UNIVERSITI PUTRA MALAYSIA

EVALUATION OF POST INFANT HEPATITIS B VACCINATION AMONG HEALTHY VACCINEES AND MOLECULAR DETECTION OF OCCULT HEPATITIS B AMONG HEALTHY VOLUNTEERS

HUDU ABDULLAHI SHUAIBU

FPSK(M) 2014 16

EVALUATION OF POST INFANT HEPATITIS B VACCINATION AMONG HEALTHY VACCINEES AND MOLECULAR DETECTION OF OCCULT HEPATITIS B AMONG HEALTHY VOLUNTEERS

By

HUDU ABDULLAHI SHUAIBU

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

JANUARY 2014

DEDICATION

This thesis is dedicated to the loving memory of my late father Mallam Hudu

His Silent inspiration, encouragement and guidance still linger on.

Abstract of thesis presented to the senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

EVALUATION OF POST INFANT HEPATITIS B VACCINATION AMONG HEALTHY VACCINEES AND MOLECULAR DETECTION OF OCCULT HEPATITIS B AMONG HEALTHY VOLUNTEERS

By

HUDU ABDULLAHI SHUAIBU

January 2014

Chair: Prof. Zamberi Sekawi, MPath

Faculty: Medicine and Health Sciences

Hepatitis B virus is the commonest chronic viral pathogen affecting about 2 billion people worldwide. Of these, 378 million are chronic carriers. In Malaysia, 2.4 million people have been estimated to be hepatitis B carriers and this population continues to be a potential source of infection, to tackle these more than 100 countries adopted National policy on infant hepatitis B vaccination including Malaysia and it has been shown to effectively control the diseases even in endemic areas. The production of hepatitis B surface antibodies (anti-HBs) has been reported to confer long-term protective immunity; these antibodies are produced in response to vaccination or exposure to HBV infection. Hepatitis B core antibody (anti-HBc) on the other hand, are detected in almost all individuals that have been previously exposed to the virus.The success of childhood vaccination against hepatitis B relies mainly on persistence of immunity to adulthood, however, mutation in the surface antigen is becoming a global challenge because these mutants seldom respond to the currently available vaccine and escaped been detected by current serological screening methods. At the moment, there is lack of information regarding the assessment of HBV vaccination in Malaysia; moreover, evidences are scanty regarding occult hepatitis B infection among Blood donors and vaccinees. It is against this background that the present study was carried out with the following objectives; (i) to evaluate the persistence of immunity following childhood hepatitis B vaccination among vaccinated undergraduate Student. (ii) to investigate the prevalence of isolated hepatitis B core antibody among vaccinated undergraduate Students cohort (iii) to detect occult hepatitis B viral infection among Blood donors and undergraduate students using serological and molecular methods and (iv) to perform molecular characterization of occult hepatitis B virus using bioinformatics tools.

This study involved two population, these are undergraduate students vaccinated at infant and blood donors. Objective one and two are related to the former while objectives three and four are related to the later. Sample size was calculated using sample size formula by Daniel, 1999 for estimating minimum sample size in cross-sectional study. Minimum sample size for each of the study population was found to be 379 and 153 for undergraduate vaccines and donors respectively. However, there were 402 undergraduate students that were eligible for this study and all were included while the sample size of the blood donor was also increase to 1000 to increase the precision of the sample.

Serum samples from both vaccinated undergraduate students and the blood donors were tested for HBsAg and anti-HBc while only the undergraduate vaccines were tested for anti-HBs pre and post vaccine booster using commercially available Enzyme-linked Immunosorbent Assay (ELISA). A booster dose of 20µg recombinant hepatitis B vaccine (Euvax B, Sanofi Aventis, Malaysia) was administered to individuals with negative anti-HBs (<10 IU/L) and their anti-HBs status was evaluated one month after the booster dose. To further elucidate the genetic characteristic of hepatitis B variant, all anti-HBc positive and HBsAg negative samples from both undergraduate vaccines and blood donors were selected for hepatitis B surface mutation analysis. The DNA was extracted from all anti-HBc positive samples and Nested PCR was done using two set of primers targeting *S*-gene region of hepatitis B envelope proteins. Samples with positive HBV DNA were purified, sequenced and subjected to bioinformatics analyses.

The results showed that none of the samples were found to be HBsAg positive, while 5.5% were found to be anti-HBc positive. For the anti-HBs of vaccinated undergraduate student the results showed that 83.9% were anti-HBs positive (> 10 IU/L) pre booster dose, majority (67.9%) of which received three doses of the vaccine and 94% anamnestic response post booster dose with only nine non-responders and were given two doses of the vaccine to receive the third dose in six month time. All the 55 anti-HBc positive samples were found to be HBV DNA positive by nested PCR. Sequence analysis of the amplified product revealed a 99% identity to HBV with most of the local isolates having close phylogenetic relationship with HBV isolate 14 (JX869999) from Panama. This isolate belongs to genotype B, serotype adw^2 and was isolated from plasma of Chinese population living in Latin region of Panama.

This study therefore, reveals the persistence of immunity for an average of 21 years post primary vaccination with recombinant hepatitis B vaccine and 67.9% of the vaccinees with protective anti-HBs (>10 IU/L) received three doses of the vaccine with a significant association between number of doses receive and persistence of immunity (P<0.05) which is similar to a recent finding in UK. Thus, the presence of immune memory post-infant vaccination has been confirmed in this study, as well as other related studies with anamnestic response, indicating persistence of memory beyond the duration of circulating antibodies. The rate of anti-HBc-positive subjects among the vaccinated undergraduate students was found to be 5% with isolated anti-HBc in 0.9% of the vaccinees. All the four isolated anti-HBc response to a single dose of recombinant hepatitis B vaccine which suggests primary infection with

mutant variant of HBV. Hepatitis B viral DNA was detected from all the 55 anti-HBc positive, HBsAg negative serum using nested PCR, which is in line with previous studies that demonstrated similar finding in serum, mononuclear cells and liver samples. Based on sequence and phylogenetic relationship of our HBV isolates and reference isolates from other parts of the world, we found that all our isolates belong to genotype B. Another interesting finding from this study is the occurrence of occult hepatitis in 3 % and 2 % of the blood donors and undergraduate students respectively. All hepatitis B core antibodies positive were found to be HBV DNA positive thus, indicating the reliability of hepatitis B core antibodies in screening for occult hepatitis B infection, especially in low endemic regions while nucleic acid amplification testing (NAT) is the recommended screening test in high endemic regions. Bioinformatics analysis revealed that mutation at position 16 of the amino acid is common to all the 55 isolates with substitution of Glutamine (Q) with Lysine (K) in 53 of the isolate, while the remaining 2 isolates have Arginine and stop codon respectively. A total of 105 amino acid mutations, were found in all the 55 isolates.

In conclusion, the evidence presented in this study, reveals persistence of both humoral and cellular immunity for an average of 21 years after infant vaccination and presence of isolated anti-HBc in vaccines which response to a single dose of recombinant hepatitis B vaccine suggesting primary infection with mutant variant of HBV despite the presence of cellular immunity against the none mutant hepatitis B virus. The presence of HBV DNA in all the anti-HBc positive samples may suggest the reliability of the use of hepatitis B core antibodies in screening for occult hepatitis B infection. All the 55 sequences in this study belong to genotype B and have various mutations in different regions of the *S* gene with the most common mutations found in this study are substitution of Glutamine at position 16 with lysine resulting in nonfunctional hepatitis B surface antigen therefore; mutation outside the *a* determinant region might play a role in hepatitis B surface antigen detection failure.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah master Sains

PENILAIAN POST BAYI HEPATITIS B VACCINATION KALANGAN VACCINEES SIHAT DAN MOLEKUL PENGESANAN GHAIB HEPATITIS B KALANGAN SUKARELAWAN SIHAT

Oleh

HUDU ABDULLAHI SHUAIBU

January 2014

Pengemsi: Prof. Dr. Zamberi Sekawi, MPath

Fakulti: Perubatan dan Sains Kesihatan

Hepatitis B virus adalah patogen utama penyakit kronik virus menjejaskan kira-kira 2 bilion orang di seluruh dunia. Daripada jumlah ini, 378 juta adalah pembawa kronik. Di Malaysia, 2.4 juta orang telah dianggarkan pembawa hepatitis B dan penduduk ini terus menjadi sumber yang berpotensi jangkitan, untuk menangani lebih 100 negara mengamalkan polisi Kebangsaan bayi vaksinasi hepatitis B termasuk Malaysia dan ia telah terbukti berkesan mengawal penyakit walaupun di kawasan endemik. Pengeluaran antibodi hepatitis B permukaan (anti -HBs) telah dilaporkan memberi imuniti perlindungan jangka panjang; antibodi ini dihasilkan sebagai tindak balas kepada suntikan atau pendedahan kepada jangkitan HBV. Hepatitis B antibodi teras (anti- HBC) di sisi lain , yang dikesan dalam hampir semua individu yang telah sebelum ini terdedah kepada kejayaan virus.The yang vaksinasi zaman kanak-kanak menentang hepatitis B bergantung terutamanya pada kegigihan imuniti kepada dewasa, bagaimanapun, mutasi dalam antigen permukaan menjadi satu cabaran global kerana mutan ini jarang bertindak balas terhadap vaksin yang sedia ada dan melarikan diri dikesan oleh kaedah pemeriksaan serologi semasa. Pada masa ini , terdapat kekurangan maklumat mengenai penilaian vaksin HBV di Malaysia; lebih-lebih lagi , bukti-bukti adalah kikir mengenai ghaib jangkitan hepatitis B di kalangan penderma darah dan vaccinees . Ia adalah bertentangan dengan latar belakang ini bahawa kajian ini telah dijalankan dengan objektif seperti berikut : (i) Menilai kegigihan imuniti berikut zaman kanak-kanak Hepatitis B vaksinasi di kalangan Pelajar ijazah pertama vaksin . (ii) Untuk menyiasat kelaziman terpencil hepatitis B antibodi teras di kalangan vaksin ijazah Pelajar kohort (iii) Untuk mengesan ghaib hepatitis B jangkitan virus di kalangan penderma darah dan sarjana muda menggunakan serologi dan kaedah molekul dan (iv) Untuk melaksanakan pencirian molekul hepatitis ghaib B virus menggunakan alat bioinformatik

Kajian ini melibatkan dua penduduk , ini adalah pelajar ijazah pertama vaksin pada bayi dan darah penderma. Satu matlamat dan dua berkaitan dengan bekas manakala objektif tiga dan empat berkaitan dengan kemudian. Saiz sampel dikira menggunakan saiz sampel formula oleh Daniel , 1999 untuk menganggarkan saiz sampel minimum dalam kajian keratan rentas. Saiz sampel minimum bagi setiap penduduk kajian didapati 379 dan 153 untuk vaksin ijazah dan penderma masing-masing . Walau bagaimanapun , terdapat 402 pelajar ijazah yang layak untuk kajian ini dan telah dimasukkan semasa saiz sampel penderma darah juga meningkat kepada 1000 untuk meningkatkan ketepatan sampel.

Serum dari kedua-dua pelajar ijazah pertama vaksin dan penderma darah telah diuji untuk HBsAg dan anti- HBC manakala hanya vaksin ijazah sarjana muda telah diuji untuk anti -HBs pra dan vaksin pasca penggalak menggunakan boleh didapati secara komersial Enzim berkaitan imunoserapan assay (ELISA). Satu dos booster 20μg rekombinan vaksin hepatitis B (Euvax B, Sanofi Aventis, Malaysia) telah diberikan kepada individu yang negatif anti -HBs (<10 IU / L) dan status anti -HBs mereka telah dinilai satu bulan selepas dos penggalak . Untuk menjelaskan lagi ciri genetik hepatitis B varian , semua anti- HBC sampel negatif positif dan HBsAg dari kedua-dua vaksin ijazah dan penderma darah telah dipilih untuk analisis hepatitis B permukaan mutasi. DNA tersebut telah diekstrak daripada semua anti- HBC sampel positif dan Bersarang PCR telah dilakukan dengan menggunakan dua set primer mensasarkan kawasan S - gen hepatitis B protein sampul surat. Sampel dengan positif HBV DNA telah disucikan , urutan dan tertakluk kepada bioinformatik analisis.

Hasil kajian menunjukkan bahawa tidak ada sampel telah didapati HBsAg positif , manakala 5.5 % telah didapati anti- HBC positif. Bagi anti -HBs daripada pelajar sarjana muda vaksin keputusan menunjukkan bahawa 83.9 % adalah anti -HBs positif (> 10 IU / L) pra dos booster, majoriti (67.9 %) di mana menerima tiga dos vaksin dan 94% anamnestic post tindak balas booster dos dengan hanya sembilan bukan balas dan telah diberi dua dos vaksin untuk menerima dos ketiga dalam enam masa bulan. Semua 55 anti- HBC sampel positif didapati HBV DNA positif oleh bersarang PCR. Analisis urutan produk dikuatkan mendedahkan identiti 99% kepada virus hepatitis B dengan kebanyakan pencilan tempatan yang mempunyai hubungan rapat dengan filogenetik HBV mengasingkan 14 (JX869999) dari Panama. Isolat ini kepunyaan genotip B, serotype adw2 dan telah diasingkan daripada plasma daripada penduduk Cina yang tinggal di kawasan Latin Panama.

Kajian ini oleh itu , mendedahkan kegigihan imuniti selama purata 21 tahun vaksinasi jawatan utama dengan vaksin hepatitis B rekombinan serupa dengan apa yang dilaporkan di Thailand. Kajian kami mendapati bahawa 67.9 % daripada vaksin dengan perlindungan anti -HBs (> 10 IU / L) menerima tiga dos vaksin dengan persatuan yang signifikan antara bilangan dos menerima dan kegigihan imuniti ($P < 0.05$) yang serupa dengan penemuan baru-baru ini di UK yang dilaporkan oleh Yates et al. , 2013. Oleh itu , kehadiran memori imun vaksin selepas bayi telah disahkan dalam kajian ini, serta lain-lain kajian yang berkaitan dengan sambutan anamnestic , menunjukkan kegigihan memori di luar tempoh antibodi . Kadar subjek anti- HBC - positif di kalangan pelajar ijazah pertama vaksin didapati 5% dengan

terpencil anti- HBC dalam 0.9 % daripada vaccinees . Semua empat terpencil anti-HBC tindak balas kepada dos tunggal rekombinan vaksin hepatitis B yang mencadangkan jangkitan utama dengan varian mutan HBV dengan kehilangan anti -HBs liar, yang berbeza dengan kajian yang dijalankan sebelum pengenalan vaksin HBV negara di Taiwan, di mana kebanyakan terpencil anti- HBC tidak menunjukkan sebarang tindak balas terhadap vaksin hepatitis B dos booster . Hepatitis B DNA virus dikesan dari semua 55 anti- HBC positif , serum negatif HBsAg menggunakan bersarang PCR , yang adalah selaras dengan kajian sebelum ini yang menunjukkan dapatan yang sama dalam serum , sel-sel mononuklear dan sampel hati. Berdasarkan turutan dan hubungan filogenetik diasingkan HBV dan rujukan diasingkan dari bahagian-bahagian lain di dunia , kami mendapati bahawa semua pencilan kami milik genotip B. Satu lagi penemuan menarik daripada kajian ini ialah berlakunya hepatitis ghaib dalam 3 % dan 2 % daripada penderma darah dan sarjana muda masing-masing . Semua antibodi teras hepatitis B positif didapati HBV DNA positif dengan itu , menunjukkan kebolehpercayaan antibodi teras hepatitis B dalam pemeriksaan untuk ghaib jangkitan hepatitis B, terutama di kawasan-kawasan endemik rendah manakala asid nukleik penguatan ujian (NAT) adalah ujian pemeriksaan yang dicadangkan di dalam kawasan endemik yang tinggi. Analisis bioinformatik mendedahkan bahawa mutasi pada kedudukan 16 asid amino adalah perkara biasa kepada semua 55 mengasingkan dengan penggantian Glutamin (Q) dengan Lysine (K) dalam 53 isolat , manakala baki 2 pencilan mempunyai Arginine dan berhenti codon masing-masing . Seramai 105 mutasi asid amino, terdapat dalam kesemua 55 pencilan .

Kesimpulannya, keterangan yang dikemukakan dalam kajian ini , mendedahkan kegigihan kedua-dua imuniti tindakbalas humoral dan sel untuk purata 21 tahun selepas suntikan bayi dan kehadiran terpencil anti- HBC dalam vaksin yang tindak balas kepada dos tunggal rekombinan vaksin hepatitis B mencadangkan jangkitan utama dengan varian mutan HBV walaupun kehadiran sel imuniti terhadap mutan tiada virus hepatitis B . Kehadiran HBV DNA dalam semua anti- HBC sampel positif mungkin mencadangkan kebolehpercayaan penggunaan antibodi teras hepatitis B dalam pemeriksaan untuk ghaib jangkitan hepatitis B . Semua 55 urutan dalam kajian ini milik genotip B dan mempunyai pelbagai mutasi di rantau yang berbeza gen S dengan mutasi yang paling biasa ditemui dalam kajian ini adalah penggantian Glutamin di kedudukan 16 dengan lisin menyebabkan nonfunctional hepatitis B antigen permukaan oleh itu, mutasi di luar rantau penentu mungkin memainkan peranan dalam hepatitis B permukaan kegagalan pengesanan antigen.

ACKNOWLEDGEMENTS

Glories are due to Almighty ALLAH for granting me the opportunity to pilot and navigate through the waves of academia. My sincere appreciation to my committee chair Professor Dr. Zamberi Sekawi, who has the substance and attitude of a genius. He constantly and convincingly conveyed a spirit of adventure in respect to scholarship and research. His guidance and persistence makes this dissertation possible. My humble appreciation goes to other committee members, Professor Dr. Yasmin A Malik and Dr. Niazlin Muhd. Taib, I appreciate all your support. In addition, a big thanks to Dr. Nabil S Harmal for his guidance and valuable suggestions during PCR optimization, Muhammad Ibrahim Saeed, for sharing his knowledge and experience in ELISA with me and Dr. Faruku Bande for his academic advices, assistance in sequence analysis and submission to GenBank. I am deeply grateful to Saeid Kadkhodaei for his kind assistance in Bioinformatics.

Furthermore I would like to acknowledge with much appreciation, the crucial role of my team mates, Nur Ayu Atika Aziz, Raihana Abu Bakar, Khairunnisa Izzati, Beh Poay Ling and Siti Noorhidayah Musa, who help me through the difficult task of sample collection and vaccination campaign, a big thank you to the entire volunteers for their enrolment in this study. I wish to also record a special regards to the Director and staff of National Blood centre for making this research a reality, Dr. Noris and Muhammad Azam deserved special mentioning. I am particularly obliged to my wife Fatima and children (Fatima aka mama, and Adeel) for their patience, courage and support during the course of our stay in Malaysia, I appreciate their moral support and throughout the course of my study. Not forgetting my lovely Mother Binta Abdullahi for her unrelenting prayers and encouragement. Finally I would like to acknowledge and thank Sanofi-Aventis, Malaysia for their financial support and to Faculty of Medicine and Health Sciences for providing me with logistic support.

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Masters of Science. The members of the Supervisory Committee were as follows:

Zamberi Sekawi, MD, MPath
Professor
Faculty of Medicine and Health Sciences,
Universiti Putra Malaysia
(Chairman)

Yasmin A Malik, FRCPath
Professor
Faculty of Medicine & Health Science
Universiti Tunku Abdul Rahman
(Member)

Niazlin Moh'd Taib, MB, Bch, MPath
Medical Lectur
Faculty of Medicine and Health Sciences,
Universiti Putra Malaysia
(Member)

_____
BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia
Date:

DECLARATION BY GRADUATE STUDENT

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (research) Rules 2012;
- written permission must be obtained from supervisor and the office of the Deputy Vice Chancelor (research and Innovation) before thesis is published in book form
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to Universiti Putra Malaysia (Graduate Studies) Rule 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: _____ Date: 29 January 2014

Name and Matric No: **Hudu Abdullahi Shuaibu (GS 31266)**

DECLARATION BY MEMBERS OF SUPERVISORY COMMITTEE

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature: _____
Name of
Chairman of
Supervisory
Committee: **Zamberi Sekawi**

Signature: _____
Name of
Member of
Supervisory
Committee: **Yasmin A Malik**

Signature: _____
Name of
Member of
Supervisory
Committee: **Niazlin Moh'd Taib**

Signature: _____
Name of
Member of
Supervisory
Committee:

TABLE OF CONTENTS

LIST OF TABLES

LISTS OF FIGURES

LIST OF ABBREVIATIONS

AIDS	Acquired Immunodeficiency Syndrome
Anti-HBc	Hepatitis B Core Antibodies
Anti-HBe	Hepatitis B e antigen
Anti-HBs	Hepatitis B Surface Antibodies
cccDNA	Covalently Closed Circular DNA
$CD4^+$	Cluster of differentiation 4
$CD8^+$	Cluster of differentiation 8
CDC	Centers for Disease Control
COV	Cut off value
^oC	Degree Celsius
HBsAg	Hepatitis B Surface Antigen
HBV	Hepatitis B Virus
HCC	Hepatocellular Carcinoma
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
HRP	Anti-Horseradish Peroxidase
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IMR	Institute for Medical Research
IU	International Unit
Kb	Kilo base
L	Litre
mg	Mili gram
MHR	Major Hydrophilic Region
ML	Maximum Likelihood
ml	Mili litre
µg	Micro gram
NAT	Nucleic Acid Amplification Test
NCBI	National Center for Biotechnology Information
NTC	No Template Control
NTCP	Sodium Taurocholate Co Transporting Polypeptide
OHBI	Occult hepatitis B infection
OD	Optical Density
ORF	Open Reading Frame
P value	Probability
PC	Positive Control
PCR	Polymerase Chain Reaction
pegINF	Pegylated Interferon
RNA	Ribo Nucleic Acid
RT	Room Temperature
SC	Sub cutaneous
SD	Standard Deviation
STIs	Sexual Transmitted Infections

TAE	Tris-Acetate EDTA
UK	United Kingdom
UPM	Universiti Putra Malaysia
USA	United State of America
WHO	World Health Organisation

CHAPTER 1

INTRODUCTION

The history of hepatitis B virus (HBV) dates back to 1967 when an unidentified antigen was recognized to be linked with hepatitis B viral infection in Australia, which was later referred to as hepatitis B surface antigen (Blumberg, 1967). Since then, hepatitis B surface antigen (HBsAg) has functioned as a significant diagnostic marker of hepatitis B viral infection qualitatively. Although, remarkable improvements in the development of quantitative HBsAg assays have been made, which allowed viral replication monitoring (Ahn, 2011). Hepatitis B virus has been classified as Hepatotropic virus, in the genus Orthohepadnavirus, belonging to the family of Hepadnaviridae.It is a DNA virus composing of; an envelope, core, viral polymerase, and DNA genome. It is a partially double-stranded circular DNA with about 3200 nucleotides (Kann and Gerlich, 2007; Scaglioni, 1996). Hepatitis B virion is spherical in shape and measured 42-45 nm in diameter (intact hepatitis B viral particle), and can be visualized using electron microscope. It has a shell which has two layers; the outer shell is called the envelope protein and also referred to as hepatitis B surface protein, the inner shell is known as the core protein. The hepatitis B surface protien is further divided into S, M and L (small, middle and large) proteins respectively (Kann and Gerlich, 2007).

Hepatitis B surface antigen is an essential marker in screening and diagnostic assay as well as the principal serological marker of HBV infection. The detection limit for HBsAg, is directly proportional to diagnostic ability and inversely proportional to the amount of HBsAg, this implies that; the lower the detection limit for HBsAg, the smaller the diagnostic ability in early infection, most especially within the 'window phase'(Nick and Scheiblauer, 2007; Scheiblauer et al., 2006) and the higher the capability to detect the smallest amounts of HBsAg in chronic carriers and asymptomatic patients (Carman et al., 1997). HBsAg contains a region that is common to all hepatitis B subtypes called the *a* determinant (Howard and Allison, 1995). The *a* determinant region (codon 124–147) which is a two loop structure located in the major hydrophilic region of the surface anigen is the key target for the neutralizing antibody produced following vaccination or through hepatitis B natural infection (Waters, 1987). The emergence of mutation in this region (*a* determinant), may lead to evading neutralization by antibody produced against hepatitis B (anti-HBs) and escaped detection by current diagnostic assay, this leads to potential threat to vaccine efficacy and increases the risk of unsafe blood transfusion. The most effective preventive measure against hepatitis B is implimenting early childhood hepatitis B vaccination.

Published data, reveals that; 95% of vaccines produced protective antibody to hepatitis B surface antigen (anti-HBs) providing long lasting immunity against hepatitis B infection in immunocompetent individuals (Gabbuti et al., 2007; Hammitt et al., 2007). However, the extent of the anti-HBs response is essentially influenced by vaccine regimen, site, route of administration, age immunocompetency and body mass (Zuckerman, 1996). Incoporating infant hepatitis B vaccine into universal immunisation strategy may lead to sustained decreased in HBV prevalence and related death as observed in countries that have successfuly eliminate it most especially countries that were classified previously as high endemic area, but now classified as low endemic (Zanetti, 2008). In Thailand, prevalence of HBV drops drastically to 0.7% 12 years after implementing universal hepatitis B vaccination for children between 6 months and 18 years of age (Chongsrisawat et al., 2006). Similarly, in Taiwan up to 87% decline in HBV seroprevalence was achieved 20 years post routine infant vaccination with 68% decreased in rate of fulminant hepatitis as well as 75% drop in hepatocellular carcinoma (HCC) (Chien, 2006). Likewise, in Malaysia the prevalence of HBV among children decreases to 0.4% from 3.0% prior to introduction universal infant hepatitis B vaccination (Ng et al., 2005). However, the prevalence in general population ranges from 1.5 to 9.8% but reported to be lower (0.4%) among repeated blood donors (Yousuf et al., 2007).

The predominant mode of transmission in Asia Pacific is perinatal, and the disease is transmitted vertically during early childhood from mother to the infant (Dwivedi et al., 2011; Shao et al., 2011), however, both horizontal and vertical transmission have been reported in the Sub Saharan Africa (Botha et al., 1984). In Southeast Asia, about 100 million people are chronic carriers of HBV, with estimated annual mortality of 300,000 mainly due to hepatocellular carcinoma (HCC) and liver cirrhosis. Therefore, all countries in the Asia Pacific Region view hepatitis B as a serious public health issue and maintain policies, goals and plans, targeting at prevention and control of hepatitis B, but in most countries, implementation is not sufficient, sometimes following a series of uncoordinated programmes rather than a consistent strategic approach (WHO 2011).

Hepatitis B viral infection affects more than 2 billion people globally, with at least 378 million chronic infections, which stand at risk of developing fulminant hepatitis, HCC and death (WHO, 2009). In the United States, nearly 78,000 people are infected with HBV in 2001 with the highest infection rate in young adults and annual motality of about 5,000 people (*www.**cdc**.gov/mmwr/preview/mmwrhtml/mm5251a3.htm*).While in Malaysia, hepatitis B carries were estimated to be 2.4 million people, constituting a potential source of infection (Malaysian Liver Foundation, 2005). In 1997, 5.24% of healthy volunteers, screened for HBsAg were found to be positive (Merican et al., 2000).

Since 1989, it has been a policy in Malaysia to vaccinate newborn, against HBV, thus HBsAg seroprevalence is expected to decline over time. However, persistence of immunity into adolescence and adulthood is the key marker for a successful infant vaccination. Mutations in the HBsAg, also called *S*-gene mutations have several clinical effects, including; (i) reduced sensitivity to available diagnostic tests, (ii) lack of immunity following vaccination with non-mutant HBV variant (vaccine escaped mutant) and (iii) failure of passive immunization with hepatitis B specific immunoglobulin (El Chaar, 2010). Failure of diagnostic assay may be a major threat in recipients of blood transfusion or organ transplant (Levicnik-Stezinar, 2004; Thakur et al., 2005). Hepatitis B surface mutants are stable and can be spread through either vertical, or horizontally transmission (Hunt et al., 2000).

WHO has set up year 2012 as target to achieve less than 2% seroprevalence of HBV for all countries of the world (WHO 2008), in order to actualize this goal, Malaysia embarked on childhood vaccination against HBV from 1989 to date, however, there is lack of information regarding the assessment of HBV vaccination as well as the seroprevalence of surface antigen mutation status of Malaysian population. Moreover, HBV surface mutation is becoming a worldwide problem and the mutants seldom respond to the currently available screening and diagnostic assays. Therefore the study of persistence of immunity post hepatitis B vaccination as well as vaccine and diagnostic escaped hepatitis B infection is essential, hence, the objectives of this study.

General Objective

To study the persistence of immunity post hepatitis B vaccination as well as occult hepatitis B infection among healthy volunteers.

Specific Objectives

1. To evaluate the persistence of immunity following childhood hepatitis B vaccination among vaccinated undergraduate student.
2. To investigate the prevalence of isolated hepatitis B core antibody and its response to vaccine booster among vaccinated undergraduate Students.
3. To detect occult hepatitis B viral infection among blood donors and undergraduate students using serological and molecular methods.
4. To perform molecular characterization of occult hepatitis B virus using bioinformatics tools.

Hypothesis

H_{A1}: There is persistence Immunity following childhood hepatitis B vaccination among undergraduate student.
H_{A2}: Isolated anti-HBc is prevalent among vaccinated undergraduate student and it response to hepatitis B vaccine booster
H_{A3}: HBsAg mutation is prevalent among among blood donors and undergraduate student in Malaysia.
H_{A4}: There is significant genetic diversity among hepatitis B surface antigen mutant detected in blood donors and undergraduate students.

BIBLOGRAPHY

Abbas, Z., Jafri, W., Shah, S., Khokhar, N., and Zuberi, S. (2004). PGS Consensus statement on management of Hepatitis B virus infection-2003. Journal-Pakistan Medical Association *54*, 150-158.

Aghakhani, A., Banifazl, M., Izadi, N., McFarland, W., Sofian, M., Khadem-Sadegh, A., Pournasiri, Z., Foroughi, M., Eslamifar, A., and Ramezani, A. (2011). Persistence of antibody to hepatitis B surface antigen among vaccinated children in a low hepatitis B virus endemic area. World J Pediatr *7*, 358-360.

Ahad, M.A., Alim, M.A., Guho, A., Islam, Q.T., and Azad, K.A.K. (2009). Role of Booster Dose on Antibody Titer after Recombinant Hepatitis B Vaccination. Journal of Medicine *10*, 67-76.

Ahn, J.M.L.a.S.H. (2011). Quantification of HBsAg: Basic virology for clinical practice. World J Gastroenterol *17*, 283-289.

Alexandre, K.V.F., Martins, R.M.B., Souza, M.M.d., Rodrigues, I.M.X., and Teles, S.A. (2012). Brazilian hepatitis B vaccine: a six-year follow-up in adolescents. Memórias do Instituto Oswaldo Cruz *107*, 1060-1063.

Allain, J.P., Mihaljevic, I., Gonzalez-Fraile, M.I., Gubbe, K., Holm-Harritshøj, L., Garcia, J.M., Brojer, E., Erikstrup, C., Saniewski, M., and Wernish, L. (2013). Infectivity of blood products from donors with occult hepatitis B virus infection. Transfusion.

Altunay, H., Kosan, E., Birinci, I., Aksoy, A., Kirali, K., Saribas, S., Aslan, M., Yuksel, P., Alan, E., and Yenen, O.S. (2010). Are isolated anti-HBc blood donors in high risk group? The detection of HBV DNA in isolated anti-HBc cases with nucleic acid amplification test (NAT) based on transcription-mediated amplification (TMA) and HBV discrimination. Transfusion and Apheresis Science *43*, 265-268.

Avellón, A., and Echevarria, J.M. (2006). Frequency of hepatitis B virus 'a'determinant variants in unselected Spanish chronic carriers. Journal of Medical Virology *78*, 24-36.

Badrawy, H., and Bakry, R. (2013). Anti-HBc and HBV-DNA detection in blood donors negative for hepatitis B virus surface antigen. American Journal of Molecular Biology *3*, 62-66.

Barash, C., Conn, M.I., DiMarino Jr, A.J., Marzano, J., and Allen, M.L. (1999). Serologic hepatitis B immunity in vaccinated health care workers. Archives of Internal Medicine *159*, 1481.

Baumert, T.F., Köck, J., and Blum, H.E. (2005). A novel target of hepatitis B virus mutations: Splicing of surface RNA. Hepatology *42*, 21-23.

Bialek, S.R., Bower, W.A., Novak, R., Helgenberger, L., Auerbach, S.B., Williams, I.T., and Bell, B.P. (2008). Persistence of protection against hepatitis B virus infection among adolescents vaccinated with recombinant hepatitis B vaccine beginning at birth: a 15-year follow-up study. The Pediatric Infectious Disease Journal *27*, 881-885.

Blumberg B. S., G.B.J., Hungerford D. A., London W. T., Sutnick, A. I. (1967). A serum antigen (Australia antigen) in Down's syndrome, leukemia, and hepatitis. Annals of internal medicine *66*, 924-931.

Botha, J., Dusheiko, G., Ritchie, M., Mouton, H., and Kew, M. (1984). Hepatitis B virus carrier state in black children in Ovamboland: role of perinatal and horizontal infection. The Lancet *323*, 1210-1212.

Bréchot, C., Thiers, V., Kremsdorf, D., Nalpas, B., Pol, S., and Paterlini-Bréchot, P. (2001). Persistent hepatitis B virus infection in subjects without hepatitis B surface antigen: clinically significant or purely "occult"? Hepatology *34*, 194-203.

Bruce, S.A., and Murray, K. (1995). Mutations of some critical amino acid residues in the hepatitis B virus surface antigen. Journal of Medical Virology *46*, 157-161.

Busch, M., Tobler, L., Saldanha, J., Caglioti, S., Shyamala, V., Linnen, J., Gallarda, J., Phelps, B., Smith, R., and Drebot, M. (2005). Analytical and clinical sensitivity of West Nile virus RNA screening and supplemental assays available in 2003. Transfusion *45*, 492-499.

But, D.Y.-K., Lai, C.-L., Lim, W.-L., Fung, J., Wong, D.K.-H., and Yuen, M.-F. (2008). Twenty-two years follow-up of a prospective randomized trial of hepatitis B vaccines without booster dose in children: Final report. Vaccine *26*, 6587-6591.

Cabrerizo, M., Bartolomé, J., Caramelo, C., Barril, G., and Carreño, V. (2000). Molecular analysis of hepatitis B virus DNA in serum and peripheral blood mononuclear cells from hepatitis B surface antigen–negative cases. Hepatology *32*, 116-123.

Cacciola, I., Pollicino, T., Squadrito, G., Cerenzia, G., Villari, D., de Franchis, R., Santantonio, T., Brancatelli, S., Colucci, G., and Raimondo, G. (2000). Quantification of intrahepatic hepatitis B virus (HBV) DNA in patients with chronic HBV infection. Hepatology *31*, 507-512.

Candotti, D., Grabarczyk, P., Ghiazza, P., Roig, R., Casamitjana, N., Iudicone, P., Schmidt, M., Bird, A., Crookes, R., and Brojer, E. (2008). Characterization of occult hepatitis B virus from blood donors carrying genotype A2 or genotype D strains. Journal of Hepatology *49*, 537-547.

Candotti, D., Lin, C.K., Belkhiri, D., Sakuldamrongpanich, T., Biswas, S., Lin, S., Teo, D., Ayob, Y., and Allain, J.-P. (2012). Occult hepatitis B infection in blood donors from South East Asia: molecular characterisation and potential mechanisms of occurrence. Gut *61*, 1744-1753.

Carman, W.F., Karayiannis, P., Waters, J., Thomas, H., Zanetti, A., Manzillo, G., and Zuckerman, A.t. (1990). Vaccine-induced escape mutant of hepatitis B virus. The Lancet *336*, 325-329.

Carman, W.F., Van Deursen, F.J., Mimms, L.T., Hardie, D., Coppola, R., Decker, R., and Sanders, R. (1997). The prevalence of surface antigen variants of hepatitis B virus in Papua New Guinea, South Africa, and Sardinia. Hepatology *26*, 1658-1666.

Chakravarty, R., Neogi, M., Roychowdhury, S., and Panda, C.K. (2002). Presence of hepatitis B surface antigen mutant G145R DNA in the peripheral blood leukocytes of the family members of an asymptomatic carrier and vidence of its horizontal transmission. Virus Research *90*, 133-141.

Chan Cho YU , L.S.D., Tsai Yang TE, Lo Kwang Juei (1995). Hepatitis B vaccination alone is not adequate for the categorizing of adult subjects with isolated anti-HBc. Journal of Gastroenterology and Hepatology *10*, 192-197.

Chang, M.-H., Chen, C.-J., Lai, M.-S., Hsu, H.-M., Wu, T.-C., Kong, M.-S., Liang, D.-C., Shau, W.-Y., and Chen, D.-S. (1997). Universal Hepatitis B Vaccination in Taiwan and the Incidence of Hepatocellular Carcinoma in Children. New England Journal of Medicine *336*, 1855-1859

Chaves, S.S., Fischer, G., Groeger, J., Patel, P.R., Thompson, N.D., Teshale, E.H., Stevenson, K., Yano, V.M., Armstrong, G.L., and Samandari, T. (2012). Persistence of long-term immunity to hepatitis B among adolescents immunized at birth. Vaccine *30*, 1644.

Chaves, S.S., Groeger, J., Helgenberger, L., Auerbach, S.B., Bialek, S.R., Hu, D.J., and Drobeniuc, J. (2010). Improved anamnestic response among adolescents boosted with a higher dose of the hepatitis B vaccine. Vaccine *28*, 2860-2864.

Chemin, I., Jeantet, D., Kay, A., and Trepo, C. (2001). Role of silent hepatitis B virus in chronic hepatitis B surface antigen (−) liver disease. Antiviral Research *52*, 117-123.

Chen, C.-Y., Hsu, H.-Y., Liu, C.-C., Chang, M.-H., and Ni, Y.-H. (2010). Stable seroepidemiology of hepatitis B after universal immunization in Taiwan: a 3-year study of national surveillance of primary school students. Vaccine *28*, 5605-5608.

Chen Chien-Jen, W.L.-Y., Yu Ming-Whei (2000). Epidemiology of hepatitis B virus infection in the Asia–Pacific region. Journal of Gastroenterology and Hepatology *15*, E3-E6.

Chen, Y., Delbrook, K., Dealwis, C., Mimms, L., Mushahwar, I.K., and Mandecki, W. (1996). Discontinuous epitopes of hepatitis B surface antigen derived from a filamentous phage peptide library. Proceedings of the National Academy of Sciences *93*, 1997-2001.

Chien R. N., L.C.Y., Yeh C. T., Liaw Y. F. (2006). Hepatitis B virus genotype B is associated with better response to thymosin α1 therapy than genotype C. Journal of Viral Hepatitis *13*, 845-850.

Chongsrisawat,V.,Yoocharoen, P., Theamboonlers, A., Tharmaphornpilas, P., Warinsathien, P., Sinlaparatsamee, S., Paupunwatana, S., Chaiear, K., Khwanjaipanich, S., and Poovorawan, Y. (2006). Hepatitis B seroprevalence in Thailand: 12 years after hepatitis B vaccine integration into the national expanded programme on immunization Tropical Medicine & International Health *11*, 1496-1502.

Clements, C.J., Baoping, Y., Crouch, A., Hipgrave, D., Mansoor, O., Nelson, C.B., Treleaven, S., van Konkelenberg, R., and Wiersma, S. (2006). Progress in the control of hepatitis B infection in the Western Pacific Region. Vaccine *24*, 1975-1982.

Cowie, B., Karapanagiotidis, T., Enriquez, A., and Kelly, H. (2010). Markers of hepatitis B virus infection and immunity in Victoria, Australia, 1995 to 2005. Australian and New Zealand journal of public health *34*, 72-78.

Dandri, M., Burda, M.R., Bürkle, A., Zuckerman, D.M., Will, H., Rogler, C.E., Greten, H., and Petersen, J. (2002). Increase in de novo HBV DNA integrations in response to oxidative DNA damage or inhibition of poly(ADP-ribosyl)ation. Hepatology *35*, 217-223.

De Paschale, M., Manco, M.T., Belvisi, L., Brando, B., Latella, S., Agrappi, C., Mirri, P., Gatti, A., and Clerici, P. (2012). Prevalence of markers of hepatitis B virus infection or vaccination in HBsAg-negative subjects. Blood Transfusion *10*, 344.

Dwivedi, M., Misra, S.P., Misra, V., Pandey, A., Pant, S., Singh, R., and Verma, M. (2011). Seroprevalence of hepatitis B infection during pregnancy and risk of perinatal transmission. Indian Journal of Gastroenterology *30*, 66-71.

Echevarría, J.M., and Avellón, A. (2006). Hepatitis B virus genetic diversity. Journal of Medical Virology *78*, S36-S42.

El Chaar, M.C., Daniel Crowther, R. Anthony Allain, Jean Pierre (2010). Impact of hepatitis B virus surface protein mutations on the diagnosis of occult hepatitis B virus infection. Hepatology *52*, 1600-1610.

Emini, E.A., Ellis, R.W., Miller, W.J., McAleer, W.J., Scolnick, E.M., and Gerety, R.J. (1986). Production and immunological analysis of recombinant hepatitis B vaccine. Journal of Infection *13*, 3-9.

Engelke, M., Mills, K., Seitz, S., Simon, P., Gripon, P., Schnölzer, M., and Urban, S. (2006). Characterization of a hepatitis B and hepatitis delta virus receptor binding site. Hepatology *43*, 750-760.

Fang, Y., Shang, Q.-L., Liu, J.-Y., Li, D., Xu, W.-Z., Teng, X., Zhao, H.-W., Fu, L.-J., Zhang, F.-M., and Gu, H.-X. (2009). Prevalence of occult hepatitis B virus infection among hepatopathy patients and healthy people in China. Journal of Infection *58*, 383-388.

Feret, E.L., Bernard Diop, Biron Sow, Moustapha London, Thomas Blumberg, Baruch S (1987). Epidemiology of hepatitis B virus infection in the rural community of Tip, Senegal. American Journal of Epidemiology *125*, 140-149.

Francis, D.P., Feorino, P.M., McDougal, S., Warfield, D., Getchell, J., Cabradilla, C., Tong, M., Miller, W.J., Schultz, L.D., and Bailey, F.J. (1986). The safety of the hepatitis B vaccine. JAMA: The Journal of the American Medical Association *256*, 869-872.

François Guido, K.M., Van Damme Pierre, Mphahlele M. Jeffrey, Meheus André (2001). Mutant hepatitis B viruses: a matter of academic interest only or a problem with far-reaching implications? Vaccine *19*, 3799-3815.

Gabbuti, A., Romanò, L., Blanc, P., Meacci, F., Amendola, A., Mele, A., Mazzotta, F., and Zanetti, A.R. (2007). Long-term immunogenicity of hepatitis B vaccination in a cohort of Italian healthy adolescents. Vaccine *25*, 3129-3132.

Geoffrey Zambezi Mutuma, M.W.M., Eberhard Zeyhle, Rana Fasana, Fredrick A. Okoth J. Maina Kabanga, Kuria Julius, Luseso Shiramba, M. Kariuki Njenga, Peter. M. Kaiguri and Vincent Osidiana (2011). Prevalence of Hepatitis B Virus (HBV) surface antigen and HBV-associated hepatocellular carcinoma in Kenyans of various ages. Afr J Health *18*, 53-61.

Gerlich, W., Glebe, D., and Schüttler, C. (2007). Deficiencies in the standardization and sensitivity of diagnostic tests for hepatitis B virus. Journal of Viral Hepatitis *14*, 16-21.

Goh, K.T., Doraisingham, S., Tan, K., Oon, C., Ho, M., Chen, A., and Chan, S.H. (1989). The hepatitis B immunization programme in Singapore. Bulletin of the World Health Organization *67*, 65.

Gomaa, A.I., Khan, S.A., Toledano, M.B., Waked, I., and Taylor-Robinson, S.D. (2008). Hepatocellular carcinoma: epidemiology, risk factors and pathogenesis. World journal of gastroenterology: WJG *14*, 4300.

Grethe, S., Monazahian, M., Böhme, I., and Thomssen, R. (1998). Characterization of unusual escape variants of hepatitis B virus isolated from a hepatitis B surface antigen-negative subject. Journal Of Virology *72*, 7692-7696.

Gu, H., Yao, J., Zhu, W., Lv, H., Cheng, S., Ling, L., Xia, S., and Chen, Y. (2013). The effects of booster vaccination on hepatitis B vaccine in anti-HBs negative infants of HBsAg-positive mothers after primary vaccination. Human vaccines & immunotherapeutics *9*, 0--1.

Hadziyannis, S.J., and Vassilopoulos, D. (2001). Hepatitis B e antigen-negative chronic hepatitis B. Hepatology *34*, 617-624.

Hakre, S., Peel, S.A., O'Connell, R.J., Sanders-Buell, E.E., Jagodzinski, L.L., Eggleston, J.C., Myles, O., Waterman, P.E., McBride, R.H., and Eader, S.A. (2011). Transfusion-transmissible viral infections among US military recipients of whole blood and platelets during Operation Enduring Freedom and Operation Iraqi Freedom. Transfusion *51*, 473-485.

Hammitt, L.L., Hennessy, T.W., Fiore, A.E., Zanis, C., Hummel, K.B., Dunaway, E., Bulkow, L., and McMahon, B.J. (2007). Hepatitis B immunity in children vaccinated with recombinant hepatitis B vaccine beginning at birth: A follow-up study at 15 years. Vaccine *25*, 6958-6964.

Hass, M., Hannoun, C., Kalinina, T., Sommer, G., Manegold, C., and Günther, S. (2005). Functional analysis of hepatitis B virus reactivating in hepatitis B surface antigen-negative individuals. Hepatology *42*, 93-103.

Hong, W.W., Ang, L.W., Cutter, J.L., James, L., Chew, S.K., and Goh, K.T. (2010). Changing seroprevalence of hepatitis B virus markers of adults in Singapore. Ann Acad Med Singapore *39*, 591-598.

Howard, C.R. (1986). The Biology of Hepadnaviruses. Journal of General Virology *67*, 1215-1235.

Howard, C.R., and Allison, L.M.C. (1995). Hepatitis B Surface Antigen Variation and Protective Immunity. Intervirology *38*, 35-40.

Hsu, H.-Y., Chang, M.-H., Ni, Y.-H., Chiang, C.-L., Chen, H.-L., Wu, J.-F., and Chen, P.-J. (2010). No Increase in Prevalence of Hepatitis B Surface Antigen Mutant in a Population of Children and Adolescents Who Were Fully Covered by Universal Infant Immunization. Journal of Infectious Diseases *201*, 1192-1200.

Hsu, H.Y., Chang, M.H., Liaw, S.H., Ni, Y.H., and Chen, H.L. (1999). Changes of hepatitis B surface antigen variants in carrier children before and after universal vaccination in Taiwan. Hepatology *30*, 1312-1317.

Hunt, C.M., McGill, J.M., Allen, M.I., and Condreay, L.D. (2000). Clinical relevance of hepatitis B viral mutations. Hepatology *31*, 1037-1044.

Huy, T.T., and Abe, K. (2004). Molecular epidemiology of hepatitis B and C virus infections in Asia. Pediatrics international *46*, 223-230.

Ie Susan, I.T.M.D., Roni Martono, and Muljono David H (2010). Prediction of conformational changes by single mutation in the hepatitis B virus surface antigen (HBsAg) identified in HBsAg-negative blood donors. Virology Journal *7*, 326.

Ito, K., Qin, Y., Guarnieri, M., Garcia, T., Kwei, K., Mizokami, M., Zhang, J., Li, J., Wands, J.R., and Tong, S. (2010). Impairment of hepatitis B virus virion secretion by single-amino-acid substitutions in the small envelope protein and rescue by a novel glycosylation site. Journal Of Virology *84*, 12850-12861.

Jafarzadeh, A., and Montazerifar, S.J. (2006). Persistence of anti-HBs antibody and immunological memory in children vaccinated with hepatitis B vaccine at birth. J Ayub Med Coll Abbottabad *18*, 4-9.

Jolivet-Reynaud, C., Lesenechal, M., O'Donnell, B., Becquart, L., Foussadier, A., Forge, F., Battail-Poirot, N., Lacoux, X., Carman, W., and Jolivet, M. (2001). Localization of hepatitis B surface antigen epitopes present on variants and specifically recognised by anti-hepatitis B surface antigen monoclonal antibodies. Journal of Medical Virology *65*, 241-249.

Juergen Beck, M.N. (2007). Hepatitis B virus replication. World Journal of Gastroenterology *7*, 48-64.

Jutavijittum, P., Yousukh, A., Samountry, B., Samountry, K., Ounavong, A., Thammavong, T., Keokhamphue, J., and Toriyama, K. (2007). Seroprevalence of hepatitis B and C virus infections among Lao blood donors.

Kabir Ali, K.M., Alavian SeyedMoayyed (2012). Effect of Vaccination Against Hepatitis B in Cases with Isolated Anti-HBc. Govaresh *12*, 86-91.

Kaech, C., Pache, I., Bürgisser, P., Elzi, L., Darling, K.E.A., and Cavassini, M. (2012). Immune response to hepatitis B vaccination in HIV-positive adults with isolated antibodies to HBV core antigen. Journal of Infection *65*, 157-164.

Kaneko, S., Miller, R.H., Feinstone, S.M., Unoura, M., Kobayashi, K., Hattori, N., and Purcell, R.H. (1989). Detection of serum hepatitis B virus DNA in patients with chronic hepatitis using the polymerase chain reaction assay. Proceedings of the National Academy of Sciences *86*, 312-316.

Kann, M., and Gerlich, W.H. (2007). Structure and Molecular Virology. In Viral Hepatitis (Blackwell Publishing Ltd), pp. 147-180.

Kann M, S.A., Rabe B (2007). Intracellular transport of hepatitis B virus. World J Gastroenterol *13*, 39-47.

Kao, J.-H., and Chen, D.-S. (2005). Hepatitis B vaccination: to boost or not to boost? The Lancet *366*, 1337-1338.

Kay, A., and Zoulim, F. (2007). Hepatitis B virus genetic variability and evolution. Virus Research *127*, 164-176.

Kazim, S.N., Sarin, S.K., Sharma, B.C., Khan, L.A., and Hasnain, S.E. (2006). Characterization of naturally occurring and Lamivudine-induced surface gene mutants of hepatitis B virus in patients with chronic hepatitis B in India. Intervirology *49*, 152-160.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of molecular evolution *16*, 111-120.

Kleinman, S.H., Kuhns, M.C., Todd, D.S., Glynn, S.A., McNamara, A., and DiMarco, A. (2003). Frequency of HBV DNA detection in US blood donors testing positive for the presence of anti-HBc: implications for transfusion transmission and donor screening. Transfusion *43*, 696-704.

Kosan, E., Kocazeybek, B., Altunay, H., Aymelek, M., Alan, E., Saribas, S., Aslan, M., Yenen, O.S., Yuksel, P., and Birinci, I. (2010). Can the nucleic acid amplification test (NAT) be an alternative to the serologic tests? A prospective study, the results of 18,200 blood donors from the Turkish Red Crescent. Transfusion and Apheresis Science *43*, 269-272.

Koyanagi, T., Nakamuta, M., Sakai, H., Sugimoto, R., Enjoji, M., Koto, K., Iwamoto, H., Kumazawa, T., Mukaide, M., and Nawata, H. (2000). Analysis of HBs antigen negative variant of hepatitis B virus: unique substitutions, Glu129 to Asp and Gly145 to Ala in the surface antigen gene. Medical science monitor: international medical journal of experimental and clinical research *6*, 1165.

Kreutz, C. (2002). Molecular, immunological and clinical properties of mutated hepatitis B viruses. Journal of cellular and molecular medicine *6*, 113-143.

Kuhns, M.C., Kleinman, S.H., McNamara, A.L., Rawal, B., Glynn, S., and Busch, M.P. (2004). Lack of correlation between HBsAg and HBV DNA levels in blood donors who test positive for HBsAg and anti-HBc: implications for future HBV screening policy. Transfusion *44*, 1332-1339.

Kwei, K., Tang, X., Lok, A.S., Sureau, C., Garcia, T., Li, J., Wands, J., and Tong, S. (2013). Impaired virion secretion by hepatitis B virus immune escape mutants and its rescue by wild-type envelope proteins or a second-site mutation. Journal Of Virology *87*, 2352-2357.

Lao, T.T., Sahota, D.S., Suen, S.S.H., Chan, P.K.S., and Leung, T.Y. (2012). Impact of neonatal hepatitis B vaccination programme on age-specific prevalence of hepatitis B infection in teenage mothers in Hong Kong. Epidemiology & Infection *FirstView*, 1-9.

Launay, O., Masurel, J., Servant-Delmas, A., Basse-Guérineau, A.L., Méritet, J.F., Laperche, S., Sogni, P., and Rosenberg, A. (2011). High levels of serum hepatitis B virus DNA in patients with 'anti-HBc alone': role of HBsAg mutants. Journal of Viral Hepatitis *18*, 721-729.

Lee, H., Kiang, P., Watanabe, P., Halon, P., Shi, L., and Church, D. (2013). Hepatitis B virus infection and immunizations among asian american college students: infection, exposure, and immunity rates. Journal of American college health: J of ACH *61*, 67.

Lee, P.I., Lee, C.Y., Huang, L.M., and Chang, M.H. (1995). Long-term efficacy of recombinant hepatitis B vaccine and risk of natural infection in infants born to mothers with hepatitis B e antigen. The Journal of pediatrics *126*, 716-721.

Lemon, S.M., Layden, T.J., Seeff, L., Suzuki, H., Nishioka, K., Mishiro, S., and Johnson, L. (2000). The 20th United States–Japan joint hepatitis panel meeting. Hepatology *31*, 800-806.

Leuridan, E., and Van Damme, P. (2011). Hepatitis B and the Need for a Booster Dose. Clinical Infectious Diseases *53*, 68-75.

Levicnik-Stezinar, S. (2004). Hepatitis B surface antigen escape mutant in a first time blood donor potentially missed by a routine screening assay. Clinical laboratory *50*, 49.

Lewis, T.L., Alter, H.J., Chalmers, T.C., Holland, P.V., Purcell, R.H., Alling, D.W., Young, D., Frenkel, L.D., Lee, S.L., and Lamson, M.E. (1973). A Comparison of the Frequency of Hepatitis-B Antigen and Antibody in Hospital and Nonhospital Personnel. New England Journal of Medicine *289*, 647-651.

Li, L., Chen, P.J., Chen, M.H., Chak, K.F., Lin, K.S., and Tsai, S.J.L. (2008). A pilot study for screening blood donors in Taiwan by nucleic acid amplification technology: detecting occult hepatitis B virus infections and closing the serologic window period for hepatitis C virus. Transfusion *48*, 1198-1206.

Li, R., Yang, J., Gong, J., Li, Y., Huang, Z., Fang, K., Xu, Z., Liu, C., Zhao, K., and Zhuang, H. (2004). Efficacy of hepatitis B vaccination on hepatitis B prevention and on hepatocellular carcinoma]. Zhonghua liu xing bing xue za zhi= Zhonghua liuxingbingxue zazhi *25*, 385.

Liang, T.J. (2009). Hepatitis B: The virus and disease. Hepatology *49*, S13-S21.

Liao, S.-s., Li, R.-c., Li, H., Yang, J.-y., Zeng, X.-j., Gong, J., Wang, S.-s., Li, Y.-p., and Zhang, K.-l. (1999). Long-term efficacy of plasma-derived hepatitis B vaccine: a 15-year follow-up study among Chinese children. Vaccine *17*, 2661-2666.

Liaw, Y.-F., Brunetto, M.R., and Hadziyannis, S. (2010). The natural history of chronic HBV infection and geographical differences. Antiviral therapy *15*, 25.

Linkins, R.W., Chonwattana, W., Holtz, T.H., Wasinrapee, P., Chaikummao, S., Varangrat, A., Tongtoyai, J., Mock, P.A., Curlin, M.E., and Sirivongrangson, P. (2013). Hepatitis A and hepatitis B infection prevalence and associated risk factors in men who have sex with men, Bangkok, 2006–2008. Journal of Medical Virology.

Liu, C.-J., Chen, D.-S., and Chen, P.-J. (2006). Epidemiology of HBV infection in Asian blood donors: emphasis on occult HBV infection and the role of NAT. Journal of clinical virology *36*, S33-S44.

Liu, Y., Li, P., Li, C., Zhou, J., Wu, C., and Zhou, Y.-H. (2010a). Detection of hepatitis B virus DNA among accepted blood donors in Nanjing, China. Virol J *7*, 193.

Liu, Y., Zhong, Y., Zou, Z., Xu, Z., Li, B., Ren, X., Bai, S., Wang, L., Li, X., and Dai, J. (2010b). Features and clinical implications of hepatitis B virus genotypes and mutations in basal core promoter/precore region in 507 Chinese patients with acute and chronic hepatitis B. Journal of clinical virology *47*, 243-247.

Livramento, A.d., Cordova, C.M.M.d., Scaraveli, N.G., Tonial, G.C., Spada, C., and Treitinger, A. (2011). Anti-HBs levels among children and adolescents with complete immunization schedule against hepatitis B virus. A cross-sectional study in Blumenau, State of Santa Catarina, Brazil, 2007-2008. Revista da Sociedade Brasileira de Medicina Tropical *44*, 412-415.

Lledó, J.L., Fernández, C., Gutiérrez, M.L., and Ocaña, S. (2011). Management of occult hepatitis B virus infection: an update for the clinician. World journal of gastroenterology: WJG *17*, 1563.

Locarnini, S. (2004). Molecular Virology of Hepatitis B Virus. Seminars in Liver Disease *24*, 3–10. .

Lok, A.S., Heathcote, E.J., and Hoofnagle, J.H. (2001). Management of hepatitis B: 2000—Summary of a workshop. Gastroenterology *120*, 1828-1853.

Lok, A.S., and McMahon, B.J. (2007). Chronic hepatitis B. Hepatology *45*, 507-539.

Lok, A.S.F. (2002). Chronic Hepatitis B. New England Journal of Medicine *346*, 1682-1683.

Lu, Chiang, B.L., Chi, W.K., Chang, M.H., Ni, Y.H., Hsu, H.M., Twu, S.J., Su, I.J., Huang, L.M., and Lee, C.Y. (2004). Waning immunity to plasma-derived hepatitis B vaccine and the need for boosters 15 years after neonatal vaccination. Hepatology *40*, 1415-1420.

Lu, F., Li, T., Liu, S., and Zhuang, H. (2010). Epidemiology and prevention of hepatitis B virus infection in China. Journal of Viral Hepatitis *17*, 4-9.

Ma, Q., and Wang, Y. (2012). Comprehensive analysis of the prevalence of hepatitis B virus escape mutations in the major hydrophilic region of surface antigen. J Med Virol *84*, 198-206.

Madeley, C.R. (1972). Virus morphology. Virus morphology.

Manzini, P., Girotto, M., Borsotti, R., Giachino, O., Guaschino, R., Lanteri, M., Testa, D., Ghiazza, P., Vacchini, M., and Danielle, F. (2007). Italian blood donors with anti-HBc and occult hepatitis B virus infection. Haematologica *92*, 1664-1670.

Martin, C.M., Welge, J.A., Rouster, S.D., Shata, M.T., Sherman, K.E., and Blackard, J.T. (2012). Mutations associated with occult hepatitis B virus infection result in decreased surface antigen expression in vitro. Journal of Viral Hepatitis *19*, 716-723.

Martinot-Peignoux, M., Boyer, N., Colombat, M., Akremi, R., Pham, B.-N., Ollivier, S., Castelnau, C., Valla, D., Degott, C., and Marcellin, P. (2002). Serum hepatitis B virus DNA levels and liver histology in inactive HBsAg carriers. Journal of Hepatology *36*, 543-546.

Marusawa, H., Uemoto, S., Hijikata, M., Ueda, Y., Tanaka, K., Shimotohno, K., and Chiba, T. (2000). Latent hepatitis B virus infection in healthy individuals with antibodies to hepatitis B core antigen. Hepatology *31*, 488-495.

Mason, W.S., Jilbert, A.R., and Summers, J. (2005). Clonal expansion of hepatocytes during chronic woodchuck hepatitis virus infection. Proceedings of the National Academy of Sciences of the United States of America *102*, 1139-1144.

McMahon, B.J. (2008). Natural history of chronic hepatitis B–clinical implications. The Medscape Journal of Medicine *10*, 91.

McMahon, B.J., Dentinger, C.M., Bruden, D., Zanis, C., Peters, H., Hurlburt, D., Bulkow, L., Fiore, A.E., Bell, B.P., and Hennessy, T.W. (2009). Antibody levels and protection after hepatitis B vaccine: results of a 22-year follow-up study and response to a booster dose. The Journal of infectious diseases *200*, 1390-1396.

McMahon, B.J., Holck, P., Bulkow, L., and Snowball, M. (2001). Serologic and clinical outcomes of 1536 Alaska Natives chronically infected with hepatitis B virus. Ann Intern Med *135*, 759-768.

Meldal, B.H.M., Bon, A.H., Prati, D., Ayob, Y., and Allain, J.P. (2011). Diversity of Hepatitis B virus infecting Malaysian candidate blood donors is driven by viral and host factors. Journal of Viral Hepatitis *18*, 91-101.

Mendy, M., Peterson, I., Hossin, S., Peto, T., Jobarteh, M.L., Jeng-Barry, A., Sidibeh, M., Jatta, A., Moore, S.E., Hall, A.J., *et al.* (2013). Observational Study of Vaccine Efficacy 24 Years after the Start of Hepatitis B Vaccination in Two Gambian Villages: No Need for a Booster Dose. PLoS ONE *8*, e58029.

Merican, I., Guan, R., Amarapuka, D., Alexander, M., Chutaputti, A., Chien, R., Hasnian, S., Leung, N., Lesmana, L., Phiet, P., *et al.* (2000). Chronic hepatitis B virus infection in Asian countries. Journal of Gastroenterology and Hepatology *15*, 1356-1361.

Milich, D.R., Schödel, F., Hughes, J.L., Jones, J.E., and Peterson, D.L. (1997). The hepatitis B virus core and e antigens elicit different Th cell subsets: antigen structure can affect Th cell phenotype. Journal Of Virology *71*, 2192-2201.

Mizokami, M., Orito, E., Ohba, K.-i., Ikeo, K., Lau, J.Y., and Gojobori, T. (1997). Constrained evolution with respect to gene overlap of hepatitis B virus. Journal of molecular evolution *44*, S83-S90.

Nassal, M. (2008). Hepatitis B viruses: Reverse transcription a different way. Virus Research *134*, 235-249.

Neau, D., Winnock, M., Galperine, T., Jouvencel, A., Castéra, L., Legrand, E., Tranchant, E., Balestre, E., Lacoste, D., and Ragnaud, J. (2004). Isolated antibodies against the core antigen of hepatitis B virus in HIV-infected patients. HIV medicine *5*, 171-173.

Ng, K.P., Saw, T.L., Baki, A., Rozainah, K., Pang, K.W., and Ramanathan, M. (2005). Impact of the Expanded Program of Immunization against hepatitis B infection in school children in Malaysia. Med Microbiol Immunol *194*, 163-168.

Nick, S., and Scheiblauer, H. (2007). Sensitivities of CE-marked HIV, HCV, and HBsAg assays. Journal of Medical Virology *79*, S59-S64.

Niederhauser, C., Graziani, M., Stolz, M., Tinguely, C., and Schneider, P. (2008). Blood donor screening: how to decrease the risk of transfusion-transmitted hepatitis B virus? Swiss medical weekly *138*, 134-141.

Niermeiier P, G.C., Huizenga JR, Ringers J, Verkerk S, Houthuff HJ, Houwen B, Snijder JA, Nielsen JO. (1978). IgM-anti-HBc as a marker of persistent and IgG-anti-HBc as a marker of past hepatitis B infection. A longitudinal study over 5 years. Acta Hepatogastroenterol (Stuttg) *25*, 360-364.

Norder, H., Couroucé, A.-M., Coursaget, P., Echevarria, J., M, e., Lee, S.-D., Mushahwar, I.K., Robertson, B.H., Locarnini, S., and Magnius, L.O. (2004). Genetic diversity of hepatitis B virus strains derived worldwide: genotypes, subgenotypes, and HBsAg subtypes. Intervirology *47*, 289-309.

Ogata, N., Zanetti, A.R., Yu, M., Miller, R.H., and Purcell, R.H. (1997). Infectivity and pathogenicity in chimpanzees of a surface gene mutant of hepatitis B virus that emerged in a vaccinated infant. Journal of Infectious Diseases *175*, 511-523.

Olinger, C.M., Jutavijittum, P., Hübschen, J.M., Yousukh, A., Samountry, B., Thammavong, T., Toriyama, K., and Muller, C.P. (2008). Possible new hepatitis B virus genotype, southeast Asia. Emerging infectious diseases *14*, 1777.

Oon, C.-J., Lim, G.-K., Ye, Z., Goh, K.-T., Tan, K.-L., Yo, S.-L., Hopes, E., Harrison, T.J., and Zuckerman, A.J. (1995). Molecular epidemiology of hepatitis B virus vaccine variants in Singapore. Vaccine *13*, 699-702.

Osiowy, C., and Giles, E. (2003). Evaluation of the INNO-LiPA HBV Genotyping Assay for Determination of Hepatitis B Virus Genotype. Journal of Clinical Microbiology *41*, 5473-5477.

Ott, J.J., Stevens, G.A., Groeger, J., and Wiersma, S.T. (2012a). Global epidemiology of hepatitis B virus infection: New estimates of age-specific HBsAg seroprevalence and endemicity. Vaccine *30*, 2212-2219.

Ott, J.J., Stevens, G.A., and Wiersma, S.T. (2012b). The risk of perinatal hepatitis B virus transmission: hepatitis B e antigen (HBeAg) prevalence estimates for all world regions. BMC Infectious Diseases *12*, 131.

Parkin, D.M., Bray, F., Ferlay, J., and Pisani, P. (2001). Estimating the world cancer burden: Globocan 2000. International Journal of Cancer *94*, 153-156.

Pokorski, R.J., and Ohlmer, U. (2001). Long-term morbidity and mortality in Chinese insurance applicants infected with the hepatitis B virus. JOURNAL OF INSURANCE MEDICINE-NEW YORK- *33*, 143-164.

Pondé, R.A.A., Cardoso, D.D.P., and Ferro, M.O. (2010). The underlying mechanisms for the 'anti-HBc alone' serological profile. Arch Virol *155*, 149-158.

Poorolajal, J., Mahmoodi, M., Majdzadeh, R., Nasseri-Moghaddam, S., Haghdoost, A., and Fotouhi, A. (2010). Long-term protection provided by hepatitis B vaccine and need for booster dose: A meta-analysis. Vaccine *28*, 623-631.

Poovorawan, Y., Chongsrisawat, V., Theamboonlers, A., Leroux-Roels, G., Crasta, P.D., and Hardt, K. (2012). Persistence and immune memory to hepatitis B vaccine 20 years after primary vaccination of Thai infants, born to HBsAg and HBeAg positive mothers. Human vaccines & immunotherapeutics *8*, 896-904.

Poovorawan, Y., Sanpavat, S., Pongpunlet, W., Chumdermpadetsuk, S., Sentrakul, P., Chitinand, S., Sakulramrung, R., and Tannirundorn, Y. (1990). Comparison of a recombinant DNA hepatitis B vaccine alone or in combination with hepatitis B immune globulin for the prevention of perinatal acquisition of hepatitis B carriage. Vaccine *8*, S56-S59.

Purdy, M.A. (2007). Hepatitis B virus S gene escape mutants. Asian journal of transfusion science *1*, 62.

Qiu, S., Zhang, J., Tian, Y., Yang, Y., Huang, H., Yang, D., Lu, M., and Xu, Y. (2008). Reduced antigenicity of naturally occurring hepatitis B surface antigen variants with substitutions at the amino acid residue 126. Intervirology *51*, 400-406.

Raimondo, G., Allain, J.-P., Brunetto, M.R., Buendia, M.-A., Chen, D.-S., Colombo, M., Craxì, A., Donato, F., Ferrari, C., and Gaeta, G.B. (2008). Statements from the< i> Taormina</i> expert meeting on occult hepatitis B virus infection. Journal of Hepatology *49*, 652-657.

Ren, X., Xu, Z., Liu, Y., Li, X., Bai, S., Ding, N., Zhong, Y., Wang, L., Mao, P., and Zoulim, F. (2010). Hepatitis B virus genotype and basal core promoter/precore mutations are associated with hepatitis B-related acute-on-chronic liver failure without pre-existing liver cirrhosis. Journal of Viral Hepatitis *17*, 887-895.

Roth, W., Busch, M., Schuller, A., Ismay, S., Cheng, A., Seed, C., Jungbauer, C., Minsk, P., Sondag-Thull, D., and Wendel, S. (2012). International survey on NAT testing of blood donations: expanding implementation and yield from 1999 to 2009. Vox sanguinis *102*, 82-90.

Roukens, A.H., and Visser, L.G. (2011). Hepatitis B vaccination strategy in vaccine low and non-responders: A matter of quantity of quality? Human Vaccines *7*, 654-657.

Roznovsky, L., Harrison, T.J., Fang, Z.L., Ling, R., Lochman, I., Orsagova, I., and Pliskova, L. (2000). Unusual hepatitis B surface antigen variation in a child immunised against hepatitis B. Journal of Medical Virology *61*, 11-14.

Sa-nguanmoo, P., Tangkijvanich, P., Tharmaphornpilas, P., Rasdjarmrearnsook, A.o., Plianpanich, S., Thawornsuk, N., Theamboonlers, A., and Poovorawan, Y. (2012). Molecular analysis of hepatitis B virus associated with vaccine failure in infants and mothers: A case–control study in Thailand. Journal of medical virology *84*, 1177-1185.

Samandari, T., Fiore, A.E., Negus, S., Williams, J.L., Kuhnert, W., McMahon, B.J., and Bell, B.P. (2007a). Differences in response to a hepatitis B vaccine booster dose among Alaskan children and adolescents vaccinated during infancy. Pediatrics *120*, e373-e381.

Samandari, T., Fiore, A.E., Negus, S., Williams, J.L., Kuhnert, W., McMahon, B.J., and Bell, B.P. (2007b). Differences in response to a hepatitis B vaccine booster dose among Alaskan children and adolescents vaccinated during infancy. Pediatrics *120*, e373-381.

Sayan, M., Şentürk, Ö., Akhan, S., Hülagü, S., and Cekmen, M. (2010). Monitoring of hepatitis B virus surface antigen escape mutations and concomitantly nucleos (t) ide analog resistance mutations in Turkish patients with chronic hepatitis B. International Journal of Infectious Diseases *14*, e136-e141.

Scaglioni, P.P., Melegari, Margherita Wands, Jack R. (1996). Recent advances in the molecular biology of hepatitis B virus. Baillière's Clinical Gastroenterology *10*, 207-225.

Scheiblauer, H., Soboll, H., and Nick, S. (2006). Evaluation of 17 CE-marked HBsAg assays with respect to clinical sensitivity, analytical sensitivity, and hepatitis B virus mutant detection. Journal of Medical Virology *78*, S66-S70.

Schmitz, A., Schwarz, A., Foss, M., Zhou, L., Rabe, B., Hoellenriegel, J., Stoeber, M., Panté, N., and Kann, M. (2010). Nucleoporin 153 arrests the nuclear import of hepatitis B virus capsids in the nuclear basket. PLoS pathogens *6*, e1000741.

Schulze, A., Gripon, P., and Urban, S. (2007). Hepatitis B virus infection initiates with a large surface protein–dependent binding to heparan sulfate proteoglycans. Hepatology *46*, 1759-1768.

Schulze, A., Mills, K., Weiss, T.S., and Urban, S. (2012). Hepatocyte polarization is essential for the productive entry of the hepatitis B virus. Hepatology *55*, 373-383.

Seddigh-Tonekaboni, S., Lim, W., Young, B., Hou, J.L., Waters, J., Luo, K.X., Thomas, H., and Karayiannis, P. (2001). Hepatitis B surface antigen variants in vaccinees, blood donors and an interferon-treated patient. Journal of Viral Hepatitis *8*, 154-158.

Seed, C., and Kiely, P. (2013). A method for estimating the residual risk of transfusion-transmitted HBV infection associated with occult hepatitis B virus infection in a donor population without universal anti-HBc screening. Vox sanguinis.

Seeger, C., and Mason, W.S. (2013). Sodium-dependent taurocholic cotransporting polypeptide: a candidate receptor for human hepatitis B virus. Gut.

Seo, D.H., Whang, D.H., Song, E.Y., Kim, H.S., and Park, Q. (2011). Prevalence of antibodies to hepatitis B core antigen and occult hepatitis B virus infections in Korean blood donors. Transfusion *51*, 1840-1846.

Shao, Z.J., Zhang, L., Xu, J.Q., Xu, D.Z., Men, K., Zhang, J.X., Cui, H.C., and Yan, Y.P. (2011). Mother-to-infant transmission of hepatitis B virus: A Chinese experience. Journal of Medical Virology *83*, 791-795.

Sheldon, J., and Soriano, V. (2008). Hepatitis B virus escape mutants induced by antiviral therapy. Journal of antimicrobial chemotherapy *61*, 766-768.

Soeung, S.C., Rani, M., Huong, V., Sarath, S., Kimly, C., and Kohei, T. (2009). Results from nationwide hepatitis B serosurvey in Cambodia using simple and rapid laboratory test: implications for National Immunization Program. The American Journal of Tropical Medicine and Hygiene *81*, 252-257.

Soldan, K., Davison, K., and Dow, B. (2005). Supply in the United Kindom, 1996 to 2003. Euro Surveill *10*, 17-19.

Srey, C.T., Ijaz, S., Tedder, R.S., and Monchy, D. (2006). Characterization of hepatitis B surface antigen strains circulating in the Kingdom of Cambodia. J Viral Hepat *13*, 62-66.

Steinhauer, D.A., and Holland, J.J. (1986). Direct method for quantitation of extreme polymerase error frequencies at selected single base sites in viral RNA. JOURNAL OF VIROLOGY *57*, 219-228.

Stirk HJ, T.J., Howard CR (1992). Atopological Model for Hepatitis B Surface Antigen. Intervirology *33*, 148–158.

Su, F.-H., Bai, C.-H., Chu, F.-Y., Lin, Y.-S., Su, C.-T., and Yeh, C.-C. (2012). Significance and anamnestic response in isolated hepatitis B core antibody-positive individuals 18 years after neonatal hepatitis B virus vaccination in Taiwan. Vaccine *30*, 4034-4039.

Szmuness, W., Stevens, C.E., Zang, E.A., Harley, E.J., and Kellner, A. (1981). A controlled clinical trial of the efficacy of the hepatitis B vaccine (heptavax B): A final report. Hepatology *1*, 377-385.

Tamura, K., Nei, M., and Kumar, S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Sciences of the United States of America *101*, 11030-11035.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., and Kumar, S. (2011). MEGA5: Molecular Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Molecular Biology and Evolution *28*, 2731-2739.

Tatematsu, K., Tanaka, Y., Kurbanov, F., Sugauchi, F., Mano, S., Maeshiro, T., Nakayoshi, T., Wakuta, M., Miyakawa, Y., and Mizokami, M. (2009). A genetic variant of hepatitis B virus divergent from known human and ape genotypes isolated from a Japanese patient and provisionally assigned to new genotype J. JOURNAL OF VIROLOGY *83*, 10538-10547.

Thakur, V., Kazim, S.N., Guptan, R.C., Hasnain, S.E., Bartholomeusz, A., Malhotra, V., and Sarin, S.K. (2005). Transmission of G145R mutant of HBV to an unrelated contact. Journal of Medical Virology *76*, 40-46.

Thakur, V., Kazim, S.N., Guptan, R.C., Malhotra, V., and Sarin, S.K. (2003). Molecular epidemiology and transmission of hepatitis B virus in close family contacts of HBV-related chronic liver disease patients. Journal of Medical Virology *70*, 520-528.

Thuy, T., Thi, L., Ryo, H., Van Phung, L., Furitsu, K., and Nomura, T. (2005). Distribution of genotype/subtype and mutational spectra of the surface gene of hepatitis B virus circulating in Hanoi, Vietnam. Journal of Medical Virology *76*, 161-169.

Ton, S., Lopez, C., and Hasnah, H. (1979). Prevalence of anti-HBc in Malaysian male blood donors and its correlation with DNA polymerase activity. The Southeast Asian journal of tropical medicine and public health *10*, 1.

Tong, W., Sun, L., He, J., He, S., and Du, F. (2010). A novel nucleotide insertion in S gene of hepatitis B virus in a chronic carrier. Virology Journal *7*, 104.

Ural O, F.D. (2001). The Response of Isolated Anti-HBc Positive Subjects to Recombinant Hepatitis B Vaccine. Journal of Infection *43*, 187–190.

Ural, O., and Findik, D. (2001). The response of isolated anti-HBc positive subjects to recombinant hepatitis B vaccine. Journal of Infection *43*, 187-190.

Urban, S., Schulze, A., Dandri, M., and Petersen, J. (2010). The replication cycle of hepatitis B virus. Journal of Hepatology *52*, 282-284.

Vitale, F., Tramuto, F., Orlando, A., Vizzini, G., Meli, V., Cerame, G., Mazzucco, W., Virdone, R., Palazzo, U., and Villafrate, M.R. (2008). Can the serological status of "anti-HBc alone" be considered a sentinel marker for detection of "occult" HBV infection? Journal of Medical Virology *80*, 577-582.

Viviani, S., Carrieri, P., Bah, E., Hall, A.J., Kirk, G.D., Mendy, M., Montesano, R., Plymoth, A., Sam, O., Van der Sande, M., *et al.* (2008). 20 Years into the Gambia Hepatitis Intervention Study: Assessment of Initial Hypotheses and Prospects for Evaluation of Protective Effectiveness Against Liver Cancer. Cancer Epidemiology Biomarkers & Prevention *17*, 3216-3223.

Wakil, S.M., Kazim, S.N., Khan, L.A., Raisuddin, S., Parvez, M.K., Guptan, R.C., Thakur, V., Hasnain, S.E., and Sarin, S.K. (2002). Prevalence and profile of mutations associated with lamivudine therapy in Indian patients with chronic hepatitis B in the surface and polymerase genes of hepatitis B virus. Journal of Medical Virology *68*, 311-318.

Waters J. A., O.R.S.M., Thomas H. C., Richardson S. C., Papaevangelou G. (1987). Qualitative analysis of the humoral immune response to the "a" determinant of HBs antigen after inoculation with plasma-derived or recombinant vaccine. Journal of Medical Virology *21*, 155-160.

Weber, B. (2005). Genetic variability of the S gene of hepatitis B virus: clinical and diagnostic impact. Journal of clinical virology *32*, 102-112.

Weinberger, K.M., Bauer, T., Böhm, S., and Jilg, W. (2000). High genetic variability of the group-specific a-determinant of hepatitis B virus surface antigen (HBsAg) and the corresponding fragment of the viral polymerase in chronic virus carriers lacking detectable HBsAg in serum. Journal of General Virology *81*, 1165-1174.

Williams, I.T., Williams, I.T., Goldstein, S.T., Tufa, J., Tauillii, S., Margolis, H.S., and Mahoney, F.J. (2003). Long term antibody response to hepatitis B vaccination beginning at birth and to subsequent booster vaccination. The Pediatric Infectious Disease Journal *22*, 157-163.

Wilson, J.N., Nokes, D.J., and Carman, W.F. (1999). The predicted pattern of emergence of vaccine-resistant hepatitis B: a cause for concern? Vaccine *17*, 973-978.

Wong, D.K.H., Huang, F.Y., Lai, C.L., Poon, R.T.P., Seto, W.K., Fung, J., Hung, I.F.N., and Yuen, M.F. (2011). Occult hepatitis B infection and HBV replicative activity in patients with cryptogenic cause of hepatocellular carcinoma. Hepatology *54*, 829-836.

Wright, T.L. (2006). Introduction to chronic hepatitis B infection. American journal of gastroenterology *101*, S1.

Wu, C., Deng, W., Deng, L., Cao, L., Qin, B., Li, S., Wang, Y., Pei, R., Yang, D., and Lu, M. (2012). Amino acid substitutions at positions 122 and 145 of hepatitis B virus surface antigen (HBsAg) determine the antigenicity and immunogenicity of HBsAg and influence in vivo HBsAg clearance. Journal of Virology *86*, 4658-4669.

Wu, J.S., Hwang, L.-Y., Goodman, K.J., and Beasley, R.P. (1999). Hepatitis B Vaccination in High-Risk Infants: 10-Year Follow-Up. Journal of Infectious Diseases *179*, 1319-1325.

Wu, Q., Zhuang, G.-H., Wang, X.-L., Wang, L.-R., Li, N., and Zhang, M. (2011). Antibody levels and immune memory 23 years after primary plasma-derived hepatitis B vaccination: Results of a randomized placebo-controlled trial cohort from China where endemicity is high. Vaccine *29*, 2302-2307.

Xiao, L., Zhou, B., Gao, H., Ma, S., Yang, G., Xu, M., Abbott, W.G., Chen, J., Sun, J., and Wang, Z. (2011). Hepatitis B virus genotype B with G1896A and A1762T/G1764A mutations is associated with hepatitis B related acute-on-chronic liver failure. Journal of Medical Virology *83*, 1544-1550.

Xu, L., Wei, Y., Chen, T., Lu, J., Zhu, C.-L., Ni, Z., Huang, F., Du, J., Sun, Z., and Qu, C. (2010). Occult HBV infection in anti-HBs-positive young adults after neonatal HB vaccination. Vaccine *28*, 5986-5992.

Yan, H., Zhong, G., Xu, G., He, W., Jing, Z., Gao, Z., Huang, Y., Qi, Y., Peng, B., Wang, H., *et al.* (2012). Sodium taurocholate cotransporting polypeptide is a functional receptor for human hepatitis B and D virus. eLife Sciences *1*.

Yang, H.-I., Yeh, S.-H., Chen, P.-J., Iloeje, U.H., Jen, C.-L., Su, J., Wang, L.-Y., Lu, S.-N., You, S.-L., Chen, D.-S., *et al.* (2008). Associations Between Hepatitis B Virus Genotype and Mutants and the Risk of Hepatocellular Carcinoma. Journal of the National Cancer Institute *100*, 1134-1143.

Yang, W., and Summers, J. (1995). Illegitimate replication of linear hepadnavirus DNA through nonhomologous recombination. Journal of Virology *69*, 4029-4036.

Yates, T.A., Paranthaman, K., Yu, L.-M., Davis, E., Lang, S., Hackett, S.J., Welch, S.B., Pollard, A.J., and Snape, M.D. (2013). UK vaccination schedule: persistence of immunity to hepatitis B in children vaccinated after perinatal exposure. Archives of Disease in Childhood.

Yousuf, R., Rapiaah, M., Ahmed, S., Rosline, H., Salam, A., Selamah, S., and Roshan, T. (2007). Trends in Hepatitis B Virus Infection among Blood Donors in Kelantan, Malaysia : a Retrospective Study. Southeast Asian J Trop Med Public Health *36*, 1070-1074.

Yu, H., Yuan, Q., Ge, S.-X., Wang, H.-Y., Zhang, Y.-L., Chen, Q.-R., Zhang, J., Chen, P.-J., and Xia, N.-S. (2010). Molecular and phylogenetic analyses suggest an additional hepatitis B virus genotype "I". PLoS ONE *5*, e9297.

Yu, M.C., Yuan, J., Ross, R.K., and Govindarajan, S. (1997). Presence of antibodies to the hepatitis B surface antigen is associated with an excess risk for hepatocellular carcinoma among non-Asians in Los Angeles County, California. Hepatology *25*, 226-228.

Yuen, M.-F., Lee, C.-K., Wong, D.K.-H., Fung, J., Hung, I., Hsu, A., But, D.Y.-K., Cheung, T.-K., Chan, P., and Yuen, J.C.-H. (2010). Prevalence of occult hepatitis B infection in a highly endemic area for chronic hepatitis B: a study of a large blood donor population. Gut *59*, 1389-1393.

Yuen, M.-F., Wong, D.K.-H., Lee, C.-K., Tanaka, Y., Allain, J.-P., Fung, J., Leung, J., Lin, C.-K., Sugiyama, M., and Sugauchi, F. (2011). Transmissibility of hepatitis B virus (HBV) infection through blood transfusion from blood donors with occult HBV infection. Clinical Infectious Diseases *52*, 624-632.

Zanetti Alessandro R., V.D.P., Shouval Daniel (2008). The global impact of vaccination against hepatitis B: A historical overview. Vaccine *26*, 6266-6273.

Zanetti, A.R., Mariano, A., Romanò, L., D'Amelio, R., Chironna, M., Coppola, R.C., Cuccia, M., Mangione, R., Marrone, F., Negrone, F.S., *et al.* (2005). Long-term immunogenicity of hepatitis B vaccination and policy for booster: an Italian multicentre study. The Lancet *366*, 1379-1384.

Zeeshan, M., Jabeen, K., Ali, A., Ali, A., Farooqui, S., Mehraj, V., and Zafar, A. (2007). Evaluation of immune response to Hepatitis B vaccine in health care workers at a tertiary care hospital in Pakistan: an observational prospective study. BMC Infectious Diseases *7*, 120.

Zervou, E.K., Dalekos, G.N., Boumba, D.S., and Tsianos, E.V. (2001). Value of anti-HBc screening of blood donorsfor prevention of HBV infection: results of a 3-year prospective study in Northwestern Greece. Transfusion *41*, 652-658.

Zheng, X., Weinberger, K.M., Gehrke, R., Isogawa, M., Hilken, G., Kemper, T., Xu, Y., Yang, D., Jilg, W., and Roggendorf, M. (2004). Mutant hepatitis B virus surface antigens (HBsAg) are immunogenic but may have a changed specificity. Virology *329*, 454-464.

Zuckerman, J.N. (1996). Nonresponse to hepatitis B vaccines and the kinetics of anti-HBs production. Journal of Medical Virology *50*, 283-288.

Zuckerman, J.N., and Zuckerman, A.J. (2003). Mutations of the surface protein of hepatitis B virus. Antiviral Research *60*, 75-78.