
Age (in years)					
4	3/91	2.95	0.83	9.91	
5	3/97	2.57	0.74	8.54	
6	3/74	3.51	0.85	13.38	
7	4/78	5.20	1.42	17.26	
8	3/72	8.54	1.66	34.10	
9	3/55	5.73	0.74	33.24	0.72
Province					
Sanaa	8/151	8.74	3.01	22.82	
Taiz	3/66	4.33	1.39	12.63	
Aden	1/90	1.11	0.14	8.48	
Shabwa	3/75	5.07	1.24	18.59	
Hodeidah	4/85	3.64	0.43	24.74	0.55
Area of residence					
Urban	9/241	6.14	2.26	15.58	
Rural	10/226	4.11	1.54	10.55	0.55
Vaccination Status					
Not Vaccinated	18/410	5.22	2.47	10.68	
Completely Vaccinated	0/4	0.00			
Partially Vaccinated	0/5	0.00			0.83
School Attendance					
Yes	8/198	4.15	1.38	11.79	
No	11/268	5.32	2.25	12.04	0.69
Socioeconomic Status					
Low	14/200	7.82	3.64	16.03	
Middle	3/161	0.83	0.18	3.69	
Upper	2/106	1.49	0.38	5.61	0.002

Only 9 of the 467 children aged 4-9 participating in the survey claimed and had evidence of receiving at least one dose of hepatitis B vaccine and none of these children had evidence of being exposed to HBV infection (i.e. these children were anti-HBc negative). The prevalence of anti-HBc positive children aged 4-9 appeared to be lower amongst males than females and lower in rural than urban areas but neither of these was statistically significant. There was no evidence of a statistically significant difference in the prevalence of anti-HBc positive children aged 4-9 between the five provinces (p=0.55) or rural provinces (p=0.93) in the survey, however, there appeared to be a difference between the urban provinces, with the prevalence of anti-HBc positive children aged 4-9 living in Sanaa (8.74%) higher than children living in Aden (1.11%) (p=0.06). Table 7.1.2 shows the prevalence of anti-HBc positivity amongst children aged 4-9 by mothers characteristics.

Table 7.1.2
Prevalence of anti-HBc positive children aged 4-9
by mother's characteristics

Prevalence anti-HBc positive	Number of Children	Prevalence (weighted) %	95% CI LL	95% CI UL	p-value (Pearson design-based)
Mothers Age (in years)					
18 to 24	5/81	11.01	2.76	35.09	
25 to 29	5/131	2.45	0.88	6.63	
30 to 34	4/127	4.10	1.05	14.70	
35 to 39	4/88	5.79	1.59	18.96	
40 to 52	1/40	2.29	0.28	16.40	0.31
Mother's Educational Status					
Educated	5/200	1.55	0.65	3.69	
Uneducated	14/267	6.59	3.02	13.77	0.02
Mother's Level of Education					
Read and Write	2/64	1.57	0.20	11.30	
Primary School	3/86	2.97	0.87	9.66	
Secondary School	0/37	0.00			
University or College	0/13	0.00			0.67
Mother's Employment					
Employed	0/25	0.00			
Not Employed	19/442	5.02	2.51	9.81	0.50

7.2 Anti-HBc univariable analysis:

The univariable analysis of the association of anti-HBc positivity amongst children aged 4-9 with exposure variables produced three types of results. The first of these had OR statistically significantly different from 1. The second had notably increased or decreased OR, by more or less than 30%, but these were not statistically significant. The third type neither had notably increased or decreased OR nor were these statistically significant. These results are presented in tables 7.2.1, 7.2.2 and 7.2.3.

Out of all the variables analysed those that had a statistically significant association with anti-HBc positivity amongst children aged 4-9 by univariable analysis were educational status of the mother and father, socioeconomic status and ownership of a fridge/freezer. The OR of anti-HBc positivity amongst children aged 4-9 born to an uneducated mother was 4.47 (95% CI 1.19 to 16.78), and the OR of anti-HBc positivity amongst children aged 4-9 born to an uneducated father was 6.14 (95% CI 1.67 to 22.57). The OR of anti-HBc positivity was 4.13 (95% CI 1.11 to 15.3) amongst children aged 4-9 who did not have a fridge/freezer in their household (p=0.04). There was a decline in the odds of anti-HBc positivity amongst children aged 4-9 with increasing socioeconomic status. The OR of anti-HBc positivity was significantly lower amongst children of middle (OR=0.1) and upper (OR=0.18) socioeconomic status compared to children of low socioeconomic status (p=0.03). Table 7.2.1 shows the results of the univariable analysis of the association of demographic variables with anti-HBc positivity amongst children aged 4-9.

Table 7.2.1
Association of anti-HBc positivity amongst children aged 4-9 with children's demographic characteristics

Variable		Number Controls	Number Cases	OR	p value	95% C.I.
Sex	Male	222	10	1		
	Female	226	9	1.77	0.38	0.47 – 6.69
Age of child (in months)	4	88	3	1		
	5	94	3	0.87	0.86	0.17 – 4.38
	6	71	3	1.20	0.79	0.29 – 4.95
	7	74	4	1.81	0.37	0.48 – 6.84
	8	69	3	3.08	0.32	0.30 – 31.33
	9	52	3	2.00	0.53	0.21 – 19.53
	Joint significance				*0.80*	
	Linear trend OR			1.24	0.25	0.85 – 1.82
Province	Sanaa	143	8	1		
	Taiz	63	3	0.47	0.35	0.09 – 2.38
	Aden	89	1	0.12	0.08	0.01 – 1.28
	Shabwa	72	3	0.56	0.52	0.09 – 3.51
	Hodeidah	81	4	0.39	0.44	0.03 – 4.52
	Joint significance				*0.55*	
Area	Urban	232	9	1		
	Rural	216	10	0.66	0.55	0.15 – 2.79
Child Birth Order	1	123	6	1		
	2	99	2	0.12	0.04	0.02 – 0.85
	3	61	4	0.64	0.61	0.11 – 3.83
	4	44	2	0.45	0.40	0.07 – 2.67
	5	37	3	0.95	0.96	0.15 – 6.00
	6 to 15	84	1	0.15	0.12	0.01 – 1.67
	Joint significance				*0.16*	
	Linear Trend OR			0.84	0.36	0.57 – 1.24
Vaccination Status	Unvaccinated	392	18	1		
	Partially	5	0	-		
	Completely	4	0	-		

Variables that had increased or decreased OR by more or less than 30% but were not statistically significantly associated with anti-HBc positivity amongst children aged 4-9 were age of the child, area and province of residence, household ownership, type, construction and source of water, availability of an electricity supply, ownership of a television and home telephone, as well as birth order of the child and mother, and age of the mother. These are presented in tables 7.2.1, 7.2.2, and 7.2.3. There appeared to be an increasing odds of anti-HBc positivity amongst children aged 4-9 with increasing age of the child with children aged 7, 8 and 9 having an higher odds of anti-HBc positivity than

children aged 4, 5, and 6 (p=0.8). Table 7.2.2 shows the results of the univariable analysis of the association of mothers and fathers characteristics with anti-HBc positivity amongst children aged 4-9.

Table 7.2.2
Association of anti-HBc positivity amongst children aged 4-9 with mothers and fathers characteristics

Variable		Number Controls	Number Cases	OR	p value	95% C.I.
Mothers Age (in years)	18 to 24	76	5	1		
	25 to 29	126	5	0.2	0.12	0.03 – 1.44
	30 to 34	123	4	0.35	0.28	0.05 – 2.57
	35 to 39	84	4	0.5	0.46	0.07 – 3.38
	40 to 52	39	1	0.19	0.21	0.01 = 2.73
	Joint significance				*0.31*	
	Linear Trend OR			0.78	0.42	0.41 – 1.47
Mothers Education	Educated	195	5	1		
	Uneducated	253	14	4.47	**0.03**	1.19 – 16.78
Mothers Birth Order	1	79	5	1		
	2	85	1	0.12	0.1	0.01 – 1.61
	3	71	5	2.56	0.27	0.45 – 14.57
	4	62	1	0.36	0.39	0.03 – 4.02
	5	55	2	0.77	0.78	0.11 – 5.22
	6 to 13	96	5	1.48	0.65	0.24 – 9.02
	Joint significance				*0.3*	
	Linear Trend OR			1.08	0.62	0.77 – 1.53
Mothers Employment	Unemployed	423	19	1		
	Employed	25	0	-		
Fathers Education	Educated	363	11	1		
	Uneducated	85	8	6.14	**0.009**	1.67 – 22.57

There were no anti-HBc positive children amongst children receiving any number of doses of hepatitis B vaccine, born from mothers in paid employment, living in households with a crowding index of 1, or living in households with a mobile telephone, and therefore no OR were computed for these variables in the univariable analysis.

Variables that did not show an increase or decrease in their OR by more or less than 30% nor evidence of a statistically significant association with anti-HBc positivity amongst children aged 4-9 were ownership of a car (p=0.86) and radio (p=0.99). Table 7.2.3 shows the association of anti-HBc positivity amongst children aged 4-9 with household characteristics and possessions.

Table 7.2.3
Association of anti-HBc positivity amongst children aged 4-9 with household characteristics and possessions

Variable		Number Controls	Number Cases	OR	p value	95% C.I.
Household Ownership	Owned	358	15	1		
	Rented	90	4	3.24	0.28	0.36 – 29.22
Household Type	House	303	12	1		
	Apartment	99	3	0.55	0.47	0.10 – 3.02
	Shack	46	4	0.99	0.99	0.28 – 3.55
	Joint significance				*0.77*	
Household Construction	Stone	113	3	1		
	Block	226	10	1.7	0.58	0.24 – 12.15
	Mud or Clay	58	2	0.71	0.78	0.06 – 8.38
	Mixed	51	4	1.21	0.84	0.18 – 8.26
	Joint significance				*0.85*	
Crowding Index	1	30	0	-		
	2	113	4			
	3	118	6			
	4	74	3			
	5	41	2			
	6 to 15	72	4			
Electricity Supply	Yes	334	12	1		
	No	114	7	1.42	0.59	0.37 – 5.39
Source of Water	Governmental	156	4	1		
	Local Project	132	3	0.16	0.08	0.02 – 1.22
	Water Truck	67	6	2.73	0.25	0.46 – 16.27
	Buckets	93	6	0.82	0.82	0.14 – 4.84
	Joint significance				*0.08*	
Freezer	Yes	232	5	1		
	No	216	14	4.13	**0.04**	1.11 – 15.30
Television	Yes	317	9	1		
	No	131	10	2.47	0.25	0.50 – 12.16
Radio	Yes	308	9	1		
	No	140	10	0.99	0.99	0.26 – 3.72
Home Phone	Yes	106	2	1		
	No	342	17	3.11	0.18	0.56 – 17.23
Mobile Phone	Yes	34	0	-		
	No	414	19			
Car	Yes	129	4	1		
	No	319	15	0.86	0.86	0.14 – 5.24
Socioeconomic Status	Lower	186	14	1		
	Middle	158	3	0.1	0.01	0.02– 0.56
	Upper	104	2	0.18	0.05	0.03 – 1.02
	Joint significance				***0.03***	

7.3 Anti-HBc multivariable analysis:

The independent variable statistically significantly associated with anti-HBc positivity amongst children aged 4-9 in the multivariable analysis was father's educational status. The adjusted OR of being anti-HBc positive amongst children aged 4-9 born from fathers who were uneducated was 7.89 (95% CI 1.55 to 40.17) compared to children born from fathers claiming to be educated. Age, sex, and area of residence did not show evidence of a statistically significant association with anti-HBc positivity amongst children aged 4-9, but being *a priori* variables they were added to the final model at the end of the process. The OR of anti-HBc positivity increased with increasing age of the child, and the OR of anti-HBc positivity appeared to be lower amongst children living in rural areas of residence, but neither of these was statistically significant. There was no evidence of interaction between the independent variables in the model. Table 7.3.1 shows the results of the multivariable analysis of the association of the independent variables with anti-HBc positivity amongst children aged 4-9.

Table 7.3.1
Adjusted logistic regression of the association of anti-HBc positivity amongst children aged 4-9 with independent exposure variables

Variable		OR*	p value**	95% C.I.***
Age of Child (in years)	4	1		
	5	0.89	0.90	0.14 – 5.54
	6	1.53	0.57	0.33 – 7.11
	7	1.98	0.34	0.46 – 8.44
	8	2.65	0.35	0.32 – 22.29
	9	2.57	0.42	0.23 – 28.32
	Joint significance	****	*0.73*	
Sex	Male	1		
	Female	1.35	0.58	0.45 – 4.09
Area	Urban	1		
	Rural	0.38	0.24	0.07 – 2.03
Fathers Educational Status	Educated	1		
	Uneducated	7.89	**0.02**	1.55 – 40.17

* adjusted OR computed by survey logistic regression multivariable analysis.
** p-value of the adjusted wald test statistic.
*** 95% Confidence Intervals.
**** p-value of the adjusted survey wald test statistic of the significance of the joint effect of the categories of the independent variable on the dependent variable.

The multivariable analysis of variables associated with anti-HBc positivity amongst children aged 4-9 was repeated excluding the socioeconomic variables and substituting them with the single socioeconomic status variable. The adjusted OR of being anti-HBc positive amongst children aged 4-9 was significantly lower amongst children of middle (OR=0.06) and upper (OR=0.1) socioeconomic status compared to children of low socioeconomic status (p=0.02). The OR and p-values of anti-HBc positivity associated with sex of the child aged 4-9 and area of residence were similar in the model including the socioeconomic status variable as the model including all the socioeconomic variables. The OR of anti-HBc positivity associated with increasing age of the child in the model including the socioeconomic status variable was slightly higher and is shown in table 7.3.2. Table 7.3.2 shows the results of the multivariable analysis of the association of independent variables and the socioeconomic status variable with anti-HBc positivity amongst children aged 4-9 participating in the survey.

Table 7.3.2
Adjusted logistic regression of the association of anti-HBc positivity amongst children aged 4-9 with independent exposure variables and socioeconomic status

Variable		OR*	p value**	95% C.I.***
Age of Child (in years)	4	1		
	5	0.98	0.98	0.16 – 6.11
	6	1.48	0.59	0.34 – 6.45
	7	1.85	0.38	0.44 – 7.72
	8	4.23	0.15	0.58 – 30.70
	9	3.49	0.29	0.31 – 39.00
	Joint significance	****	*0.45*	
Sex	Male	1		
	Female	1.33	0.65	0.37 – 4.80
Area	Urban	1		
	Rural	0.36	0.14	0.09 – 1.41
Socioeconomic Status	Low	1		
	Middle	0.06	0.01	0.01 – 0.38
	Upper	0.10	0.04	0.01 – 0.88
	Joint significance		***0.02***	

* adjusted OR computed by survey logistic regression multivariable analysis.
** p-value of the adjusted wald test statistic.
*** 95% Confidence Intervals.
**** p-value of the adjusted survey wald test statistic of the significance of the joint effect of the categories of the independent variable on the dependent variable.

HBV Chronic Infection:

7.4 Prevalence of HBV chronic infection:

The prevalence of HBV chronic infection amongst children aged 4-9 participating in the survey in Yemen was 3.08% (95% CI 1.10 to 8.32). Out of the 467 children participating in the survey only 9 were HBV chronic carriers.

There was no evidence of a statistically significant difference in the prevalence of HBV chronic infection amongst children aged 4-9 by any of the variables under investigation. The prevalence of HBV chronic infection amongst children aged 4-9 participating in the survey born to uneducated women (4.35%) was higher than the prevalence of HBV chronic infection amongst children born to women claiming to have any level of education (0.89%) (p=0.07). There were no HBV chronic carrier children aged 4-9 participating in the survey born from women who completed secondary school, college or university, or women in paid employment. The prevalence of HBV chronic infection declined with increasing socioeconomic status (p=0.12). The prevalence of HBV chronic infection was 5.46% amongst children of low socioeconomic status, 0% amongst children of middle socioeconomic status, and 0.65% amongst children of upper socioeconomic status. The higher prevalence of HBV chronic infection amongst children of upper compared to middle socioeconomic status was not statistically significant (p=0.23). There appeared to be an increase in the prevalence of HBV chronic infection with increasing age across the six age-groups of children participating in the survey (p=0.72). Figure 7.4.1 shows the distribution of HBV chronic infection amongst children aged 4-9 by age-group amongst children participating in the survey.

Figure 7.4.1
Prevalence of HBV chronic infection amongst children aged 4-9

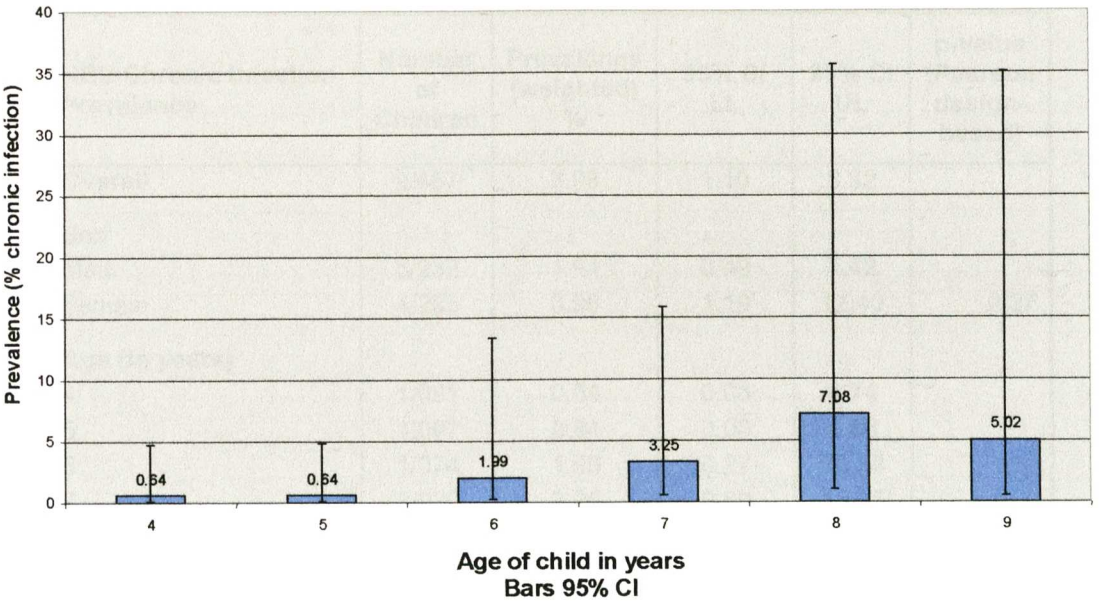


Table 7.4.1 shows the prevalence of HBV chronic infection amongst children aged 4-9 by demographic characteristics.

Table 7.4.1
Prevalence of HBV chronic infection amongst children aged 4-9 by children's demographic characteristics

HBV Chronic Infection Prevalence	Number of Children	Prevalence (weighted) %	95% CI LL	95% CI UL	p-value (Pearson design-based)
Overall	9/467	3.08	1.10	8.32	
Sex					
Male	5/232	1.94	0.49	7.42	
Female	4/235	3.96	1.19	12.40	0.37
Age (in years)					
4	1/091	0.64	0.08	4.74	
5	1/097	0.64	0.08	4.86	
6	1/074	1.99	0.27	13.38	
7	3/078	3.25	0.60	15.85	
8	2/072	7.08	1.04	35.63	
9	1/055	5.02	0.51	35.09	0.44
Province					
Sanaa	5/151	7.10	1.69	25.34	
Taiz	1/066	2.50	0.30	17.89	
Aden	0/90	0.00			
Shabwa	0/75	0.00			
Hodeidah	3/085	2.73	0.33	19.31	0.51
Area of residence					
Urban	5/241	4.68	1.13	17.34	
Rural	4/226	2.35	0.53	9.84	0.48
Vaccination Status					
Not Vaccinated	9/410	3.49	1.24	9.43	
Completely Vaccinated	0/4	0.00			
Partially Vaccinated	0/5	0.00			0.88
School Attendance					
Yes	4/198	3.16	0.79	11.82	
No	5/268	3.03	0.80	10.77	0.96
Socioeconomic Status					
Low	8/200	5.46	1.89	14.75	
Middle	0/161	0.00			
Upper	1/106	0.65	0.08	5.37	0.12

Out of the 9 children aged 4-9 participating in the survey whom received at least one dose of hepatitis B vaccine none was a HBV chronic carrier. The prevalence of HBV chronic infection amongst children aged 4-9 appeared to be lower amongst males than females and lower amongst children aged 4-9 living in rural areas than children living in urban areas but these were not statistically significant. There was no evidence of a statistically significant difference in the prevalence of HBV chronic infection amongst children aged 4-9 between the five provinces (p=0.51), rural provinces (p=0.88), or urban provinces (p=0.29) in the survey. Table 7.4.2 shows the prevalence of HBV chronic infection amongst children aged 4-9 by mother's characteristics.

Table 7.4.2
Prevalence of HBV chronic infection amongst children aged 4-9 by mothers characteristics

HBV Chronic Infection Prevalence	Number of Children	Prevalence (weighted) %	95% CI LL	95% CI UL	p-value (Pearson design-based)
Mothers Age (in years)					
18 to 24	3/081	8.48	1.48	36.39	
25 to 29	3/131	1.62	0.41	6.12	
30 to 34	1/127	2.78	0.35	18.76	
35 to 39	2/088	3.17	0.43	19.98	
40 to 52	0/040	0.00			0.48
Mother's Educational Status					
Educated	3/200	0.89	0.21	3.64	
Uneducated	6/267	4.35	1.42	12.56	0.07
Mother's Level of Education					
Read and Write	1/064	0.79	0.10	5.85	
Primary School	2/086	1.81	0.22	13.28	
Secondary School	0/037	0.00			
University or College	0/013	0.00			0.78
Mother's Employment					
Employed	0/025	0.00			
Not Employed	9/442	3.26	1.18	8.69	0.61

7.5 HBV chronic infection univariable analysis:

The univariable analysis of the association of HBV chronic infection amongst children aged 4-9 with exposure variables produced three types of results. The first had OR that were statistically significantly different from 1. The second had OR that were increased or decreased by more or less than 30%, but did not reach statistical significance. The third neither had increased or decreased OR nor did they reach statistical significance. These results are shown in tables 7.5.1, 7.5.2 and 7.5.3.

In the univariabe analysis the variables that had a statistically significant association with HBV chronic infection amongst children aged 4-9 were educational status of the father and ownership of a fridge/freezer. The OR of HBV chronic infection amongst children aged 4-9 born to an uneducated father was 12.67 (95% CI 1.67 to 22.57) and the OR of HBV chronic infection was 12.51 (95% CI 1.17 to 133.4) amongst children aged 4-9 who did not have a fridge/freezer in their household (p=0.04). Table 7.5.1 shows the results of the univariable analysis of the association of demographic variables with HBV chronic infection amongst children aged 4-9.

Table 7.5.1
Association of HBV chronic infection amongst children aged 4-9 with children's demographic characteristics

Variable		Number Controls	Number Cases	OR	p value	95% C.I
Sex	Male	227	5	1		
	Female	231	4	2.09	0.38	0.38 – 11.32
Age of child (in years)	4	90	1	1		
	5	96	1	0.99	0.94	0.72 – 1.36
	6	73	1	3.13	0.43	0.17 – 58.09
	7	75	3	5.19	0.22	0.34 – 80.48
	8	70	2	11.78	0.1	0.60 – 231.7
	9	54	1	8.17	0.16	0.42 – 160.4
	Joint significance				*0.34*	
	Linear trend OR			1.57	0.08	0.94 – 2.63
Province	Sanaa	146	5	1		
	Taiz	65	1	0.34	0.4	0.02 – 5.12
	Aden	90	0	-		
	Shabwa	75	0	-		
	Hodeidah	82	3	0.37	0.44	0.02 – 5.62
	Joint significance				*0.63*	
Area	Urban	236	5	1		
	Rural	222	4	0.49	0.49	0.06 – 3.99
Child Birth Order	1	125	4	1		
	2	100	1	0.1	0.07	0.01 – 1.23
	3	64	1	0.14	0.16	0.01 – 2.36
	4	45	1	0.41	0.43	0.04 – 4.27
	5	39	1	0.41	0.47	0.03 – 5.14
	6 to 15	85	0	-		
	Joint significance				*0.4*	
	Linear Trend OR			0.63	0.23	0.29 – 1.37
Vaccination Status	Unvaccinated	401	9	1		
	Partially	5	0	-		
	Completely	4	0	-		

Variables with increased or decreased OR associated with HBV chronic infection amongst children aged 4-9 failing to reach statistical significance were age and sex of the child, area and province of residence, mothers educational status, socioeconomic status, household ownership, type, construction and source of water, availability of an electricity supply, ownership of a television, home telephone, car and radio, as well as birth order of the child and mother, and age of the mother. These are presented in tables 7.5.1, 7.5.2 and 7.5.3. There appeared to be an increase in the OR of HBV chronic infection amongst children aged 4-9 with increasing age of the child with children aged 7, 8 and 9 having an higher OR of HBV chronic infection than children aged 4, 5, and 6 (p=0.34). Children

born to uneducated women appeared to have a higher OR of HBV chronic infection [5.07 (95% CI 0.72 to 35.69)] but this was not statistically significant (p=0.1). Table 7.5.2 shows the results of the univariable analysis of the association of mothers and fathers characteristics with HBV chronic infection amongst children aged 4-9.

Table 7.5.2
Association of HBV chronic infection amongst children aged 4-9 with mothers and fathers characteristics

Variable		Number Controls	Number Cases	OR	P value	95% C.I.
Mothers Age (in years)	18 to 24	78	3	1		
	25 to 29	128	3	0.18	0.16	0.01 – 2.14
	30 to 34	126	1	0.31	0.38	0.02 – 4.69
	35 to 39	86	2	0.35	0.46	0.02 – 6.09
	40 to 52	40	0	-		
	Joint significance				*0.25*	
	Linear Trend OR			0.62	0.37	0.21 – 1.81
Mothers Education	Educated	197	3	1		
	Uneducated	261	6	5.07	0.1	0.72 – 35.69
Mothers Birth Order	1		5	1		
	2		1	0.12	0.1	0.03 – 1.61
	3		5	2.56	0.27	0.45 – 14.57
	4		1	0.36	0.39	0.03 – 4.02
	5		2	0.77	0.78	0.11 – 5.22
	6 to 13		5	1.48	0.65	0.24 – 9.02
	Joint significance				*0.3*	
	Linear Trend OR			1.08	0.62	0.77 – 1.53
Mothers Employment	Unemployed	423	19	1		
	Employed	25	0	-		
Fathers Education	Educated	363	11	1		
	Uneducated	85	8	12.67	**0.005**	2.41 – 66.52

There were no cases of HBV chronic infection amongst children receiving any number of doses of hepatitis B vaccine, born from mothers in paid employment, living in households with a crowding index of 1, living in households with a mobile telephone, or in Aden and Shabwa provinces, and therefore no OR were computed for these variables in the univariable analysis. Table 7.5.3 shows the association of HBV chronic infection amongst children aged 4-9 with household characteristics and possessions.

Table 7.5.3
Association of HBV chronic infection amongst children aged 4-9 with household characteristics and possessions

Variable		Number Controls	Number Cases	OR	p value	95% C.I.
Household Ownership	Owned	367	6	1		
	Rented	91	3	5.64	0.17	0.45 – 71.5
Household Type	House	309	6	1		
	Apartment	100	2	0.48	0.52	0.05 – 4.96
	Shack	49	1	0.19	0.18	0.02 – 2.23
	Joint significance				*0.37*	
Household Construction	Stone	115	1	1		
	Block	229	7	1.89	0.61	0.14 – 24.97
	Mud or Clay	60	0	-		
	Mixed	54	1	0.24	0.32	0.01 – 4.25
	Joint significance				*0.33*	
Crowding Index	1	30	0	-		
	2	115	2			
	3	122	2			
	4	75	2			
	5	43	0			
	6 to 15	73	3			
Electricity Supply	Yes	341	5	1		
	No	117	4	1.41	0.73	0.19 – 10.57
Source of Water	Governmental	158	2	1		
	Local Project	135	0	-		
	Water Truck	69	4	2.82	0.33	0.31 – 26.12
	Buckets	96	3	0.47	0.54	0.04 – 6.11
	Joint significance				*0.36*	
Freezer	Yes	236	1	1		
	No	222	8	12.51	**0.04**	1.17 – 133.4
Television	Yes	322	4	1		
	No	136	5	1.92	0.56	0.20 – 18.8
Radio	Yes	312	5	1		
	No	146	4	0.45	0.33	0.08 – 2.39
Home Phone	Yes	107	1	1		
	No	351	8	4.65	0.21	0.4 – 53.49
Mobile Phone	Yes	34	0	-		
	No	424	9			
Car	Yes	131	2	1		
	No	327	7	0.66	0.7	0.07 – 5.95
Socioeconomic Status	Lower	192	8	1		
	Middle	161	0	-		
	Upper	105	1	0.11	0.08	0.01 – 1.34

7.6 HBV chronic infection multivariable analysis:

The independent variable statistically significantly associated with HBV chronic infection amongst children aged 4-9 in the multivariable analysis was father's educational status. The adjusted OR of HBV chronic infection amongst children aged 4-9 born from uneducated fathers was 23.1 (95% CI 1.99 to 269) compared to children born from fathers claiming to be educated. There were no cases of HBV chronic infection amongst children of upper and middle socioeconomic status and therefore this variable was dropped from the multivariable analysis and a model was not fitted substituting the remaining socioeconomic variables with the socioeconomic status variable. Age, sex, and area of residence did not show evidence of a statistically significant association with HBV chronic infection amongst children aged 4-9, but being *a priori* variables, they were added to the final model. There was no interaction between the independent variables in the model. Table 7.6.1 shows the results of the multivariable analysis of the association of independent variables with HBV chronic infection amongst children aged 4-9.

Table 7.6.1
Adjusted logistic regression of the association of HBV chronic infection amongst children aged 4-9 with independent exposure variables

Variable		OR*	p value**	95% C.I.***
Age of Child (in years)	4	1		
	5	1.04	0.88	0.62 – 1.74
	6	4.75	0.3	0.22 – 103
	7	6.35	0.21	0.33 – 121
	8	10.01	0.1	0.63 – 159
	9	14.42	0.12	0.49 – 428
	Joint significance		*0.45*	
Sex	Male	1		
	Female	1.41	0.64	0.31 – 6.29
Area	Urban	1		
	Rural	0.2	0.18	0.02 – 2.18
Fathers Educational Status	Educated	1		
	Uneducated	23.1	**0.02**	1.99 – 269

* adjusted OR computed by survey logistic regression multivariable analysis.
** p-value of the adjusted wald test statistic.
*** 95% Confidence Intervals.
**** p-value of the adjusted survey wald test statistic of the significance of the joint effect of the categories of the independent variable on the dependent variable.

7.7 Discussion:

The reason this age-group of children aged 4-9 years was targeted in the survey was in order to obtain a baseline measure of the prevalence of HBV infection amongst an unvaccinated cohort of children aged 4-9 prior to the effect of universal hepatitis B vaccination on the prevalence of HBV infection amongst these children. Such an estimate will allow the evaluation of the impact of routine hepatitis B vaccination following its introduction to the Yemeni EPI on the prevalence of HBV infection amongst children aged 4-9 in the future when it is compared to the baseline measure obtained in this survey. It has already been discussed in previous chapters that no surveys have been conducted to investigate the prevalence of HBV infection amongst children, and therefore there is a lack of data and information on the epidemiology of HBV infection amongst children in the Republic of Yemen. When the children aged 4-9 participating in this survey were recruited there was no intention or plan to perform an analysis of factors associated with HBV infection amongst these children. Nevertheless, because data was already collected from these children it was decided to perform a case-control analysis to investigate factors associated with anti-HBc positivity and HBV chronic infection amongst these children to add more strength to information resulting from the analysis of factors associated with anti-HBc positivity and chronic infection amongst children aged 1-3.

The overall prevalence of anti-HBc positivity (4.75%) and HBV chronic infection (3.08%) amongst children aged 4-9 participating in the survey was lower than expected. The prevalence of HBV chronic infection amongst children aged 4-9 was expected to be 5% to 10% based on a few factors. First of all, the majority of studies conducted in neighbouring countries in the region have suggested that most of HBV chronic infections occur during childhood in the Middle-East. Secondly, previous studies conducted in Yemen have reported a high prevalence of HBV chronic infection amongst adults in the general population and it is expected that these are mainly the result of childhood transmission. Thirdly, earlier studies have reported a high prevalence of HBV chronic infection as well as a high HBeAg positivity amongst HBV chronic carrier women of childbearing age in Yemen suggesting a potentially high rate of perinatal transmission of HBV infection. Taking these factors into consideration it was expected that the survey

would obtain at least 47 anti-HBc positive and 23 HBV chronic carrier children aged 4-9. Nevertheless, contrary to expectations, what the survey did obtain was 19 anti-HBc positive and 9 HBV chronic carrier children aged 4-9, which is less than half the number that was expected. Obviously, one of the limitations resulting from the small number of anti-HBc and HBsAg positive children aged 4-9 would be an insufficient power to detect a statistically significant difference or association even if one existed. This was despite the successful sampling process that resulted in a total actual sample size (467 children aged 4-9) exceeding the total planned sample size (420 children aged 4-9) by over 10%. Nevertheless, this was not of major concern because the main objective of sampling children aged 4-9 in the first place, as mentioned above, was to examine the pattern of HBV infection and provide a baseline measure of the prevalence of HBV infection amongst children aged 4-9, not to investigate associations by performing univariable and multivariable analyses.

This survey demonstrated an increase in the prevalence of anti-HBc and HBsAg positivity amongst children aged 4-9 with increasing age of the child. The prevalence of anti-HBc positivity was 3%, 2.6%, 3.5%, 5.2%, 8.5% and 5.7% amongst children aged 4, 5, 6, 7, 8 and 9 years, respectively (p=0.72), whereas, the prevalence of HBV chronic infection was 0.64%, 0.64%, 1.99%, 3.25%, 7.08%, and 5.02% amongst children aged 4, 5, 6, 7, 8 and 9 years, respectively (p=0.44). In the multivariable analysis there was a clear increasing OR of anti-HBc positivity and HBV chronic infection with increasing age of the child from 4 to 9 years, although these did not reach statistical significance. Failure of these associations to reach statistical significance does not mean that these associations are not true but only, as mentioned above, that the power of the study may have been insufficient to provide evidence of a statistically significant association. The increasing prevalence of anti-HBc positivity and HBV chronic infection with increasing age across the age-groups of children aged 4-9 suggests that these children are becoming infected with HBV through childhood transmission, regardless of the initial extent or magnitude of perinatal transmission of HBV infection amongst these children. This is supported by the higher prevalence of HBV chronic infection throughout childhood above the baseline prevalence of 1% HBV chronic infection amongst children aged 1-3

years, which is likely to be the result of mother-to-child transmission. Nevertheless, it has already been discussed that the low prevalence of HBV infection amongst children aged 1-3 and the low prevalence of HBeAg positive chronic carrier women of childbearing age provides evidence that mother-to-child transmission of HBV infection is not a major problem in Yemen.

Similar to findings with anti-HBc positivity amongst children aged 1-3 there was a significant decline in the prevalence of anti-HBc positivity amongst children aged 4-9 with increasing socioeconomic status (p=0.002) and amongst children aged 4-9 born from women claiming to be educated (1.55%) compared to uneducated (6.59%) mothers (p=0.02). The association between socioeconomic status and anti-HBc positivity amongst children aged 4-9 was also statistically significant in the multivariable analysis and showed a decline in anti-HBc positivity with increasing socioeconomic status. There were also differences in the prevalence of HBV chronic infection amongst children aged 4-9 years by mothers educational and socioeconomic status but these did not reach statistical significance, probably due to the small number of HBV chronic carrier children aged 4-9. There were no cases of HBV chronic infection amongst children of middle and upper socioeconomic status, and therefore, the socioeconomic status variable was not added to the multivariable analysis model of HBV chronic infection amongst children aged 4-9. An interesting association not found in the multivariable analysis of anti-HBc and HBsAg positivity amongst children aged 1-3 years was the statistically significant association of fathers educational status with anti-HBc and HBsAg positivity in the multivariable analysis of children aged 4-9. There was a higher adjusted OR of being anti-HBc positive [OR=4.1 (95% CI 1.05 to 15.96)] and HBV chronically infected [OR=23.1 (95% CI 1.99 to 269)] amongst children of uneducated fathers compared to children of fathers claiming to be educated. Although this was not consistent with findings amongst children aged 1-3 it confirms the significance of the association of educational status specifically and socioeconomic variables in general with HBV infection.

An unexplainable finding was the higher prevalence of HBV infection amongst children aged 4-9 living in urban areas and females. This was contrary to what was expected and found amongst children aged 1-3 participating in the survey. This may be due to the small number of anti-HBc positive and HBV chronic carrier children in the survey resulting in inaccurate prevalence estimates as demonstrated by their wide 95% confidence intervals. Not only did Sanaa, one of the two urban provinces in the survey, have a higher prevalence of anti-HBc positivity (8.74%) amongst children aged 4-9 than Aden (1.11%) (p=0.06), it also had the highest prevalence of anti-HBc positive and HBV chronic carrier children aged 4-9 compared to all the provinces in the survey. This might be the result of the sampling process of children aged 4-9 participating in the survey. It was initially planned to sample an equal number of children from all age-groups extending from 4-9 and five provinces in the survey, however, with children aged 4-9 being a secondary objective this was not strictly adhered to during the survey. Actually, the sample size of children aged 4-9 from Sanaa was greater than the sample size of children from the remaining provinces in the survey. Table 7.7.1 shows the governorate sampling distribution of children aged 4-9 participating in the survey. While this should have produced more accurate point estimates with narrower confidence intervals it may have also obtained more cases of HBV infection in Sanaa.

Table 7.7.1
Governorate sampling distribution of children aged 4-9 participating in the cross-sectional household sero-epidemiological survey

Province (Survey Domain)	Planned Sample Size	Actual Sample Size (%)
Sanaa	84	151 (32%)
Aden	84	66 (14%)
Taiz	84	90 (20%)
Shabwa	84	75 (16%)
Hodeidah	84	85 (18%)
Total	420	467 (100%)

It is expected that the prevalence estimates of anti-HBc positivity and HBV chronic infection amongst children aged 4-9 in Yemen participating in this survey are reliable estimates obtained from a representative sample of the general population. All children aged 4-9 participating in the survey were selected from within the same households of participants in the survey of children aged 1-3. Seventy-six percent (356/467) of the children aged 4-9 were older siblings of children aged 1-3 years participating in the survey. The remaining 24% were either relatives or were living in the same household. Considering all children participating in the survey of children aged 1-3 were randomly selected, which gave all children aged 1-3 and consequently all children aged 4-9 in the population an equal chance of being selected, this makes selection bias unlikely. A group of children aged 4-9 at risk of not being selected or included in the sample population are children that did not have a child aged 1-3 living in their household. However, there is no apparent reason such children differ from children who did have a child aged 1-3 living in their household. Another group of children aged 4-9 at risk of not being selected are children living away from home or those at school. Nevertheless, it is uncommon for young children aged 4-9 to live away from home or their parents, and considering that households were visited during the morning and afternoon this should give children attending schools an equal chance of being selected. Finally, a more general discussion of the possible effect and sources of bias or confounding follows in chapter 9.

Chapter 8: Results Vaccine Coverage and Effectiveness:

In this chapter the results of the prevalence, univariable analysis, and multivariable analysis of hepatitis B vaccine coverage as well as the effectiveness of hepatitis B vaccination in preventing HBV infection amongst children aged 1-3 participating in the survey are presented. It is important to note that in all subsequent comparisons of the prevalence of hepatitis B vaccination coverage, as well as factors associated with hepatitis B vaccination coverage investigated by univariable and multivariable analysis, the two comparison groups examined were hepatitis B vaccinated and unvaccinated children, with hepatitis B vaccinated children referring to all vaccinated children regardless of number of doses received. When analysing vaccine effectiveness the two comparison groups were completely vaccinated and unvaccinated children, whereas, all children of unknown vaccination status were excluded from the analysis.

Hepatitis B Vaccine Coverage:

8.1 Prevalence of hepatitis B vaccination coverage:

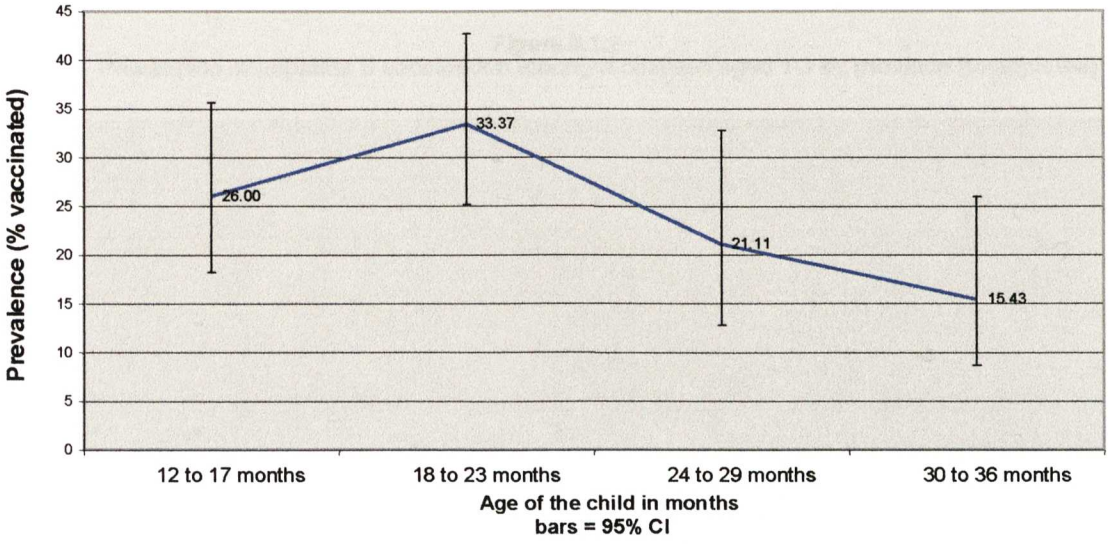
The overall prevalence of hepatitis B vaccine coverage, with at least one dose of hepatitis B vaccine, amongst children aged 1-3 participating in the survey was 21.1% (95% CI 15.81 to 27.58). The classification of the vaccination status of children aged 1-3 participating in the survey was carried out primarily according to what their vaccination card records showed their hepatitis B vaccination status to be, and/or secondarily according to the response of their parents to questions specifically asking about the vaccination status of their child. Children who had evidence of receiving three doses of hepatitis B vaccine were classified as completely vaccinated, children who had evidence of receiving one or two doses of hepatitis B vaccine were classified as partially vaccinated, and children who had vaccination records showing they did not receive any doses of hepatitis B vaccine or whose parents confidently confirmed that their child did not receive hepatitis B vaccine were classified as unvaccinated. Finally, children whose vaccination status was unknown by their parents or children who were reported to have received hepatitis B vaccine but did not have documentary evidence supporting this were classified as unknown vaccination status. Out of all the children aged 1-3 participating in the survey 8.63% (95% CI 5.1 to 14.24) were completely vaccinated with hepatitis B

vaccine, 12.47% (95% CI 9.72 to 15.85) were partially vaccinated with hepatitis B vaccine, 65.94% (95% CI 54.32 to 75.92) were unvaccinated with hepatitis B vaccine, and 12.96% (95% CI 8.09 to 20.11) were of unknown hepatitis B vaccination status. As for the number of doses of hepatitis B vaccine received by children aged 1-3 participating in the survey 8.63% (95% CI 5.1 to 14.24) received all three doses, 6.2% (95% CI 4.51 to 8.48) received two doses, 6.27% (95% CI 4.34 to 8.98) received one dose, 65.94% (95% CI 54.32 to 75.92) did not receive any doses, and 12.96% (95% CI 8.09 to 20.11) had an unknown dose status.

There was a statistically significant difference in the prevalence of hepatitis B vaccination coverage amongst children aged 1-3 by demographic, health care, and educational characteristics.

Demographic characteristics showing a significant difference in the prevalence of hepatitis B vaccination amongst children aged 1-3 were sex, age, province, area of residence, and socioeconomic status. The prevalence of hepatitis B vaccination was 26.28% amongst males aged 1-3 and 22.02% amongst females (p=0.04). There was a decline in hepatitis B vaccine coverage with increasing age of the child. The prevalence of hepatitis B vaccination was lowest amongst children aged 30 to 36 months (15.43%) and highest amongst children aged 18 to 23 months (33.37%) (p=0.0009). The prevalence of hepatitis B vaccination amongst children aged 1-2 (30.32%) was significantly higher than children aged 2-3 (17.85%) (p=0.0007). Figure 8.1.1 shows the prevalence of hepatitis B vaccination coverage amongst children aged 1-3 by age.

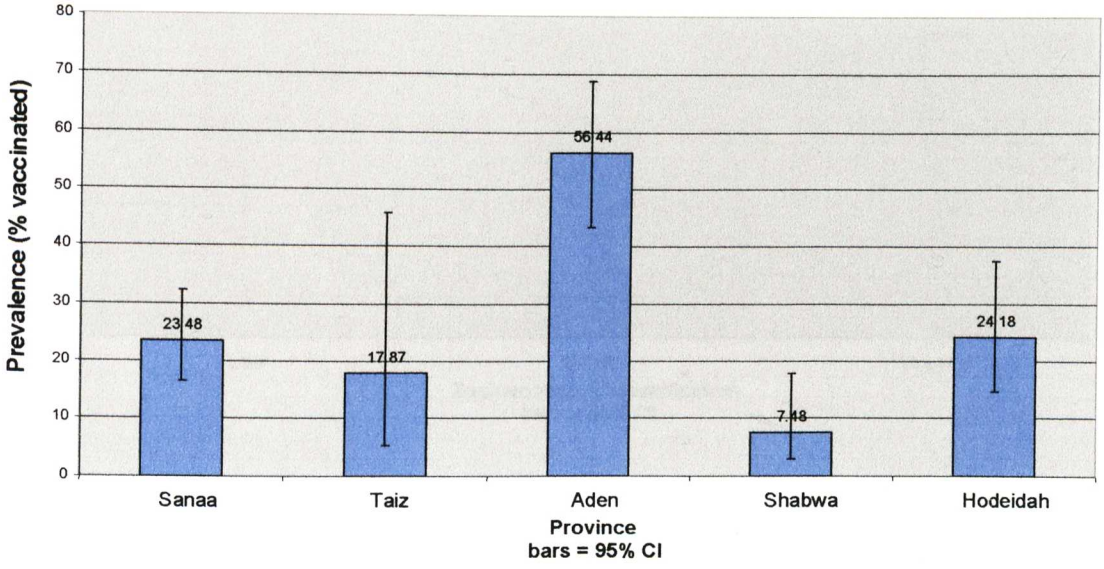
Figure 8.1.1
Prevalence of hepatitis B vaccination amongst children aged 1-3 by age (unadjusted)



There was a statistically significant difference in the prevalence of hepatitis B vaccine coverage amongst children aged 1-3 living in the five provinces of the survey (p=0.04). Hepatitis B vaccine coverage was highest in Aden (56.44%), one of the two urban provinces, and lowest in Shabwa (7.48%), one of the three rural provinces in the survey. There was also evidence of a statistically significant difference in the prevalence of hepatitis B vaccination between the two urban provinces in the survey. Hepatitis B vaccine coverage was 23.48% (95% CI 15.79 to 33.44) in Sanaa and 56.44 (95% CI 41.85 to 69.99) in Aden (p=0.002). On the other hand, there was no evidence of a statistically significant difference in the prevalence of hepatitis B vaccination between the three rural provinces in the survey (p=0.41). The prevalence of hepatitis B vaccine coverage amongst children aged 1-3 living in urban areas (35.33%) was higher than children living in rural areas (19.53%) and this was statistically significantly different (p=0.03). When the prevalence of hepatitis B vaccine coverage was compared by urban versus rural area of birth of child aged 1-3 the vaccination results were similar to those found according to area of residence of the child. The prevalence of hepatitis B vaccination was 36.93% (156/380) amongst children aged 1-3 born in urban areas and 18.96% (113/704) amongst children aged 1-3 born in rural areas, and this difference was

also statistically significant (p=0.009). Figure 8.1.2 shows the prevalence of hepatitis B vaccination coverage amongst children aged 1-3 by province unadjusted for age or sex.

Figure 8.1.2
Prevalence of hepatitis B vaccination amongst children aged 1-3 by province (unadjusted)



There was a statistically significant difference in the prevalence of hepatitis B vaccine coverage amongst children aged 1-3 between the three socioeconomic classes in the survey (p<0.0001). The prevalence of hepatitis B vaccination coverage was positively related to socioeconomic status. In other words, there was an increase in the prevalence of hepatitis B vaccination coverage with increasing socioeconomic status. Figure 8.1.3 shows the prevalence of hepatitis B vaccination coverage amongst children aged 1-3 according to socioeconomic status.

Figure 8.1.3
Hepatitis B vaccination amongst children aged 1-3 by socioeconomic status

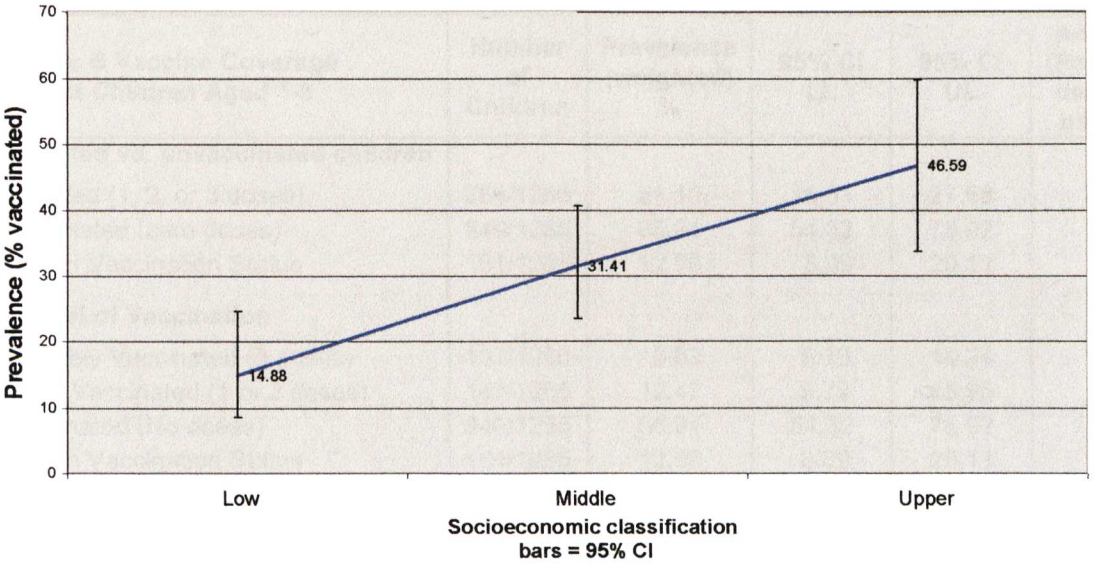


Table 8.1.1 shows the prevalence of hepatitis B vaccination coverage amongst children aged 1-3 by level of vaccination and number of doses according to demographic characteristics of children aged 1-3 participating in the survey.

Table 8.1.1
Hepatitis B vaccine coverage amongst children aged 1-3 by children's demographic characteristics

Hepatitis B Vaccine Coverage Amongst Children Aged 1-3	Number of Children	Prevalence (weighted) %	95% CI LL	95% CI UL	p-value (Pearson design-based)
Vaccinated vs. unvaccinated children					
Vaccinated (1, 2, or 3 doses)	284/1285	21.10	15.81	27.58	
Unvaccinated (zero doses)	840/1285	65.94	54.32	75.92	
Unknown Vaccination Status	161/1285	12.96	8.09	20.11	
By Level of Vaccination					
Completely Vaccinated (3 doses)	137/1285	8.63	5.10	14.24	
Partially Vaccinated (1 or 2 doses)	147/1285	12.47	9.72	15.85	
Unvaccinated (No doses)	840/1285	65.94	54.32	75.92	
Unknown Vaccination Status	161/1285	12.96	8.09	20.11	
By Number of Doses of Vaccine					
Completely Vaccinated (3 doses)	137/1285	8.63	5.10	14.24	
Partially Vaccinated (2 Doses)	81/1285	6.20	4.51	8.48	
Partially Vaccinated (1 Dose)	66/1285	6.27	4.34	8.98	
Unvaccinated (No doses)	840/1285	65.94	54.32	75.92	
Unknown Vaccination Status	161/1285	12.96	8.09	20.11	
Sex					
Male	155/570	26.28	19.01	35.12	
Female	129/554	22.02	14.76	31.53	0.04
Age-group (in months)					
12 to 17 months	67/241	26.00	18.20	35.69	
18 to 23 months	111/346	33.37	25.17	42.71	
24 to 29 months	54/230	21.11	12.79	32.79	
30 to 36 months	52/307	15.43	8.69	25.93	0.0009
Province					
Sanaa	48/207	23.48	16.42	32.40	
Taiz	39/215	17.87	5.32	45.75	
Aden	121/214	56.44	43.22	68.80	
Shabwa	19/251	7.48	2.94	17.73	
Hodeidah	57/237	24.18	14.50	37.48	0.04
Area					
Urban	169/421	35.33	28.29	43.07	
Rural	115/703	19.53	10.95	32.38	0.03
Socioeconomic Status					
Low	66/471	14.88	8.59	24.55	
Middle	127/439	31.41	23.47	40.60	
Upper	91/213	46.59	33.78	59.85	<0.0001

Health care characteristics showing a statistically significant difference in the prevalence of hepatitis B vaccination coverage amongst children aged 1-3 were qualifications of the person managing delivery of the child, setting of delivery, receiving antenatal care during pregnancy, type of delivery, and parental knowledge of hepatitis B vaccine.

The prevalence of hepatitis B vaccination amongst children whose delivery was managed by a medically qualified individual (36.65%) was twice as high as the prevalence of hepatitis B vaccination amongst children whose delivery was managed by an unqualified individual (17.52%) ($p<0.0001$). Similarly, the prevalence of hepatitis B vaccination amongst children delivered in a health setting (43.19%) was twice as high as the prevalence of hepatitis B vaccination amongst children delivered in a home setting (19.89%) ($p<0.0001$). A significantly higher percentage of children delivered by caesarean section (41.64%) were vaccinated than children delivered by normal delivery (23.9%) ($p=0.006$). Children whose mothers received antenatal care during their pregnancy (35.93%) had a higher prevalence of hepatitis B vaccination than children whose mothers did not (15.84%) ($p<0.0001$). Less than 1% of children of parents who did not know of hepatitis B vaccine were vaccinated compared to 32% of children of parents who knew of hepatitis B vaccine ($p<0.0001$). Table 8.1.2 shows the prevalence of hepatitis B vaccination coverage amongst children aged 1-3 by children's health care characteristics.

Table 8.1.2
Hepatitis B vaccination coverage amongst children aged 1-3 by children's health care characteristics

Hepatitis B Vaccination Coverage Amongst Children Aged 1-3	Number of Children	Prevalence (weighted) %	95% CI LL	95% CI UL	p-value (Pearson design-based)
Person Managing Delivery					
Medically Qualified Personnel	179/465	36.65	26.27	48.45	
Unqualified Personnel	103/638	17.52	11.67	25.44	<0.0001
Setting of Delivery					
Health Setting	123/277	43.19	33.54	53.38	
Home Setting	152/802	19.89	13.26	28.73	<0.0001
Type of Delivery					
Normal	270/1094	23.90	16.71	32.95	
Caesarian	14/30	41.64	31.60	52.41	0.006
Antenatal Care During Pregnancy					
Yes	192/506	35.93	26.32	46.82	
No	90/614	15.84	10.28	23.62	<0.0001
Parents Know of Vaccine					
Yes	283/819	32.14	23.78	41.82	
No	1/299	0.52	0.06	4.19	<0.0001

The prevalence of hepatitis B vaccination amongst children aged 1-3 born from women claiming to be educated (39.59%) was more than double the prevalence amongst children born from uneducated women (16.69%) (p=0.0001). Likewise, the prevalence of hepatitis B vaccination amongst children aged 1-3 whose fathers claimed to be educated (27.88%) was nearly double the prevalence of vaccination amongst children aged 1-3 of uneducated fathers (15.29%) (p=0.003). There was a statistically significant difference in the prevalence of hepatitis B vaccine coverage amongst children aged 1-3 by the level of mothers (p=0.0001) or fathers (p=0.003) education. The prevalence of hepatitis B vaccination amongst children aged 1-3 born to women or fathers claiming to have attended University/College or secondary school was higher than the prevalence of hepatitis B vaccination amongst children aged 1-3 born to women or fathers claiming to have completed primary school, which was higher than the prevalence of hepatitis B vaccination amongst children aged 1-3 born to women or fathers claiming they could

read and write. The prevalence of hepatitis B vaccination amongst children aged 1-3 born from women in paid employment (54.13%) was higher than the prevalence of hepatitis B vaccination amongst children aged 1-3 born from women who were not in paid employment (23.45%) (p=0.0004). Table 8.1.3 shows the prevalence of hepatitis B vaccine coverage amongst children aged 1-3 by mothers and fathers characteristics.

Table 8.1.3
Hepatitis B vaccination coverage amongst children aged 1-3 by mother's and father's characteristics

Hepatitis B Vaccination Coverage Amongst Children Aged 1-3	Number of Children	Prevalence (weighted) %	95% CI LL	95% CI UL	p-value (Pearson design-based)
Mothers Age					
15 to 24 years	91/341	25.55	18.39	34.33	
25 to 29 years	86/302	27.94	19.60	38.16	
30 to 34 years	51/241	19.74	12.09	30.56	
35 to 39 years	38/158	23.86	14.06	37.49	
40 to 49 years	18/82	18.29	9.54	32.20	0.16
Mothers Educational Status					
Reads and Writes	178/445	39.59	30.51	49.45	
Does not Read and Write	106/679	16.69	10.37	25.77	0.0001
Mothers Level of Education					
Reads and Writes	33/151	25.44	19.21	32.88	
Completed Primary School	79/180	39.24	26.86	53.18	
Completed Secondary School	53/91	61.17	48.11	72.80	
Completed University or College	13/23	61.68	44.66	76.24	0.0001
Mother's Employment					
Employed	23/41	54.13	38.70	68.80	
Unemployed	261/1083	23.45	16.29	32.53	0.0004
Father's Educational Status					
Reads and Writes	250/873	27.88	20.34	36.92	
Does not Read and Write	34/251	15.29	8.49	25.99	0.003
Father's Level of Education					
Reads and Writes	38/195	18.88	13.61	25.58	
Completed Primary School	80/295	27.00	18.55	37.53	
Completed Secondary School	75/238	32.16	21.39	45.23	
Completed University or College	57/145	35.78	21.11	53.71	0.05

8.2 Hepatitis B vaccination coverage univariable analysis:

The univariable analysis of the association of hepatitis B vaccination amongst children aged 1-3 participating in the survey with exposure variables produced results that were classified into three categories. The first of these had OR that were statistically significantly different from 1. The second category had notably increased or decreased OR, by more or less than 30%, but these were not statistically significant. The third category consisted of results that neither had notable increased or decreased OR nor were these significant. These results of the univariable analysis are presented in tables 8.2.1, 8.2.2 and 8.2.3.

The demographic and health care variables of children aged 1-3 (table 8.2.1) that had a statistically significant association with hepatitis B vaccination of children aged 1-3 in the univariable analysis were sex and age of child, province and area of residence, birth order of child, qualifications of the person managing delivery, setting and type of delivery, and having received antenatal care during pregnancy. Mother's and father's variables that had a statistically significant association with hepatitis B vaccination of children aged 1-3 (table 8.2.2) by univariable analysis were mothers education, mothers employment, fathers education, and parents knowledge of hepatitis B vaccine. Household characteristics and possessions that had a statistically significant association with hepatitis B vaccination of children aged 1-3 (table 8.2.3) by univariable analysis were type of household, material used in the construction of household, availability of an electricity supply, household source of water, socioeconomic status, and the ownership of a fridge/freezer, television, home telephone, mobile telephone, and radio.

Starting with demographic and health care variables females aged 1-3 were significantly less likely be vaccinated with hepatitis B vaccine [OR=0.79 (95% CI 0.64 to 0.98)] compared to males aged 1-3 participating in the survey. Children aged 12 to 17 (OR=1) and 18 to 23 months (OR=1.43) had a higher OR of receiving hepatitis B vaccine than children aged 24 to 29 (OR=0.76) and 30 to 36 months (OR=0.52) (p=0.0004). Province of residence was significantly associated with having received hepatitis B vaccine (p=0.001). Children aged 1-3 living in Aden (OR=4.22) were four times more likely to have received hepatitis B vaccine than children in Sanaa (p<0.001). Children living in

rural areas [OR=0.44 (95% CI 0.21 to 0.94)] had less than half the odds of being vaccinated compared to children living in urban areas. Child birth order was significantly associated with hepatitis B vaccination (p=0.02) and there was a decline in the odds of being vaccinated with increasing birth order of the child.

Children whose delivery was managed by an unqualified individual [OR=0.37 (95% CI 0.25 to 0.53)], children delivered in a home setting [OR=0.33 (95% CI 0.22 to 0.48)], or children whose mothers did not receive antenatal care during pregnancy [OR =0.34 (95% I 0.23 to 0.48)] had a statistically significantly lower odds of receiving hepatitis B vaccine. On the other hand, children delivered by caesarean section [OR=2.27 (95% CI 1.29 to 4)] had a higher odds of receiving hepatitis B vaccine than children delivered by normal vaginal delivery. Table 8.2.1 shows the results of the univariable analysis of the association of demographic and health care characteristics with hepatitis B vaccination coverage amongst children aged 1-3.

Table 8.2.1
Association of hepatitis B vaccination coverage amongst children aged 1-3 with demographic and health care characteristics

Variable		Number Controls	Number Cases	OR	p value	95% C.I.
Sex	Male	415	155	1		
	Female	425	129	0.79	**0.04**	0.64 – 0.98
Age of child (in months)	12 to 17	174	67	1		
	18 to 23	235	111	1.43	0.008	1.11 – 1.83
	24 to 29	176	54	0.76	0.32	0.43 – 1.34
	30 to 36	255	52	0.52	0.02	0.30 – 0.89
	Joint significance				***0.0004***	
	Linear trend OR			0.77	0.003	0.65 – 0.90
Province	Sanaa	159	48	1		
	Taiz	176	39	0.71	0.62	0.17 – 2.95
	Aden	93	121	4.22	<0.001	2.11 – 8.45
	Shabwa	232	19	0.26	0.02	0.09 – 0.77
	Hodeidah	180	57	1.04	0.92	0.48 – 2.25
	Joint significance				***0.001***	
Area	Urban	252	169	1		
	Rural	588	115	0.44	**0.04**	0.21 – 0.94
Child Birth Order	1	115	81	1		
	2	132	47	0.49	0.005	0.30 – 0.78
	3	122	46	0.60	0.06	0.35 – 1.02
	4	93	31	0.44	0.02	0.22 – 0.86
	5	94	23	0.39	0.002	0.23 – 0.67
	6 to 18	284	56	0.32	<0.001	0.18 – 0.56
	Joint significance				***0.02***	
	Linear Trend OR			0.82	0.001	0.74 – 0.91
Breastfed	Yes	830	274	1		
	No	9	10	2.49	0.17	0.66 – 9.33
Duration Breastfed	Up to 1 year	304	110	1		
	1 to 2 years	452	147	1.00	0.99	0.70 – 1.44
	More than 2 yrs	71	17	0.57	0.09	0.30 – 1.09
	Joint significance				*0.23*	
Person Managing Delivery	Qualified Medic	286	179	1		
	Unqualified	535	103	0.37	**<0.001**	0.25 – 0.53
Setting of Delivery	Health Setting	154	123	1		
	Home Setting	650	152	0.33	**<0.001**	0.22 – 0.48
Type of Delivery	Normal	824	270	1		
	Caesarian	16	14	2.27	**0.007**	1.29 – 4.00
Antenatal Care During Pregnancy	Yes	314	192	1		
	No	524	90	0.34	**<0.001**	0.23 – 0.48

With reference to mother's and father's characteristics, knowledge of hepatitis B vaccine by either parent of the child aged 1-3 was strongly associated with being vaccinated with

hepatitis B vaccine. Children whose parents did not know of hepatitis B vaccine [OR=0.01 (95% CI 0.001 to 0.08)] were extremely unlikely to be vaccinated with hepatitis B vaccine compared to children of parents who did know of the vaccine. Children aged 1-3 born to uneducated mothers [OR=0.31 (95% CI 0.18 to 0.51)] and uneducated fathers [OR=0.47 (95% CI 0.29 to 0.76)] experienced a 70% and 50% reduction, respectively, in the odds of being vaccinated with hepatitis B vaccine compared to children of mothers and fathers claiming to be educated. On the other hand, children of women in paid employment were nearly four times more likely [OR=3.85 (95% CI 1.88 to 7.88)] to be vaccinated with hepatitis B vaccine than children of women who were not employed. Table 8.2.2 shows the results of the univariable analysis of the association of mothers and fathers characteristics with hepatitis B vaccination coverage amongst children aged 1-3.

Table 8.2.2
Association of hepatitis B vaccination coverage amongst children aged 1-3 with mothers and fathers characteristics

Variable		Number Controls	Number Cases	OR	p value	95% C.I.
Mothers Age (in years)	15 to 24	250	91	1		
	25 to 29	216	86	1.13	0.40	0.84 – 1.52
	30 to 34	190	51	0.72	0.1	0.48 – 1.07
	35 to 39	120	38	0.91	0.71	0.55 – 1.51
	40 to 49	64	18	0.65	0.23	0.32 – 1.35
	Joint significance				*0.25*	
	Linear Trend OR			0.91	0.14	0.80 – 1.04
Mothers Education	Educated	267	178	1		
	Uneducated	573	106	0.31	**<0.001**	0.18 – 0.51
Mothers Birth Order	1	188	58	1		
	2	137	37	1.18	0.56	0.65 – 2.16
	3	126	52	1.57	0.12	0.89 – 2.77
	4	104	35	1.20	0.57	0.62 – 2.33
	5	86	23	0.88	0.57	0.56 – 1.39
	6 to 19	199	79	1.33	0.17	0.88 – 2.03
	Joint significance				*0.24*	
	Linear Trend OR			1.03	0.46	0.95 – 1.11
Mothers Employment	Unemployed	822	261	1		
	Employed	18	23	3.85	**0.001**	1.88 – 7.88
Fathers Education	Educated	623	250	1		
	Uneducated	217	34	0.47	**0.004**	0.29 – 0.76
Parents know of Vaccine	Yes	536	283	1		
	No	298	1	0.01	**<0.001**	0.001 –0.08

When examining socioeconomic variables, children of upper socioeconomic status [OR=4.99 (95% CI 2.71 to 9.19)] and middle socioeconomic status [OR=2.62 (95% CI 1.44 to 4.75), were 5 times and 2.5 times, respectively, more likely to receive hepatitis B vaccine than children of low socioeconomic status (p=0.0002). Children aged 1-3 living in households that did not have an electricity supply [OR=0.3 (95% CI 0.14 to 0.62)], fridge/freezer [OR=0.39 (95% CI 0.22 to 0.69)], radio [OR=0.43 (95% CI 0.27 to 0.68)], home telephone [OR=0.34 (95% CI 0.19 to 0.6)], or mobile telephone [OR=0.32 (95% CI 0.12 to 0.86)] in their household had a statistically significantly lower odds of receiving hepatitis B vaccine than children aged 1-3 who did have these commodities in their household. Children living in apartments had a higher odds [OR=2.76 (95% CI 1.59 to 4.77)] and children living in shacks had a lower odds [OR=0.29 (95% CI 0.18 to 0.45)] of receiving hepatitis B vaccine than children living in houses. Children living in households built of mud/clay [OR=0.38 (95% CI 0.13 to 1.14)] or mixed materials [OR=0.23 (95% CI 0.1 to 0.51)] had a lower odds of receiving hepatitis B vaccine than children living in households built of stone. Children living in households obtaining water through by a local project (OR=0.34), water truck (OR=0.37), or buckets (OR=0.23) had a statistically significantly lower odds of receiving hepatitis B vaccine than children obtaining water by a governmental water supply (p=0.0003). Table 8.2.3 shows the association of household characteristics and possessions with hepatitis B vaccination coverage amongst children aged 1-3.

Table 8.2.3
Association of hepatitis B vaccination coverage amongst children aged 1-3 with household characteristics and possessions

Variable		Number Controls	Number Cases	OR	p value	95% C.I.
Household Ownership	Owned	736	240	1		
	Rented	104	44	1.23	0.39	0.75 – 2.03
Household Type	House	644	184	1		
	Apartment	91	91	2.76	0.001	1.59 – 4.77
	Shack	104	9	0.29	<0.001	0.18 – 0.45
	Joint significance				***<0.0001***	
Household Construction	Stone	215	85	1		
	Block	346	170	1.16	0.71	0.51 – 2.59
	Mud or Clay	157	19	0.38	0.08	0.13 – 1.14
	Mixed	122	10	0.23	0.001	0.10 – 0.51
	Joint significance				***<0.0001***	
Crowding Index	1	60	18	1		
	2	197	75	1.26	0.38	0.74 – 2.12
	3	192	81	1.04	0.91	0.48 – 2.24
	4	153	48	0.79	0.48	0.41 – 1.55
	5	83	32	0.86	0.77	0.31 – 2.39
	6 to 18	155	30	0.45	0.04	0.21 – 0.96
	Joint significance				*0.12*	
	Linear Trend OR			0.82	0.03	0.69 – 0.98
Electricity Supply	Yes	526	238	1		
	No	314	46	0.30	**0.002**	0.14 – 0.62
Source of Water	Governmental	200	167	1		
	Local Project	343	60	0.34	0.005	0.17 – 0.70
	Water Truck	73	19	0.37	<0.001	0.23 – 0.60
	Buckets	223	38	0.23	0.001	0.10 – 0.49
	Joint significance				***0.0003***	
Freezer	Yes	321	177	1		
	No	519	107	0.39	**0.003**	0.22 – 0.69
Television	Yes	502	234	1		
	No	338	50	0.35	**0.006**	0.17 – 0.72
Radio	Yes	551	231	1		
	No	289	53	0.43	**0.001**	0.27 – 0.68
Home Phone	Yes	114	101	1		
	No	726	183	0.34	**0.001**	0.19 – 0.60
Mobile Phone	Yes	20	24	1		
	No	820	260	0.32	**0.03**	0.12 – 0.86
Car	Yes	191	71	1		
	No	649	213	0.75	0.14	0.51 – 1.11
Socioeconomic Status	Lower	405	66	1		
	Middle	312	127	2.62	0.003	1.44 – 4.75
	Upper	122	91	4.99	<0.001	2.71 – 9.19
	Joint significance				***0.0002***	

Variables that had notably increased or decreased OR, by more or less than 30%, but were not statistically significantly associated with hepatitis B vaccination amongst children were having breastfed the child aged 1-3 and duration of breastfeeding (table 8.2.1). Variables that neither showed notably increased or decreased OR nor evidence of a statistically significant association with hepatitis B vaccination amongst children aged 1-3 were age of the child's mother, birth order of the child's mother, household ownership, household crowding index and the ownership of a car.

8.3 Hepatitis B vaccination coverage multivariable analysis:

The independent variables that had a statistically significant association with hepatitis B vaccination coverage amongst children aged 1-3 in the multivariable analysis were age of the child, parents knowledge of hepatitis B vaccine, having received antenatal care during pregnancy, mothers educational status, and ownership of a radio.

The adjusted OR of hepatitis B vaccination amongst children aged 1-3 born from families that did not know of hepatitis B vaccine was 0.01 (95% CI 0.002 to 0.11) indicating that it is extremely unlikely for children born in families that do not know of hepatitis B vaccine to be vaccinated. Children born from women who did not receive antenatal care during their pregnancy with the child aged 1-3 participating in the survey had half the odds (OR= 0.5) of being vaccinated with hepatitis B vaccine after adjusting for the remaining variables in the model (p=0.001). Similarly, children born from uneducated women had half the odds (OR=0.55) of receiving hepatitis B vaccine compared to women claiming to be educated (p=0.02). The adjusted OR of hepatitis B vaccination was 0.67 amongst children living in households that did not have a radio indicating that children living in households without a radio had an approximately 30% lower likelihood of being vaccinated compared to children living in households that did have a radio (p=0.02). Age of the child was statistically significantly associated with hepatitis B vaccination after adjusting for the remaining significant variables in the model (p=0.02). There was a significant difference in the odds of receiving hepatitis B vaccine according to age. Children aged 12 to 17 months and 18 to 23 months had a higher odds of receiving hepatitis B vaccine compared to children aged 24 to 29 and 30 to 36 months (p=0.006). Female children (OR=0.81) and children living in rural areas (OR=0.91) aged 1-3 had a

lower odds of receiving hepatitis B vaccine but sex and area of residence did not show evidence of a statistically significant association with hepatitis B vaccination amongst children aged 1-3, but because these were *a priori* explanatory variables these were added to the model at the end of the process. There was no evidence of interaction between the independent variables in the model. Table 8.3.1 shows the results of the multivariable analysis of the association of the independent variables with hepatitis B vaccination coverage amongst children aged 1-3.

Table 8.3.1
Adjusted logistic regression of the association of hepatitis B vaccination amongst children aged 1-3 with independent exposure variables

Variable		OR*	p value**	95% C.I.***
Age of Child (in months)	12 to 17	1		
	18 to 23	1.51	0.06	0.98 – 2.32
	24 to 29	0.79	0.5	0.38 – 1.62
	30 to 36	0.51	0.02	0.29 – 0.90
	Joint significance		***0.02***	
Sex	Male	1		
	Female	0.81	0.09	0.63 – 1.04
Area	Urban	1		
	Rural	0.91	0.81	0.42 – 1.99
Parents know of hepatitis B vaccine	Yes	1		
	No	0.01	**<0.001**	0.002 – 0.11
Antenatal care during pregnancy	Yes	1		
	No	0.5	**0.001**	0.34 – 0.74
Mothers Educational Status	Educated	1		
	Uneducated	0.55	**0.02**	0.34 – 0.89
Radio Ownership	Yes	1		
	No	0.67	**0.02**	0.48 – 0.94

* adjusted OR computed by survey logistic regression multivariable analysis.
** p-value of the adjusted wald test statistic.
*** 95% Confidence Intervals.
**** p-value of the adjusted survey wald test statistic of the significance of the joint effect of the categories of the independent variable on the dependent variable.

The multivariable analysis was repeated excluding all the socioeconomic variables that contributed to the socioeconomic index constructed in the survey and substituting them with the single socioeconomic status variable. The OR and p-values of the age of the child, sex, and area of residence associated with hepatitis B vaccination were extremely similar in this model in table 8.3.1 ending up with mothers educational status and radio

ownership, expect for the p-value of sex, which changed from 0.09 to 0.05. Socioeconomic status, on the other hand, was significantly associated with hepatitis B vaccine coverage, and there was a clear increased OR of hepatitis B vaccination associated with increasing socioeconomic status. Table 8.3.2 shows the results of the multivariable analysis of the association of independent variables and socioeconomic status with hepatitis B vaccination coverage amongst children aged 1-3.

Table 8.3.2
Adjusted logistic regression of association of hepatitis B vaccination amongst children aged 1-3 with exposure variables including socioeconomic status

Variable		OR*	p value**	95% C.I.***
Age of Child (in months)	12 to 17	1		
	18 to 23	1.50	0.05	0.98 – 2.32
	24 to 29	0.78	0.47	0.38 – 1.62
	30 to 36	0.50	0.02	0.29 – 0.90
	Joint significance		***0.01***	
Sex	Male	1		
	Female	0.78	0.05	0.61 – 1.00
Area	Urban	1		
	Rural	0.94	0.87	0.43 – 2.04
Parents know of hepatitis B vaccine	Yes	1		
	No	0.01	**<0.001**	0.001 – 0.11
Antenatal care during pregnancy	Yes	1		
	No	0.5	0.001	0.34 – 0.71
Socioeconomic Status	Low	1		
	Middle	1.72	0.07	0.95 to 3.09
	Upper	2.53	0.004	1.39 to 4.59
	Joint significance		***0.02***	

* adjusted OR computed by survey logistic regression multivariable analysis.
** p-value of the adjusted wald test statistic.
*** 95% Confidence Intervals.
**** p-value of the adjusted survey wald test statistic of the significance of the joint effect of the categories of the independent variable on the dependent variable.

Hepatitis B Vaccine Effectiveness:

8.4 Hepatitis B vaccine immunogenicity:

The immunogenicity of hepatitis B vaccine amongst children aged 1-3 participating in the survey was measured in terms of the ability of hepatitis B vaccine to stimulate an immune response amongst vaccinated children resulting in hepatitis B surface antibody (anti-HBs) concentrations greater than 10 milli-international units per litre (MiU/L). Children with anti-HBs concentrations > 10 MiU/L were classified as anti-HBs positive and were considered protected against HBV infection.

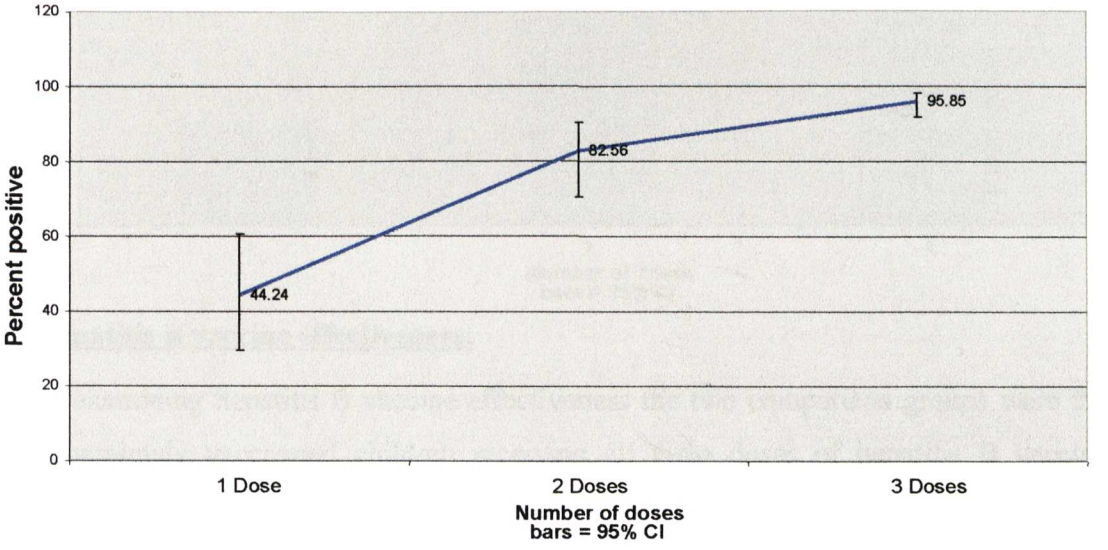
Out of 1285 children aged 1-3 participating in the survey, 284 children received one, two or three doses of hepatitis B vaccine, as discussed in section 8.1. Six out of these 284 children were excluded from the analysis of immunogenicity. One of these 6 children had insufficient serum samples to test for anti-HBs. The remaining five children excluded from the analysis were anti-HBc positive indicating that they were exposed to HBV, and were excluded with the intention of removing the effect of HBV infection in inducing natural immunity amongst these 5 children (because it was not known if they responded to vaccination). The final analysis of the immunogenicity of hepatitis B vaccine included 278 vaccinated children aged 1-3. Overall, 77.03% (95% CI 67.16 to 84.62) of vaccinated children (completely and partially) were anti-HBs positive. However, this varied significantly according to the number of doses of hepatitis B vaccine given to the child. Table 8.4.1 shows vaccine induced immunity amongst children aged 1-3 according to number of doses of hepatitis B vaccine.

Table 8.4.1
Vaccine induced immunity amongst children aged 1-3
by number of doses of hepatitis B vaccine

Number of Doses of Hepatitis B Vaccine	% anti-HBs Positive (anti-HBs > 10 miU/L) Weighted	Number	95% CI LL	95% CI UL	p-value
1 Dose	44.24	29/63	29.53	60.64	
2 Doses	82.56	66/80	70.39	90.41	
3 Doses	95.85	130/135	91.62	97.99	<0.0001

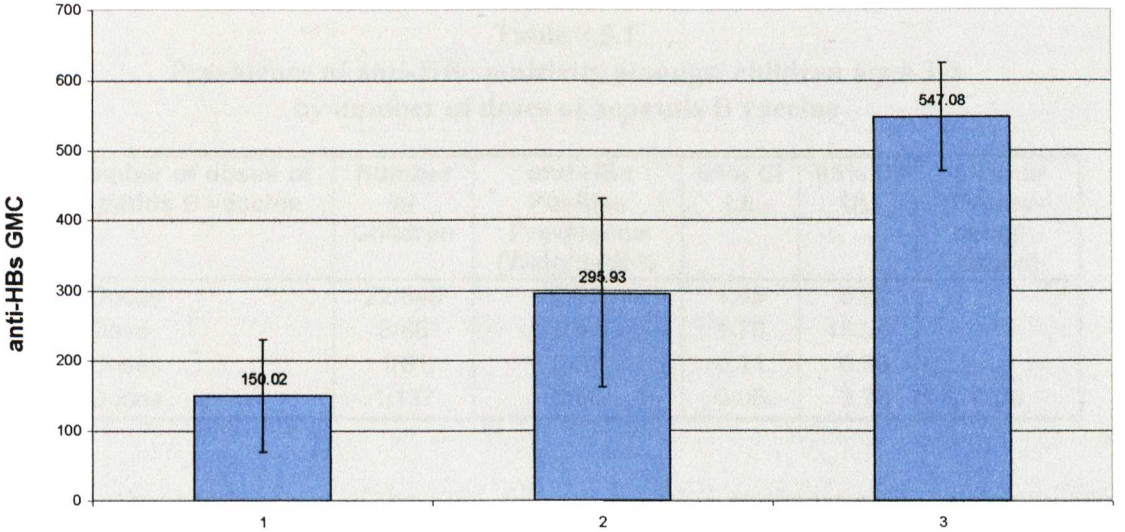
There was a clear increase in the proportion of anti-HBs positive children with increasing number of doses of hepatitis B vaccine received and this difference was statistically significant ($p<0.0001$). Figure 8.4.1 shows the increase in anti-HBs positivity amongst vaccinated children aged 1-3 by number of doses of hepatitis B vaccine received.

Figure 8.4.1
Anti-HBs positivity (> 10 MiU/L) amongst children aged 1-3 by number of doses of hepatitis B vaccine



Similar to the increasing proportion of anti-HBs positive children with an increase in the number of doses of hepatitis B vaccine, there was also an increase in the anti-HBs geometric mean concentration with an increase in number of doses hepatitis B vaccine received by children aged 1-3 participating in the survey. Figure 8.4.2 shows the geometric mean concentration of anti-HBs amongst children aged 1-3 by number of doses of hepatitis B vaccine.

Figure 8.4.2
Anti-HBs Geometric Mean Concentration (GMC) amongst children aged 1-3 (anti-HBc-/anti-HBs+) by number of doses of hepatitis B vaccine



8.5 Hepatitis B vaccine effectiveness:

When examining hepatitis B vaccine effectiveness the two comparison groups were the 137 completely vaccinated children receiving all three doses of hepatitis B vaccine regardless of vaccine schedule and dose intervals, and the 840 unvaccinated children aged 1-3 participating in the survey. Two aspects of vaccine effectiveness were examined, one was the effectiveness of hepatitis B vaccine in preventing anti-HBc positivity and the other was the effectiveness of hepatitis B vaccine in preventing HBV chronic infection.

Overall, 27 of the 1285 children aged 1-3 participating in the survey were anti-HBc positive. 81.6% (22/27) of these anti-HBc positive children were unvaccinated with hepatitis B vaccine, 14.3% (3/27) received one dose, 2.4% (1/27) received two doses, and 1.8% (1/27) received all three doses of hepatitis B vaccine.

The prevalence of anti-HBc positivity was 2.95% (95% CI 1.45 to 5.92) amongst children unvaccinated with hepatitis B vaccine compared to 0.5% (95% CI 0.06 to 3.78) amongst children completely vaccinated with hepatitis B vaccine (p=0.06). There was a decline in the prevalence of anti-HBc positivity according to the number of doses of hepatitis B vaccine given to children aged 1-3 participating in the survey. Table 8.5.1 shows the

prevalence of anti-HBc positivity amongst children aged 1-3 according to the number of doses of hepatitis B vaccine received by children aged 1-3 participating in the survey.

Table 8.5.1
Prevalence of anti-HBc positivity amongst children aged 1-3 by number of doses of hepatitis B vaccine

Number of doses of hepatitis B vaccine	Number of Children	anti-HBc Positive Prevalence (Weighted) %	95% CI LL	95% CI UL	p-value (Pearson design-based)
0 Doses	22/840	2.95	1.45	5.92	
1 Dose	3/66	5.42	1.70	15.98	
2 Doses	1/81	0.91	0.11	6.96	
3 Doses	1/137	0.50	0.06	3.78	0.06

On the other hand, 11 out of the 1285 children aged 1-3 participating in the survey were HBV chronic carriers. All of these HBV chronic carrier children were unvaccinated with hepatitis B vaccine and there were no HBV chronic carriers amongst children receiving one, two or three doses of hepatitis B vaccine. The prevalence of HBV chronic infection was 1.5% (95% CI 0.53 to 4.12) amongst children unvaccinated with hepatitis B vaccine. Table 8.5.2 shows the prevalence of HBV chronic infection amongst children aged 1-3 according to the number of doses of hepatitis B vaccine received by children aged 1-3 participating in the survey.

Table 8.5.2
Prevalence of HBV chronic infection amongst children aged 1-3 by number of doses of hepatitis B vaccine

Number of doses of hepatitis B vaccine	Number of Children	HBV Chronic Infection Prevalence (Weighted) %	95% CI LL	95% CI UL	p-value (Pearson design-based)
0 Doses	11/840	1.5	0.53	4.16	
1 Dose	0/66	0			
2 Doses	0/81	0			
3 Doses	0/137	0			0.69

The OR of anti-HBc positivity amongst children aged 1-3 completely vaccinated with hepatitis B vaccine compared to unvaccinated children was 0.17 (95% CI 0.03 to 1.09).

This OR suggests an 80% reduction in anti-HBc positivity amongst children receiving all three doses of hepatitis B vaccine (p=0.06). There were no cases of HBV chronic infection amongst children completely vaccinated with hepatitis B vaccine. Consequently the OR of HBV chronic infection amongst children aged 1-3 completely vaccinated with hepatitis B vaccine compared to unvaccinated was 0. Vaccine effectiveness (VE) is computed as 1 – relative risk. It is well established that in rare diseases the risk ratio approximates the odds ratio and rate ratio. HBV infection amongst children aged 1-3 in Yemen was clearly found to be a rare disease, therefore, vaccine effectiveness was computed as VE = 1 – OR. Accordingly, hepatitis B vaccine had a vaccine effectiveness of 83% (95% CI -9 to 97) in preventing anti-HBc positivity and a vaccine effectiveness of 100% (95% CI 97% lower limit) in preventing HBV chronic infection amongst completely vaccinated children aged 1-3 participating in the survey. Table 8.5.3 shows hepatitis B vaccine effectiveness in preventing anti-HBc positivity and HBV chronic infection amongst completely vaccinated children aged 1-3 participating in the survey.

Table 8.5.3
Hepatitis B vaccine effectiveness in preventing HBV infection amongst completely vaccinated children aged 1-3

Completely vaccinated versus unvaccinated children aged 1-3	OR (95% CI)	p-value (Pearson design-based)	Vaccine Effectiveness (95% CI)
Anti-HBc positivity	0.17 (0.03 to 1.09)	0.06	83% (-9 to 97%)
HBV Chronic Infection	0 (0.03 Upper Limit)	0.50	100% (97% Lower Limit)

8.6 Discussion:

Overall, the percentage of children aged 1-3 participating in this survey receiving at least one dose of hepatitis B vaccine in Yemen was 21.1% (95% CI 15.81 to 27.58). Out of all children aged 1-3 participating in the survey 8.63% (95% CI 5.1 to 14.24) were completely vaccinated (received 3 doses), 12.47% (95% CI 9.72 to 15.85) were partially vaccinated (received 1 or 2 doses), and 65.94% (95% CI 54.32 to 75.92) did not receive any dose of hepatitis B vaccine. The percentage of children aged 1-3 with unknown hepatitis B vaccination status was 12.96% (95% CI 8.09 to 20.11) and the potential effect

of this proportion of children on the hepatitis B vaccine coverage prevalence estimates of this survey will be discussed later in this section.

These are the first independent estimates of hepatitis B vaccine coverage in Yemen. The prevalence estimates of this survey show hepatitis B vaccine coverage to be much lower than initially expected and much lower than vaccine coverage with the remaining routine EPI vaccines in Yemen. These estimates of hepatitis B vaccine coverage are also much lower than hepatitis B vaccine coverage in the majority of Middle-Eastern countries which are presented in Chapter 1.

The EPI reports that vaccine coverage with BCG, DPT (3 doses), OPV (3 doses), measles and hepatitis B vaccine (3 doses) was 78.2%, 72.8%, 72.8%, 71.4% and 14.8%, respectively, by one year of age (MOPHP(B), 2001). Hepatitis B vaccine coverage with three doses of hepatitis B vaccine was reported to be 9% in 1999 (MOPHP(A), 2001) which when compared to vaccine coverage in 2001 indicates that the proportion of children completely vaccinated with hepatitis B vaccine is increasing. The EPI in Yemen reports that 42% of children under the age of 1 year receive at least one dose of hepatitis B vaccine but this figure has been occasionally incorrectly reported as the prevalence of hepatitis B vaccine coverage with all three doses of hepatitis B vaccine by the age of 1 year.

The estimates of hepatitis B vaccine coverage amongst children aged 1-3 obtained from this survey are expected to be more reliable than those reported by the EPI at the MOPHP in Yemen. It is unknown how the EPI obtained its estimate of hepatitis B vaccine coverage but being responsible for the vaccination programme in Yemen the EPI may have provided biased high vaccine coverage estimates. This survey, on the other hand, is independent of the EPI and MOPHP, and there is no motive or reason for study investigators to underestimate or overestimate hepatitis B vaccine coverage. It is noteworthy to mention that the reported high coverage with BCG vaccine should be encouraging had it been proven that a birth dose of hepatitis B vaccine was necessary

These results of low hepatitis B vaccine coverage are not encouraging, especially when 78% of parents claimed to have knowledge of hepatitis B vaccine and 32% of parents said their child received hepatitis B vaccine. Nevertheless, this survey did not investigate the attitude and practice of parents toward hepatitis B vaccination and it is unknown if

parents of unvaccinated children were willing but unable to have their child vaccinated or if they refused to do so.

The only independent variables that remained statistically significantly associated with hepatitis B vaccine coverage amongst children aged 1-3 in the multivariable analysis after adjusting for the remaining variables in the model were age of the child, receiving antenatal care during pregnancy, parents knowledge of hepatitis B vaccine, mothers educational status, and the ownership of a radio. All of these variables, except for age of the child, are related to health awareness, health attitudes and practices, and socioeconomic status.

The OR of receiving hepatitis B vaccine was 0.5 amongst children aged 2-3 compared to children aged 1-2 (p=0.001), indicating that children aged 2-3 had half the odds of being vaccinated with hepatitis B vaccine compared to children aged 1-2. The higher prevalence of hepatitis B vaccine coverage amongst children aged 1-2 (30.32%) compared to children aged 2-3 (17.85%) (p=0.0007) is probably because the hepatitis B vaccination programme was gradually introduced (phased introduction) starting with central health centres in 1998 targeting children less than 1 year old, and then extended to peripheral health centres and units. Initially children older than 1 year could pay for hepatitis B vaccine but this was discontinued later due to legal constraints on selling childhood vaccines by the rolling fund established by the MOPHP for this purpose. This meant that younger cohorts of children at the time the survey was carried out in 2001 had a better chance of receiving the vaccine and consequently higher hepatitis B vaccine coverage.

Knowledge of hepatitis B vaccine by either parent had the most significant association with hepatitis B vaccine coverage [OR=0.01 (95% CI 0.002 to 0.11)] and it was extremely unlikely for children born to parents who had no knowledge of hepatitis B vaccine to be vaccinated. Nevertheless, it is not known for sure if being vaccinated is the result of knowledge of hepatitis B vaccine or vice versa (reverse causality). Actually, having a child vaccinated with hepatitis B vaccine may have resulted in the parents becoming more knowledgeable about hepatitis B vaccine. In this survey the parents of only 1 child out of 284 children receiving hepatitis B vaccine did not know of it.

However, due to the cross-sectional nature of this survey it is difficult to establish a causal link between parents knowledge and receiving hepatitis B vaccine.

There was a 50% reduction in hepatitis B vaccine coverage amongst children aged 1-3 born from uneducated mothers [adjusted OR=0.55 (95% CI 0.34 to 0.89)] compared to educated mothers. The risk in investigating the association between mother's educational status as an exposure and hepatitis B vaccine coverage as an outcome is the potential for misclassification of mother's educational status. Mother's educational status was recorded when completing the interviewer administered questionnaire and later in the database according to the response of the person interviewed. 63% of mothers participating in this survey were uneducated and the remaining 37% claiming to be educated did not show the study investigators evidence of their educational status. Moreover, 75% of women claiming to be educated could only read or write or had completed primary school at the best. It may have been difficult for mothers to recall precisely the number of years spent in education and this was not verified because they were not requested to show study investigators evidence of educational attainment. As a result mothers may have underestimated or overestimated their educational status with study investigators unable to validate their claims to educational status and attainment. This is of concern because educational differences are commonly reported to be associated with low vaccination coverage, and therefore it is important to obtain information on educational status as precisely and accurately as possible. There was also a lower OR [adjusted OR=0.67 (95% CI 0.48 – 0.94)] of receiving hepatitis B vaccine amongst children living in households that did not have a radio compared to households that had a radio. The association of radio ownership and mothers educational status with hepatitis B vaccine coverage is most probably explained by these being indicators of socioeconomic status. In the second multivariable model (table 8.2.2), where radio ownership, mothers educational status, and all other socioeconomic variables were substituted with socioeconomic status, this variable was significantly associated with hepatitis B vaccine coverage (p=0.02) and showed an increased odds of vaccine coverage with increasing socioeconomic status.

The odds of hepatitis B vaccine coverage was significantly lower amongst children aged 1-3 of mothers who did not receive antenatal care during their pregnancy [adjusted

OR=0.5 (95% CI 0.34 to 0.74)] compared to mothers who received antenatal care during their pregnancy. There was also lower prevalence of hepatitis B vaccine coverage according to availability of health care factors. Children receiving better health care as suggested by delivery managed by a qualified medical individual, a hospital-based delivery, or caesarean section (which was most likely to be done by a qualified individual in a hospital setting) had approximately twice as high hepatitis B vaccine coverage compared to children who did not receive this health care. Parents of children delivered by medically qualified personnel, in health settings, or receiving antenatal care, are likely to be of a higher educational status, socioeconomic status, and have better health awareness or access to health services. Such parents come into close contact with health care professionals allowing them to receive better health education and whether on their own or according to medical personnel's recommendations are more likely to subsequently visit health centres for postnatal checkups and to vaccinate their child and follow up on their children's vaccination schedule.

Hepatitis B vaccine coverage was not the same in the provinces and areas of residence in the survey. Children living in rural areas had a 50% lower hepatitis B vaccine coverage (unadjusted OR= 0.44) than children living in urban areas (p=0.04). The higher vaccine coverage amongst children aged 1-3 living in urban areas compared to children living in rural areas suggests better availability of hepatitis B vaccine and accessibility to health services and health care in urban areas, which is in accordance with the phased introduction of hepatitis B vaccination starting with central health centres. Nevertheless, when the two urban provinces, Sanaa and Aden, were compared, children living in Aden were four times more likely (OR=4.22) to receive hepatitis B vaccine than children living in Sanaa (p=0.001), and children aged 1-3 in Aden (56.44%) had more than double the hepatitis B vaccine coverage than children in Sanaa (23.48%) (p=0.002). This cannot be explained by health service factors alone such as better availability of vaccine or accessibility to health services, but is likely to be influenced by individual factors such as socioeconomic status, higher educational status of mother and father, better attitude and practice by parents, and better compliance with health care advice. An important finding supporting this was the significant increase in the prevalence of hepatitis B vaccine coverage with increasing socioeconomic status (p<0.0001). In accordance, there was a

significantly higher proportion of study participants of upper socioeconomic status in Aden than Sanaa (p=0.01). In Aden, 91% of mothers claimed to be educated compared to 49% of mothers in Sanaa (p<0.0001) and 98% of fathers in Aden claimed to be educated compared to 81% of fathers in Sanaa (p<0.0008). It is noteworthy to mention that female children appeared to have lower hepatitis B vaccination coverage (22%) compared to male children (26%) (p=0.04). The adjusted OR of being vaccinated amongst females aged 1-3 was 0.81 (95% CI 0.63 to 1.04) indicating a 20% reduction in hepatitis B vaccine coverage amongst females compared to males. This did not reach statistical significance but may suggest a bias towards vaccinating males or a bias towards taking more males to health centres than females increasing their chance of being vaccinated.

Hepatitis B vaccine was highly immunogenic and there was an increase in anti-HBs positivity and anti-HBs geometric mean concentrations with an increase in the number of doses of hepatitis B vaccine received. The study investigators did not ask every study participant about the site hepatitis B vaccine was administered on the body but it was assumed that all the children received the vaccine on the antero-lateral aspect of the thigh or biceps muscle. The vaccine effectiveness estimates lead to the conclusion that hepatitis B vaccination was highly effective in preventing anti-HBc positivity and HBV chronic infection amongst completely vaccinated children aged 1-3 participating in the survey. Only one of the completely vaccinated children became anti-HBc positive [0.5% (95% CI 0.06 to 3.78)] and the OR of becoming anti-HBc positive amongst completely vaccinated children was 0.17 (95% CI 0.03 to 1.09) compared to unvaccinated children, which suggests a highly significant reduction (80%) in anti-HBc positivity indicating that hepatitis B vaccine is highly effective in preventing HBV infection [VE=83% (95% CI -9% to 97%)] even though this barely reached statistical significance (p=0.06). There were no cases of HBV chronic infection amongst completely vaccinated children which is suggestive of a vaccine effectiveness of 100%, given the assumption that this protection was the result of hepatitis B vaccination and that this was not the result of the small number of HBV chronically infected children found in the survey. This was not demonstrated in the multivariable case-control analysis of HBV chronic infection amongst children aged 1-3 because none of the HBV chornic carrier children received

hepatitis B vaccine, and consequently no odds ratio could be computed to compare them with the controls. Nevertheless, the high vaccine immunogencitiy and the increasing anti-HBc GMC with increasing number of doses received is consistent with previous knowledge and reassuring of hepatitis B vaccine effectiveness. This survey did not address the time interval been hepatitis B vaccine doses or adherence to the EPI routine vaccination schedule and its effect on hepatitis B vaccine effectiveness. Actually, vaccine effectiveness may have been higher than what this survey found if the vaccine schedule had been strictly adhered to. This survey also did not investigate causes of delayed or incompletely vaccinated children such as hepatitis B vaccine availability and accessibility to health services.

An important factor that may have biased the estimate of hepatitis B vaccine coverage in this survey is the number of children aged 1-3 participating in the survey with no vaccine information available, especially when vaccine information was related to the independent variable being examined, which was hepatitis B vaccine coverage. Out of all the children aged 1-3 participating in the survey 12.96% (161/1285) were of unknown hepatitis B vaccination status. These were children of parents who did not know if their child participating in the survey received hepatitis B vaccine and did not have a vaccination card, or children of parents who said that their child did receive hepatitis B vaccine but did not have written documentation to show the study investigators to confirm this. To avoid misclassification of hepatitis B vaccination status, children reported to have an unknown hepatitis B vaccination status were left as a separate category in the prevalence estimates presented in section 8.1 of this chapter, and were excluded from the univariable and multivariable analysis of hepatitis B vaccine coverage. If, in the unlikely event, a large proportion of these children are hepatitis B vaccinated, this may result in the survey underestimating hepatitis B vaccine coverage. If on the other hand, most of these children are unvaccinated against HBV infection this may result in the survey overestimating hepatitis B vaccine coverage.

Other potential sources of error in the survey estimates of hepatitis B vaccine coverage may be mistakes in vaccination cards or in parent's memory and response to their child's hepatitis B vaccination status. At the time of conducting the survey attempts were made

to keep these errors minimal by combining all children with and unknown or unconfirmed vaccination status in a separate category (children of unknown vaccination status) and excluding such children from the univariable and multivariable analysis, and the analysis of hepatitis B vaccination coverage and effectiveness. In order to assess the extent of mistakes occurring in vaccination cards, on some occasions, these were verified by referring to the vaccinating health centre. In a few districts in the survey the vaccination status of children participating in the survey with doubtful vaccination cards was checked and compared to their vaccination records in their vaccinating health centres. The finding of this exercise were reassuring. Recall and interviewer bias was unlikely considering study participants and investigators were blind regarding the child's HBV infection status at the time of completing the interviewer administered questionnaire. Moreover, the consistency in the immunologic response (anti-HBs positivity) amongst vaccinated children according to the number of doses of hepatitis B vaccine received is highly reassuring with regard to the correct classification of study participants according to number of doses of vaccine received as well as serological assay predictability.

Given the hepatitis B vaccination coverage estimates of this survey it is concluded that hepatitis B vaccine coverage in Yemen is low. A few factors have been found to be significantly associated with hepatitis B vaccine coverage and it is important that these factors are taken into consideration by the Yemeni MOPHP in its necessary attempt to increase vaccination coverage. Firstly, the MOPHP must acknowledge and voice that the low educational status of women is an obstacle hindering high hepatitis B vaccine coverage and emphasise the importance of improving the educational status of women in Yemen if vaccine coverage is to be increased. This is an extremely difficult task which is not directly under the control and influence of the MOPHP, and requires political commitment and multi-sectoral governmental collaboration. Secondly, health education programmes and messages must have two main objectives; increasing awareness about vaccines generally and hepatitis B vaccine specifically, and increasing antenatal visits and the proportion of women attending for antenatal care during pregnancy (currently 45%), with an emphasis on rural areas, and targeting these women with the appropriate

health care and education programmes ensuring that they understand the importance of hepatitis B vaccination so that they return after delivery to vaccinate their children. Thirdly, when designing any health education programmes or messages the educational status and attainment of the target population must be taken into consideration in order to design messages appropriate to the target population. Additionally, health education programmes must utilise the appropriate method, which in this case is radio broadcasting, to convey its message to the target population. There was 33% lower hepatitis B vaccine coverage in households that did not have a radio [adjusted OR=0.67 (95% CI 0.48 to 0.94)]. With over 65% of all households and 60% of rural households possessing a radio in Yemen, this is a highly attractive way of conveying messages to the population which is dispersed over 65,000 rural villages and hamlets. Finally, all of this will not have a sustainable impact unless the MOPHP ensures a continuous supply of hepatitis B vaccine in all its health centres. Availability and accessibility to health centres with an equitable distribution for the general population is essential. This survey was not conducted with the intention of analysing health care infrastructure, provision, or accessibility to health services in Yemen nor is there an intention to claim that it did. With regard to the target population for hepatitis B vaccination, the MOPHP must reconsider its objective of vaccinating children under 1 year because by not offering the vaccine to older children the MOPHP is missing out on the opportunity of vaccinating children who did not visit health centres when they were less than 1 year old but did visit later in childhood. This will have financial implications but the feasibility of this should be investigated.

Recently, the MOPHP decided to introduce a new pentavalent vaccine to its routine immunisation programme which will result in changing the current hepatitis B vaccination schedule. Considering the pentavalent vaccine will reduce the number of injections given to a child during each visit, this new vaccine is likely to increase parental acceptance and uptake and consequently vaccine coverage. This will depend on the availability of a sustainable supply of vaccines, minimal side effects of the vaccine, and no widely publicised vaccine scares, such as what happened previously with hepatitis B vaccine. If the introduction of the pentavalent vaccine is successful, due to health service and population factors, this is likely to increase hepatitis B vaccine and haemophilus

influenzae vaccine coverage to the same level of vaccine coverage as DPT, if not increasing overall vaccine coverage to levels better than current levels. If, on the other hand, introduction is not successful this may compromise the existing vaccines provided in the routine EPI. This indicates the importance of careful planning, preparation and campaigning for the introduction of the pentavalent vaccine to the EPI in Yemen. Currently, there is already a delay in introducing the vaccine because there is a delay in provision of the pentavalent vaccine by the manufacturer until 2004.

Chapter 9: Discussion and Recommendations:

This chapter starts with a discussion of the methodological issues in the study. This is followed by a discussion of the most important research findings of this survey, their interpretation and implications. A summary of the research findings is presented, fulfilling the research objectives. Finally a number of recommendations are made based on the research findings in the context of national and international evidence.

9.1 Discussion:

This is the first community-based household seroepidemiological survey to investigate HBV infection in Yemen. Previous studies investigating HBV infection in Yemen were mainly hospital-based surveys conducted in urban areas of the country. This is also the first survey of its nature to estimate hepatitis B vaccine coverage and vaccine effectiveness amongst children in Yemen.

This survey also investigated the association of demographic factors and variables with anti-HBc positivity and HBV chronic infection with the intention of providing a better understanding of the epidemiology of HBV infection in Yemen, information that is essential for guiding health policy formulation. Some of the factors that were investigated, for example, educational status, age, and socioeconomic status, were found associated with HBV infection, which was consistent with research findings carried out in the Middle East. Other well-recognized risk factors associated with HBV infection identified in previous studies amongst hospital populations in Yemen, and internationally, such as history of blood transfusion or surgery, injection practices, haemodialysis, and history of jaundice were not investigated in this survey because these are more specific to high risk groups and less relevant to the general population especially children.

It is expected that the estimates of anti-HBc positivity and HBV chronic infection amongst children aged 1-3, children aged 4-9, and women of childbearing age obtained from this survey in Yemen are reliable, accurate, valid, and generalisable. There is no reason to believe that the results of this study are biased. Selection bias is expected to be minimal considering all children participating in the survey were randomly selected based on probability proportional to size giving all children of the population an equal

chance of being selected. All women of childbearing age participating in the survey were mothers of children aged 1-3 participating in the survey. There were two categories of women who could not be included in the sample population of this survey. The first category were women without a child aged 1-3 and the second were women with a child aged 1-3 but who could not be recruited because they or their child were not present at home at the time the study investigators visited their household. The main reason women might not have a child is infertility. This could be related to a history of sexually transmitted infection and thus might be associated with hepatitis B infection. However, this would not produce a major selection bias. The second category of women, women absent from home on the day their household was surveyed, were uncommon, probably due to the small number of women in paid employment. Throughout the survey study investigators only came across a few households within this category. The women who were not in their households at the time their household was surveyed were either visiting neighbours, were shortly gone shopping or on errands, or farming. Study investigators waited while household members went to call mothers/children visiting neighbouring households, or marked and visited households of women away on errands or farming later on that day. Study investigators did come across a few households where mothers and their children travelled away to attend a wedding or visit relatives, and households where there was a child aged 1-3 whose mother had unfortunately died, and consequently, these could not be included in the survey, but this was uncommon. Other factors contributing to minimising selection bias were the high response rate attained (over 80%), comprehensive training of study investigators on household sampling methodology, and the blindness of study investigators to the HBV infection status of potential study participants.

On the other hand, self-selection bias was avoided because study participants were randomly included in the survey based on probability proportional to size, and not according to potential study participants wishes. Study investigators did come across potential study participants who refused to participate in the survey for various reasons such as suspecting that study investigators intended to sell their blood after it was collected, suspecting the injections used to withdraw the blood samples caused infertility,

or not wanting to give personal information or details of their household and possessions to an unknown person. Nevertheless, the presence of local facilitators, health workers and pre-arrangements with local leaders minimized refusal in rural areas whereas higher educational attainment helped minimize refusal in urban areas.

Strict laboratory procedures and quality control minimised measurement bias. Two other potential sources of bias in this survey were information and recall bias. Interviewer bias was minimized because before carrying out the survey study investigators were trained in conducting and interpreting responses to an interviewer administered questionnaire. Throughout the interview study investigators, systematically asked identical questions. Recall and interviewer bias with reference to the HBV infection estimates of the survey were avoided because both interviewer and respondent were blind to the HBV infection status of the study participant. Recall and interviewer bias resulting in misclassification with reference to hepatitis B vaccine coverage were avoided because data on vaccination status was only based on what was recorded on vaccination cards not on what respondents reported. An important factor that may have biased the estimate of hepatitis B vaccine coverage in this survey is that 13% of children aged 1-3 participating in the survey had no vaccine information available. If, in the unlikely event, a large proportion of these children had received hepatitis B vaccine, this would result in the survey underestimating hepatitis B vaccine coverage. If on the other hand, most of these children did not receive hepatitis B vaccine this may result in the survey overestimating hepatitis B vaccine coverage.

A major concern, as described previously, was the reduction in the study's power to detect statistically significant differences or associations even when there were increased or decreased odds ratios. This was the result of the much lower number of anti-HBc positive and HBV chronically infected study participants amongst children aged 1-3 and children aged 4-9 participating in the survey than was initially expected.

Another important concern was potential HBV chronic carrier misclassification. This resulted from a few cases (three cases) of repeatedly reactive HBsAg samples that were simultaneously repeatedly anti-HBc negative. These were considered non-specific

reactive false HBsAg positives and were classified HBsAg negative in subsequent analysis. This was based on information from Abbott Laboratories, the low S/N ratio of these samples, and advice from Professor Richard Tedder virologist at University College London. Another source for potential misclassification was in measuring mothers and fathers educational status and level of education. Mothers and fathers level of education was recorded according to what they claimed their level of education to be. Study investigators did not request study participants to show them their educational certificates or evidence of their educational attainment. If there was a tendency for study participants to misinform study investigators this may have passed unnoticed. However, parents with high educational attainment were clearly noticed during interviews and study investigators carefully investigated parents claims to their level of education or educational status if they appeared to be less or more educated during the interview than they claimed to be.

Representative controls were included in the analysis of anti-HBc positivity and HBV chronic infection amongst children aged 1-3, children aged 4-9, and mother's. The controls in the case-control univariable and multivariable analysis of anti-HBc positivity and HBV chronic infection were all children or mothers uninfected with HBV participating in the survey, and thus a subset of the sample population. These are unlikely to differ from the cases considering they were selected at the same time, from the same population, and based on the same criteria as children and mothers who were subsequently diagnosed as anti-HBc positive or HBV chronic carrier women and therefore are considered an appropriate comparative group.

A potential weakness resulting from the cross-sectional nature of this survey is that associations found in the case-control analysis between outcome variables and exposure variables were measured at the same point in time. This potentially led to misinterpretation of the causal relationship between exposure and outcome. For example, mothers HBV infection status and HBV infection amongst children aged 1-3 were measured at the same time, and based on the association between these two variables the proportion of childhood infections arising from the mother was determined.

Generally speaking, and relative to pre-survey expectations, the results of this study are encouraging with regard to the prevalence of anti-HBc positivity and HBV chronic infection amongst all three age-groups investigated in the study but disappointing with regard to hepatitis B vaccine coverage.

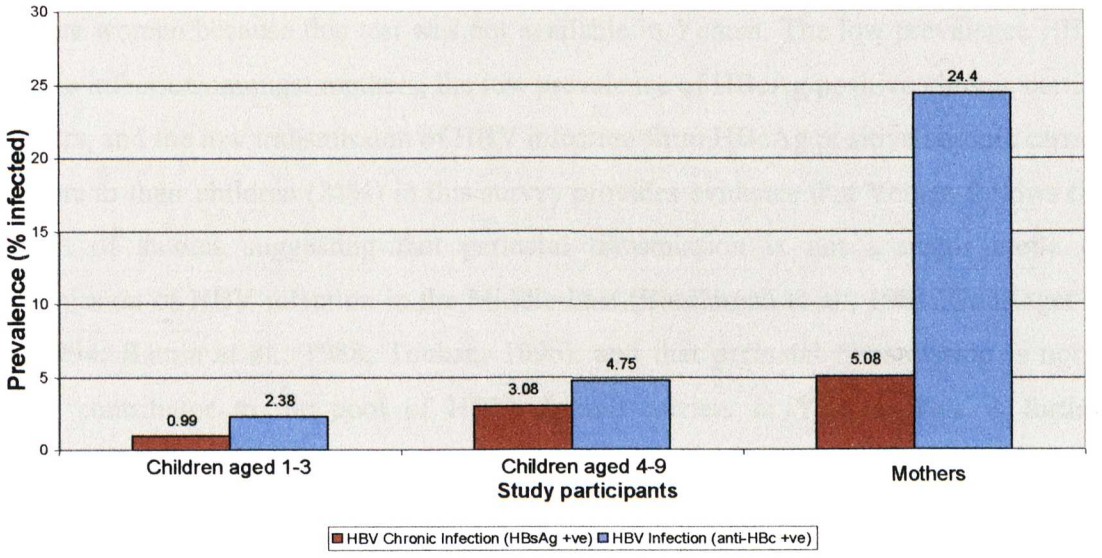
The prevalence estimate of HBV chronic infection amongst women of childbearing age participating in the survey is considered representative of the prevalence of HBV chronic infection amongst adults in the general population. This prevalence estimate is much lower than estimates from previous hospital-based studies in Yemen showing the prevalence of HBV chronic infection to range from 12.5% to 16.6% amongst pregnant women (Abdul Raheem et al., 1991; El-Guneid et al., 1993; Scott et al., 1990). However, these results are not surprising when considering that the population targeted in this community-based survey is different from the hospital-based populations targeted in previous surveys in Yemen who may have a higher risk of HBV infection than the general population, as well as laboratory quality control procedures that may have differed between this survey and the earlier surveys in Yemen. Based on the results of this survey it cannot be ruled out, however, that some pocket populations such as the African community in Sanaa or inhabitants of Socotra island did not have a higher prevalence of HBV infection, and therefore, may require specific vaccination policies. This surveys prevalence estimate barely places Yemen amongst the group of countries with a high endemicity of HBV infection. This is contrary to earlier estimates suggesting Yemen is amongst the group of countries in the upper range of highly endemic or hyperendemic for HBV infection (El-Guneid et al., 1993; Scott et al., 1990). In fact, this prevalence estimate of HBV chronic infection amongst mothers in Yemen makes the endemicity of HBV infection in Yemen consistent with the endemicity of HBV infection in other Middle-Eastern countries in the region such as Saudi Arabia, Kuwait, Lebanon, Tunisia and Iran (Al-Nakib et al., 1986; Amini et al., 1993; Basalamah et al., 1984; Coursaget et al., 1994; Nabulski et al., 1997; Ramia et al., 1984).

The prevalence estimates of anti-HBc positivity and HBV chronic infection amongst the three groups of study participants show an increasing risk of exposure to HBV infection with increasing age. It is not possible in this cross sectional survey to know whether this

is due to a fall in childhood infection with time (a birth cohort effect) or evidence of increasing infection with age. However the fact that HBV chronic infection is reasonably constant by age in women whilst cumulative infection increases suggests that it is an effect of continuing exposure to the virus in adult life. Regardless of the initial extent of perinatal transmission of HBV infection amongst children aged 1-3, there appears to be childhood transmission of HBV infection occurring amongst the children aged 4-9, as indicated by the higher prevalence amongst this age-group of children relative to the baseline prevalence amongst children aged 1-3, and by the increasing prevalence of HBV infection across the age-groups extending from 4-9. The importance of childhood transmission has been supported by other studies in the Middle East (Amini et al., 1993; Arya et al., 1985; Ramia, 1990). The higher prevalence of HBV infection amongst mothers relative to children aged 4-9 suggests that there is transmission of HBV infection during adult life. The mode of transmission of HBV infection in adult life is not known. This may be through sexual transmission between married couples, at the time of childbirth through contaminated equipment (e.g. used razors to cut umbilical cord) used by unqualified individuals in non-health setting, or unsafe injection practices. Anecdotal evidence suggests that health facilities do not incinerate waste products posing a health and safety haphazard, especially to children living near health facilities. For example, in one health centre visited exposed used needles were seen on the ground outside the windows of health centre consulting rooms. No further comments can be made about the extent of sexual and parenteral transmission based on this survey because it was not initially designed to investigate these modes of transmission. It is recommended that studies be undertaken to investigate these modes of transmission as well as emphasising on the importance of enforcing the basic health and safety guidelines to prevent them. The prevalence of anti-HBc positivity and HBV chronic infection amongst children aged 1-3, children aged 4-9, and mothers in Yemen is shown in figure 9.1.1.

Figure 9.1.1

Prevalence of anti-HBc positivity and HBV chronic infection amongst children aged 1-3, children aged 4-9, and mothers in Yemen



The low prevalence of HBV chronic infection (0.99%) amongst children aged 1-3 participating in this survey suggests that perinatal transmission is unlikely to be a major mode of HBV transmission in Yemen. This is contrary to earlier studies suggesting that perinatal transmission may play an important role in HBV transmission in Yemen (Abdul Raheem et al., 1991; El-Guneid et al., 1993). The low rate of perinatal transmission of HBV infection is mainly the result of both the low prevalence of HBV chronic infection amongst women of childbearing age and the low prevalence of HBeAg positivity amongst these HBV chronic carrier women (i.e. HBV chronic carrier women with low infectivity). In this survey, 5.08% of mothers were HBV chronic carriers (only one-third the prevalence suggested by earlier studies), which clearly reduces the prevalence of HBV chronic carrier mothers in Yemen. Moreover, only 12.84% (95% CI 6.12 to 24.99) of HBV chronic carrier mothers were HBeAg positive, which reduces the pool of highly infectious HBV chronic carrier mothers in Yemen to one-third of what was previously reported (El-Guneid et al., 1993). Additionally, only 21% (2/8) of HBeAg positive chronic carrier mothers had HBV infected children confirming a low rate of perinatal transmission of HBV infection from these mothers. This is in contrast to HBeAg positive mothers in Southeast Asia where 70 – 90% of children born to these women became HBV chronic carriers (CDC, 1991; Hall, 1994; Hwang et al., 1985). This lower risk of

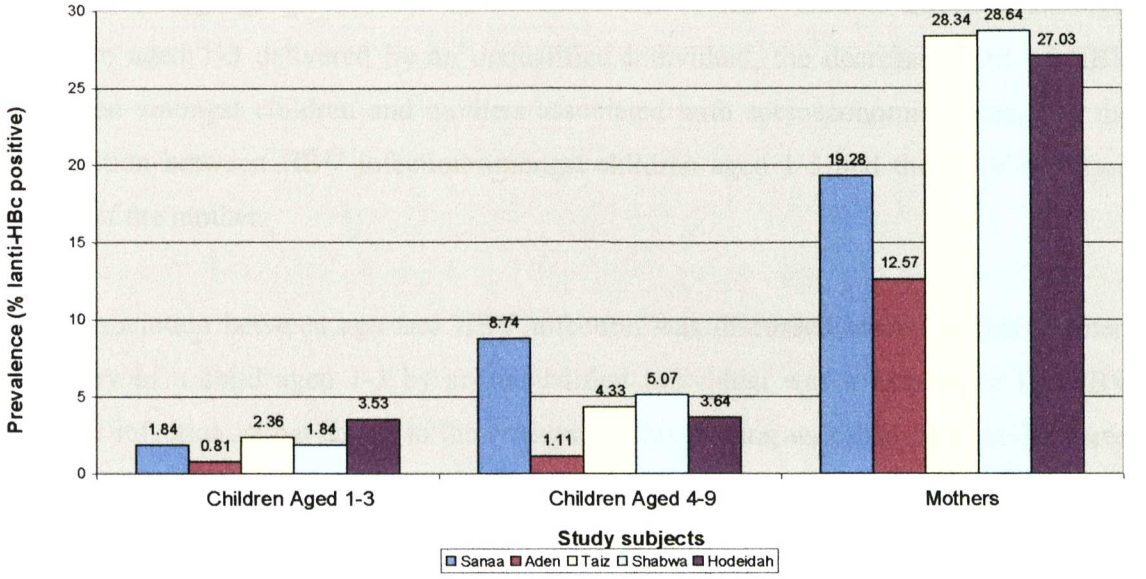
transmitting HBV infection to the offspring of HBeAg positive women in Yemen follows a pattern similar that observed amongst HBeAg positive African women (Hall, 1994), however, this survey did not measure HBV DNA concentrations amongst the HBeAg positive women because this test was not available in Yemen. The low prevalence HBV chronic infection amongst mothers, the low prevalence of HBeAg positive chronic carrier mothers, and the low transmission of HBV infection from HBeAg positive chronic carrier mothers to their children (21%) in this survey provides evidence that Yemen follows the pattern of studies suggesting that perinatal transmission is not a major mode of transmission of HBV infection in the Middle East (Basalamah et al., 1984; Coursaget et al., 1994; Ramia et al., 1988; Toukan, 1996), and that perinatal transmission is not a major contributor to the pool of HBV chronic carriers in Yemen. This is further supported by the low prevalence of HBV chronic infection amongst children aged 1-3.

This indicates there is no need to introduce an additional birth dose of hepatitis B vaccine or hepatitis B immunogloblulin (HBIG) nor is there need to amend the current schedule of hepatitis B vaccine administered in the EPI in order to prevent perinatal transmission of HBV infection. This solves for the MOPHP the problem of having to provide the funds and logistics involved in introducing a birth dose of hepatitis B vaccine or HBIG, and avoids the incremental cost resulting from the introduction of a fourth dose of hepatitis B vaccine. The Yemeni EPI states in its plan for the introduction of hepatitis B and Haemophilus influenzae type b vaccines to its routine vaccination schedule that it is unknown if a birth dose of hepatitis B vaccine is necessary to prevent perinatal transmission of HBV infection (MOPHP(A), 2001). It also states that if it is proven that a birth dose is needed this will be extremely difficult to implement considering only 16 out of every 100 children are delivered at a health facility. This makes it extremely difficult to ensure a birth dose of hepatitis B vaccine for children delivered at home within 24 hours of delivery to prevent mother-to-child transmission of HBV infection. In this plan it was recommended that a study needed to be done to investigate perinatal transmission of HBV infection. Considering that this survey has already been conducted and has answered the questions needed for the MOPHP to make an evidence-based policy decision, it is no longer necessary for the MOPHP to conduct such a survey.

The prevalence of anti-HBc positivity amongst males was higher than females aged 1-3 but this was not significant, whereas, the prevalence of HBV chronic infection was similar amongst males and females aged 1-3. On the other hand, there was a higher prevalence of HBV infection amongst females aged 4-9 compared to males but this was not significant. These observed differences are unlikely to be due to better access to hepatitis B vaccination by males. First of all, hepatitis B vaccination was not available when these children received their routine vaccinations, and secondly, there was no evidence suggesting a major difference in hepatitis B vaccine coverage between males and females participating in the survey. A similar finding was observed in Colombia amongst girls older than 10 years and a suggested explanation was that these girls had a higher exposure to HBV infection as a result of initiating sex early in life (De La Hoz, 2002). This is unlikely to be the explanation in this survey considering the younger age of the females (aged 4-9) participating in it. This is consistent with results found in a number of studies reported from Saudi Arabia that did not find significant evidence of higher anti-HBc positivity or HBV chronic carrier rates amongst males (Al-Faleh., 1992; El-Hamzi, 1989; Parande et al., 1986). Similarly, a WHO collaborative study in 20 countries did not find a significant difference in the prevalence of HBV chronic infection by sex (Sobeslavsky, 1980).

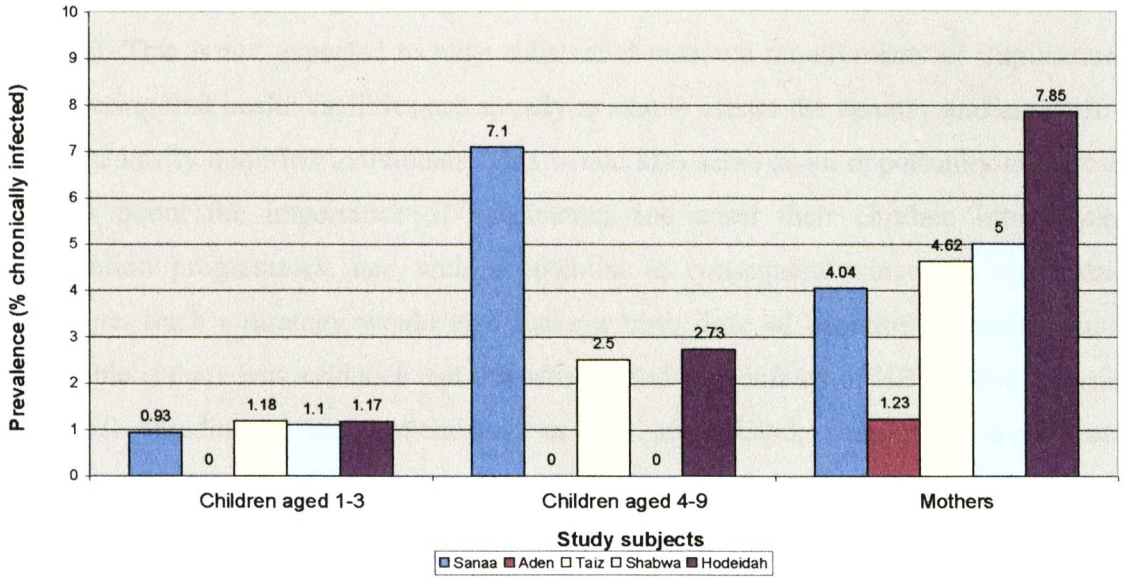
This survey did not show evidence of a statistically significant difference in the prevalence of anti-HBc positivity and HBV chronic infection amongst mothers, children aged 1-3, and children aged 4-9 when comparing all five provinces, three rural provinces, or two urban provinces in the survey. This was most probably the result of the small number of anti-HBc and HBsAg positive study participants, which when divided across the five provinces diminished the power of the study to detect a statistically significant difference, not the result of the absence of a true difference. Figure 9.1.2 shows the prevalence of anti-HBc positivity amongst children aged 1-3, children aged 4-9, and mothers by province of residence in Yemen. It seems likely that had sample sizes been greater the rates in Aden would have been statistically significantly lower than other provinces.

Figure 9.1.2
Prevalence of anti-HBc positivity amongst children aged 1-3, 4-9, and mothers by province



There were no cases of HBV chronic infection amongst the 247 children aged 1-3 in Aden, 90 children aged 4-9 in Aden, or 75 children aged 4-9 in Shabwa. Figure 9.1.3 shows the prevalence of HBV chronic infection amongst children aged 1-3, children aged 4-9, and mothers by province in Yemen.

Figure 9.1.3
Prevalence of HBV chronic infection amongst children 1-3, 4-9, and mothers by province



Variables found to be statistically significantly associated with HBV infection by multivariable analysis were the increasing OR of anti-HBc positivity amongst mothers with increasing age (p=0.0001), the increased OR of HBV chronic infection amongst children aged 1-3 delivered by an unqualified individual, the decreasing OR of HBV infection amongst children and mothers associated with socioeconomic status, and the association between HBV infection amongst children aged 1-3 and the HBV infection status of the mother.

The association between age and HBV infection was discussed earlier in this chapter. Delivery of a child aged 1-3 by an unqualified individual was a risk factor for HBV chronic infection. What added to the strength of this finding was that children delivered in a home setting had an increased risk of HBV infection. A similar association between the increased OR of HBV chronic infection and delivery by an unqualified individual was observed in Colombia, and it was suggested that practices at the time of birth carried-out by unqualified persons such as cutting the umbilical cord may increase the child's risk of HBV infection (De La Hoz, 2002). Another possible explanation was that such children were less likely to receive hepatitis B vaccine. There is room to intervene and reduce the risk of HBV infection amongst children delivery by unqualified individuals. This can be reduced by designing massive health education campaigns promoting delivery in health facilities and by medically qualified personnel (whether this may be at a health or home setting). This is not expected to have substantial resource requirements or implications considering that health facilities are already available across the country and are staffed with medically qualified individuals. This would also serve as an opportunity to educate parents about the importance of vaccination and enrol their children into routine vaccination programmes, and with a potential to consequently increase vaccination coverage. Such a strategy would also make a birth dose of hepatitis B vaccine more attainable if there was evidence that this was needed (e.g. infants of HBV chronic carrier mothers). Traditional birth attendants, on the other hand, should be invited and encouraged to attend training courses on sterile, aseptic, and safe delivery methods, and motivated to do so by issuing them training certificates and reimbursing their travel and accommodation expenses.

In the multivariable analysis of HBV infection amongst children aged 1-3 and mothers, fridge/freezer, home telephone, and television were three household possessions found to be statistically significantly associated with HBV infection after adjusting for the remaining variables in the model. Fridge/freezer ownership was significantly associated with anti-HBc positivity and HBV chronic infection amongst mothers, home telephone ownership was significantly associated with anti-HBc positivity amongst mothers, and television ownership was significantly associated with anti-HBc positivity amongst children aged 1-3. Not owning these household possessions was associated with a statistically significantly increased OR of HBV infection. There is no known direct causal relationship between these three household possessions and HBV infection. Nevertheless, their association with HBV infection was most probably indirect, by these variables being indicators of socioeconomic status and well-being. When these variables, as well as the remaining socioeconomic indicator variables were removed from the multiple logistic regression analysis and substituted with the socioeconomic status variable, there was a statistically significant association between this variable and anti-HBc positivity amongst mothers and children aged 4-9, as well as HBV chronic infection amongst mothers. In other words, the socioeconomic status variable had a similar association with anti-HBc positivity and HBV chronic infection amongst mothers and children aged 1-3 as the three socioeconomic indicator variables mentioned above. Moreover, the socioeconomic status variable was also found to be significantly associated in the multivariable analysis with anti-HBc positivity amongst children aged 4-9. Failure of the association between anti-HBc positivity and HBV chronic infection amongst children aged 1-3, and the socioeconomic status variable reaching statistical significance was probably due to an insufficient power to detect a significant association and not due to the absence of a true association. There were only 11 and 9 HBV chronic carrier children aged 1-3 and 4-9, respectively, which is less than half the number of cases that were expected to be identified in the survey. On the other hand, there were 59 HBV chronic carrier mothers, and this larger number of mothers, relative to the number of HBV chronic carrier children, provided a power sufficient to detect a statistically significant association of HBV chronic infection with the socioeconomic status variable. The association found between hepatitis B vaccination coverage and the socioeconomic status variable

described later in the discussion adds to the strength and reliability of this variable in socioeconomic classification. This is consistent with findings from studies conducted in other Middle East countries that show a clear association between HBV infection and socioeconomic status (Awidi et al., 1984; Ghaffar et al., 1989 Toukan et al., 1990; Toukan, 1996; WHO, 1995).

Despite the small number of anti-HBc positive and HBV chronic carrier children aged 1-3 identified in this survey, the study did demonstrate the important and significant association between HBV infection amongst children aged 1-3 and the HBV infection status of the mother. The adjusted OR of anti-HBc positivity amongst children aged 1-3 born from anti-HBc positive mothers was 3.97 (95% CI 1.38 to 11.43) and the OR of anti-HBc positivity amongst children aged 1-3 born from mothers chronically infected with HBV was 7.84 (95% CI 3.78 to 16.25). Preventing HBV infection amongst children delivered from HBV infected mothers is a more difficult task than reducing the risk of HBV infection amongst children associated with delivery by unqualified individuals. First, of all, there is no means of identifying who are the children at risk, at risk children becoming infected before they are protected, and the extreme difficulty in reaching at risk children widely dispersed across the country. A possible solution for identifying at risk children would be to screen all pregnant women for HBV chronic infection. Considering that less than half the pregnant women receive antenatal care during pregnancy in Yemen and the enormous costs involved in introducing an antenatal screening programme in a country with one of the highest fertility rates in the world, a screening programme will not appear to be an attractive option for the government of Yemen or international donors. Nevertheless, and regardless of HBV infection, the government of Yemen must act, quickly and effectively, to reduce the total fertility rate and consequently the population growth rate that will only put more strain on the already struggling public and natural resources in the country.

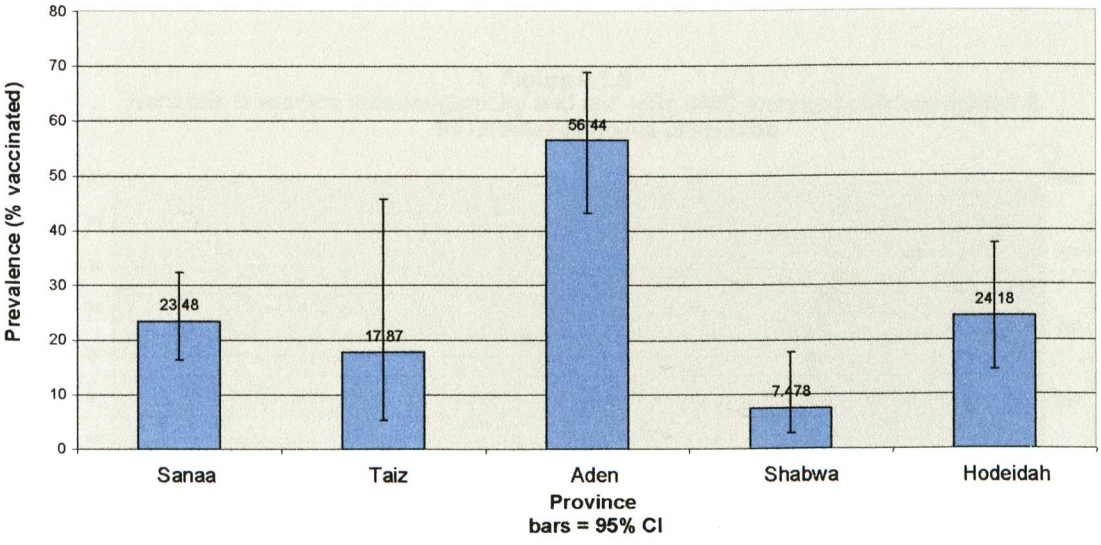
This survey provides the first as well as the only independent estimate of hepatitis B vaccine coverage in Yemen. The only estimates of hepatitis B vaccine coverage prior to this survey are those presented by the Yemeni EPI. The EPI reported that 42% of children below the age of one year received at least one dose of hepatitis B vaccine and 14.8% of

children were completely vaccinated (MOPHP(B), 2001). The overall prevalence of children aged 1-3 participating in this survey completely vaccinated with hepatitis B vaccine [8.63% (95% CI 5.1 to 14.24)] or receiving at least one dose of hepatitis B vaccine [21.1% (95% CI 15.81 to 27.58)] was lower than what was reported and expected. These estimates are much lower than vaccine coverage with the remaining EPI vaccines in Yemen (MOPHP(B), 2001), and much lower than hepatitis B vaccine coverage estimates from other countries in the Middle East (Toukan, 1997).

Also disappointing was the large proportion of incompletely vaccinated children [12.47% (95% CI 9.72 to 15.85)], that is, children receiving one or two doses of hepatitis B vaccine failing to complete their vaccination schedule. If all these children were completely vaccinated, this would have increased the proportion of completely vaccinated children to more than double its current level. It is unknown why these children did not complete their vaccination schedule nor was this survey designed to investigate this. Possible causes may be divided into health service factors (such as availability of vaccine and accessibility to health services) and individual factors (such as knowledge, attitude, and practices). These factors are likely to be responsible for the observed marked variation in hepatitis B vaccine coverage between the five provinces in the survey, between the urban versus rural provinces in the survey, and between the two urban provinces in the survey. The variation in hepatitis B vaccination coverage by province and area of residence suggests an inequitable distribution, availability or accessibility to primary health care services in Yemen. It has already been pointed out that this survey did not investigate health services in Yemen and nor did this survey investigate the attitude and practice of parents regarding hepatitis B vaccination. For example, it is not known if parents of unvaccinated children were willing but unable to go to vaccination centres or if these parents refused to do so because of religious or cultural beliefs, or otherwise. However, vaccination coverage estimates indicate a bias towards better provision, or at least uptake, of hepatitis B vaccine, and possibly all EPI services, in urban areas. After more than four years of hepatitis B vaccination this cannot be attributed to the phased introduction of hepatitis B vaccination which started with the major health centres in governorates. Figure 9.1.4 shows hepatitis B vaccine coverage

with at least one dose of hepatitis B vaccine amongst children aged 1-3 participating in the survey by province of residence.

Figure 9.1.4
Prevalence of hepatitis B vaccination amongst children aged 1-3 by province

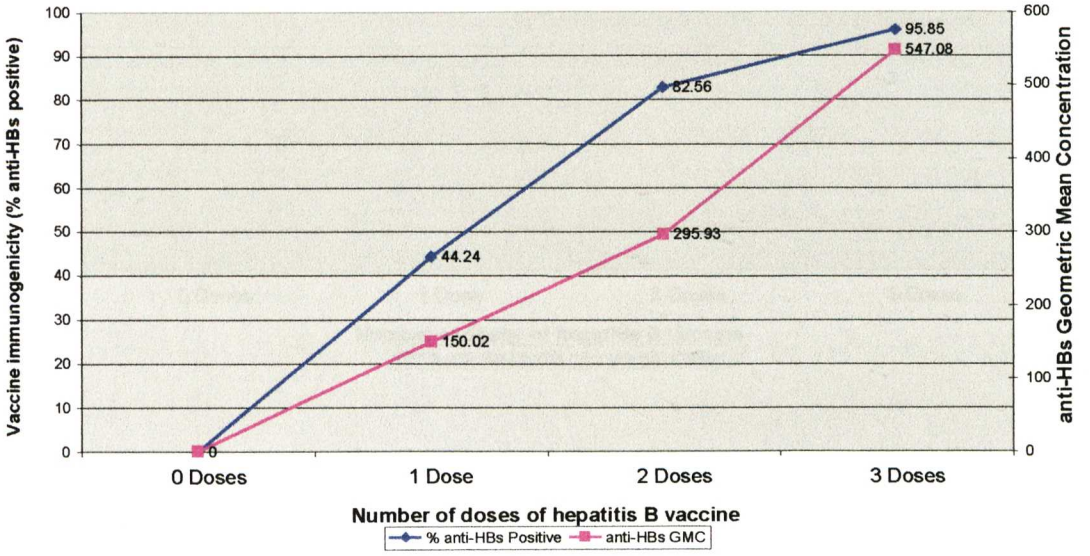


The prevalence of hepatitis B vaccination amongst children aged 2-3 (17.85%) was lower than vaccine coverage amongst children aged 1-2 (30.32%) (p=0.0007) and is most probably the result of the EPI policy of providing hepatitis B vaccine free of charge only to children under 1 year old. This was confirmed in the multivariable analysis which showed that younger children had a higher odds of being vaccinated with hepatitis B vaccine (p=0.006). On the other hand, the overall prevalence of hepatitis B vaccine coverage amongst children aged 4-9 participating in the survey was only 1.91% (95% CI 0.7 to 5.1) (with at least one dose of hepatitis B vaccine), which is simply explained by the fact that these children were not targeted or offered hepatitis B vaccine.

The number of children anti-HBs positive and their anti-HBs geometric mean concentration (GMC) was proportional to the number of doses of hepatitis B vaccine received. There was a clear increase in the proportion of anti-HBs positive children and anti-HBs geometric mean concentration with increasing number of doses of hepatitis B vaccine received. This validates the recording of hepatitis B vaccination doses. It also

indicates that hepatitis B vaccine was highly immunogenic amongst children aged 1-3 participating in the survey and that there is no need to change the current dose or schedule of hepatitis B vaccination in Yemen. Figure 9.1.5 shows hepatitis B vaccine immunogenicity and anti-HBs GMC amongst children aged 1-3 by number of doses of hepatitis B vaccine.

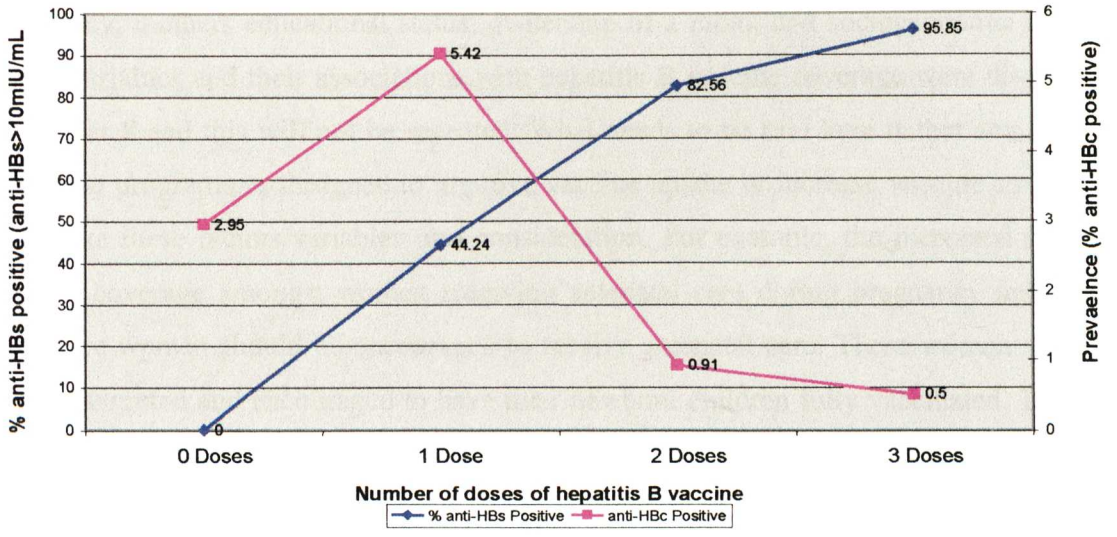
Figure 9.1.5
Hepatitis B vaccine immunogenicity and anti-HBs GMC amongst children aged 1-3 by number of doses of vaccine



The effectiveness of hepatitis B vaccine was 83% (95% CI -9 to 97%) in preventing anti-HBc positivity and 100% (95% CI lower limit is 97%) in preventing HBV chronic infection amongst completely vaccinated children aged 1-3 participating in the survey. Although this did not reach statistical significance, what is reassuring is the high immunogenicity of hepatitis B vaccine and GMC presented in figure 9.1.5. This indicates that the vaccine effectiveness failing to reach statistical significance was the result of the low number of anti-HBc positive and HBV chronic carrier children not because of vaccine ineffectiveness. Ideally, this should be repeated on a sample involving a larger number of vaccinated children, but meanwhile if a high percentage of hepatitis B vaccine coverage is achieved, the difficulty may then become identifying unvaccinated children of the same age-group. What this survey did not measure was the effect of dose intervals or site of administration of vaccine on the immunogenicity and effectiveness of hepatitis

B vaccine. Figure 9.1.6 shows HBV infection amongst children aged 1-3 participating in the survey by number of doses of hepatitis B vaccine.

Figure 9.1.6
HBV infection amongst children aged 1-3 by number of doses of hepatitis B vaccine



Given the above, the current level of hepatitis B vaccine coverage is unsatisfactory. It is extremely important to increase hepatitis B vaccine coverage and achieve a pre-specified target of high vaccine coverage. What is encouraging with regard to increasing hepatitis B vaccine coverage in the near future is the introduction of the pentavalent vaccine. This will reduce the number of injections and visits needed for children to receive all three doses of hepatitis B vaccine. Considering that these children will receive all doses of hepatitis B vaccine according to the new pentavalent vaccine schedule (6 weeks, 10 weeks and 14 weeks) at the same time as DPT, this should increase coverage with hepatitis B vaccine to a level similar to coverage with DPT vaccine which is currently 72.8% (MOPHP(A), 2001; MOPHP(B), 2001).

Equally important to increasing vaccination coverage is ensure that once achieved, this level of coverage is sustainable. This requires strong managerial, financial and political commitment, all of which are unlikely to be readily available considering the worsening economic situation in Yemen. The situation will probably be more complicated in the

future when support provided from the global alliance for vaccines and immunisation (GAVI) ends unless the MOPHP designs a sound financial sustainability plan well in advance. Independent variables found to be significantly associated with hepatitis B vaccine coverage amongst children aged 1-3 after adjusting for the remaining variables were age of the child, knowledge of hepatitis B vaccine, receiving antenatal care during pregnancy, mothers educational status, ownership of a radio, and socioeconomic status. These variables and their associations with hepatitis B vaccine coverage were discussed in chapter 8 and this will not be repeated. What needs to be said here is that any health education programmes designed to improve vaccine uptake or increase vaccine coverage must take these factors/variables into consideration. For example, the increased OR of vaccine coverage amongst women receiving antenatal care during pregnancy indicates that more women should be encouraged to receive antenatal care. These women should then be targeted and encouraged to have their newborn children fully vaccinated. Taking this one step further, what would be the best way to reach these women? The associations above suggest that health education programmes utilising the radio should be given priority over other media considering radio ownership was associated with higher hepatitis B vaccine coverage whereas television ownership was not. Moreover, 65% of households participating in the survey had radios whereas 59% of households had a television. Additionally, only 45% of rural households had a television compared to 60% or rural households that had a radio. Nevertheless, one must not forget that radio ownership is also an indicator of socioeconomic status, which showed an increase in vaccine coverage with increasing socioeconomic status. The strong association between knowledge of hepatitis B vaccine and vaccine coverage may lead one to believe that the solution to increase vaccine coverage is increasing knowledge about hepatitis B vaccine. Why then, although 78% of parents claimed to have knowledge of hepatitis B vaccine and 32% of parents said their child received hepatitis B vaccine, in reality a lower percentage of children were really vaccinated. These questions remain unanswered and qualitative surveys are needed to investigate these issues as well as the health seeking behaviour of the Yemeni population.

9.2 Summary:

1- HBV infection in Yemen is not as major a public health problem as was originally expected. Initially, it was expected that approximately 15% of adults in the general population would be HBV chronic carriers. There was an increase in the prevalence of HBV infection with increasing age. The prevalence of HBV chronic infection amongst adults (women of childbearing age) in the general population barely places Yemen amongst the group of countries with a high endemicity of HBV infection (> 5%).

2- There was no evidence of a statistically significant difference in the prevalence of anti-HBc positivity and HBV chronic infection amongst women of childbearing age, children aged 1-3, and children aged 4-9 when comparing the five provinces in the survey.

3- There was evidence that perinatal transmission is not a major mode of transmitting HBV infection in Yemen. This is mainly the result of both a low prevalence of HBV chronic infection amongst women of childbearing age, a low prevalence of HBeAg amongst these HBV chronic carrier women (i.e. chronic carrier women with low infectivity), and low transmission of HBV infection from HBeAg positive mothers.

4- Variables associated with HBV infection amongst mothers were age of the mother and socioeconomic status. Variables associated with HBV infection amongst children were mothers HBV status, socioeconomic status, and qualifications of the person managing delivery of the child.

5- The overall level of hepatitis B vaccination coverage amongst children aged 1-3 participating in the survey was low.

6- A high proportion of children were incompletely vaccinated (one or two doses) and had an unknown vaccination status.

7- There was a significant difference in the prevalence of hepatitis B vaccine coverage amongst children aged 1-3 between the five provinces and areas of residence in Yemen. The figures of vaccination coverage suggest an inequitable distribution, availability or accessibility to primary health care services, with a

bias towards better provision, or at least uptake, of primary health care services in urban areas.

8- Hepatitis B vaccine was found to be highly immunogenic amongst children aged 1-3. The number of children anti-HBs positive and their anti-HBs GMC was proportional to the number of doses of hepatitis B vaccine received.

9- Hepatitis B vaccine had a vaccine effectiveness of 83% (95% CI -9 to 97%) in preventing anti-HBc positivity and a vaccine effectiveness of 100% (95% CI lower limit is 97%) in preventing HBV chronic infection amongst completely vaccinated children aged 1-3 participating in the survey.

9.3 Recommendations:

9.3.1 Recommendations to the MOPHP/EPI in Yemen:

Recommendations based on the findings of this survey:

1- The MOPHP should quote the prevalence estimates of HBV infection found in this survey when presenting estimates of the prevalence of anti-HBc positivity and HBV chronic infection amongst the general population and children in Yemen.

2- The MOPHP should disseminate the prevalence estimates of HBV infection and vaccine coverage found in this survey to the relevant national and international bodies such as WHO, international organisations and donors, educational institutions, health care personnel and workers, and health authorities. This can be achieved through the mass media, MOPHP health education programmes, and national and international conferences with the objective of updating Yemen's profile of HBV infection and hepatitis B vaccine coverage, and correcting the current widespread false belief that 18% of adults in the general population are chronically infected with HBV.

3- There is no need to introduce a birth dose of hepatitis B vaccine or HBIG considering the evidence from this survey suggesting that perinatal transmission is not a major contributor to HBV transmission in Yemen.

4- The EPI should increase its unsatisfactory current hepatitis B vaccine coverage. The MOPHP should set targets to be achieved as well as process indicators to

monitor progress towards meeting these targets. The current EPI 5-year-plan set a target to achieve 90% hepatitis B vaccine coverage by the end of 2006. This appears to be an optimistic target but may be more realistic following introduction of the new pentavalent vaccine which is likely to improve hepatitis B vaccine uptake to levels similar to DPT and OPV, which are currently reported to be over 70%.

5- The EPI should set as a process indicator the objective of increasing the proportion of children completely vaccinated with hepatitis B vaccine to 60% two years after introduction of the pentavalent vaccine.

6- Two years after introducing the pentavalent vaccine an independent survey should be performed on a representative sample of the children in order to estimate vaccine coverage amongst children by 1 year of age and evaluate progress towards the final target (assuming this is before 2006).

7- The MOPHP should proceed with introduction of the pentavalent vaccine as soon as possible. This will reduce the number of injections and time spent per visit, thereby increasing parents acceptance, as well as improving record keeping at health centres.

8- The PHC/EPI must ensure that children receive all three doses of hepatitis B vaccine. Children receiving only one dose of hepatitis B vaccine are a missed opportunity of becoming completely vaccinated children. EPI staff and health care workers must be professionally trained to emphasise to parents/guardians the importance of receiving subsequent vaccine doses and the importance of their child returning to receive the remaining doses of hepatitis B vaccine according to the hepatitis B vaccination schedule.

9- There is currently no need for a nationwide immunisation survey to be performed to estimate hepatitis B vaccine coverage as suggested in the EPI 5-year-plan considering this survey has already provided the data needed on hepatitis B vaccine coverage.

10- The PHC/EPI should ensure that every child receives a vaccination card and that health centre records are up-to-date with children vaccination records. EPI/PHC health workers should educate parents/guardians about the importance of having

and retaining vaccination cards in order to have a full vaccination history and for school entry and registration.

11- The PHC/EPI should ensure equitable distribution, availability, and accessibility to hepatitis B vaccine in all provinces, with an emphasis on rural regions, in Yemen. The PHC/EPI should investigate the reasons behind the significant variation in hepatitis B vaccination coverage between geographic regions in Yemen.

12- The PHC/EPI should ensure a sufficient supply of hepatitis B vaccine to cover potential increase in demand for vaccine triggered by the MOPHP health education programmes.

13- Ideally, hepatitis B vaccine should be provided free of charge to all children requiring it. The MOPHP ought to revise the current EPI hepatitis B vaccination policy of providing hepatitis B vaccine free only to children under 1-year-old. The MOPHP needs to consider and evaluate the cost-effectiveness of providing hepatitis B vaccine to children over 1-year-old. Funding and provision of hepatitis B vaccine should be discussed with international organisations and donors such as WHO and GAVI. However, due to the low hepatitis B vaccination coverage amongst children aged 1-3, it is recommended that the implementation of this should be delayed until hepatitis B vaccine coverage amongst children less than one-year reaches target levels.

Recommendations based on the findings of this research and for which there have been previous similar suggestions made to the MOPHP/EPI by its own reports, plans or consultations but have not yet been implemented (MOPHP(A), 2001; MOPHP(B), 2001; MOPHP(C), 2001):

14- The MOPHP needs to ensure that a realistic financial sustainability plan is designed and prepared so that immunisation practices continue after support by GAVI ends.

15- The MOPHP needs to strengthen its department of statistics and health information systems in order to have basic data essential for planning activities.

16- The PHC/EPI needs to strengthen its logistic capability with an emphasis on upgrading its cold chain systems. Issues such as difficulty of storage of hepatitis B vaccine at the periphery due to lack of electricity and refrigerators need to be addressed and necessary action must be taken by PHC, EPI and district health authorities to solve these problems.

17- EPI should reinforcing vaccine management and quality at the district level, especially with the developing district health systems, and train its staff to minimise vaccine wastage and monitor vaccine wastage at the central level.

18- The EPI should establish a vaccine adverse events monitoring system and design the appropriate forms and database for this purpose and ensure that health workers at the periphery provide this information to the central level.

19- PHC/EPI health care staff need to be trained to educate women of childbearing age attending health centres for antenatal care or otherwise about the importance and benefits of early childhood vaccination and encourage them to attend health centres for postnatal visits.

20- The MOPHP and district health authorities need to ensure that safe injection practices are implemented and that used materials are safely disposed of and/or incinerated.

21- The department of reproductive health and the National Population Council need to act to reduce the total fertility rate by promoting family planning, providing family planning methods, and encouraging childbirth spacing. The recommendations set by the second national population conference should be implemented and progress evaluated.

22- Unplanned building of health units must be stopped as this puts strain on the EPI to provide equipment and supplies to these units.

9.3.2 Recommendations requiring multi-sectoral collaboration in Yemen:

1- The health education department in the MOPHP needs to develop Information, Education, and Communication (IEC) campaigns, mainly utilising radio to educate the population about the importance and benefits of antenatal care during pregnancy, vaccination in general, and hepatitis B vaccination specifically. The MOPHP in collaboration with the Ministry of Information need to select the prime-time for broadcasting these programmes on television and radio in order to produce the greatest impact and reach the highest number of the target population. The MOPHP should review 1 year after instructing the health education department to do so, how many programmes were developed, frequency of broadcasting, and evaluate the impact of these programmes and campaigns on attendance for antenatal care and postnatal vaccination at the same time as when the vaccination survey is conducted.

2- The MOPHP in collaboration with the Ministry of Education (MOE) needs emphasise to the cabinet that adult literacy in Yemen is low, with an emphasis on female education, and the negative impact this low education has on health status in general, and vaccination coverage specifically. It needs to be clearly demonstrated that improving educational status has positive externalities on health, socioeconomic status, and many other demographic characteristics. The MOPHP and MOE need to present the cabinet with a bilaterally developed project proposal for improving educational status (with an emphasis on mothers education), providing a more equitable distribution of schools in Yemen, with an emphasis on rural areas, and lobby the government and Ministry of Finance to increase its expenditure on education and health.

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Annex I

Hepatitis B Virus Infection in Yemen
Household Survey Questionnaire
Children Aged 1 – 3 Years Old

Baseline Information:

No			Key	Code
1.	Record Number		*0-00-00-00-00*	
2.	Child's Identification Number			
3.	Mother's Identification Number			
4.	Province		*1-5*	
5.	District		*1-5*	
6.	City or Village		*1-5*	
7.	Street			
8.	Household Number		*0000*	
9.	Area (Urban or Rural)		*1-2*	
10.	Interview Date			
11.	Interview Time			
12.	Interviewer			
13.	Interviewer Code	----------	*1-4*	
14.	Person Interviewed			
15.	Relation to Child			

Characteristics of Individuals from whom blood was drawn:

Child Aged 1-3 years old:

No	Question			Key	*Code*
16.	What is the Child's Name?			*Tertiary*	
17.	Family Name (Surname)?				
18.	When was the child born? (What is the child's DOB?)				
19.	How old is the child?			*In months*	
20.	What is the source of the child's DOB/ Age?	Approximately Vaccination Records Birth Certificate Parents Memory Parents Records Passport or ID Card Other, specify	1 2 3 4 5 6 7	*1-7*	

21.	Is the child male or female?	Male Female	1 2	*1-2*	
22.	In what village/city, district and province was the child born?	Village/City District Province		*Province 1-6*	
23.	Where was the child delivered?	Public Hospital Private Hospital Health Centre Husbands Home Parents Home Other, specify	1 2 3 4 5 6	*1-6*	
24.	Who delivered the child?	Doctor Nurse Midwife TBA Relative Alone or with husband Other, specify	1 2 3 4 5 6 7	*1-7*	
25.	What was the type of delivery?	Vaginal Delivery Caesarean Section	1 2	*1-2*	
26.	Did you receive antenatal care during your pregnancy with this child before you started delivery?	Yes No Don't Know	1 2 3		
27.	Have you breastfed this child?	Yes No → go to 29	1 2	*1-2*	
28.	For how long have you breastfed this child?			*Calculate in months*	
29.	How many children have you ever given birth to (delivered)?				
30.	How many of these children were born before this child?			*Birth Order*	
31.	How many children other than your own children live in the household?				

Child's Vaccination History

32.	Does the child have a vaccination card?	Yes No ⟶ go to 34	1 2	*1-2*	
33.	Is the vaccination card available? *(fill in vaccination details)*	Yes ⟶ request card No	1 2	*1-2*	
34.	Have you ever heard of Hepatitis B vaccine?	Yes No Don't Know	1 2 3	*1-3*	
35.	Has the child been vaccinated against HBV?	Yes No ⟶ Go to 37 Don't Know	1 2 3	*1-3*	
36.	How many doses of vaccine has the child received?	One Two Three Four Don't Know	1 2 3 4 5	*1-5*	
37.	What is the source of the child's vaccination status?	Vaccination Card Parents Memory Parents Records Other, specify	1 2 3 4	*1-4*	

To be filled from vaccination card or record if available

Date	BCG	DPT	OPV	MMR	HBV
1st dose **Location**					
2nd dose **Location**	---------- ----------			---------- ----------	
3rd dose **Location**	---------- ----------			---------- ----------	

38.	*Child's hepatitis B vaccination Status*	Vaccinated Incompletely Vaccinated Unvaccinated Unknown	1 2 3 4	*1-4*	
39.	*Number of doses received according to vaccination card*				
40.	*Need to confirm vaccination status at health centre*	Yes No	1 2	*Check 1-2*	

Mother of Child Aged 1-3 Years:

41.	What is your name?			
42.	How old were you when you first got married?			
43.	How long after your marriage did you give birth to your first child?			
44.	What is the age of your oldest child?			
45.	What is your age?		*Compare*	
46.	How do you know your age?	Approximate 1 Birth Certificate 2 Health Records 3 Family Records 4 ID Card or Passport 5 Other, specify 6	*1-6*	
47.	In what village/city, district and province were you born?	Village/city District Province	*Province 1-6*	
48.	How many brothers and sisters have you ever had?			
49.	How many of your brothers and sisters were born before you?		*Birth Order*	
50.	Can you read and write?	Yes 1 No → go to 52 2	*1-2*	
51.	What is your level of education?	Read and write 1 Completed 1ry School 2 Completed 2ry School 3 Completed University 4	*1-4*	
52.	Are you employed? (Do you work)	Yes 1 No → go to 54 2	*1-2*	
53.	What is your occupation?			

Household Characteristics and Socioeconomic Status:

54.	Is the household owned or rented?	Owned 1 Rented 2	*1-2*	
55.	What is the type of the household? *(filled by investigator)*	House 1 Apartment 2 Hut or Shack 3	*1-3*	
56.	What is the household built of? *(filled by investigator)*	Stone 1 Brick or Block 2 Clay 3	*1-6*	

		Mud Mixed Undefined	4 5 6		
57.	What is the number of rooms in the household? *(excluding kitchen and bath)*			*Count*	
58.	What is the number of people living in the household?			*Specify Household*	
59.	*Crowding Index*	*XXXXXXXXXX*		*58/57*	
60.	What is the number of individuals sleeping in the same room as the child?				
61.	Does the household have an electrical supply?	Yes No → go to 63	1 2	*1-2*	
62.	Excluding electrical failures is the electricity available?	Continuously After Sunset Specific hours only	1 2 3	*1-3*	
63.	What is the house's main source of water?	Governmental supply Local project supply Purchase water truck Buckets local source Other, specify	1 2 3 4 5	*1-5*	
64.	Is the father/legal guardian of the child educated?	Yes No → go to 66	1 2	*1-2*	
65.	What is the level of the father/legal guardian's education?	Reads and writes Completed 1ry School Completed 2ry School Completed University	1 2 3 4	*1-4*	
66.	What is the father/legal guardian's occupation?	Farmer Civil Servant Shop Owner Manual Labourer Businessman Professional Other, specify	1 2 3 4 5 6 7	*1-7*	
67.	Does the household own a fridge/freezer?	Yes No	1 2	*1-2*	
68.	Does the household own a Television?	Yes No	1 2	*1-2*	
69.	Does the household own a Radio?	Yes No	1 2	*1-2*	
70.	Does the household own a	Yes	1	*1-2*	

	home telephone?	No	2		
71.	Does the household own a mobile telephone?	Yes No	1 2	*1-2*	
72.	Does the household own a Car, Truck or Tractor?	Yes No	1 2	*1-2*	
73.	Does the household own a Motorcycle?	Yes No	1 2	*1-2*	
74.	Does the household own a Bicycle?	Yes No	1 2	*1-2*	

Annex II

Hepatitis B Virus Infection in Yemen
Household Survey Questionnaire
Children Aged 4 -9 Years Old

Baseline Information:

No			Key	Code
1.	Record Number		*0-00-00-00-00*	
2.	Identification number			
3.	Province		*1-5*	
4.	District		*1-5*	
5.	City or Village		*1-5*	
6.	Street			
7.	Household Number		*0000*	
8.	Area (Urban or Rural)		*1-2*	
9.	Interview Date			
10.	Interview Time			
11.	Interviewer			
12.	Interviewer Code	----------	*1-4*	
13.	Person Interviewed			
14.	Relation to Child			

Characteristics of Individuals from whom blood was drawn:

Child Aged 4-9 years old:

No	Question	Response		Key	*Code*
15.	What is the Child's Name?			*Tertiary*	
16.	Family Name (Surname)?				
17.	When was the child born? (What is the child's DOB?)				
18.	How old is the child?			*In Years*	
19.	What is the source of the child's DOB/ Age?	Approximately Vaccination Records Birth Certificate Parents Memory Parents Records Passport or ID Card Other, specify	1 2 3 4 5 6 7	*1-7*	

20.	Is the child male or female?	Male Female	1 2	*1-2*	
21.	In what village/city, district and province was the child born?	Village/City District Province		*Province 1-6*	
22.	How many older brothers and sisters does this child have?			*Birth Order*	

Child's Vaccination History

23.	Does the child have a vaccination card?	Yes No ⟶ go to 25	1 2	*1-2*	
24.	Is the vaccination card available?	Yes⟶ request card No	1 2	*1-2*	
25.	Have you ever heard of Hepatitis B vaccine?	Yes No Unknown	1 2 3	*1-3*	
26.	Has the child been vaccinated against HBV?	Yes No ⟶ Go to 28 Don't Know	1 2 3	*1-3*	
27.	How many doses of vaccine has the child received?	One Two Three Four Don't Know	1 2 3 4 5	*1-5*	
28.	What is the source of the child's vaccination status?	Vaccination Card Parents Memory Parents Records Other, specify	1 2 3 4	*1-4*	

To be filled from vaccination card or record if available

Date	BCG	DPT	OPV	MMR	HBV
1st dose **Location**					
2nd dose **Location**	---------- ----------			---------- ----------	
3rd dose **Location**	---------- ----------			---------- ----------	

29.	*Child's hepatitis B vaccination status*	Vaccinated Incompletely Vaccinated Unvaccinated Unknown	1 2 3 4	*1-4*	
30.	*Number of doses received according to vaccination card*				
31.	*Need to confirm vaccination status at health centre*	Yes No	1 2	*Check 1-2*	

Education of Child Aged 4-9 Years:

32.	Does the child go to school?	Yes No	1 2	*1-2*	

IDNO of younger sibling (if applicable):

IDNO of mother (if applicable):

Mother of Child Aged 4-9 Years:

33.	What is your name?				
34.	How old were you when you first got married?				
35.	How long after your marriage did you give birth to your first child?				
36.	What is the age of your oldest child?				
37.	What is your age?			*Compare*	
38.	How do you know your age?	Approximate Birth Certificate Health Records Family Records ID Card or Passport Other, specify	1 2 3 4 5 6	*1-6*	
39.	In what village/city, district and province were you born?	Village/city District Province		*Province 1-6*	
40.	How many brothers and sisters have you ever had?				

41.	How many of your brothers and sisters were born before you?			*Birth Order*	
42.	Can you read and write?	Yes No → go to 44	1 2	*1-2*	
43.	What is your level of education?	Read and write Completed 1ry School Completed 2ry School Completed University	1 2 3 4	*1-4*	
44.	Are you employed? (Do you work)	Yes No → go to 46	1 2	*1-2*	
45.	What is your occupation?				

Household Characteristics and Socioeconomic Status:

46.	Is the household owned or rented?	Owned Rented	1 2	*1-2*	
47.	What is the type of the household? *(filled by investigator)*	House Apartment Hut or Shack	1 2 3	*1-3*	
48.	What is the household built of? *(filled by investigator)*	Stone Brick or Block Clay Mud Mixed Undefined	1 2 3 4 5 6	*1-6*	
49.	What is the number of rooms in the household? *(excluding kitchen and bath)*			*Count*	
50.	What is the number of people living in the household?			*Specify household*	
51.	*Crowding Index*	*XXXXXXXXXX*		*50/49*	
52.	What is the number of individuals sleeping in the same room as the child?				
53.	Does the household have an electrical supply?	Yes No → go to 55	1 2	*1-2*	
54.	Excluding electrical failures is the electricity available?	Continuously After Sunset Specific hours only	1 2 3	*1-3*	
55.	What is the house's main source of water?	Governmental supply Local project supply Purchase water truck Buckets local source Other, specify	1 2 3 4 5	*1-5*	

56.	Is the father/legal guardian of the child educated?	Yes No → go to 58	1 2	*1-2*	
57.	What is the level of the father/legal guardian's education?	Reads and writes Completed 1ry School Completed 2ry School Completed University	1 2 3 4	*1-4*	
58.	What is the father/legal guardian's occupation?	Farmer Civil Servant Shop Owner Manual Labourer Businessman Professional Other, specify	1 2 3 4 5 6 7	*1-7*	
59.	Does the household own a fridge/freezer?	Yes No	1 2	*1-2*	
60.	Does the household own a Television?	Yes No	1 2	*1-2*	
61.	Does the household own a Radio?	Yes No	1 2	*1-2*	
62.	Does the household own a home telephone?	Yes No	1 2	*1-2*	
63.	Does the household own a mobile telephone?	Yes No	1 2	*1-2*	
64.	Does the household own a Car, Truck or Tractor?	Yes No	1 2	*1-2*	
65.	Does the household own a Motorcycle?	Yes No	1 2	*1-2*	
66.	Does the household own a Bicycle?	Yes No	1 2	*1-2*	

Annex III

Hepatitis B Virus Infection in Yemen
Governorate Household Survey

Information Sheet

Dear Citizen,

We are currently doing a survey on vaccination and liver diseases (viral hepatitis) in Yemen. The survey is supervised by the University of London in the United Kingdom, Taiz University and is approved by the Yemeni Ministry of Public Health.

In this survey we are concentrating on liver diseases (viral hepatitis) and vaccination amongst children between 1-3 years old and their mothers, and children between 4 – 9 years old.

The survey intends to investigate the extent of spread of this liver disease (viral hepatitis) in Yemen and its transmission from mother-to-child, the availability of hepatitis B vaccine provided by the Expanded Programme on Immunisation, and how well hepatitis B vaccine works in preventing liver disease (hepatitis B virus infection).

For this survey, we are randomly (by chance) choosing people from different governorates in the country, and from within different cities, towns and villages in these governorates. Your household has been randomly chosen (chosen by chance) to be part of the population on which this survey will be carried-out.

All information you provide us with will be very confidential and only the individuals carrying-out the survey will have access to your personal and household details. All your personal details and blood samples will be given a secret number and kept in a locked and safe place. Any information you give us will only be used for this survey. None of your personal or household details will be given to anybody else unless you allow us to do so.

We encourage you to participate in the survey because the more people participate and co-operate with the survey, the more the results of the survey become correct, useful and accurate.
The results of the survey will benefit the country and its citizens and will allow us to understand and estimate the extent of spread of liver disease (viral hepatitis) in Yemeni governorates and the chance of mothers passing on this disease to their newborn children. It will also allow us to know the extent of vaccine availability for infants in the population and how well it prevents liver disease. This will allow the Ministry of Health and the Expanded Programme on Immunisation to provide better health services and prevention programmes against the disease.

You will also directly benefit from participating in the survey because our survey will be able to know if the children or mothers in your household have this liver disease. If you have no objection, we will notify the health authorities of the children's and mother's results. The health authorities can then let you know these results and can advise you to have all the close contacts and family members of infected individuals vaccinated against this liver disease (hepatitis B virus infection).

During our visit to your household we will do two things. First, we will fill in a questionnaire about the child aged 1-3 years old, mother, and some household characteristics. Secondly, we will take a blood sample from the child aged 1 – 3 and the mother, which will be tested in a laboratory. On some occasions we might also fill in a questionnaire and take a blood sample from a child aged 4 – 9 in the household.
All the equipment used for blood sampling is of the highest standard and the staff withdrawing the blood samples have excellent experience.

If you have any questions about this survey or viral hepatitis we are happy to answer them. You can contact us in Sanaa at Telephone number 203834, or write to us at

Viral Hepatitis in Yemen
PO Box 11351
Sanaa
Republic of Yemen.

Your verbal consent to participate in this survey is very important for the completion of this survey and for everybody to benefit.

Yours sincerely,

Dr. Naseeb Qirbi
Principal Investigator

Annex IV

Hepatitis B Virus Infection in Yemen
Population Based Cross Sectional Survey

Household Survey
Study Investigators Manual

Sanaa
Republic of Yemen
May 2001

Dear study investigator,

It is with great pleasure that I present to you this study investigator manual for the household survey of hepatitis B virus infection in Yemen you will be participating in.

This is the first household survey investigating hepatitis B virus infection in Yemen.

This Manual provides the necessary information and guidelines that you must study, understand and practice prior to initiating the survey. All procedures outlined in this manual should be strictly implemented in the order they appear by study investigators participating in the household survey. Kindly study this manual carefully and keep it with you throughout the fieldwork for reference. I am aware that over the years of your professional experience you will have most probably practised techniques for procedures such as blood sampling that may differ from the ones outlined in the manual. The basic principles of blood sampling are universal and whatever technique you have been implementing is open for discussion during the workshop. However, when you adopt a blood sampling protocol you should be consistent throughout the survey.

If after reading the manual you have any questions, comments or suggestions I will be happy to discuss these with you.

Our aim is to conduct research of the highest standard and quality. To achieve this we must be accurate, punctual, devoted to our work and obsessional throughout its conduct.

During the next few months we are likely to encounter difficulties and obstacles but with good communication and teamwork we will achieve our targets and goals.

I look forward to working with you and truly believe that by working together we can ensure a better understanding of this disease in Yemen essential for future health policy formulation.

Yours sincerely

Dr. Naseeb Qirbi

Hepatitis B Virus Infection

1 Background:

Hepatitis B virus infection is a major public health problem worldwide. An individual exposed to hepatitis B virus can either become infected with the virus or become a chronic carrier. Approximately 2 billion individuals worldwide (a third of the world's population) have been infected with the hepatitis B virus. Being infected means that an individual was either previously infected or is currently infected with the virus. It does not mean that the individual will suffer from liver disease or is a risk for infecting others. It is estimated that there are over 350 million chronic carriers (an individual who is positive for a serological marker called the hepatitis B surface antigen HBsAg for longer than six months) of the hepatitis B virus worldwide. An individual who is a chronic carrier of the virus is at risk of developing complications and a risk for infecting others. The course of infection depends on the age at exposure to hepatitis B virus, with age at infection being the major determinant of whether a person will become a chronic carrier. The risk of becoming a chronic carrier and consequently developing complications of hepatitis B virus infection is inversely proportional to age (i.e. the younger the age of the individual at the time of infection the higher the risk of becoming a chronic carrier). Approximately 25% of chronic carriers of hepatitis B virus develop the three main complications of chronic hepatitis B virus infection which are chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. Hepatocellular carcinoma is the fourth most common cause of death from cancer and the eighth most common cancer in the world. The hepatitis B virus is the world's second most common cause of cancer. It is estimated that 54% - 80% of liver cancers result from chronic hepatitis B virus infection. The majority of chronic carriers are found in developing countries. Liver cancer is also mainly a problem of developing countries, which contribute 81% of all deaths resulting from liver cancer. The global distribution of hepatocellular carcinoma follows the distribution of hepatitis B virus chronic carriers and occurs predominantly in regions where hepatitis B virus infection is highly endemic, particularly China and Sub-Saharan Africa.

2 Epidemiological Classification:

Based on the prevalence of hepatitis B virus chronic carriers (individuals positive for hepatitis B virus surface antigen HBsAg for longer than six months) amongst adults in the general population, countries are divided into three main groups. Countries with a prevalence of chronic carriers less than 2% are classified as countries with a low endemicity of infection; those with a prevalence of chronic carriers between 2 – 5 % are classified as having an intermediate endemicity of infection; and countries with a prevalence of chronic carriers exceeding 5% are classified as having a high endemicity of infection.

3 Hepatitis B Virus Infection in the Region:

Hepatitis B virus infection is a major public health problem in the Middle East. Countries of the region fall into the three groups of low, intermediate and high endemicity of hepatitis B virus infection. However, the majority of countries in the region have an intermediate and high endemicity of chronic carriers. Nevertheless, there are marked geographical differences in the prevalence of HBV chronic carriers within these countries. In Saudi Arabia, for example, there are significant geographic differences in the prevalence of chronic carrier females, ranging from 7.3% in Najran to 25% in Jaizan.

4 Hepatitis B Virus Infection in Yemen:

There are a few studies that have investigated hepatitis B virus infection in Yemen. These studies were mainly hospital-based surveys carried out in the cities of Sanaa, Taiz, Hodeidah and Hajja. Study subjects were mainly blood donors, pregnant women and outpatients. All these cities are located in the northern governorates of Yemen. There is a lack of information on hepatitis B virus infection in southern, eastern, and rural parts of the country. Whether geographical differences exist between these regions is unknown.
Yemen has a high endemicity of hepatitis B virus infection. Surveys in Yemen show the prevalence of chronic carriers to range from 12% to 18%. The prevalence of hepatitis B virus infection ranges from 45% to 60%. It is the prevalence of chronic carriers that is important not the prevalence of hepatitis B virus infection.
The prevalence of chronic carriers amongst pregnant women in Yemen ranges from 12% to 17%. This indicates a potentially high rate of mother-to-child transmission of hepatitis B virus from chronic carrier mothers of hepatitis B virus to their infants.
Hepatitis B virus infection is the most frequent cause of acute viral hepatitis in Yemen, being responsible for 27% of cases. This is followed by hepatitis E virus responsible for 14% of cases. Hepatitis A virus, hepatitis C virus and hepatitis D virus are each responsible for 5.1%, 6.4% and 2.6%, respectively, of cases of acute viral hepatitis. These cases of acute viral hepatitis resulting from hepatitis B virus infection are likely to be only the tip of the iceberg. In Yemen, the majority of infections occur during childhood and only 10% of infected children develop symptoms of acute viral hepatitis. 90% of infected children will have had sub-clinical infections and have no symptoms. Additionally, only 30 – 40% of infected adults develop symptoms with the remainder also passing unnoticed.

5 Mode of Transmission:

HBV infection can be transmitted at 3 stages in life; around the time of birth (perinatal), during childhood, and in adult life. The main modes of transmission are mother-to-child, child-to-child (horizontal), sexual and parenteral. The role of each of these modes varies from one country to another. In developed countries (also countries with low endemicity of hepatitis B virus infection) sexual transmission and intravenous drug abuse in adolescence and adult life account for the majority of cases of hepatitis B virus transmission. In developing countries (countries with intermediate and high endemicity of hepatitis B virus infection), such as Yemen, mother-to-child and child-to-child

transmission during the early years of life are the major modes of transmission of hepatitis B virus infection.
Many people in the general population incorrectly believe that hepatitis B virus infection results from ingesting contaminated water and food. While these are sources of infection for hepatitis A and E viruses they are not sources of hepatitis B virus infection.
The main factors determining mother-to-child transmission are the mothers serological status for two markers, hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg). Being positive for hepatitis B surface antigen for longer than six months means that the mother is a chronic carrier of hepatitis B virus, and can therefore potentially infect her newborn infant. Being positive for hepatitis B e antigen means that the mother is highly infectious and indicates that there is a high risk of the infant acquiring a chronic infection. Approximately 70 - 90% of infants born to mothers who are hepatitis B e antigen positive become carriers. Determining the role either mode of transmission plays has important policy implications and may influence vaccination schedules. If mother-to-child transmission is found to be a common mode of transmitting HBV infection, this will indicate that vaccinating children against HBV at 6-8 weeks of age with the first dose of polio/DPT will be too late to prevent mother-to-child infection. Under such circumstances, one must aim to vaccinate newborns at birth or within 48 hours of delivery at the latest. In Yemen, where the majority of deliveries occur at home, introducing such a procedure will encounter numerous obstacles. One of the major concerns will be the difficulty of delivering vaccines to home deliveries occurring in remote areas considering that 75% of the Yemeni population lives in rural areas and is dispersed over approximately 65,000 rural hamlets.
Sexual and parenteral transmission are not major modes of infection in Yemen. Sexual transmission is probably uncommon due to the conservative culture of the Yemeni society. The role of parenteral transmission of hepatitis B virus infection in health institutions is expected to be currently limited as a result of routine screening of blood products. The number of intravenous drug abusers in Yemen remains low when compared to other regions.

6 Factors Associated with HBV Infection:

In Yemen increasing age, a history of jaundice, and a combined history of transfusion and surgery are associated with hepatitis B virus infection. Other factors found to be associated with HBV infection and carrier status in the Middle East are large family size, low socio-economic status, low educational status or contact with a jaundiced person.

7 Prevention:

The most common methods employed for the control of hepatitis B virus infection in the Middle East are active immunisation (universal and targeted), routine blood screening for hepatitis B virus, and ensuring safe injection practices. Due to beliefs that childhood transmission accounts for the majority of cases of hepatitis B virus infection, its high cost and the large proportion of household deliveries in many Middle Eastern countries, passive immunisation using Hepatitis B Immunoglobulin (HBIG) is uncommon in the Middle East. Due to cultural sensitivity safe sex is not officially promoted in most countries of the region.

7.1 Active Vaccination:

In 1982, a vaccine was licensed for active vaccination against hepatitis B virus infection. Hepatitis B vaccine is the first vaccine effective in preventing cancer or a sexually transmitted disease. Two types of vaccine are currently available, plasma derived and recombinant hepatitis B vaccine. Both types of vaccine have proven to be safe, highly effective, recently cost-effective, and available at a cost most countries can afford. As the success of treatment for hepatitis B virus infection remains low and antiviral therapy expensive, prevention of infection by vaccinating against hepatitis B virus infection remains the best option.

In 1991 the World Health Assembly recommended the integration of HBV vaccines to national immunisation programmes in countries with an intermediate to high endemicity by 1995, and to all countries by 1997. The main challenge facing developing countries is reaching highly deprived populations through a primary health care infrastructure that is in many cases exhausted and short of funds.

Currently recommended and implemented schedules of HBV vaccination vary, but generally follow a pattern of 2 –3 injections at monthly intervals followed by a booster dose 6 – 12 months after the initial dose. Immunisation is considered to have occurred if the concentration of antibody to hepatitis B surface antigen (anti-HBs) is greater than 10mIU/L. Most individuals should remain protected for 5 – 10 years with no need for booster doses. There is no need to do any post- vaccination tests.

In order to prevent mother-to-child transmission, infants born to carrier mothers should receive hepatitis B immunoglobulin immediately following delivery or hepatitis B vaccine as early as possible within the first 48 hours following delivery. Due to high cost, unavailability and logistical considerations of delivery, Hepatitis B immunoglobulin is not administered in most developing countries. On the other hand, vaccination at delivery appears to be an economically attractive option, particularly in developing countries. The most difficult challenge facing this option is how to identify children at risk and vaccinate children within 48 hours following delivery in developing countries with a high rate of mother-to-child transmission where the majority of deliveries occur at home.

The effectiveness of hepatitis B vaccine alone within 48 hours following delivery is nearly as effective as hepatitis B vaccine with hepatitis B immunoglobulin.

7.2 Active Vaccination in Yemen:

In 1998, the Ministry of Public Health introduced plasma derived hepatitis B vaccine then changed to recombinant vaccine both purchased from Cheil-Jedang Corporation in South Korea. The vaccine manufacturer Cheil-Jedang recommends giving double the dose to infants of carrier mothers. However, considering the high chronic carrier rates amongst women of childbearing age in Yemen and the lack of routine screening for chronic carriers of hepatitis B virus infection amongst pregnant women allowing the identification of children at risk, it is highly unlikely that the vaccine manufacturer's recommendations will be implemented. This leaves infants born to chronic carrier mothers at potential risk of acquiring perinatal infection.

The first, second and third doses of hepatitis B vaccine, in Yemen, are administered at 2, 3 and 9 months respectively. The first and second doses are administered at the same time as the first and second doses of DPT/Polio vaccines and the third dose with Measles, Mumps and Rubella. Ideally the first and second doses of hepatitis B vaccine should be given one month apart and the third dose given 7 months following the first dose. The recommendations for dose intervals should be implemented in order maximise vaccine effectiveness. If a dose is not given by its recommended date (due to parents forgetting, not going to a vaccination centre, or unavailability of vaccine), it should be given as soon as possible.

Data available from Yemen suggests that vaccine coverage has reached 42% for the first dose and 9% for the third dose of hepatitis B vaccine by one year of age. The lower uptake for the third dose may be due to the later administration of the third dose of hepatitis B vaccine given with MMR at nine months of age.

8 Study Objectives:

8.1 Primary Objectives:

1- Measure the prevalence of hepatitis B virus chronic carriers amongst Yemeni children aged 1-3 years old and estimate the proportion of these cases resulting from mother-to-child transmission.
2- Measure the prevalence of hepatitis B virus chronic carriers and highly infectious chronic carriers amongst Yemeni women of childbearing age and estimate the potential risk of mother-to-child transmission of hepatitis B virus from these women.
3- Investigate whether the prevalence of hepatitis B virus chronic carriers amongst children and mothers differs between geographic regions and governorates in Yemen.

8.2 Secondary Objectives:

1- Evaluate the effectiveness of the hepatitis B vaccination programme in preventing hepatitis B virus infection and carrier status amongst children vaccinated in the country's Expanded Programme on Immunisation (EPI).
2- Measure hepatitis B vaccination coverage amongst children aged 1-3 years old participating in the study.
3- Examine the pattern of hepatitis B virus infection and carrier status amongst children aged 4-9 years old in Yemen prior to the introduction of hepatitis B vaccination to these age groups and its influence on these cohorts of children.

9 Study Investigators:

This survey will be conducted by four study investigators in Yemen. Dr. Naseeb Qirbi is the principal study investigator and will be assisted by three other study investigators (one of which is you).
The study investigators will be divided into two teams during the household survey. Each team will consist of a qualified medical doctor and a qualified nurse or laboratory technician. One of the qualified medical doctors is the primary investigator. The second qualified medical doctor will be recruited based on having a minimum of three years medical experience and a reasonable relation and background with the governorates and villages surveyed. A different medical doctor may be selected for each of the governorates surveyed. Nurses or laboratory technicians will be recruited based on having a minimum of three years experience in a paediatric ward or active involvement in withdrawing blood samples from children and infants. A different nurse or laboratory technician may be selected for each of the governorates and/or primary sampling units surveyed. Study investigators must be trained on selecting households, filling questionnaire's, using vaccutainers for blood sampling, and all other aspects of the survey during a two-week workshop in Sanaa prior to initiating the survey.
The research is supervised by Professor Andy Hall, Head of Infectious Disease Epidemiology Unit, Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, University of London. Professor Andy Hall will come to Yemen to attend the first two or three weeks of the survey in order to supervise the

fieldwork and ensure that it is being conducted as planned. He will also advise on how to deal with unforeseen difficulties.

10 Study Methodology:

The research will consist of two parts. First, a population based cross-sectional household seroepidemiological survey of a representative sample of children aged 1-3 and their mothers. Study subjects participating in the cross-sectional survey will be children aged 1-3 years old and their mothers selected from five governorates representative of the country's overall population. A total of 1100 children and their mothers will be included in the sample population. After obtaining informed witnessed verbal consent from study participants an interviewer administered questionnaire will be completed and both mother and child will be bled and tested for markers of hepatitis B virus infection. This will provide information on the prevalence of infection and carrier status amongst children aged 1-3 and women of childbearing age, mother-to-child transmission of hepatitis B virus infection, vaccine coverage with hepatitis B vaccine, and geographic distribution of hepatitis B virus infection and carrier status. An analysis of individuals participating in the cross sectional survey of children aged 1-3 will evaluate the effectiveness of Expanded Programme of Immunisation hepatitis B vaccination in preventing hepatitis B virus infection and carrier status.
Secondly a population based cross sectional survey will be conducted amongst children aged 4-9 years old, randomly drawn from within the same households of children aged 1-3 in order to examine the pattern of hepatitis B virus infection and carrier status in unvaccinated children in Yemen. A total of 420 children will participate in the survey of the children aged 4-9 years old with 70 children randomly selected from each one of the six age groups extending from 4-9.

Study Settings:

The survey will be carried out in the following five Yemeni governorates:

1- Sanaa city municipality (political capital).
2- Aden city (economic capital).
3- Taiz governorate (excluding Taiz city).
4- Shabwa governorate.
5- Hodeidah governorate.

These governorates have been selected because they are considered representative of Yemen's geographic regions, tribes and socio-economic classes; include governorates from the Southern and Eastern regions of the country, and because of the feasibility of doing a household survey in these governorates.
Households will be selected from within these five governorates and any child aged 1-3 years old, regardless of sex, meeting the inclusion criteria whose parents witnessed verbal consent is obtained will be eligible to participate in the study.

Inclusion criteria for children aged 1 - 3:

All children aged 1-3 years and their mothers willing to participate in the survey from whom witnessed verbal consent is obtained.

Exclusion criteria for children aged 1 - 3:

1- Children and mothers refusing to participate in the study (children's exclusion will be based on their guardians refusal to allow them to participate in the study).
2- Seriously ill children.
3- Children and mothers with a history of bleeding disorders.

11 Household Sampling Methodology:

An equal number of children (220 children) will be selected from each one of the five governorates (see table).

Table
Governorate Sampling Distribution

Governorate	Total Sample Size
Sanaa	220
Aden	220
Taiz	220
Shabwa	220
Hodeidah	220
Total	1,100

Sanaa and Aden will represent urban populations whereas Taiz (excluding Taiz city), Shabwa and Hodeidah (excluding Hodeidah city) will represent rural populations. Within each governorate, five districts (in rural areas) or enumeration areas (in urban areas) will be randomly selected as primary sampling units with a fixed number of individuals selected from each district or enumeration area. Fourty four children aged 1-3 and their mothers will be randomly selected from each one of the five enumeration areas or districts in each governorate.

Different procedures will be followed for selecting children aged 1-3 in Sanaa and Aden (urban populations) than in Taiz, Shabwa and Hodeidah (rural populations).

11.1 Urban Populations:

In Sanaa City municipality and Aden, which are urban areas and for which maps are available, five enumeration areas will be randomly selected (based on probability proportional to size) as primary sampling units with a fixed number of 44 children aged 1-3 and their mothers selected from each enumeration area. Within these enumeration areas, sampling will be initiated from a street blindly pointed out from a map of this enumeration area. The first household to be sampled in the street will be selected based on numbering the households on the street and randomly selecting a household from the street. If the household is not inhabited by a child aged 1-3 or consent is not obtained (an eligible household), a coin will be tossed (to determine whether to go right of left) and every third household will be visited until an eligible household is identified. Once in an eligible household and after obtaining consent an interviewer administered questionnaire will be completed and both child aged 1 – 3 years and the mother will be bled and tested for serological markers of hepatitis B virus infection. Only one child aged 1 – 3 per mother will be included in the study sample. If more than one child aged 1 –3 per mother is present in the household, one of these children will be randomly selected, by tossing a coin, for inclusion to the sample population. If there is more than one mother with a child aged 1 –3 in the household, all mothers with a child aged 1 –3 present in the household will be included in the study sample. Upon the completion of surveying the household, neighbouring households will be surveyed including to the study population all eligible consenting households inhabited by a 1 –3 year old child. If no more eligible households can be identified within the neighbourhood another street in the same enumeration area will be blindly selected from the map of this enumeration area and the same process of household selection will be repeated until the target number of 44 children from this enumeration area has been reached. The same process will be repeated in all five enumeration areas of each of these urban survey domains (Sanaa City municipality and Aden).

Procedures for household selection from within enumeration areas in urban governorates:

1- Randomly select a street from the enumeration area by blindly pointing on a map of the enumeration area. If an intersection is pointed on randomly select one of these streets (by giving each street a number and randomly selecting one of these numbers using a calculator generating random numbers).
2- Number the households on that street starting with the first household on the right hand side of the street and ending with the first household on the left hand side of the street (relative to the study investigator standing at the beginning of the street).
3- Randomly select one of these households by using a calculator generating random numbers within the range of the number of houses on the street.
4- Approach the randomly selected household. After introducing yourselves to household inhabitants explain why you are there and ask the screening question (if the household is inhabited by a child aged 1-3 with the child's mother). If the household is inhabited by a child aged 1-3 and witnessed verbal consent is obtained take this household as the first household to be sampled. If the household is not

inhabited by a child aged 1-3 or consent is not given, leave the household, toss a coin to determine whether to go right or left (heads-right and tails-left) and visit every third household in that direction until an eligible consenting household inhabited by a child aged 1-3 is sampled (which will become the first household to be sampled).

5- Within households, only take one child aged 1-3 per mother. If more that one child aged 1-3 per mother is present, toss a coin to determine which child to include to the sample (heads-younger child and tails-older child).
6- If there is more than one mother with a child aged 1-3 in the household, sample all mothers with a child aged 1-3 present in the household.
7- Upon leaving the first household to be sampled, toss a coin to determine whether to go right or left, and visit neighbouring households in that direction until the target number of 44 children from that enumeration area has been reached.
8- If the target number of 44 children from that enumeration area (primary sampling unit) is not reached, blindly select another street from that enumeration area and repeat steps 1-7 until the target number of children aged 1-3 has been reached.
9- In non-consenting households inhabited by a child aged 1-3 (i.e. eligible but refusing to participate), always ask and write down the reason for refusal to participate in the survey.

11.2 Rural Populations:

Once the five districts from each of Taiz, Hodeidah and Shabwa have been randomly selected (based on probability proportional to size) as primary sampling units, villages and towns will be randomly selected from within these districts based on probability proportional to size. Following the selection of a village or town a suitable local facilitator will be identified and recruited to ensure the co-operation of the village inhabitants. Within these villages and towns households will be randomly selected. The first household to be sampled in villages or towns will be selected based on selecting a central point or street in the village, choosing a random direction from the central point, numbering households from the central point to the outer boundary of the village and randomly selecting a household from these numbered households. If the household is not inhabited by a child aged 1-3 or witnessed verbal consent is not obtained, a coin will be tossed (to determine whether to go right or left) and every third household in that direction will be visited until an eligible household is identified. Once in an eligible household and after obtaining consent an interviewer administered questionnaire will be completed and children aged 1 – 3 years and their mothers will be bled to test for serological markers of hepatitis B virus infection. Only one child aged 1 – 3 per mother will be included to the study sample. If more than one child aged 1 –3 per mother is present in the household, one of these children will be randomly selected, by tossing a coin, for inclusion to the sample population. If there is more than one mother with a child aged 1 –3 in the household, all mothers with a child aged 1 –3 present in the household will be included in the study sample. Once the first consenting household has been surveyed all the neighbouring households (starting with the nearest) in the village or town will be approached until the target number of 44 children and their mothers from this primary sampling unit has been reached. If village or town maps are available these will be used to mark surveyed households and streets in the village or town. Otherwise, you

should draw a map roughly showing surveyed streets and households in the village or town so that study investigators do not waste time revisiting and surveying households and streets that have already been surveyed. If the village or town has been completely surveyed and the targeted sample size from the primary sampling unit has not been reached, the team will move on to the next village or town selected from within the primary sampling unit based on probability proportional to size. The same process for selecting the first and subsequent households for sampling will be repeated in the next village or town.

Procedures for selection of households from within districts in rural governorates:

1- Go to central point in village. If there is more than one central point in the village randomly select one of these (by numbering the central points in the village and randomly selecting one using a calculator generating random numbers).
2- Randomly select a direction from the central point of the village by spinning an arrow.
3- Number the households in that direction from the central point to the outer boundary of the village.
4- Randomly select one of these households by using a calculator generating random numbers.
5- Approach the randomly selected household. After introducing yourselves explain to household inhabitants why you are there and ask the screening question (if the household is inhabited by a child aged 1-3 with the child's mother). If the household is inhabited by a child aged 1-3 and witnessed verbal consent is obtained take this household as the first household to be sampled. If the household is not inhabited by a child aged 1-3 or consent is not given, leave the household, toss a coin to determine whether to go right or left (heads-right and tails-left) and visit every third household in that direction until an eligible consenting household inhabited by a child aged 1-3 is sampled (which will be the first household to be sampled).
6- Within households, only take one child aged 1-3 per mother. If more that one child aged 1-3 per mother is present, toss a coin to determine which child to include (heads-younger child and tails-older child).
7- If there is more than one mother with a child aged 1-3 in the household, sample all mothers with a child aged 1-3 present in the household.
8- Upon leaving the first household to have been sampled, toss a coin to determine whether to go right or left, and visit the nearest neighbouring household in that direction until the target number of 44 children from that village or town has been reached.
9- If the target number of 44 children from that district is not reached, move on to the next village or town selected based on probability proportional to size in that district and repeat the steps from 1-8 until the target number of children aged 1-3 has been reached.
10- In towns (which are larger than villages) it may not be possible to count the number of households from the central point of the town (and actually there may be more than one central point) to the outer boundary of the town. In this case, count the number of mosques in the town, randomly select one of these (by numbering the mosques and

randomly selecting one of them using a calculator) to be the central point and repeat the steps from 1-8.

11- In non-consenting households inhabited by a child aged 1-3 (i.e. eligible but refusing to participate), always ask and write down the reason for refusal to participate in the survey.

12 Approaching and Accessing Households:

Upon approaching a household study investigators must first of all state that they are conducting a survey on hepatitis B virus infection and vaccination of children. They must then ask if there is a child aged 1-3 living in the household. If there is a child aged 1-3 living in the household the study team must request to meet the parents or guardian of the child.

Upon identifying and gaining access to a household occupied by a child aged 1-3 years old the study team (consisting of two individuals) must firstly introduce themselves to the head of the household and/or the mother of the child. In order to ensure that the same correct and complete information is given to each study participant, study investigators must use the information sheet to give the head of the household and/or the mother of the child a brief explanation that they are conducting a survey on hepatitis B virus infection and vaccination. They must explain how the household was selected, reassure on confidentiality and safety issues and encourage household inhabitants to participate in the survey. Potential study participants must be provided with sufficient information on potential risks and benefits to enable them to make an informed decision on whether or not to participate in the study. Study participants must also be informed that the visit to their household will involve two main procedures. The first is the completion of an interviewer administered questionnaire. The second is obtaining a blood sample from both mother and child to test for markers of HBV infection. Following this, household members should be encouraged to bring forward any questions they may have which should be answered by the study investigators. Literate parents/ guardians may be given a copy of the information sheet specifically prepared for the study.

Following the above study participants will be asked for their witnessed verbal consent for the mothers and child's inclusion in the study population of the survey. They must also be asked for permission to notify the health authorities of the results of identified chronic carriers of the virus.

Individuals participating in the survey will be subject to the completion of an interviewer administered questionnaire and the withdrawal of a blood specimen for investigating markers of HBV infection and carrier status. The mother should be the target person to be interviewed. If this is not possible the father or legal guardian of the child should be interviewed. The questionnaire will be filled by one of the study investigators. The study investigator will systematically go over the questions reading them out as written and fill the form according to the response of the person interviewed. Before starting to fill in the questionnaire the person interviewed should be asked about his/her preferences on the presence or absence of any individual in the room. During and after filling the questionnaire strict confidentiality must be ensured for the study participants responses and details. Completed questionnaires must be labelled with a unique identification

number for mother and child separately, and kept in a safe and inaccessible place until returning to base where they will be kept under lock and key.
Once the questionnaire has been completed first the mother and then the child should be bled aseptically using sterile disposable needles. Samples should be immediately labelled with the identical identification number that was placed on the questionnaire. The code number will ensure the anonymity of study participants when samples are transported, tested for markers of HBV infection, stored, and at the analysis stage.
In some of the households parents will be asked if a child aged 4-9 lives in the household. Any child 4-9 years old, regardless of sex, whose parents consent is obtained will be considered eligible to be included in this survey of children aged 4-9 years old. 420 children will be equally selected from the five provinces included in the cross-sectional survey and from the six age groups extending from 4-9 years (i.e. 70 children from each of the six age groups extending from 4-9 years).

Procedures upon gaining access to households:

1- When approaching a household state that you are there for the purpose of hepatitis B virus infection and vaccination.
2- Ask household inhabitants if the household is inhabited by a child aged 1-3 and mother.
3- Ask for permission to enter the household (for both study investigators in the team). If there is sensitivity toward entry of a male member of the team to the household the male member should wait outside.
4- Ask to meet the head of the household, father, or mother of the child.
5- The study investigators must introduce themselves to the head of the household and/or the parents of the child.
6- Use the information sheet to explain that you are there to survey hepatitis B virus infection (liver disease) and vaccination, and the survey's objectives.
7- Use the information sheet to explain to the head of the household and/or the mother or father of the child on how their household was selected.
8- Use the information sheet to reassure the head of the household and/or the mother or father of the child on confidentiality of their details, responses and results.
9- Use the information sheet to encourage the head of the household and/or the mother or father of the child to participate in the survey.
10- Use the information sheet to inform the head of the household and/or mother or father of the child about the general and personal benefits from the study.
11- Use the information sheet to inform study participants that the visit to their household will involve two main procedures. The first is the completion of an interviewer administered questionnaire. The second is obtaining a blood sample from both mother and child to test for markers of HBV infection. Inform study participants that in some cases you might also fill in a questionnaire and take a blood sample from a child aged 4 – 9 in the household.
12- If the head of the household and/or parents are literate give them the information sheet.
13- Encourage household members to ask any questions they may have.

14- Request witnessed verbal consent for the inclusion of the mother and child aged 1-3 to the study.
15- Request witnessed verbal consent for notifying the health authorities of the results of identified chronic carriers of the virus.
16- After completing the questionnaire and blood sampling of mother and child 1-3 ask if there is a child aged 4-9 in the household and request witnessed verbal consent for the inclusion of this child to the study population.

13 Who to request witnessed verbal consent from:

In order to ensure that the same correct and complete information is given to each study participant, study investigators must use an information sheet for the procedures above.
Study participants must be asked for their witnessed verbal consent for the mothers and child's inclusion in the study population of the cross-sectional survey. The reason verbal consent has been selected is because approximately 75% of the Yemeni population is illiterate and this is more pronounced in rural areas. Requesting the study participating to provide written consent will be difficult and impractical.
It must be attempted to obtain the consent of both mother and father of the child. If the father is not present the mother should be asked if the father is likely to object to their inclusion in the sample population, in which case an attempt should be made to obtain the fathers consent. In the event of either parent refusing inclusion of the child and mother in the study population, efforts should be made to address their concerns and alleviate their fears. If however, following this either parent refuses to participate in the study, the household members should not be included in the study population, with subjects refusing to participate being free to do so at their own wish. However, subjects should be asked for the reason of their refusal in order to assess if non-participants differ in any aspects from participants in the study population.
In some of the households of children aged 1-3, parents should be asked if a child aged 4-9 lives in the household. Any child 4-9 years old, regardless of sex, whose parents witnessed verbal consent is obtained should be considered eligible to be included in this survey of children aged 4-9 years old. A total of 420 children will be equally selected from the five provinces included in the cross-sectional survey and from the six age groups extending from 4-9 years.

14 Questionnaire:

Individuals participating in the survey of children aged 1-3 will be subject to an interviewer administered questionnaire. The questionnaire focusing on the child aged 1-3 and the mother asks about the child's details and vaccination history, mother, father, and the household's characteristics (refer to attached questionnaire for children aged 1-3, Annex I). It is divided into a section on baseline information, a section on characteristics of individuals from whom blood was drawn (mother and child), and a section on household characteristics and socio-economic status. The questionnaire does not include any sensitive or embarrassing questions.
Individuals participating in the survey of children aged 4-9 will also be subject to an interviewer administered questionnaire. The questionnaire focuses on the child aged 4-9 and asks about the child's details and vaccination history, mother, father, and the

household's characteristics (refer to attached questionnaire for children aged 4-9, Annex II). It is divided into a section on baseline information, a section on characteristics of the child, and a section on household characteristics and socio-economic status. This questionnaire also does not include any sensitive or embarrassing questions.
The mother must be the target person to be interviewed. If this is not possible the father or legal guardian of the child should be interviewed. The interview will be conducted in the study subjects household and the questionnaire will be administered and filled by one of the study investigators participating in the survey. During the interview and prior to filling the questionnaire respondents should be asked about their preferences on the presence or absence of subjects in the room. No more than two study investigators should be present in the room at the same time. Whilst the interview is being carried out individuals present from the study subjects side should be kept to a minimum, unless this runs contrary to the study subjects wishes. The study investigator must systematically go over the questions reading them out as written and filling the form according to the response of the person interviewed. When filling in the questionnaire you must be as consistent as possible in the way you read out the questions and avoid leading questions.
Completed questionnaires must be labelled with a unique identification number for mother and child separately (or child alone in the case of children aged 4-9), and kept in a safe and inaccessible place until returning to base where they will be kept under lock and key. Strict confidentiality must be ensured for the respondents personal and household characteristics and details and you must not discuss or reveal any of the study participants identity, details, characteristics or responses to anyone else other than the study investigators.
During the workshop prior to the survey you will be trained on how to conduct the interview and filling the questionnaire. You will receive a full explanation and description of every question in the questionnaire. If you find anything unclear regarding the questionnaire please discuss this with the primary investigator.

Procedures for Questionnaire:

1- No more than two study investigators should be present in the room at the same time (unless the additional person present is the primary investigator or his supervisor).
2- Aim to interview the mother of the child aged 1-3. If this is not possible interview the father/legal guardian of the child.
3- Before starting to fill in the questionnaire ask the mother about her preferences on the presence or absence of subjects in the room and take these into consideration.
4- Whilst the interview is being carried out individuals present from the study subjects side should be kept to a minimum, unless this runs contrary to the study subjects wishes.
5- Fill in the questionnaire with a black or blue pen. Do not use pencils or red pens to fill in the questionnaire.
6- Write clearly and neatly on the questionnaire (write numbers in English. Do not confuse 1 and 7, 4 and 9, 6 and 9, 2 and z, 5 and s).
7- Do not write or mark on the coding column on the right of the questionnaire.
8- The study investigator conducting the interviewer administered questionnaire must systematically go over the questions reading them out as written and fill- in the form

according to the response of the person interviewed. When filling in the questionnaire you must be as consistent as possible in the way you read out the questions and avoid leading questioning unless absolutely necessary.

9- When a questionnaire has been completed it must be labelled with a unique identification number for child and mother separately. These same identification numbers will be used to label the child's and mothers blood specimens.

10- After completing the questionnaire revise it and check for any missing information or skipped questions.

11- Place the questionnaire in the folder for completed questionnaires during fieldwork and keep it in a safe and inaccessible place until returning to base where questionnaires will be kept under lock and key.

12- Strict confidentiality must be ensured for the respondents personal and household characteristics and details and you must not discuss or reveal any of the study participants identity, details, characteristics or responses to anyone else other than the study investigators.

13- If more than one mother with a child aged 1-3 is present in the household, do not allow the other mothers who will be interviewed to attend the interviewer administered questionnaire with other mothers.

15 Blood Sampling:

Following the completion of the interviewer administered questionnaire both mother and child aged 1-3 must be bled to test for serological markers of hepatitis B virus infection. Venesection is the main procedure that may cause discomfort to subjects participating in the study. This is more likely amongst children than adults. Children will be more likely to perceive the study investigators in their household as strangers and dislike contact with them. This is expected to reach its peak when the child is held to obtain the blood sample. Attempts should be made to minimise this occurring especially whilst the questionnaire is being completed. During the interview the study team should attempt to be friendly, nice and playful with the child. Before the blood sample is withdrawn the mother should be instructed to hold the child giving him/her a sense of security. When withdrawing blood samples from children both study investigators with the co-operation and permission of the mother should calmly and friendly hold the child in a secure and fixed position. This is to prevent injury that may result from sudden movements or attempts by the child to remove the needle that may occur at the time of blood withdrawal or due to the sting of the needle.

Procedures for withdrawing the blood sample:

1- 5 millilitre and 2 millilitre vaccutainers, respectively, will be used for obtaining blood samples from mother and child.

2- If vaccutainers are not used for collecting blood samples, syringes of the same capacity will be used for collecting the blood specimen, which will then be transferred into a vaccutainer. 23 gauge needles 15 millimetres in length should be used in children.

3- Any venepuncture must be performed using a sterile needle or butterfly and vaccuntainer (or syringe).
4- According to circumstances aim to obtain the first specimen from the mother and the second specimen from the child.
5- Label vaccutainer (or blood container) with the corresponding patient identification number (for children ID NO 1001 – 1200, for mothers 4001 – 4200).
6- Attach needle to holder. Push the evacuated tube into the holder, but do not fully penetrate the rubber seal with the back end of the needle. The front of the needle should still be sheathed (if using a needle and syringe attach the needle to syringe with needle still sheathed).
7- Position the patient, sitting or lying comfortably, with one arm outstretched and supported. The elbow should be fully extended, and the hand should be lower than the elbow.
8- Before bleeding the child, ask the mother to hold the child in her lap firmly with the child's facing away from the study investigators and, with one arm outstretched and supported. The elbow should be fully extended, and the hands should be lower than the elbow. The other hand and both lower limbs should be held and fixed in position by the second study investigator not withdrawing the blood sample.
9- Apply the tourniquet just above the bend in the elbow.
10- Locate a vein near the bend in the elbow. It is best to use lateral veins to avoid injuring the artery or nerve on the medial side of the arm.
11- The vein should be felt not just seen. If veins are not prominent, gently slap, rub or warm the area of skin over them. This will cause them to stand out. Also, tell the study participant (if mother) to alternatively close and open her fist until a vein becomes more prominent. Feel along the path of the vein with your fingers. The needle should be inserted at the first point (closer to the child's or mothers hand than to shoulder)) at which the vein can be felt.
12- Once a vein has been located and the site of venepuncture determined, clean the skin over the vein with an antiseptic swab. Rubbing well with an antiseptic soaked swab will also make the vein more prominent. Do not touch the area of skin after it has been disinfected. If that area of skin is touched again (e.g. in order to feel the vein) it must be wiped again with an antiseptic soaked swab.
13- Leave the disinfected skin to dry for 1 minute.
14- Wear disposable gloves.
15- Pick up the needle and vaccutainer (or syringe) and remove the sheath from the needle. Hold the needle and vaccutainer (or syringe) in one hand. With the other hand, hold the skin taut just below the vein to prevent the vein from moving.
16- Hold the needle in line with the vein (pointing towards the shoulder) and at a 15 degrees angle with the skin push the needle carefully and directly into the vein (5 – 10 millimetre).
17- Release the tourniquet before withdrawing the blood sample.
18- If using a vaccutainer steady the holder with one hand, and with the other hand push the vaccutainer all the way into the holder). The tube will automatically fill, withdrawing a pre-determined volume of blood, when the rubber seal is penetrated by the back end of the needle. If another tube is to be filled (e.g. EDTA or Heparin container for mothers), remove the first tube (without removing the needle from the

vein), and with one hand steady on the holder, push the second tube all the way into the holder.

19- If using a syringe keep the syringe steady with one hand and slowly (to avoid the vein collapsing) draw back the plunger with the other hand. Blood will enter the syringe if the needle is in the vein. Continue to withdraw the plunger until the syringe has filled (with 5 millilitres of blood in mother and 2 millilitre of blood in children).
20- If you have not already done so, MAKE SURE that you release the tourniquet before withdrawing the needle from the vein.
21- After collecting the targeted amount of blood slowly withdraw the needle from the vein, maintaining the same angle with the skin.
22- Firmly apply a clean cotton wool to the site of venepuncture for 3 minutes, preferably with the blood sampled subjects arm above their head.
23- Ensure that bleeding has stopped from the site of venepuncture and that the household has not become contaminated with any drops of blood.
24- Apply a bandage to the site of venepuncture if bleeding has not stopped after 3 minutes.
25- If you are not successful at your first attempt of venepuncture, relax and repeat the attempt once again after 5 minutes (if the child is crying wait until s/he calms down). Meanwhile reassure the parents, especially the mother.
26- Do not reuse needles, syringes or vaccutainers.

16 Procedures for handling blood sample and sampling equipment in the household:

1- Remove holder from vaccutainer or needle from syringe.
2- Gently handling blood specimens in order to prevent haemolysis.
3- If a syringe has been used, expel the blood into the labelled vaccutainers (labelled with the study participants identification number) by slowly pushing the plunger into the syringe (do not squirt blood), and securely put cap on blood container.
4- Dispose of needle, syringe, holder, cotton wool, packaging and all equipment used in safe disposable box. The disposable box should be taken with the team back to base and disposed of in the local health centre at the end of each working day.
5- In the case of EDTA or heparin holders for mothers serum mix blood with anticoagulant by gently inverting the tube several times.
6- Place the blood samples in racks.
7- Place racks in normal temperature transportation box maintaining temperature between 10 – 40 degrees centigrade for 30 minutes until clotting has occurred (to protect the stability of immunoglobulins).
8- Transfer the samples into icebox after 30 minutes have passed from the collection of the blood specimen. If clotting has not occurred yet, leave blood samples in normal transportation temperature box for another 30 minutes.
9- Count all remaining and make sure that you have not left any equipment in the household. Under no circumstances should used or unused items used in the blood sampling process be left in the household.

17 Vaccinating children and mothers:

1- If vaccine is available and when eligible vaccinate mother and child (children will be considered eligible if they have not received all three doses of hepatitis B vaccine). Give priority of vaccination to children.
2- Hepatitis B vaccine should be administered intramuscularly.
3- Mothers receive double the dose of children (mothers receive 3 micrograms and children receive 1.5 micrograms of hepatitis B vaccine).
4- Mother's should receive the vaccine in the deltoid muscle and children on the lateral aspect of the thigh.
5- Take hepatitis B vaccine out of the cold-box and make sure it has not frozen.
6- Withdraw the corresponding vaccine dose into the syringe.
7- Before vaccinating the child, ask the mother to hold the child in her lap firmly with the child facing away from the study investigators and, with the lower limb extended and supported. The other lower limb and both upper limbs should be held and fixed in position by the second study investigator not giving the injection (i.e. implement the same procedures as when withdrawing the blood sample).
8- The lower limb to be injected should be fully exposed.
9- Disinfect the skin at the site of injection using an antiseptic swab.
10- Leave the skin to dry for 1 minute.
11- Check that the syringe with the correct dose of vaccine has no air in it.
12- Perpendicular to the surface of the skin, insert the needle 2 centimetres deep into the muscle.
13- Pull on the plunger slightly, to ensure that the needle in not in a vein. If it is in a vein blood will appear in the syringe. If this happens, slowly and slightly pull the needle backwards and pull on the plunger again to ensure that the needle is not in a vein.
14- After ensuring needle is not in a vein push on the plunger to inject the dose of the vaccine.
15- Carefully withdraw the needle from the muscle, maintaining the same angle of the needle with the skin.
16- Firmly apply a clean cotton wool to the site of injection for 1 minute.
17- Ensure that bleeding has stopped from the injection site.
18- Apply a bandage to the injection site if bleeding has not stopped.
19- If the child has a vaccination record card available write the date and dose of the hepatitis B vaccine given to the child. If the child does not have a vaccination record available s/he should be given one with the date and the number of the dose the child has been given.
20- Dispose of needle, syringe, cotton wool, packaging and all equipment used in safe disposable box. The disposable box should be taken with the team back to base and disposed of in the local health centre at the end of each working day.
21- Count and make sure that you have not left any equipment in the household. Under no circumstances should used or unused items used in the blood sampling process be left in the household.

18 Procedures for field transportation and field storage of blood:

In order to ensure the safety of study subjects, study investigators and environment the following measures should be strictly implemented:

1- For transportation, blood samples will be collected in a sealed vaccutainer (or plain sealed blood container) and/or sealed EDTA/heparin blood container. This will prevent any spillage, leaking or bacterial contamination of blood.
2- After allowing 30 minutes for blood clotting at temperatures between 10 – 40 degrees centigrade in a normal room temperature storage box, vaccutainers and/or blood containers should be transferred into pre-cooled iceboxes.
3- If 30 minutes after collection the blood has not clotted, allow another 30 minutes before transferring to an icebox. Blood should not be refrigerated until a firm clot has formed.
4- Sealed vaccutainers and/or EDTA blood containers will be held in racks and stored in a mobile icebox. The lower half of the icebox will be filled with ice.
5- In order to ensure that the blood samples do not freeze, the racks will be kept stacked above and avoiding contact with the ice packs placed in the bottom of the icebox.
6- Stability of blood specimens at any constant temperature from –196 to 20 degrees centigrade is better than at changing temperatures, especially freeze thaw changes. Try to maintain the temperature of specimen storage at a constant temperature and avoid exposing samples to heat.
7- During transportation care must be taken to avoid shaking of the blood samples on bumpy roads, in order to minimise mixing of serum with cells and products of haemolysis. Stability of immunoglobulins is also reduced if they are subject to shaking or bouncing.
8- During transportation care must be taken to avoid exposing iceboxes to sunlight (by covering and concealing them).
9- During transportation care must be taken to keep iceboxes upright.
10- During transportation checks should be made to make sure that the ice in the iceboxes has not melted.

19 Procedures for base handling and base storage of blood specimens:

1- Upon arrival to base, blood samples should be centrifuged for 15 minutes at 700 x g.
2- Serum should be separated from the residual blood clot using pipettes. Two serum samples should be collected from each container with an equal volume of serum placed in each of the two serum holders (labelled with the identification number).
3- The two serum specimens from each study subject should be stored in a freezer at – 20 degrees centigrade.
4- Unused hepatitis B vaccine should be placed in a refrigerator.
5- The freezer must be in a safe inaccessible place and locked with a key when possible.
6- The freezer must be checked that it is working properly every morning before the survey and when adding the new batch of serum samples to it in the evening.
7- No items other than the serum samples should be stored in the freezer.
8- Throughout the process of handling blood study investigators should be wearing disposable gloves.

9- Residual products and containers must be disposed of safely at the local health centre when possible.
10- Serological tests on the children's serum will be carried out according to the following plan:
All children's sera will be tested for hepatitis B surface antigen (HBsAg). Children positive for HBsAg will be further tested for Immunoglobulin M antibody to hepatitis B core antigen (IgM anti-HBc). Children negative for HBsAg will be tested for total antibody to hepatitis B core antigen (anti-HBc).
Vaccinated children will be tested for antibody to hepatitis B surface antigen (anti-HBs), which will be measured in milli-international units per litre (mIU/L).
11- Serological tests on the mother's serum will be carried out according to the following plan:
All mother's sera will be tested for HBsAg. Mothers positive for HBsAg will be further tested for hepatitis B e antigen (HBeAg) and hepatitis B virus DNA (HBV DNA Quantitative) concentration. Mothers negative for HBsAg will be tested for anti-HBc.

20 Safety of study investigators:

1- Study investigators participating in the study will be screened for hepatitis B surface antigen to exclude them being carriers, and thereby reducing the risk of infecting study participants.
2- Study investigators, particularly those handling blood will be asked if they have been vaccinated against HBV. Those who have not been vaccinated will be advised to get vaccinated as early as possible before the fieldwork is initiated, thereby reducing the risk of becoming infected.
3- Study investigators who have been vaccinated will be tested for anti-HBs. If their anti-HBs level is lower than 10 mIU/L they will be given a booster dose of hepatitis B vaccine.
4- Study investigators must wear disposable gloves whenever handling blood or blood products in the households, during transportation, or at base.
5- White coats should be worn when handling blood or blood products in the households, during transportation, or at base.
6- Extra care must be taken when handling sharp objects such as needles.
7- Antiseptic (eg. Dettol) must be available at all times of handling blood or blood products and must be used for immediate decontamination of hands if exposed to blood or pricked by needles.
8- Hands should be washed using soap or antiseptic after blood sampling or vaccine administration.
9- All unpacked needles, whether new or used, should be re-sheathed and discarded into safety boxes.
10- All syringes, holders, and packaging used in the blood sampling process must be discarded into safety boxes.
11- Study investigators (mainly the medical doctor) must check that no blood sampling equipment (new or used) is left in the household before leaving it.

12- When travelling to malaria endemic areas you are advised to take the necessary anti-malarial prophylaxis prior to travelling to high risk areas.

21 Daily Checklist before departing from base:

On the night before the day in which household visits will be carried out, all the items and equipment needed on next days survey should be prepared and confirmed using a checklist.
Each day's fieldwork requires the following:

1- 20 questionnaires for children aged 1-3 years old.
2- 20 questionnaires for children aged 4-9 years old.
3- 50 vaccutainers, holders and needles.
4- 50 two millilitre syringes, 50 fifteen millilitre needles and 50 butterflies.
5- 50 five millilitre syringes and 50 needles.
6- 20 labels for children aged 1-3, 20 labels for mothers, and 20 labels for children aged 4-9.
7- 60 antiseptic swabs.
8- 60 bandages.
9- 40 infant doses of hepatitis B vaccine.
10- Cotton wool.
11- 2 racks.
12- 6 blue pens.
13- 4 writing pads.
14- Antiseptic disinfectant (Dettol).
15- 12 bottles of water.
16- 1 normal room temperature storage box.
17- Icebox filled with ice on the morning of the journey.
18- Folder of new questionnaires for children aged 1-3.
19- Folder of new questionnaires for children aged 4-9.
20- Folder for filled in questionnaires of children aged 1-3.
21- Folder for filled in questionnaires of children aged 4-9.
22- Paper for drawing maps.
23- Log book and survey diary.
24- One 20 litre container of water.

22 Responsibilities:

The person in-charge of the fieldwork is the primary investigator. He has the right to monitor and evaluate study investigators conduct. He also has the right to excuse study investigators of their duties and participation in the survey if he has reason to do so.

It is the responsibility of the medical doctor to do the following:

1- Prepare the equipment and supplies on the night before everyday of the survey and go over the supplies checklist.

2- Check that the freezer is working properly every morning before the survey and when adding the new batch of blood samples to it in the evening.
3- Fill the icebox with ice on the morning of the survey just prior to departure.
4- Fill in the household survey questionnaire (unless the medical doctor is a male and has been refused entry to the household).
5- Answer any health related questions household members may raise whilst visiting the household.
6- Hold the limbs of the child aged 1-3 and 4-9 whilst the second study investigator is performing venepuncture (unless it is the doctor who is performing the venepuncture, in which case the nurse or laboratory technician should hold the child's limbs).
7- Make sure that there is no new or used equipment used for blood sampling left in the household prior to leaving the household.
8- Check that questionnaires and blood samples have been correctly labelled before leaving the household.
9- Draw a map of surveyed parts and households of villages.
10- Observe and monitor the serum container labelling process at base.

It is the responsibility of the nurse or technician to do the following:

1- Prepare the blood sampling equipment in the household (vaccutainers, holders and needles, syringes and needles, butterfly, swabs, bandages and labels).
2- Withdraw the blood sample from the children and mothers in the household.
3- Ensure that bleeding has stopped from the site of venepuncture.
4- Apply a bandage if necessary.
5- Dispose of used equipment in safety disposable box.
6- Place the blood samples in racks and then in the room temperature storage box and leave them in it for 30 minutes to allow clotting.
7- Administer hepatitis B vaccine to children and mothers.
8- After 30 minutes check that blood clotting has occurred and transfer the samples from the storage box to the icebox (if clotting has not occurred leave the samples in the room temperature storage box for a further 30 minutes).
9- Check on the samples and make sure that they have not been tipped over, broken or spilt, and that the ice has not thawed.
10- Upon arrival to base they must centrifuge the samples and place them is two labelled serum holders.
11- Place the serum holders in the freezer.
12- Safely dispose of used vaccutainers.

23 Quality Control:

1- Everyday a minimum of 12 eligible and consenting households should be surveyed and samples from these households obtained (6 households per study team).
2- The progress and quality of work of each study team and investigator will be monitored and evaluated.
3- Each investigator should check the questionnaire after filling it for missing information or skipped questions.

4- Everyday upon returning to base the primary investigator should check and code questionnaires.
5- Study investigators are obliged to consider all information obtained from study participants strictly confidential and must not disclose of such information to other parties.
6- Study investigators are obliged to consider all the procedures related to the survey strictly confidential and must not disclose such information to any other party.

24 Working hours and holidays:

Work will be carried out 6 days a week (Saturday to Thursday).
Friday is a holiday although meetings may be held with study investigators on this day during the fieldwork in rural areas.
Work starts at 8:30 am and ends at 6:30 pm (working hours may be longer if there is a delay in transportation or a backlog of work).

25 Accommodation:

You will be provided with accommodation during the fieldwork. This will not include cost of any phone calls, meals or room orders you make. If accommodation includes any free meals you will be informed of this.

26 Salary:

Each study investigator will be paid a salary on a per diem basis (based on days spent in the field). Study investigators will not be paid for transit days spent between surveys in separate governorates.

27 Supervision:

Approval for conducting the survey has been obtained from the Ethics Committee at the London School of Hygiene and Tropical Medicine (University of London) and The Minister of Public Health.

28 Disclamier:

The primary study investigator, University of London, and University of Taiz will not be held responsible for any illness, injuries, losses, or death that may occur to study investigators participating in the survey throughout the period.