SEROPREVALENCE OF HUMAN PARVOVIRUS B19 AND HEPATITIS B SURFACE ANTIGEN AMONG PATIENTS IN SELECTED HOSPITALS IN KANO METROPOLIS, NIGERIA

BY

JEGEDE ADELOLA

MSc/SCIENCE/18316/2007-2008

A THESIS SUBMITTED TO THE SCHOOL OF POST GRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MASTERS OF SCIENCE IN MICROBIOLOGY

DEPARTMENT OF MICROBIOLOGY

FACULTY OF SCIENCE

AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA.

FEBUARY, 2013

DECLARATION

I declare that the work in the thesis entitled "**SEROPREVALENCE OF HUMAN PARVOVIRUS B19 AND HEPATITIS B SURFACE ANTIGEN AMONG PATIENTS IN SELECTED HOSPITALS IN KANO METROPOLIS, NIGERIA**" has been performed by me in the Department of Microbiology under the supervision of Dr (Mrs).M Aminu-Mukhtar and Dr E.E Ella. The information derived from the literature has been duly acknowledged in the text and the list of references provided. No part of this thesis was previously presented for another degree or diploma at any university.

JEGEDE, Adelola

.....	.....	.....
Name of Student	Signature	Date

CERTIFICATION

This thesis entitled "SEROPREVALENCE OF HUMAN PARVOVIRUS B19 AND HEPATITIS B SURFACE ANTIGEN AMONG PATIENTS IN SELECTED HOSPITALS IN KANO METROPOLIS, NIGERIA" by Jegede Adelola meets the regulations governing the award of the degree of Master of Science in the Department of Microbiology, Faculty of Science, Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

Dr (Mrs).M. Aminu-Mukhtar.	.....	.....
Chairperson Supervisory Committee,	Signature	Date
Department of Microbiology,		
Faculty of Science,		
Ahmadu Bello University., Zaria.		
Dr .E. E.Ella,	.....	.....
Member Supervisory Committee,	Signature	Date
Department of Microbiology,		
Faculty of Science,		
Ahmadu Bello University., Zaria.		
Dr . S.A Ado,	.....	.....
Head, Department of Microbiology,	Signature	Date
Faculty of Science,		
Ahmadu Bello University., Zaria.		
Prof A.A. Joshua,	.....	.....
Dean, School of Post Graduate Studies,	Signature	Date
Ahmadu Bello University., Zaria.		

DEDICATION

This work is dedicated to my dear parents Mr and Mrs Benjamin.S. Jegede for their constant love, support and understanding.

ACKNOWLEDGEMENT

To Almighty God be all the glory, honour and praise for giving me a beautiful life, sound health and perseverance to carry out this work. I love you more.

During my years in graduate school I have shared with very special people some good times that I would like to acknowledge, and say thank you to. First and foremost my unreserved gratitude goes to my supervisor Dr. (Mrs).M. Aminu-Mukhtar for having the patience to accommodate me as her student, condoned and tolerated me always and in all ways encouraging. My appreciation to Dr E.E Ella for being attentive, supportive and whose contribution made this study smooth and fast, may God reward you immensely.

My profound gratitude goes to my loving parents for all the understanding, love and support during the course of this study thank you for all the sacrifices you made in giving a solid foundation i needed to reach this point. To my angel Fiyinfoluwa, thanks for always understanding despite my long absence from home, I love you.

My regards to the patients who gave their consent to be included in the study and also to all the hospital staff that assisted in the course of my study most especially Mr Uche, the Chief Medical Scientist in Medical Microbiology Laboratory, Mallam Ahmad and all staff of the G.O.P.D unit of Aminu Kano Teaching Hospital (A.K.T.H). Mallam Aliyu, the Head of the Laboratory at the Hasiya Bayero Paediatric Hospital Kano. I am grateful to all the laboratory staff of Microbiology Department.

I am grateful to my siblings Oluseun, Funmi, Segun and Tosin for their love and concern. Thank you to my relatives and friends who were there supporting me all the way.

ABSTRACT

This study determined the seroprevalence of Human Parvovirus B19 (HPVB19) and Hepatitis B surface Antigen (HBsAg) among patients attending Aminu Kano Teaching Hospital and Hasiya Bayero Paediatric Hospital Kano. It also determined some sociodemographic factors and some possible risk factors associated with acquiring the viruses. A total of 460 blood samples were collected and analysed for HPVB19 IgG by ELISA and HBsAg using the rapid test strip. Prevalence of 41.5% (191/460) and 8.5% (39/460) was found for HPVB19 and HBsAg respectively. For HPVB19 IgG seroprevalence increased with age with the highest prevalence (51%) recorded among patients ≥51 years old while the lowest was among those < 1 year old (χ^2=13.130, df=6, P=0.04). Higher prevalence was found in female (42.6%:98/230) than male patients (40.4%:93/230), though not statistically significant. With respect to occupation the highest seropositivity was observed among farmers (64.2%:18/28) while the lowest was among retired persons (27.2%:3/11). There was significantly associated between level of formal education attained and HPVB19 (χ^2 =10.363, df=4, P=0.03) and number of persons living in a house (χ^2 =14.303, df=1, P=0.00) respectively but no statistically significant association was observed with marital status and type of residence (P=0.3, P=0.5) respectively. History of transfusion was an important risk factor for HPVB19 transmission (OR=2.0:1; P=0.08). The seroprevalence among pregnant female patients was 41.5% (17/41). Despite the availability of a vaccine a HBsAg prevalence rate of 8.5% (39/460) was reported in this study. Patients in age group 21-30 years old had the highest prevalence of 15.5% (16/103) but the virus was not detected in patients <1 year old and seropositivity was significantly associated with age (χ^2=16.620, df=6, P=0.01). Prevalence in male patients (8.2%:19/230) did not differ significantly from prevalence in female (8.7%:20/230). In relation to marital status, level of education, type of residence and number of persons living in a house, there was no statistically significant association with Hepatitis B Virus. Similarly there was no statistically significant relationship between blood transfusion, history of jaundice, organ transplant and HBV infection among the study population. Overall, age was a significant factor in seroprevalence of both viruses in the population.

TABLE OF CONTENTS

TITLE PAGE.....i

DECLARATION.....ii

CERTIFICATION.....iii

DEDICATION.....iv

ACKNOWLEDGEMENT.....v

ABSTRACT.....vi

TABLE OF CONTENTS.....vii

LIST OF TABLES.....xi

LIST OF FIGURES.....,xii

LIST OF APPENDICES.....xiii

ABBREVIATIONS.....xiv

CHAPTER ONE.....1

1.0 INTRODUCTION.....1

1.1 Statement of Research Problem.....4

1.2 Justification.....5

1.3 Aim.....7

1.4 Objectives.....7

CHAPTER TWO.....8

2.0 LITERATURE VIEW.....8

2.1 Historical Background.....8

2.2 Virology of Human Parvovirus B19.....8

2.2. Taxonomy.....9

2.2.2 Structure of Human Parvovirus B19.....10

2.2.3 Genomic Organization.....14

2.2.4 Viral Proteins.....15

2.2.5 Viral Replication.....16

2.3 Epidemiology.....17

2.4 Pathogenesis.....19
2.5 Clinical Manifestations.....20
2.5.1 Asymptomatic.....20
2.5.2 Erythema Infectiosum.....21
2.5.3 Arthopathy.....21
2.5.4 Hematologic Disorders: Thrombocytopenia.....22
2.5.5 Neurologic Disorders.....22
2.5.6 Hepatitis.....22
2.5.7 Myocarditis.....23
2.5.8 Vasculitis.....23
2.5.9 Hydrops foetalis.....23
2.6 Human Parvovirus Infection complication.....24
2.6.1 Immunocompromised Individuals.....24
2.7 Human Parvovirus B19 Infection and Pregnancy.....26
2.8 Immunity.....27
2.9 Mode of Transmission.....29
2.9.1 Infectious Dose.....29
2.10 Diagnosis of HPV B19.....30
2.10.1 Immunological Assays.....30
2.10.2 Cytopathology.....31
2.10.3 B- Cell Memory.....31
2.10.4 Detection of B19 Viral Antigen 31
2.10.5 Direct Hybridization.....32
2.10.6 Immunohistochemistry.....32
2.10.7 Polymerase Chain Reaction (PCR).....32
2.10.8 Tissue Culture.....33
2.11 Treatment.....33
2.12 Prevention and Control.....34
2.13 Historical Background of Hepatitis B Virus.....35

2.14 Structure of the Virus.....36
2.15 Replication of Hepatitis B Virus.....39
2.16 Genotypes.....40
2.17 Pathogenesis.....41
2.18 Clinical Manifestations.....42
2.18.1 Acute HBV Infection.....42
2.18.2 Chronic HBV Infection.....42
2.19 Epidemiology.....42
2.20 Mode of Transmission.....43
2.20.1 Sexual Intercourse.....43
2.20.2 Percutaneous Transmission.....44
2.20.3 Household and Interhousehold Transmission.....44
2.20.4 Vertical Transmission.....44
2.21 Diagnosis of HBV Infection.....45
2.21.1 HBV Infection Diagnosis.....45
2.22 Immunity.....46
2.23 Treatment.....46
2.24 Prevention.....46
2.24.1 Immunoglobulin.....47
2.24.2 Management of Chronic HBV Infection.....47
CHAPTER THREE.....49
3.0 MATERIALS AND METHODS.....49
3.1 Study Area.....49
3.2 Ethical Approval and Consent.....49
3.3 Questionnaires.....49
3.4 Inclusion and Exclusion Criteria.....50
3.5 Sample Size.....50
3.6 Study Population.....50
3.7 Study Design.....51

3.8 Sample Collection and Processing.....51
3.9 Analysis of Samples.....51
3.9.1 Detection of HPVB19 IgG Antibody by ELISA.....52
3.9.2 Detection of Hepatitis B Surface Antigen by Rapid Test Strip.....53
CHAPTER FOUR.....55
4.0 RESULTS.....55
4.1 Analysis of Total Population.....55
4.2 Analysis of Human Parvovirus B19 IgG.....55
4.3 Analysis of Hepatitis B Surface Antigen.....65
4.4 Analysis of HPVB19 Infection among Women of Child Bearing Age.....70
CHAPTER FIVE.....72
5.0 DISCUSSION......72
5.1 CONCLUSION.....79
5.2 RECOMMENDATIONS.....80
REFERENCES......82
APPENDICES.....96

LIST OF TABLES

Table 4.1: Socio-demographic Characteristics of Patients.....57

Table 4.2: Distribution of Respondents by Possible Risk Factors.....58

Table 4.3: Seroprevalence of HPVB19 IgG.....61

Table 4.4: Sociodemographic Factors Associated with HPVB19 IgG.....62

Table 4.5: Possible Risk Factors Associated with HPVB19 IgG.....64

Table 4.6: Seroprevalence of HBsAg.....67

Table 4.7: Sociodemographic Factors Associated with HBs 68

Table 4.8: Possible Risk Factors Associated with HBsAg.....69

Table 4.9: Distribution of HPV B19 IgG among Women of Child Bearing Age...71

LIST OF FIGURES

Figure 2.1: Transcription of Human Parvovirus B19.....14

Figure 2.2: Structure of Hepatitis B Virus.....38

LIST OF APPENDICES

APPENDIX I: Aminu Kano Teaching Hospital Ethical Approval.....96

APPENDIX II: Kano State Hospitals Management Board Ethical Approval.....97

APPENDIX III: Consent Form.....98

APPENDIX IV: Questionnaire.....99

ABBREVIATIONS

A.A	Amino acid
AIDS	Acquired Immune Deficiency Syndrome
AKTH	Aminu Kano Teaching Hospital
ALT	Alanine Amino Transferase
CD4	Cluster of Differentiation 4
CFS	Chronic Fatigue Syndrome
CI	Confidence Interval
DNA	Deoxyribonucleic Acid
ELISA	Enzyme Linked Immunosorbent Assay
EI	Erythema Infectiosum
G1	Gap 1 phase
G2	Gap 2 phase
Gb4	Globotetraosylceramide
GOPD	General Outpatient Department
HBsAg	Hepatitis B Surface Antigen
HBIG	Hepatitis B Immune globulin
HBPH	Hasiya Bayero Paediatics Hospital
HIV	Human Immunodeficiency Virus
HSP	Henoch Schonlein Purpura
HPV B19	Human Parvovirus B19
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IU	International Unit
IUFD	Intrauterine Foetal Death
ITP	Idiopathic Thrombocytopenic Purpura
IVIG	Intravenous Immunoglobulin G
kDa	Kilodalton

NS1	Non Structural Protein
NT(nt)	Nucleotide
NIHF	Non–Immune Hydrops Foetalis
Mmol	Millimole
OD	Optical Density
PCR	Polymerase Chain Reaction
RBC	Red Blood Cells
RHA	Receptor-mediated Haemagglutination Assay
RNA	Ribonucleic Acid
SCID	Severe Combined Immune Deficiency
SPSS	Special Program for Social Science
TAC	Transient Aplastic Crisis
VAHS	Virus Associated Hemophagocytic Syndrome

CHAPTER ONE

1.0 INTRODUCTION

Human parvovirus B19 (HPVB19) was accidentally discovered during screening of healthy blood donors for hepatitis B (Cossart *et al.,* 1975). It is a non enveloped single stranded DNA virus which belongs to *Erythrovirus* genus and it is pathogenic to humans. Since its accidental discovery about 37 years ago, parvovirus B19 has increasingly been recognized as an important human pathogen causing significant morbidity and mortality in various patient population groups (Anderson, 1987; Girei *et al*., 2010). The cellular receptor for B19 is blood group P antigen (globoside or globotetraosylceramide, Gb4) which is expressed on mature erythrocytes or erythroid progenitors of most individuals, megakarocytes, endothelial cells, placenta, foetal liver, heart and other cell types (Labau *et al.*, 1996) which explains the narrow tissue tropism of the virus. Immature cells in the erythroid lineage are principal target for human B19 Parvovirus. Hence the major sites of virus replication in patients are assumed to be the adult marrow, some blood cells and the foetal liver. Viral replication causes cell death and interruption of red cell production (Jawetz *et al.,* 2007).

Parvovirus B19 can be found in the blood and respiratory secretion of infected patients. The virus can be transmitted parenterally by blood transfusions, infected blood products (clotting and immunoglobulin concentrates), packed red cells from blood collected during short viremic phase (Jordan *et al.,* 1998) and vertically from mother to foetus (Jawetz *et al*., 2007). In immunocompetent recipients, Human parvovirus B19 exposure by transfusion is mostly inconsequential, since a large

proportion of the population is immuned (Jordan *et al*., 1998). The virus is responsible for various clinical manifestations whose characteristics depend on the interplay between the viral properties and the physiological and immune status of the infected individuals (Salimi *et al*., 2008).

Although typically a mild, self-limiting disease, infection with the virus may have a serious clinical outcome in 3 categories of susceptible recipients: Patients with shortened red cell survival (e.g. thalassemia major, sickle cell disease and other haemolytic diseases), pregnant women and immunocompromised individuals such as AIDS and organ transplant patients (previously exposed to B19 or not). Persistent B19 infections have been documented, but their pathogenicity remains unclear (Heegaard *et al*., 2002; Candotti *et al*., 2004; Lefrere *et al*., 2005).

Parvovirus B19 infection is a common childhood illness which usually runs a mild course in immunocompetent individuals, producing a characteristic rash known as *Erythema infectiosum*, fifth disease or slapped–cheek syndrome, an innocuous exanthematous rash that affects the face, trunks and limbs of the body of school age children which was discovered in 1983. Clinically, *Erythema infectiosum* presentation is similar to rubella or measles and can be reliably distinguished only by laboratory tests. In adult population, the rash is less common but arthralgia and arthritis are the most common manifestations of primary B19 infection in adults particularly in adult females (Cohen, 1995). Human Parvovirus B19 virus may cause transient anaemia amongst healthy adults, aplastic crises in infected persons with an underlying blood disorder and prolonged anaemia in immunocompromised persons. In addition, during pregnancy the virus may be transmitted to the foetus which can result in hydrops foetalis, spontaneous abortion or intrauterine foetal death (IUFD), most commonly during the second trimester of pregnancy (Tolfvenstam, 2001).

Research indicates that there is approximately 33% transmission rate of the virus from mother to foetus and about 9% of maternal Parvovirus cases result in foetal death. It accounts for 10% of all cases of nonimmune hydrops foetalis increasing to 36% during epidemic periods. Approximately, 53% of maternal Parvovirus infections are asymptomatic and foetal distress may go unnoticed in the first 18 weeks of pregnancy (van Elsacker-Niele and Kroes, 1999). More recently the virus has been associated with a number of other diseases which includes a range of cardiovascular, dermatologic, hematologic, hepatic, neurologic, renal (Brown, 2010) and rheumatic disorders. Fever and prodomal symptoms may develop in the last few days of the incubation period but many people may remain asymptomatic. The rash associated with *Erythema infectiosum* is at least partly immune complex mediated. IgG antibodies to parvovirus B19 can be detected in serum 2-3 weeks after acquisition of infection and last for life, providing immunity against re-infection (Chin, 2000).

Viral hepatitis is an inflammation of the liver due to viral infections. There are groups of viruses that affect the liver of which hepatitis B Virus (HBV) is a causative agent of severe form of liver disease with high mortality (Dienstang, 2008). Viral hepatitis is a major cause of morbidity and mortality in humans from acute infection and its chronic sequelae, which include hepatitis B and hepatitis C infection, chronic hepatitis, cirrhosis and primary liver cancer (Al-Shamahy, 2000). The World Health Organization has estimated that more than 2 billion people have been infected with HBV including 350 million chronically infected (Agbede *et al.,* 2007). In countries where HBV is highly endemic, prevalence rate of Hepatitis B surface antigen (HBsAg) is 8% or higher with most infections occurring during infancy and early childhood. Infection occurs commonly in all age groups, although

the high rate of chronic infection is primarily maintained by transmission during infancy and early childhood. In low endemic regions (HBsAg prevalence rate of below 2%), infection occurs in young adults, especially those belonging to the known risk groups (Agbede *et al.,* 2007).

1.1 Statement of Research Problem

In general, infection with Parvovirus B19 may be mild, with most of the infections remaining asymptomatic and self-limiting but it can cause significant morbidity and occasional persistent infections or mortality in humans particularly in pregnant women or those who are immunocompromised. Severe symptoms such as acute or persistent arthropathies, critical failures of red cell production, hydrops foetalis, foetal loss, myocarditis or hepatitis (Kaufmann *et al.,* 2004) leading to serious complication or health problems can be linked to the virus. While most individuals experience transient infection in childhood, there is mounting evidence that the virus persists following acute infection in certain tissue types, including liver, synovium, and skin (Eis-Hübinger *et al*., 2001; Soderlund-Venermo *et al*., 2002; Norja *et al*., 2006; Norja *et al*., 2008).

Hepatitis B virus is endemic in Nigeria (Opaleye *et al*., 2011). The viral infection and its sequelae have become significant in our environment, most importantly owing to the high mortality rate associated with liver cirrhosis and hepatocellular carcinoma (Agbede *et al.,* 2007). Recent finding showed that HPVB19 is involved possibly in the disease progression of Asian patients infected with hepatitis B and that there is an association between infection with HPVB19 and the clinical course of HBV-associated liver disease (Toan *et al*., 2006). It has also been reported that B19-DNA could be detected in the liver-tissues of 10/30 (30%) patients with HBV and or HCV infection (Wong *et al*., 2003). However, the role of

this virus as a possible pathogen causing fulminant, chronic, or acute hepatitis is still controversial (Wong *et al*., 2003; Hsu *et al*., 2005; Toan *et al*., 2006). Since receptors for B19 are expressed in the kidney and the heart, its role in myocarditis, liver and renal transplant complications and/or rejection should not be underestimated (Shashwat *et al.*, 2005). Therefore, the virus may play a role in aggravating the liver disease resulting from hepatitis B and C virus infection as reported previously (Hsu *et al.*, 2005).

1.2 Justification

With current attempts on maximizing the safety of blood and its products with focus on entirely eliminating the risk of transmission of infectious agents, the introduction of screening tests for hepatitis B virus, hepatitis C virus and HIV have greatly reduced the risk of transmission (Kleinman *et al*., 2002). It would also be possible, though expensive to include tests for the presence of viruses such as HPVB19 and cytomegalovirus, the transmission of which is a risk for only some of those using these products.

Certain reports have suggested the existence of persistently B19-infected blood donors (Cassinotti *et al.,* 2000) and 1% of blood donors carrying B19 DNA in the presence of specific IgG antibodies (Candotti *et al*., 2004). Persistence of infection in the bone marrow has also been reported in immunocompetent individuals with or without symptoms (LaMonte *et al*., 2003; Lefrere *et al*., 2005).

Though the association between Parvovirus B19 and *Erythema infectiosum* has been established since 1983 (Anderson *et al*., 1984), there is dearth of information and published data on seroprevalence of Human Parvovirus B19 in Nigeria and Kano in particular. It would therefore be of immense benefit to have information about the status of the general population to the HPVB19 virus, the risk

of pregnant women to the virus as it can cross the placenta to cause severe foetal infections and since there is no approved vaccine yet. Thus primary prevention is not feasible which might probably initiate a policy to include screening for Parvovirus B19 for those particularly at risk thus aiding in the control and spread of this virus. Parvovirus B19 is important as a marker virus for potential transmission of more pathogenic viruses even though B19 infection is generally asymptomatic or causes very mild disease (Gaboulaud *et al*., 2002).

In most countries blood is screened for HBsAg prior to transfusion which reduces the risk but this is still a problem in most developing countries and there is an increase of HBV infection in Nigeria (Aminu *et al*., 2013). HBsAg has been shown to be hyperendemic in Nigeria and recent review showed an increasing trend in the prevalence of the virus in Nigeria (Aminu *et al*., 2013). Although HBV has been vaccine-preventable since 1981, it remains a significant public health problem.

1.3 Aim

The aim of this research is to determine the seroprevalence of HPV B19 IgG antibodies and HBsAg among patients in selected hospitals in Kano metropolis, Kano State.

1.4 Objectives

Specific objectives of the study are:

I. To determine the seroprevalence of Parvovirus B19 IgG antibodies among patients attending some hospitals in Kano Metropolis.

II. To determine the seroprevalence of Hepatitis B surface antigen among patients attending some hospitals in Kano Metropolis.

III. To determine the effect of some sociodemographic variables on the seropositivity of individuals to HPVB19 and HBsAg.

IV. To determine the possible risk factors associated with Parvovirus B19 and HBsAg infection.

V. To determine the percentage of women of childbearing age among the study population who are susceptible to HPVB19.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Historical Background

The HPVB19 virus, generally referred to as parvovirus B19 (Servey *et al.*, 2007) or sometimes erythrovirus B19 (Kahn *et al.*, 2008) was the first (until 2005) and only known human virus in the family *Parvoviridae*. It is a member of the *Erythrovirus* genus and was discovered accidentally about 37 years ago by an Australian virologist, Yvonne Cossart and her colleagues when screening healthy blood donors for hepatitis B (Cossart *et al.,* 1975). It gained its name from the coding of serum sample, number 19 in panel B that gave anomalous results when tested by counterimmunoelectrophoresis and radioimmunoassay (Heegaard and Brown, 2002). Although it was originally labelled "serum parvovirus-like" particle or human parvovirus, it was officially recognized in 1985 as a member of the *Parvoviridae* and given the name B19 by the International Committee on Taxonomy of viruses (Siegl *et al.,* 1985).

The virus was first associated with disease in 1981 when it was linked to an aplastic crisis in a patient with sickle-cell disease (Heegaard and Brown, 2002). Subsequently, B19 has since been shown to be the causative agent of *Erythema infectiosum* (EI) (Fifth disease of childhood), spontaneous abortion and some forms of acute arthritis (Corcoran and Doyle, 2005).

2.2 Virology of Human Parvovirus B19

Erythroviruses belong to the *Parvoviridae* family of small DNA viruses. It is a non-enveloped, icosahedral virus that contains a single-stranded linear DNA genome. The Parvovirus B19 has a small icosahederal capsid, surrounded by a lipid bilayer.

The capsid is composed of two structural proteins, VP1 and VP2. VP2 is the major capsid protein (95%) whereas the minor capsid protein is VP1 (5%). The latter is a 84kDa low abundance structural protein while the former is made up of a 58kDa a major structural protein. Furthermore, two regions encode two small non-structural proteins of 7.5kDa and 11.5 kDa unknown function (Shade *et al.*, 1986; Tolfvenstam, 2001).

2.2.1 Taxonomy

The classification of the family *Parvoviridae* relies on the morphology and functional characteristics. Parvoviruses include small, non envelope single stranded DNA viruses which infect a range of animals. Based on the ability to infect vertebrate or invertebrate cells the *Parvoviridae* are divided into *Parvovirinae* that infect vertebrates and *Densovirinae* that infect invertebrates (Muzyczka and Berns, 2001; Heegaard and Brown, 2002; Corcoran and Doyle, 2005). The sub family *Parvovirinae* is made up of three genera: Dependovirus whose members require helper viruses such as adenovirus or herpesvirus for replication, Parvovirus whose members replicate autonomously, Erythrovirus whose members replicate autonomously and have tropism for erythroid progenitor cells (Heegaard and Brown, 2002; Corcoran and Doyle, 2005).

Most of the parvoviruses have a relatively simple genome with two large open reading frames encoding the non-structural (NS or Rep) proteins and capsid (VP or Cap) proteins. The short parvovirus genome does not encode a DNA polymerase, so all parvoviruses are dependent on either host cell enzymes (transiting S phase) or co-infection with another virus for efficient DNA replication.

In molecular detection of parvovirus B19 a number of new erythrovirus genotypes have been identified (Servant *et al.*, 2002) and has suggested that B19 be

classified as a genotype 1 erythrovirus with newly identified strains A6 (Nguyen *et al*., 2002) and K71 (Brown, 2010) classified as genotypes 2 and erythrovirus V9 (Nguyen *et al.,* 1998) as the prototype genotype 3 each of which differ about 10% in nucleotide sequence from B19 type 1 (Jawetz *et al*., 2007). Based on inter-genotype phylogenetic relationships between NS1 and VP1u regions, erythrovirus genotype 3 (erythrovirus variant V9) has been shown to be the most prevalent erythrovirus in Ghana and appears to divide into two sub-types (Candotti *et al.,* 2004). These strains are immunologically indistinguishable from B19, although they exhibit up to 10–12% sequence divergence at the DNA level (Nguyen *et al*., 1999; Corcoran and Doyle, 2004; Ekman *et al.,* 2007). These genotypes generally cross react serologically; PCR amplification may require specific primers (Servant *et al.,* 2002).

2.2.2 Structure of Human Parvovirus B19

Human Parvovirus B19 is a small DNA virus with single stranded, linear genomes approximately 5.5 kb in length (Heegaard and Brown, 2002). The single-stranded DNA linear genome of B19 is packaged into a nonenveloped, icosahedral protein shell of ≈280 Å in diameter (Kaufmann *et al.,* 2004) and composed of only two proteins. Mature infectious viral particles have a molecular weight of 5.6 $x10^6$ and a buoyant density in a Caesium Chloride gradient of 1.41 g/ml (Berns, 1996; Kerr, 2000). The virion is composed of 60 copies of capsomer, and both negative and positive strands of DNA are packaged (Berns, 1996). The capsid consists of 60 structural subunits, ≈95% which is the major viral protein VP2 (58 kDa) (Ozawa *et al.,* 1987). The other structural protein, VP1, differs from VP2 only in a N-terminal "unique region" composed of 227 additional amino acids, which are mostly located outside the virion therefore, accessible to antibody binding (Kaufmann *et al.,* 2004).

In addition to these two structural proteins the virus is thought to have a non structural protein (NS1) which encodes a 77kDa protein.

The sequences of the two proteins are co linear and the entire VP2 sequence is identical to the carboxyl terminal of the VP1. X-ray crystallography has shown that the surface of B19 is significantly different from those of other Parvoviruses by lacking prominent spikes on the threefold icosahedral axes involved in host recognition and antigenicity (Berns, 1996).

Since HPVB19 lacks a lipid envelope it is very stable and cannot be destroyed by dry heat (56^{o}C for 60 mins) treatment or solvent detergents that inactivate enveloped viruses, some clotting factors concentrates end up contaminated. The inactivation is achieved by β-propiolactone, gamma irradiation and formaldehyde (Cohen and Brown, 1992). Researchers have confirmed that during human serum albumin and human immunoglobulin preparation, the liquid-heating step (60°C for 10 hours) rapidly inactivated B19 (Baddaaur, 2006). Nevertheless, its heat sensitivity differs depending on the specific stabilizer used in the inactivation treatment and some authors have found that B19 DNA appears to be very stable and low levels of B19 DNA can persist in serum and a range of tissues for months following acute infection (Soderlund-Venermo *et al*., 2002).

2.2.3 Genomic Organization

The single-stranded genome contains 5,596 nucleotides (nt) composed of an internal coding sequence of 4,830 nt flanked by the terminal repeat sequences of 383 nt at each (Deiss *et al*., 1990). The terminal sequences are palindromic and capable of assuming hairpin duplex configurations, serving as primers for the synthesis of the complementary strand. As in most animal parvoviruses, the B19 genome has two large open reading frames and transcription of the genome is controlled by a single

promoter P6 located at map unit 6 that regulates the synthesis of all nine viral transcripts (Gilbert *et al.,* 2005). The 5'end of the genome encodes a single non-spliced transcript for the production of the nonstructural protein (NS1) and the 3'end is responsible for eight other transcripts that through a combination of different splicing events encode two capsid proteins (VP1 and VP2) and two smaller proteins of unknown function (Vafaie and Scwartz, 2004; Gilbert *et al.*, 2005). The most important viral proteins include the major nonstructural protein NS1 and the two structural proteins VP1 and VP2 (Ozawa *et al*., 1987; Heegaard and Brown, 2002). To the left of these sequences on the B19 genome is the open-reading frame for a non-structural protein, NS1 which encodes a 77 kDa protein. NS1 is a phosphoprotein with important regulatory functions including transcriptional control (Momoeda *et al*., 1994a, Momoeda *et al*., 1994b), virus replication and also plays a role in host cell death (Ozawa *et al*., 1988). In addition, while other parvoviruses coterminate RNA species at polyadenylation sites at the far right side, several B19 transcripts terminate in the middle of the genome and use an unusual polyadenylation signal. These RNAs are derived from the left side of the genome and share an open reading frame with NS1. All transcripts of the human parvovirus B19 identified so far are regulated by a single promoter at map unit 6 of the viral genome, the so-called p6 promoter (Figure 2.1). This promoter is active in a wide variety of different cells. Interaction of the virus NS1 protein with cellular transcription factors that have not been identified may increase promoter activity further (Momoeda *et al*., 1994a).

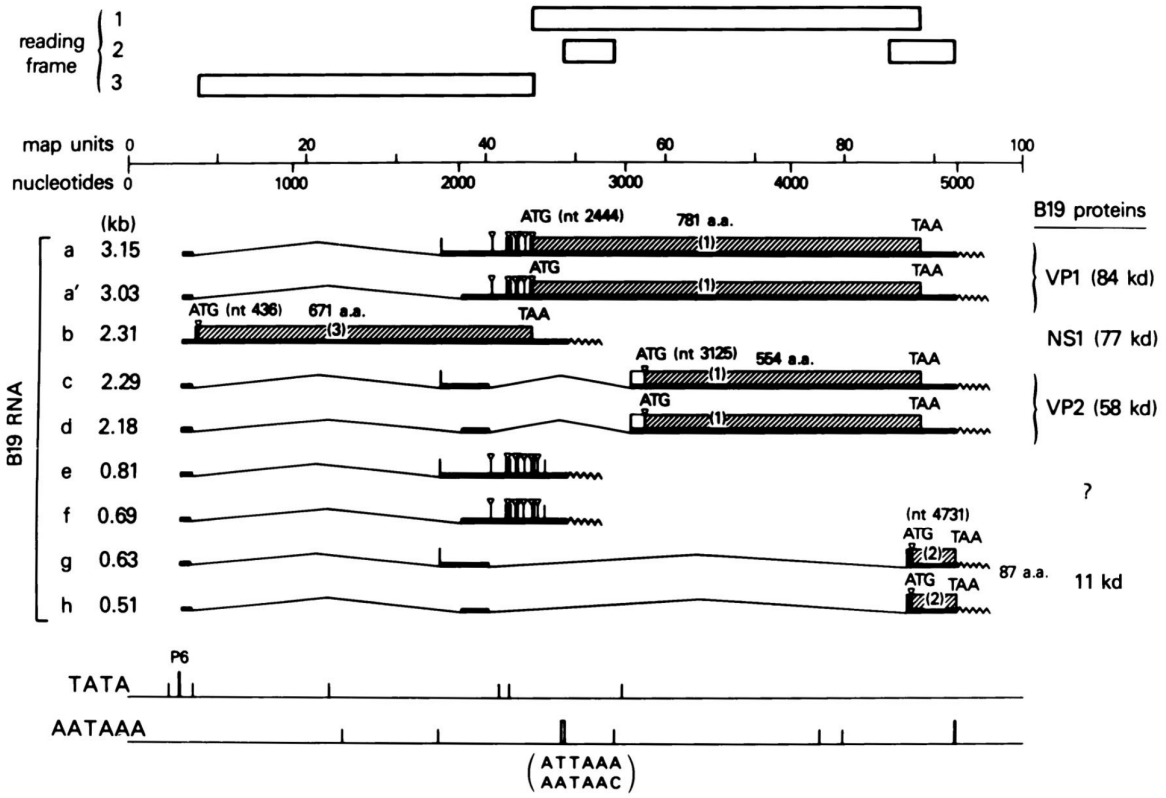


Figure 2.1: Transcription of B19 genome

2.2.4 Viral Proteins

Capsid Proteins: The HPVB19 capsid is composed of two capsomer proteins.VP1 and VP2 which are encoded by overlapping reading frames (Ozawa *et al*., 1987; Heegaard and Brown, 2002). Each capsid consists of an icosahedral structure with a

total of 60 capsomers: VP2 is the major structural protein, accounting for 96% of total capsid protein (Ozawa *et al*., 1987). The VP2 protein is encoded by sequences from nt 3125 to 4786 and has a molecular mass of 58 kDa (Shade *et al*., 1986; Ozawa *et al*., 1987). The main structure motif of VP2 is an 8-stranded β barrel protein and contains the dominant antigenic determinants (Ooi *et al*., 2001). The minor capsid protein, VP1, is encoded by the sequence from nt 2444 to 4786 and is identical to VP2 with the addition of 227 amino acids (termed the VP1 unique region) at the amino terminus (Ozawa *et al*., 1987; Heegaard and Brown, 2002). The VP1 protein has a molecular mass of 84 kDa and makes up the remaining 4% of the total capsid protein (Ozawa *et al*., 1987; Heegaard and Brown, 2002). VP2 and VP1 can be expressed in bacterial, mammalian, and insect cells. In mammalian and insect cells, expression of VP2 can self-assemble in the absence of viral DNA to produce virus-like particles that are physically, antigenically and immunogenically similar to native virions (Heegaard and Brown, 2002).

Nonstructural proteins: A number of non structural proteins have been identified (Ozawa *et al.,* 1987, Ozawa and Young, 1987). The major non-structural protein, NS1 (nt 435 to 2448), has a molecular mass of 77 kDa (Heegaard and Brown, 2002). The function of the NS1 protein is not fully characterized, although, based on data from other parvoviruses, it is thought to possess site-specific DNA-binding, DNA-nicking, ATPase, transcriptional and helicase activities (Heegaard and Brown, 2002), which may explain its pronounced cytotoxicity (Ozawa *et al.,* 1988; Moffatt *et al*., 1998). Accordingly, it was demonstrated that NS1 cytotoxicity is closely related to apoptosis by a pathway involving caspase 3, whose activation may be a key event during NS1-induced cell death (Moffatt *et al*., 1998). NS1 contains a well-conserved nucleoside triphosphate-binding motif, which is essential for a variety of biological functions

(Momoeda *et al*., 1994). Other open reading frames have been discovered but the roles of the derived proteins are not known.

2.2.5 Viral Replication

Parvoviruses have an absolute requirement for actively dividing cells. The virus may enter the cell at any time but virus replication cannot occur until the cell reaches the S-phase of the cell cycle. It has been postulated that initial replication of Parvovirus B19 takes place in the respiratory tract. The mechanisms of virus transmission from the oropharyngeal mucosa to the target cells are unknown as is the mechanism of virus release from the respiratory tract. HPVB19 needs actively dividing cells in order to replicate, hence its target cells are erythroid precursor cells in the bone marrow. HPVB19 enters these cells via the blood group 'P' antigen receptor. The 'P' antigen is found mainly on red blood cell membranes but can also be found on platelets and on tissue from the heart, liver and lungs. It is a globoside that contains two common antigens: P1 and P and one that is less common Pk. Only 1 in 100,000 humans is P negative and those persons are resistant to Parvovirus B19 infection (Brown *et al.*, 1993).

Although globoside is necessary for cell infection it is not sufficient, and additional receptors are required for cell entry. Alpha5 beta1 (Brown, 2010) and Ku80 (Brown, 2010) have been proposed as additional putative receptors. In addition to cell entry, efficient DNA processing and replication only occurs in erythroid cells, although as with most parvoviruses this can be overcome by co-infection with helper virus such as adenovirus (Guan *et al.,* 2009; Brown, 2010). Viral replication in these cells causes a cessation of erythropoesis (van Elsacker-Niele and Kroes, 1999). It is necessary for the host cell to go through S phase, but the parvoviruses do not have the ability to stimulate resting cells to initiate DNA synthesis. Parvovirus B19 is a potent

inhibitor of the erythroid cell differentiation and is cytotoxic for erythroid precursor cells. Direct toxic cell injury or cytolytic effect of Parvovirus B19 as well as Parvovirus B19 – induced apoptosis may be involved in the pathogenesis of erythroid aplasia in high risk patients.

The virus induces a cell cycle arrest at either Gap 1 (G1) or Gap 2 (G2) phase. Chisaka and others found that NS1 protein of Parvovirus B19 plays a critical role in the G1 arrest and apoptosis induction, while the G2 arrest is induced even in the absence of Parvovirus B19 gene expression, suggesting the possible involvement of Parvovirus B19 viral DNA in the G2 arrest (Chisaka *et al*., 2003). The cytopathic effect of Parvovirus B19 infection on the erythroid progenitor cells is manifested as giant pronormoblasts. Transmission electron microscopy of the cells reveals cytotoxic ultrastructural changes that include pseudopod formation, marginated chromatin and virus particles in the nucleus (Young *et al*., 1984).

2.3 Epidemiology

Parvovirus B19 infection occurs worldwide and is a common cause of infection in humans, who are the only known hosts. Parvovirus B19 infections are ubiquitous with cases reported in all seasons, age groups and as outbreaks or as sporadic cases. In temperate climates, epidemic manifestations are more common in late winter, spring, and early summer, with epidemic peaks every 3 to 7 years in a given community. Persons with blood group P antigen (the receptor for B19 erythroid cells) are universally susceptible to Parvovirus B19 (Heymann, 2004). Virtually all the infection is with HPVB19 genotype 1 (the original genotype identified). Only rarely has acute genotype 2 infection been identified, although the HPVB19 genotype 2 has been found in tissue samples (Corcioli *et al*., 2008; Brown, 2010). It has been proposed that genotype 2 was more prevalent in the past, but that it was replaced by

genotype 1 in the 1960's (Norja *et al*., 2008; Brown, 2010). In contrast, HPVB19 genotype 3 is found predominantly in parts of Africa where it seems to be the main circulating genotype (Candotti *et al.,* 2004; Brown, 2010). Virus strains are found in Western Europe, United States, and Brazil, with a lower prevalence of genotype 1 strain compared to the other strains.

Following acute infection HPVB19 DNA persists in tissue, probably for the rest of the person's life, and it has been proposed that examining tissues can give a 'Bioportfolio' of the viruses an individual has been exposed to (Norja *et al.,* 2006). The virus is readily transmitted by close contact, secondary spread among susceptible household members is common, with infection occurring in approximately 50% of susceptible contacts in some studies. The transmission rate in schools is less, but infection can become an occupational risk for school and child care personnel, approximately 20% of susceptible contacts becoming infected.

Seroprevalence increases with age, in industrialized countries 2-10% of children younger than 5 years have an infection and 40-60% of individuals older than 20 years are B19 IgG seropositive and more than 85% in the geriatric population (Heegaard and Brown, 2002). The annual seroconversion rate in women of childbearing age has been reported to be approximately 1.5%. Although antibody is prevalent in the general population, viremia or presence of viral DNA is rare. The source of maternal infection during pregnancy is often the mother's older child. The infectiousness of the virus has also been observed in the occupational setting among hospital staff and research laboratories (Cohen and Brown, 1992; Heymann, 2004).

2.4 Pathogenesis

The life cycle of B19, follow those of other non enveloped DNA viruses, which involve binding of the virus to host cell receptors, internalization, translocation

of the genome to the host nucleus, DNA replication, RNA transcription, assembly of capsids and packaging of the genome, and finally cell lysis with release of the mature virions (Heegaard and Brown, 2002). Both immunoglobulin M (IgM) and IgG antibodies are produced during B19 infection. Infection leads to a biphasic clinical course: One week after intranasal inoculation with B19 in healthy adult volunteers' viremia was detected in seronegative individuals accompanied by a mild illness with pyrexia, malaise, myalgia, itching, and excretion of virus from the respiratory tract. About 17 to18 days after infection, a second phase of symptoms commenced and was characterized by rash, itching, or arthralgia (Heegaard and Brown, 2002).

Recovery involves production of IgM antibody 10 to 12 days postinfection, coinciding with a peak in virus level. IgM usually persists in serum samples for approximately 3 months but may be found for several months (Anderson *et al.*, 1986; Heegaard and Brown, 2002). IgG antibody is detectable in volunteers about 2 weeks after inoculation and presumably persists for life and protects against secondary infections. In immunocompetent persons, viraemia is usually cleared a few weeks after infection. However, a persistent infection may be observed in immunocompromised patients unable to produce neutralizing antibodies and to clear the virus leading to chronic B19 carriage with or without anaemia (LaMonte *et al.*, 2003). Persistence of infection in the bone marrow has also been reported in immunocompetent individuals with or without symptoms (LaMonte *et al.*, 2003). During an acute infection B19V DNA is mainly found in blood and reaches levels of 10^{14} particles/ml. It is reported that immunocompetent individuals who have evidence of current or recent infection may lack detectable levels of B19 DNA in their blood when tested by PCR (Jordan, 2001).

During viremia reticulocyte numbers fall to undetectable levels, recovering 7 to 10 days later, with a resultant temporary drop in haemoglobin of 1 g/dl (0.6mmol/litre) in a healthy person (Heegaard and Brown, 2002). Clinically non significant lymphopenia, neutropenia, and thrombocytopenia occur 6 to 10 days after inoculation. All hematologic parameters may exhibit a brief overshoot prior to stabilizing at preincubation levels.

2.5 Clinical Manifestations

The spectrum of disease linked to Human Parvovirus B19 primarily involves infection in the healthy host manifested as E*rythema infectiosum*, arthralgia, and hydrops, as well as a number of hematologic symptoms in predisposed individuals (Heegaard and Brown, 2002).

2.5.1 Asymptomatic

About 20-30% of infections are asymptomatic. Asymptomatic sero-conversion following viremia with Parvovirus B19 is common in both children and adults.

2.5.2 Erythema Infectiosum

The major manifestation of B19 infection is *Erythema infectiosum* also referred to as 'slapped cheek' or 'fifth disease' as the fifth of the historical six infectious diseases of childhood associated with rash, the others being measles (first), scarlet fever (second), rubella (third), Duke's disease, now no longer recognised as a distinctive entity (fourth) and *Roseola subitum* (sixth) (Brown, 2010). The characteristic feature of this illness is erythema of the face that is often accompanied by circumoral pallor and a maculopapular, lacelike, and often pruritic rash of the trunk and limbs. The rash can fluctuate in intensity and recur with environmental changes, such as temperature and exposure to sunlight, for weeks to months (Tovari *et*

al., 2002). Emotion and exercise may also intensify the rash (Young and Brown, 2004). The onset of the rash has been shown to coincide with the production of neutralising antibodies. This erythematous illness is more common in children of early school age and occasionally affects adults. Both sporadic cases and epidemics have been described.

2.5.3 Arthropathy

Parvovirus B19 has also been associated with acute forms of arthritis. Approximately 5% of children with Parvovirus B19 infection suffer joint pain. Girls are more severely affected than boys, and in infected adults more women suffer arthritic symptoms than men (van Elsacker-Niele and Kroes, 1999). Possibly up to 15% of all new cases of arthritis are due to parvovirus, and a history of recent contact with a patient and positive serology generally confirms the diagnosis (Corcoran and Doyle, 2004). Symptoms may persist for 1-3wks but in 20% of the affected women the arthropathy may persist longer may be for years (Kirshesch, 1990). The arthropathy is presumably immunologically mediated since the onset coincides with the appearance of circulating antibodies.

2.5.4 Hematologic Disorders: Thrombocytopenia

Parvovirus B19 infection may precede the appearance of idiopathic thrombocytopenic purpura (ITP) in children (Scheurlen *et al*., 2001). The virus can cause *in vitro* bone marrow suppression by inhibiting the megakaryocytic colony formation (Ergaz and Ornoy, 2006) or by increased platelet destruction.

2.5.5 Neurologic Disorders

Meningoencephalitis has been associated with infection with Parvovirus B19. It was estimated that the incidence of undiagnosed meningoencephalitis that can be

attributed to Parvovirus B19 infection during an outbreak in the United Kingdom was 4.3% (Barah *et al.*, 2001). Chronic fatigue syndrome (CFS) may also follow Parvovirus B19 infection (Kerr and Tyrrell, 2003).

2.5.6 Hepatitis

The role of B19 in hepatitis remains unclear. (Heegaard and Brown, 2002). Transient self-limited elevation of liver aminotransferases may be associated with Parvovirus B19 infection (Yoto *et al.*, 1996). Detection of this viral DNA was reported in livers from patients requiring transplantation for acute fulminant liver failure (Karentyi *et al.*, 1999) but it is still not clear whether it is the cause of the hepatic failure or an incidental finding. An immunologically mediated mechanism for B19 induced hepatocytes destruction postulating the presence of an unsuspected co-factor that could be associated with B19 that is deleterious to the liver cells (Bernau *et al.,* 1999).

2.5.7 Myocarditis

Parvovirus B19 has been identified as a possible cause of myocarditis and heart failure in both children and adult patients. The viral DNA is present within the myocardium of patients with suspected myocarditis and idiopathic left ventricular dysfunction and can be detected and quantified in endomyocardial specimens via real time PCR (Klein *et al.*, 2004). Schowengerdt *et al* (1997) reported that Parvovirus B19 genome was found through PCR in 0.8% of children suffering from myocarditis, in 3% of children suffering from cardiac transplant rejection, and none in control cases (Schowengerdt *et al.,* 1997).

2.5.8 Vasculitis

Parvovirus B19 has been implicated in various vasculitic syndromes including Henoch Schonlein Purpura (HSP), Wegener's Granulomatosis and microscopic polyarteritis (Cioc *et al*., 2002). These are mostly single case reports without corresponding case control studies.

2.5.9 Hydrops foetalis

Research indicates that there is approximately 33% transmission rate of the virus from mother to foetus and about 9% of maternal Parvovirus cases result in foetal death. The virus accounts for 10% of all cases of nonimmune hydrops foetalis increasing to 36% during epidemic periods. Approximately, 53% of maternal Parvovirus infections are asymptomatic and foetal distress may go unnoticed in the first 18 weeks of pregnancy (van Elsacker-Niele and Kroe, 1999).

2.6 Human Parvovirus Infection Complication

Infection with HPVB19 can lead to the following complication depending on the immune status of the individual. Immunocompromised patients are particularly at risk for HPVB19 related complications and both congenital and acquired immunosuppression predisposes to the development of persistent HPV B19 infection (Frickhofen *et al*., 1990).

2.6.1 Immunocompromised Individuals

In immunocompromised hosts, chronic infection with B19 leads to severe bone marrow suppression and arthritis as the humoral immune responses are impaired in these subjects.

2.6.1.1 Transient Aplastic Crisis (TAC)

Transient aplastic crisis in Parvovirus B19 infection is involved in short life span of red blood cells. There is an abrupt cessation of erythroid progenitors in the bone marrow with the disappearance of reticulocytes in the circulatory blood with normal granulopoesis and megakaryopoesis (Ergaz and Ornoy, 2006). It usually occurs as a result of B19 infection with underlying haemolytic disorders such as hereditary spherocytosis, thalassemia, autoimmune haemolytic anaemia and red cell enzymopathies. Thus individuals suffering from decreased red blood cell production or increased destruction or loss, may be in danger of developing aplastic crisis following Parvovirus B19 infection. Concurrent thrombocytopenia, neutropenia and rarely pancytopenia can accompany the red blood cell aplasia. Reticulocytes in normal individuals can fall to zero but haemoglobin levels usually remain stable because the erythrocyte has a long life span but compared to individuals with short life span of the red blood cells that depend on normal reticulocytosis (Ergaz and Ornoy, 2006). Typically, TAC presents with anaemia, erythroid hypoplasia in bone marrow, dyspnoea, lassitude, confusion, congestive heart failure and in the most severe cases even bone marrow necrosis. Patients with transient aplastic crisis may have a prodromal illness with fever, malaise, and myalgia, but rash usually is absent.

2.6.1.2 Chronic Red Blood Cell Aplasia

In the absence of anti-viral immunity red blood cell aplasia can persist. Immunocompromised patients are unable to mount a neutralizing antibody response due to a persistent bone marrow insufficiency; HPVB19 infection may cause chronic anaemia. Predisposing conditions include immune deficiency syndromes, acute and chronic leukaemia (D'Eufemia *et al.*, 2000), lymphomas (Heegaard *et al*., 1995), neoplastic disorders, HIV infection (Crook *et al*., 2000), severe combined immune

deficiency (SCID) (Ergaz and Ornoy, 2006), bone marrow, organ transplantation and immunosuppressive therapy (D'Eufemia *et al.*, 2000). Parvovirus B19 infection can mimic a leukemic relapse or therapy induced cytopenia when anaemia and thrombocytopenia develops. There is severe anaemia without reticulocytes in the peripheral blood and in the bone marrow. The finding of giant pronormoblasts is typical (McNall *et al*., 2001). The antibodies to Parvovirus B19 are low or absent and the viral load is high (Chen *et al.*, 2001).

2.6.1.3 Virus Associated Haemophagocytic Syndrome (VAHS)

This is usually a benign self-limited condition accompanying viral, bacterial, rickettsial, fungal and parasitic infections. Parvovirus B19 was found in the bone marrow of some of the patients suffering from the syndrome (Hoang *et al*., 1998) in many of them an underlying immune-suppression condition exists. It is characterized by histiocytic hyperplasia, hemophagocytosis, and cytopenia (Ergaz and Ornoy, 2006).

2.7. Human Parvovirus B19 Infection and Pregnancy

Chronic infection with HPVB19 may be of significance in pregnancy, because it can be speculated that the altered maternal immune status may facilitate the reactivation of viral infection (Bűtlmann *et al*., 2005). However, clinical significance is often unclear.

Seroprevalence studies have shown that approximately 30-40% of women are not immuned therefore do not possess the HPVB19 antibodies and are therefore consequently at risk of the viral infection. Exposure and infection with this virus can lead to serious complications during pregnancy which may result in foetal anaemia, spontaneous abortion and non–immune hydrops foetalis (NIHF). The critical time of

infection has since been narrowed down to the 16th week of gestation (Corcoran and Doyle, 2006) with most cases of foetal loss due to HPVB19 infection reported in the second trimester (Wattre *et al*., 1998). This susceptibility can be attributed, at least in part, to the relative immaturity of the foetal immune response at this stage. More importantly though, is the tropism B19 has for erythroid progenitor cells (Corcoran and Doyle, 2006) and the fact that in the second trimester of pregnancy the lifespan of foetal red blood cells (RBCs) is shortened and the RBC mass increases 3- to 4-fold during this period of gestation (Corcoran and Doyle, 2006).

Transmission of the virus across the placenta and subsequent foetal infection may cause an abrupt inhibition of the erythropoiesis, which may lead to severe anaemia and foetal death. The former can lead to congestive heart failure and the development of hydrops foetalis which is the presence of foetal generalized subcutaneous tissue oedema accompanied by serous effusion in one or more body cavities. These manifestations however, seem to be rare and in several of the reported cases a causal relationship between infection with Parvovirus B19 and the foetal damage have not been definitely established (Ergaz, and Ornoy, 2006).

Pregnant women are more susceptible to HPVB19 infection during epidemics and also when exposed to recently infected children in the home (valeur- Jensen *et al.,* 1999). It is somewhat unfortunate that most pregnant women acutely infected with the virus remain asymptomatic although some experience symptoms such as exanthema and arthralgia (Enders *et al*., 2004). As these symptoms are commonly associated with pregnancy, acute HPVB19 infection can be overlooked, however routine analysis of maternal immune status with respect to the viral infection can check this problem.

2.8. Immunity

Following infection, specific immunoglobulins IgM, IgG and IgA are produced. The clinical course is biphasic in correlation with the immune response. Humoral immune response to B19 seems to be a crucial factor in disease resolution, and cell mediated immunity plays either none or very little role. In healthy HPVB19 infected individuals the predominant immune response is humoral (Kurtsman *et al.,* 1989). The early antibody response consists of IgM and is directed against VP2, while the mature response is characterized by an increased avidity that involves IgG as the major antibody subclass and VP1 as the primary target, despite its less-abundant relative concentration in the virion (Kurtzman *et al*., 1989; Heegaard and Brown, 2002) occurring about 2 weeks from inoculation and presumably lasts for life (Anderson *et al*., 1986). IgA is detected in about 90% of infected individuals and may play a role in protection against infection by the nasopharyngeal route (Erdman *et al*., 1991).

A cellular immune response to HPVB19 has been much harder to detect, although it must be present to illicit the humoral response (Kurtzman *et al*., 1989; Heegaard and Brown, 2002). Studies have suggested that individuals mount a classic Th1 response to the virus (Corcoran *et al*., 2000), with capsid proteins presented to CD4 T cells through class II molecules (Franssila *et al*., 2001). Corcoran and colleagues (2004) showed that B cell memory is established and maintained against conformational epitopes of VP2 and against linear epitopes of VP1 not against linear epitopes of VP2 (Corcoran *et al.,* 2004). T lymphocyte response against NS1 protein in human Parvovirus was reported by Klenerman and others (Ergaz and Ornoy, 2006).

The pattern of clinical disease is strongly influenced by the hematologic and immunologic status of the host. In healthy individuals, the infection may cause self

limiting subclinical erythroid aplasia, followed by rash or arthralgia mediated by the immune response (Heegaard and Brown, 2002). In patients suffering from diminished production or increased destruction of erythrocytes, infection can result in a dramatic decrease of haemoglobin, leading to aplastic crisis whereas immunocompromised individuals might fail to eradicate virus, thereby generating a state of chronic anaemia (Heegaard and Brown, 2002).

2.9 Mode of Transmission

Transmission of HPVB19 infection mostly occurs by personal contact via aerosol, respiratory secretions or saliva droplets. It can also be transmitted via blood transfusion and contaminated blood products such as clotting factor concentrates (especially pooled factor VIII and factor IX concentrates) while organ transplantation are a source of iatrogenic transmission (Heymann, 2004). Significantly, B19 can also be transmitted transplacentally from an infected mother to the foetus, leading to non immune foetal hydrops (NIHF), spontaneous abortion or intrauterine foetal death (IUFD) (Skjodebrand-Sparre *et al.*, 2000).

The viruses are stable in the environment and contaminated surfaces may be involved in transmission. Nosocomial infections have also been described, although infrequently (Heegaard and Brown, 2002**)** and transmission has also been reported among staff in laboratories handling native virus (Heegaard and Brown, 2002). With respect to transmission occurring through blood and blood-derived products, the risk of infection from single donor products is reported to be as low 1:500, 000 (Alao *et al.*, 2010).

2.9.1 Infectious Dose

In a study of HPVB19 seronegative plasma pool-recipients, it was shown that only those recipients who received plasma containing greater than 10^7 genome copies/ml became infected or seroconverted (Brown *et al.,* 2001; Brown, 2004). However, it has been proposed that products with much lower virus DNA concentrations (e.g., 10^1geq/ml or even lower) would transmit the virus (Blumel *et al*., 2002).

2.10 Diagnosis of HPV B19

The most common methods used to detect B19V infection are ELISA, Western Blot and PCR. These assays contain B19V antigen which is used to detect the presence of Parvovirus B19 IgG and IgM antibodies in serum or plasma.

2.10.1 Immunological Assays

Extremely accurate laboratory diagnosis of recent B19 infection or past exposure relies on testing serum or plasma specimens for either specific antibody reactivity against viral capsid proteins, VP2 or VP1. Using VP2 antigens is of particular interest for IgM capture assays. IgM is considered to be the first serological marker for B19V infection and can be detected 6 to 10 days after virus production. In the immunocompetent host, detection of serum parvovirus B19 specific immunoglobulin IgM antibody is the preferred diagnostic test. It is indicative of recent infection and is present for 2-3 months after infection. IgG antibodies are produced approximately 12 days after infection and persist for life therefore the presence of IgG is not necessarily indicative of acute infection. During the early stages the level of viremic particles in the blood can reach between 10^{12} or 10^{13} particles per ml. Antibodies against linear epitopes of the B19 VP2 protein and to

some extent VP1, disappear abruptly the viral infection whereas IgG reactivity against conformational epitopes of both VP1 and VP2 persist. Antibody may not be found in immunodeficient patients with chronic B19 infections. In such patients, chronic infection is diagnosed by detecting viral DNA (Jawetz *et al*., 2007).

2.10.2 Cytopathology

Giant pronormoblasts in either bone marrow or peripheral blood is suggestive but not diagnostic of Parvovirus B19 (Boctor and Schreiber, 2002; Ergaz and Ornoy, 2006). Their presence or absence should not be used alone to make a diagnosis of the viral infection. These cells are often absent in patients with human immunodeficiency virus (HIV) infection or other chronic infections (Heegaard and Brown, 2002).

2.10.3 B-Cell Memory

In the absence of robust cell culture methods to detect HPVB19 in test specimens, alternative techniques can be used to confirm HPV B19 IgG detection and immune status. One such technique is determination of the HPVB19-specific memory B-cell population in the patient and a memory B-cell ELISpot technique has been developed for this application.

2.10.4 Detection of B19 Viral Antigen

Two techniques have been proposed to detect B19 antigen in human serum and plasma, namely HPVB19 antigen detection immunoassay and red blood cell agglutination assay also termed receptor-mediated haemagglutination assay (RHA). The latter system (i.e. RHA) occasionally fails to detect the viral antigen in the presence of HPVB19 IgG, thereby producing false negative results. However, HPVB19 antigen assays have been shown to be easy to perform and, in addition, one assay is capable of detecting all three genotypes of human parvovirus B19.

2.10.5 Direct Hybridization

This procedure can reveal the presence of all variants of the human parvovirus, including the new V9. But it may not be effective for immuno-suppressed patients. Less-sensitive nucleic acid hybridization assay results usually are positive for only 2 to 4 days after onset of illness.

2.10.6 Immunohistochemistry

Immunohistochemistry has been used to detect HPV B19 antigens in foetal tissues and in bone marrow. It is perhaps the least frequently used technique for this as it requires extensive optimisation. It is also dependent on many factors such as tissue type or source, previous tissue treatment, available instrumentation and most significantly, the quality of anti-parvovirus B19 antibody utilised for virus detection.

2.10.7 Polymerase Chain Reaction (PCR)

Human Parvovirus B19 DNA detection by PCR is now routinely used to complement patient serology profile and viral DNA can be detected in serum, plasma, tissue extracts, and amniotic fluid (Jordan, 2001). Molecular testing is considered to be a useful diagnostic tool in determining when foetal death is associated with the viral infection, or when trying to diagnose the infection in immunosuppressed individuals with low immune response. The optimal method for detecting chronic infection in the immunocompromised patient is demonstration of virus by nucleic acid hybridization or polymerase chain reaction (PCR) assays, because HPVB19 antibody is present variably in persistent infection. As HPV B19 DNA can be detected at low levels by PCR assay in serum for up to 9 months after the acute viremic phase, detection of the viral DNA by PCR assay does not necessarily indicate acute infection.

2.10.8 Tissue Culture

Human parvovirus B19 cannot be grown in conventional cell cultures making laboratory detection and isolation of the virus extremely difficult. Virus isolation is not used to detect infection.

2.11 Treatment

Infection with HPVB19 is self limiting in the immunocompetent host and therefore, no specific therapy is required for such individuals. However, in cases where individuals suffer from arthritic complications, symptoms can be treated with non steroidal anti inflammatory drugs. The administration of high titre (IVIG) has proven successful in the treatment of patients with chronic infection but it is expensive and remission may be temporary in the immunocompromised host (Lui *et al.,* 2001). Usually given (0.4g/kg of body weight/day for 5 days or 1g/kg/day for 2 to 3 days), which is a good source of neutralizing antibodies as the majority of the adult population has been exposed to the virus. This treatment is usually ameliorative and very often curative (Kurtzman *et al*., 1998), leading to a marked increase in reticulocyte count and corresponding rise in haemoglobin. It is recommended that patients suffering from transient aplastic crisis (TAC) as a result of HPVB19 infection should be given blood transfusions (Modrow *et al*., 1997).

For cases of foetal infection, intrauterine blood transfusions may be beneficial especially in the case of hydrops and this procedure does involve additional risks to the outcome of pregnancy (Bousquet *et al.,* 2000; Enders *et al*., 2004; Corcoran and Doyle, 2005).

In patients receiving immunosuppressive agents, temporarily decreasing the dose of immunosuppressive agents usually enables the immune system to produce sufficient immunoglobulin G (IgG) to eradicate the infection and confer lifelong

protection. In some individuals with human immunodeficiency virus (HIV) infection, highly active antiretroviral therapy restores immune function, enabling resolution of chronic parvovirus B19 infection (Broliden *et al.,* 2006).

2.12 Prevention and Control

There is no vaccine developed yet against human parvovirus, although prospects are good that a vaccine can be developed. A specific vaccine is important in order to prevent aplastic crises in patients with underlying disorders, and pregnancy complications in sero-negative women at child-bearing age.

Women who are exposed to children at home or at work (e.g teachers or child care providers) are at increased risk of infection with parvovirus B19. However, because school or childcare centre outbreaks often indicate wider spread in the community, including inapparent infection, women are at some degree of risk of exposure from other sources at home or in the community. Women of childbearing age who are concerned can undergo serologic testing for IgG antibody to parvovirus B19 to determine their susceptibility to infection.

Transmission of Parvovirus B19 is likely to be decreased through use of routine infection control practices like good hygiene practices, such as hand washing and not sharing drinks, should help prevent the spread through respiratory secretions, aerosols and fomites. Isolation of infected persons is impractical, with the exception of hospitalized patients (Young and Brown, 2004). Health care workers should be advised on the importance of following good infection control measures (Heymann, 2004).

2.13. Historical Background of Hepatitis B Virus.

The early evolution of the Hepatitis B, like that of all viruses is difficult to establish. The divergence of Orthohepadnavirus and avihepadnavirus occurred approximately 125,000 years ago (van Hemert *et al.,* 2011). Both the Avihepadna and Orthohepadna viruses began to diversity about 25,000 years ago (van Hemert *et al*., 2011).

Hepatitis is characterized by distortion of the normal hepatic lobular architecture due to varying degree of necrosis of individual liver cells or group of liver cell acute, (co- inflation) or chronic (super inflection) inflammation and Kupffer cell enlargement and proliferation (super infection). There is usually some degree of disruption of normal bile flow, which cause, jaundice. The severity of the disease is highly variable often unpredictable (Taura *et al*, 2008). The disease has an enormous impact on health and national economy of many countries.

Hepatitis B virus (Fig 2.2) is classified in the family *Hepadnaviridae.* It occurs as eight distinct genotypes, designated A to H (Kramvis *et al*., 2005), but it is controversial as to whether the outcome of the infection is influenced by the genotype (Krajden *et al*., 2005). The genotypes have a distinct geographical distribution and are used in tracing the evolution and transmission of the virus (Kramvis *et al*., 2005). Differences between genotypes affect the disease severity, course and likelihood of complication, and response to treatment and possibly vaccination (Kramvis *et al*., 2005). The hepatitis B virus is a blood-borne pathogen approximately 75 - 200 times more infectious than the human immunodeficiency virus (HIV), and hyper-endemic in most parts of sub-Saharan Africa (Bowyer *et al*., 2011).

Hepatitis B virus causes liver infection that can be life-threatening and often leads to chronic liver disease, liver cirrhosis, and liver cancer. The viral infection is a major global health problem which account for about two billion infected cases and

400 million chronic infections worldwide (Bello *et al*., 2011). Despite the existence of a safe and effective vaccine HBV is hyperendemic in Asia and sub-Saharan Africa. Nigeria remains a hyperendemic area for Hepatitis B virus infection with an estimated 12% of the total population being chronic carriers (Ugwuja, 2010). Many HBV infections are either asymptomatic or never reported, so the actual number is estimated to be approximately ten-fold higher. There are about 50 million infections in Africa and 150,000 to 450,000 persons newly infected each year in the United States of America (Bello *et al*., 2011).

The risk of death from HBV-related liver cancer or cirrhosis is approximately 25% for persons who acquire chronic infection at childhood (Adoga *et al*., 2010). Moreover, 8% to 10% of people in the general population in the developing world become chronically infected and most acquire infection with HBV at childhood (Adoga *et al*., 2010).

2.14. Structure of the Virus

Hepatitis B Virus has a double-stranded DNA genome of approximately 3200 base pairs organized into four partially overlapping open reading frames, which encode the envelope, core (precore/core), polymerase and X proteins (Figure 2.2). The envelope proteins are surface glycoproteins collectively designated as Hepatitis B surface antigen (HBsAg). The core open reading frame encodes a polypeptide that is expressed as either the hepatitis B e antigen (HBeAg) or the viral capsid protein (HBcAg). The presence of detectable HBeAg in serum or plasma is associated with high levels of HBV replication, greater infectivity and an increased risk of hepatic fibrosis. Mutations in the core promoter and precore regions result in decreased levels or an absence of detectable HBeAg in the serum, but this may not alter the sequelae of chronic infection.

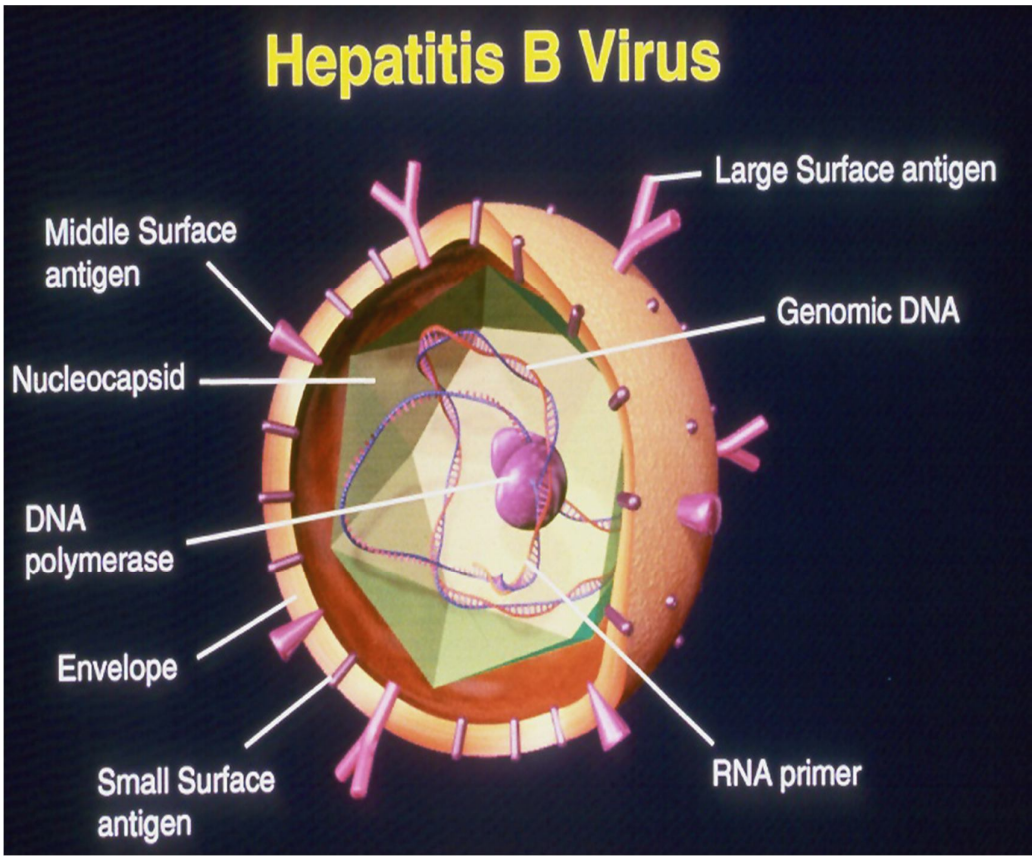


Figure 2.2: Structure of Hepatitis B Virus (Ibl, 2012)

The polymerase protein functions as a reverse transcriptase, a DNA polymerase and a terminal protein. The X protein functions as a transactivator, which may have an important role in hepatocellular carcinogenesis (Krajden *et al*., 2005). The virus is one of the smallest enveloped animal viruses with a virion diameter of 42nm but the pleomorphic forms exist, including filamentous and spherical bodies lacking a core (Howard *et al*., 1986). These particles are not infectious and are composed of the lipid and protein that forms part of the surface of the virion, which is called the surface antigen (HBsAg), and is produced in excess during the life cycle of the virus (Howard *et al*., 1986).

2.15 Replication of Hepatitis B Virus

The infectious virion attaches to cells and becomes uncoated. In the nucleus, the partially double-stranded viral genome is converted to covalently closed circular double-stranded DNA (cccDNA). The cccDNA serves as template for all viral transcripts, including a 3.5-kb pregenome RNA. The pregenome RNA becomes encapsidated with newly synthesized HBcAg. Within the cores, the viral polymerase synthesizes by reverse transcription a negative-strand DNA copy. The polymerase starts to synthesize the positive DNA strand, but the process is not completed. Cores bud from the pre-Golgi membranes, acquiring HBsAg-containing envelopes, and may exit the cell. Alternatively, cores may be reimported into the nucleus and initiate another round of replication in the same cell (Jawetz *et al*., 2007).

2.16 Genotypes

There are eight known genotypes labelled A through H (Kramvis *et al*., 2005). A possible new "I" genotype has been described (Olinger *et al*., 2008) but acceptance of this notation is not universal (Kurbanov *et al*., 2008). Different genotypes may respond to treatment in different ways (Palumbo, 2007). The genotypes differ by at least 8% of the sequence and have distinct geographical distributions and this has been associated with anthropological history. Type F which diverges from the other genomes by 14% is the most divergent type known. Type A is prevalent in Europe, Africa and South East Asia including the Philippines. Type B and C are predominant in Asia; type D is common in the Mediterranean area, the Middle East and India; type E is localized in sub-Saharan Africa; type F (or H) is restricted to Central and South America. Type G has been found in France and Germany. The E and F strains appear to have originated in aboriginal populations of Africa and the New World respectively. Within genotypes 24 subtypes have been described which differ by 4-8% of the genome.

Type A has two subtypes: Aa (A1) in Africa/Asia and the Philippines and Ae (A2) in Europe/United States. Type B has two distinct geographical distributions: Bj/B1('j'- Japan) and Ba/B2('a'-Asia). Type Ba has been further subdivided into four clades (B2-B4).

Type C has two geographical subtypes: Cs (C1) in South-east Asia and Ce (C2) in East Asia. The C subtypes have been divided into five clades (C1-C5). A sixth clade (C6) has been described in the Philippines but only in one isolate to date (Cavinta *et al*., 2009). Type C1 is associated with Vietnam, Myanmar and Thailand; type C2 with Japan, Korea and China; type C3 with New Caledonia and Polynesia;

C4 with Australia; and C5 with the Philippines. A further subtype has been described in Papua, Indonesia (Lusida *et al*., 2008).

Type D has been divided into 7 subtypes (D1-D7). Type F has been subdivided into 4 subtypes (F1-F4). F1 has been further divided into 1a and 1b. In Venezuela subtypes F1, F2, and F3 are found in East and West Amerindians. Among south Amerindians only F3 was found. Subtypes 1a, III, and IV exhibit a restricted geographic distribution (Central America, the North and the South of South America respectively) while clades Ib and II are found in all the Americas except in the Northern South America and North America respectively.

2.17 Pathogenesis

The small envelope protein, HBsAg, is the first viral marker to appear after HBV infection and may be found before symptoms or abnormalities in blood chemistry (Bowyer *et al*., 2011). The soluble e antigen, HBeAg, becomes detectable shortly thereafter. The first indirect marker, IgM antibody to the nucleocapsid or core antigen (HBcIM), develops approximately 2 weeks after the appearance of HBsAg, and disappears some 24 weeks later, being gradually replaced by IgG antibodies to the core (Bowyer *et al*., 2011). The time to HBeAg seroconversion may be short (a few weeks) or greatly delayed (10 - 30 years). Persistence of this phase for >20 weeks has been shown to increase the risk of progression to chronic HBV infection. The latter is defined by persistence of HBsAg and failure to develop neutralising surface antibodies (HBsAb) more than 6 months after infection.

2.18 Clinical Manifestations

The incubation period for HBV ranges from 45 to 180 days. Clinical features of the disease vary considerably.

2.18.1 Acute HBV Infection

This condition presents generally after an incubation period of six weeks to several months with an onset of nonspecific symptoms that may include fever, malaise, anorexia and nausea, followed by the onset of jaundice, dark urine and pale stools. In nine out of ten adults, a new ('acute') hepatitis B infection goes away on its own in the first six months. Approximately 25% to 40% of infected adults will be symptomatic, and most will demonstrate elevations in Alanine aminotransferase (ALT); however, infants, toddlers and immunosuppressed individuals may not manifest signs (eg, jaundice) or symptoms of infection (Krajden *et al*., 2005).

2.18.2 Chronic HBV Infection

Five to ten per cent of acutely infected adults, 90% of neonatally infected infants and 25 -30% of infected children become persistently infected with HBV and develop chronic liver disease of varying severity. Ultimately the dominant cause of viral persistence is a weak antiviral immune response to the HBV antigens, and this has been shown to depend on the specific viral isolate, sex and human leucocytes antigen (HLA) background of the host, age at time of infection and infectious dose of the virus (Bowyer *et al.*, 2011).

2.19 Epidemiology

HBV is worldwide in distribution. Transmission modes and response to infection vary, depending on the age at time of infection. Most individuals infected as

infants develop chronic infections. As adults they are subject to liver disease and are at high risk of developing hepatocellular carcinoma (Jawetz *et al*., 2007).

There is no seasonal trend for HBV infection and no high predilection for any age group, although there are definite high-risk groups such as parenteral drug abusers, institutionalized persons, healthcare personnel, multiple transfused patients, organ transplant patients, hemodialysis patients and staff, highly promiscuous persons, and newborn infants born to mothers with hepatitis B. Since mandatory screening of blood donors for HBsAg was instituted, the number of cases of transfusion-associated hepatitis has been dramatically reduced. People have been infected by improperly sterilized syringes, needles, or scalpels and even by tattooing or ear piercing. The estimated ratio of anicteric to icteric infections is reported to be as high as 4:1 (Jawetz *et al*., 2007).

Currently about 18 million Nigerians are infected (Bello *et al*., 2011) and has reached hyper-endemic levels with the sero-prevalence of HBsAg estimated to range from 10 to 40%.

2.20 Mode of Transmission

Blood is the most important vehicle for transmissions but other body fluids have also been implicated including semen and saliva (Hou *et al*., 2005). HBV is resistant to breakdown, can survive outside the body, and is easily transmitted through contact with infected body fluids (MacLean *et al*.,2012).

2.20.1 Sexual Intercourse

This is the major source of infection in all areas of the world especially in low endemic areas. Hepatitis B is considered to be a sexually transmitted disease (STD). Homosexual men have been considered to be at the highest risk of infection.

However, heterosexual transmission accounts for an increasing proportion of HBV infection (Hou *et al.,* 2005).

2.20.2 Percutaneous Transmission

This includes sharing of injection drug use equipment, needle stick injury, ear-piercing, body piercing, tattooing, inadequate sterilization of medical equipment, scarification, frequent transfusions or hemodialysis. In the United States and western Europe injection drug use remain a very important mode of HBV transmission (Hou *et al*., 2005).

2.20.3 Household and Interhousehold Transmission

Less risk but significant - can occur in settings such as shared toothbrushes, razors, combs, washcloths. Passed from child to child by biting, shared objects, oozing cuts, impetigo or by mastication of food by mothers before feeding to infants is common in some cultures and might lead to infection of infants in this population (Agbede *et al*., 2007).

2.20.4 Vertical Transmission

This mode of transmission perinatal transmission from a carrier mother to her baby; transplacental (rare), during delivery and post natal (breast feeding/close contact). It appears to be the most important factor in determining the prevalence of infection in high endemicity area (Hou *et al*., 2005).

2.21 Diagnosis of HBV Infection

Screening plays a vital role in the detection, diagnosis and monitoring of HBV. Screening allows for the identification of infected persons so that they can receive lifesaving care and treatments, which can break the cycle of transmission, slow disease progression and prevent deaths. According to the CDC, serologic testing

for hepatitis B surface antigen (HBsAg) is the primary way to identify persons with chronic HBV infection (CDC, 2009).

Serological tests for viral antigens and antibodies are typically used for diagnostic screening and can be performed on either serum or plasma. Both HBV antigens and antibody are stable at room temperature for days, at 4°C for months, and frozen at -20°C to -70°C for many years. Hepatitis B surface antigen (HBsAg) in the blood is the most useful marker of active HBV infection which appears in the blood exclusively as a component of the virus and as incomplete viral forms (Nwokedi *et al*., 2006).

2.21.1 HBV Infection Diagnosis

Hepatitis B is diagnosed by detecting several markers of the virus serologically as follows:

Hepatitis B Surface Antigen: If negative, chronic HBV infection is typically ruled out. If positive, the patient is considered HBV-infected. Chronic infection is diagnosed when the HBsAg remains detectable for greater than six months.

Total Antibody to Hepatitis B core Protein: If negative, past infection with HBV is typically ruled out. If positive, the patient has been infected with HBV. Infection may be resolved (HBsAg-negative) or ongoing (HBsAg-positive). If the infection is resolved, the person is considered naturally immune to HBV infection.

Antibody to Hepatitis B Surface Protein: If negative, the patient has no apparent immunity to HBV. If positive, the patient is considered immune to HBV (either because of resolved infection or as the result of prior vaccination). Very rarely (less than 1%) can chronic carriers be positive for HBsAg and antibody to hepatitis B

surface protein (anti-HBs) at the same time. In such cases, the patient is considered infectious.

2.22 Immunity

Immunity to HBV is acquired from a resolved infection or from vaccination. The HBV vaccine has been shown to induce protective immunity in 90% to 95% of vaccines. Most vaccines will have protective levels of anti-HBs for 5 to 10 years after vaccines, although the exact duration of immunity remains undefined (Krajden *et al*., 2005).

2.23 Treatment

Drugs currently licensed include Interferon-alpha, Lamivudine and Famciclovir. Adefovir dipivoxil has been licensed in the United States for chronic HBV infection.Other promising drugs, such as Entecavir, Emtricitabine, Clevudine, Tenofovir, Telbivudine and Pegylated interferons are in clinical trials or under development. These drugs, perhaps used in combination, hold the promise of resolving chronic infection and its sequelae.

2.24 Prevention

Hepatitis B vaccination is the most effective measure to prevent HBV and its consequences. There are safe and effective vaccines for use with vaccination programs targeted on eliminating perinatal transmission to neonates, where the risk of developing chronic infection is 70% to 95%, and to young infants (toddlers), where the risk of developing chronic infection is estimated at 30% (Bowyer *et al*., 2011). Additional preventive measures include routine screening of all pregnant women for HBsAg and post-exposure immunoprophylaxis of infants born to

HBsAg-positive women (CDC, 2009). Virtually all infections can be completely prevented by vaccination (Krajden *et al.*, 2005).

2.24.1 Immunoglobulin

People who have been exposed to HBV should be immediately given an injection of antibodies called hepatitis B immune globulin (HBIG) as well as the hepatitis B vaccine simultaneously at different sites to provide immediate protection with passively acquired antibody followed by active immunity generated by the vaccine (Jawetz *et al.,* 2007) Immunoglobulin should ideally be given with 48 hours.

2.24.2 Management of Chronic HBV Infection

Chronically infected patients with sustained Alanine aminotransferase (ALT) elevations greater than the upper limit of normal may be candidates for treatment and should undergo additional prognostic hepatitis marker testing. Chronic carriers whose ALT is within the normal range should be tested for this marker periodically because ALT levels can fluctuate with this infection. Determination of ALT levels and HBV serological and nucleic acid markers, although valuable in following infection, is not adequate to assess the severity of infection and the resulting liver damage. Before appropriate treatment can be initiated, consultation with a specialist and assessment of the disease through histological examination of a liver biopsy is strongly recommended (Krajden *et al.,* 2005).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

This study was conducted in Kano State. Kano state occupies part of the North West region of Nigeria. It shares boundaries with Kaduna, Bauchi, Jigawa and Katsina States. The location of Kano state is between $11^{0}30$ N and $8^{0}30$ E of the equator and occupies an area of approximately 20,131 square kilometres. The state is divided into three senatorial zones and has 44 Local Government Areas. It has a population of about 9.4million (NPC, 2006). Kano is cosmopolitan in outlook and the urban drift from rural areas within the state, other states in Nigeria and West Africa, has provided a steady stream of migrants adding to the growing population of the state. Kano city is characterised by overcrowding and overburden social amenities.

3.2 Ethical Approval and Consent

Approval for the study was obtained from the ethical committee of the Hospitals Management board Kano and ethical committee of the Teaching Hospital (Appendix I and II). Consent for the participation of the Individuals in the study was obtained from patients and parents or caregivers of patients younger than 18 years of age (Appendix III).

3.3 Questionnaires

Prior to the sample collection, information and data on socio-demographic and possible risk factors of the patients were obtained using self designed questionnaires (Appendix 1V) administered to patients, parents or care givers who consented to the study.

3.4 Inclusion and Exclusion Criteria

The Inclusion criteria for the study were individuals 0 – 70 years of age, male and female patients, parents and/or caregivers who gave their consent, while the exclusion criteria were individuals 0 – 70 years aged patients, parents and /or caregivers who did not give consent.

3.5 Sample Size

The sample size was determined using the formula by sar Mukaddam and Garad (2006):

$$n = \frac{z^2pq}{l^2}$$

Where n = sample size

z = standard normal distribution at 95% confidence interval =1.96

l = allowable error taken as 0.05

p = prevalence rate = 50%

q = 1- p = 0.5

Thus: $n = \frac{(1.96)^2 \times 0.5 \times 0.5}{(0.05)^2} = 384$

The calculated sample size was 384 hence; the least number of samples that could be used for the study was 384.

3.6 Study Population

The study population comprised of male and female individuals 0-70 years old from all walks of life who reported at two healthcare facilities in Kano from January to August 2009. The healthcare facilities were Aminu Kano Teaching Hospital (AKTH) and Hasiya Bayero Paediatric Hospital (HBPH) (both in Kano State).

3.7 Study Design

Approximately 30-40 patients attended the G.O.P.D unit of AKTH daily but samples were collected only every Monday and Wednesday during the period of the study while samples were collected in HSBH on Thursdays only. Every 5^{th} patient was selected randomly. If a selected patient did not give consent, the next willing patient was selected.

3.8 Sample Collection and Processing

Using a sterile disposable syringe, 3ml of venous blood was collected aseptically from each patient by a clinician, dispensed into a labelled plain sterile sample bottles and were transported safely to the laboratory at AKTH. Three hundred and sixty eight (368) samples were collected from AKTH and ninety two (92) from HSBH making a total of 460. The blood samples were centrifuged at 2,500 rpm and serum collected into clean, sterile, dry, plain sample bottles using a clean dry Pasteur pipette. The sera were stored at -20^0C in the Laboratory Department of Microbiology ABU, Zaria until needed for analysis (Cheesbrough, 2005).

3.9 Analysis of Samples

All samples were analysed for HPV- B19 IgG and Hepatitis B surface Antigen (HbsAg) using Enzyme Linked Immunosorbent assay (Serion Elisa Classic Institut Virion/Serion, Gmbh Germany) and Hepatitis B surface Antigen Acon rapid test kit respectively. All protocol was carried out according to manufacturer's instruction at the Ahmadu Bello University Microbiology Department Laboratory.

3.9.1 Detection of HPVB19 IgG Antibody by ELISA

The Principle of the ELISA for detecting IgG to HPVB19 is as follows: The HPVB19 IgG antibody test kit is based on the principle of the enzyme Immunoassay

(EIA). Microtiter wells are coated with HPVB19 antigens. This constitutes the solid phase. Diluted patient's serum is added to the wells and any antibodies specific for the antigen present will bind to the solid phase. After one hour incubation at 37oC the plate is rinsed with wash solution (NaCl with tween 20mm Tris) in order to remove unbounded material. After removal of unbounded material, anti human IgG conjugated to an enzyme (alkaline phosphatase) is allowed to react with the immune complex. After removal of excess conjugate by washing, an appropriate substrate (Paranitrophenylphosphate) is added, with which the conjugated enzyme reacts producing a coloured derivative of the substrate. The colour development is terminated by the addition of stop solution. The colour intensity is proportional to the level of specific antibody bound and can be quantified spectrophotometrically at wavelength of 405nm.

3.9.1.1 Test Procedure of HPVB19 Assay

All patients sample were diluted in the dilution buffer. The required numbers of cavities were placed in the frame and a protocol sheet was prepared. Diluted sample and ready to use controls of 100 µl were added into the appropriate wells of microtest strips sparing one well for the substrate blank. The plates were incubated in a moist chamber for 60 minutes at 37^{o}C. After incubation the solution was poured and the wells washed. During the washing each well was filled with 300µl of the washing solution, gently swirled and poured out into a container with a disinfectant. The washing procedure was repeated three times (four times altogether). The microtest plates were dried by tapping on a paper towel; 100µl of IgG conjugate was added to the appropriate wells except the substrate blank and incubated in a moist chamber for 30 minutes at 37^{o}C. After incubation all the wells were washed with washing solution and 100µl of the substrate solution was added to each well including the well for the

substrate blank. The plate was incubated at 37°C for 30 minutes in the moist chamber, after which 100µl of stopping solution was added to each well, shaken gently to mix and the optical density was read at 405nm against substrate blank using ELISA reader (Sigma Diagnostics EIA Multi well Reader II) and the results printed out.

3.9.1.2 Interpretation of Results

Results were interpreted according to the manufacturer's recommendation. To fix the cut-off ranges multiply the mean value of the measured standard OD with the numerical data of the quality control certificate and the standard curve sheet provided:

OD = 0.326 x MV (STD) with upper cut-off 5

OD = 0.216 x MV (STD) with lower cut-off 3

Sample values were calculated from the standard curve using the mean OD. A titre of >3 was considered positive.

3.9.2 Detection of Hepatitis B Surface Antigen by Rapid Test Strip

The Principle behind Hepatitis B Surface Antigen (HBsAg) rapid test strip is that it is a qualitative, solid phase, two-site sandwich immunoassay for the detection of HBsAg in whole blood, serum or plasma. The membrane is pre-coated with anti HBsAg antibodies on the test line region of the strip. During testing, whole blood, serum or plasma reacts with anti HBsAg antibodies conjugated particles. The mixture migrates upward on the membrane chromatographically by capillary action to react with anti-HBsAg antibodies on the membrane and generate a coloured line.

3.9.2.1 Test Procedure for Hepatitis B Surface Antigen.

The test was carried out at room temperature ($18^{o}C - 30^{o}C$). The test strip was removed from the foil pouch by tearing at the notch and placed on a level surface. The

strip was immersed into the specimen with the arrow pointing towards the specimen. It was taken out after 10 seconds, laid flat on a clean, dry and non absorbent surface (such as the mouth of the specimen container). The result was read after 15 minutes (Results after 30 minutes are not valid).

3.9.2.2 Interpretation of Results

The presence of a coloured line in the test region indicates a positive result, while its absence indicates a negative result. To serve as a procedural control, a second coloured line always appears in the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred.

3.10 Data analysis

Data generated from questionnaires and result obtained from laboratory analysis were entered into Microsoft excel and reduced to percentages by descriptive SPSS (15.0) which was used to analysis the data. Chi square was used to determine association between the viral infections and the various parameters studied at 95% confidence Interval and p value ≤ 0.05 was considered significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Analysis of the Total Population.

Data obtained using the questionnaire was analysed and the results showed that majority of the patients studied (22.4%:103/460) were between the ages of 21-30 years old while those < 1 year old constituted the least population (9%: 4/460) (Table 4.1). It was also observed that about 55% (253/460) were married while 45% (207/460) were single as at the time of the study. Looking at the educational background, most of the patients, 32% (145/460) had no formal education while only 17% (78/460) had higher education.

Gender, number of persons living in a house and occupations were also considered important demographic information. Analysis of these variables (Table 4.1) indicated that 50% (230/460) of the patients were female while 50% (230/460) were male. Also 58.2% (269/460) of the patients lived with less than 5 persons in same house while 41.5% (191/460) lived with more than 5 persons in same house. With respect to employment, 20.4% (94/460) were unemployed, 17.8% (82/460) were fulltime housewives while 2.4% (11/460) were retirees. Analysis of type of residence showed that majority of the patients were living in family compound 43.4% (200/460) while only 1.7% (8/460) were living in single room as at the time of the study.

Further analysis of the population by organ transplant revealed that only a female patient had kidney transplant (0.22%:1/460). The distribution of the population by history of transfusion showed that 93.5% (430/460) of the patients have never been transfused with blood while the history of jaundice showed that only 2.2% (10/460)

have had jaundice. Considering sickle cell anaemia, the result shows that patients with sickle cell disease was 22(4.8%) among the 460 studied (Table 4.2).

Table 4.1: Socio-demographic Characteristics of Patients from Selected Hospitals in Kano Metropolis.

Variable	Frequency	Percentage
Age(yrs)		
<1	4	0.9
1-10	91	19.8
11-20	59	12.8
21-30	103	22.4
31-40	97	21.1
41-50	59	12.8
≥51	47	10.2
Gender		
Male	230	50
Female	230	50
Marital Status		
Single	207	45
Married	253	55
Formal educational level		
None	145	32
Primary	93	20
Secondary	144	31
Higher	78	17

Occupation		
Civil Servant	58	12.6
Unemployed	94	20.4
Self employed	50	10.9
Farmer	28	6.1
Full time housewife	82	17.8
Skilled labourer	31	6.7
Student	63	13.7
Retired	11	2.4
Others	43	9.4
Type of residence		
Single room	8	1.7
Room and parlour	47	10.2
1-3 Bedroom	156	33.9
Family compound	200	43.5
Duplex	49	10.7
No of persons in a house		
≤5	269	58.5
>5	191	41.5

Key: **Mean age** = 28.8; **S.D** = 1.6; Others=Unskilled labourers, bike riders.

Table 4.2: Distribution of Patients by Possible Risk Factors

Variable	Frequency	Percentage
Pregnancy		
Yes	41	8.9
No	419	91.1
Blood transfusion		
Yes	30	6.5
No	430	93.5
Sickle cell Disease		
Yes	22	4.8
No	438	95.2
Organ transplantation		
Yes	1	0.22
No	459	99.8
History of Jaundice		
Yes	10	2.2
No	450	97.8

4.2 Analysis of Human Parvovirus B19 IgG

Of the 460 samples screened, 191 (41.5%) were positive for HPVB19 IgG, seroprevalence was not significantly different between the two hospitals (χ^2=1.513,df=1, P=0.2) with AKTH having the highest prevalence (42.9%:158/368) (Table 4.3). The distribution in relation to age showed that seropositivity increased with age with the lowest prevalence observed in patients <1year of age (1/4: 25%) and highest among patients ≥51years old (51%:24/47). This difference was statistically significant (χ^2=13.130, df=6, P=0.04) and was also significant across the different age groups (P=0.001).

The overall prevalence was higher in female patients (42.6%: 98/460) than male patients (40.4%: 93/460) although this difference was not statistically significant (P=0.3). Similarly, marital status was not significantly associated with the viral infections even though married patients had a higher prevalence (45.4%: 115/253) than the unmarried (36.7%: 76/207).

There was a significant association with the level of formal education (χ^2=10.487: df=4, P=0.03) with the highest prevalence detected among those without formal education (44%: 64/145) while the lowest prevalence was among those with higher education (33%:26/78). With respect to occupation, farmers had the highest prevalence of 64% (18/28) while the lowest prevalence (27.2%:3/11) was observed among retirees (χ^2=17.831; df=8, P=0.02).

The result was analysed according to the living conditions of the patients and it was observed that those living in a family compound had the highest prevalence of 45% (90/200) while the lowest prevalence (28.5%; 14/49) was detected among those living in duplex type of residential houses (χ^2=5.336; df=6,P= 0.5). Human Parvovirus B19 was highly associated with number of persons living in a house, with patients

living in houses with more than five persons having highest prevalence (51.8%: 99/191) while lowest prevalence was observed in patients living in houses with less than five persons (34.2%: 92/269) (Table 4.4).

Table 4.3: Seroprevalence of HPVB19 IgG Among Patients 0-70 years old in Selected Hospitals in Kano Metropolis

Hospital	No. Screened	No. Positive (%)	95%CI
AKTH	368	158 (42.9)	37-48
HBPH	92	33 (35.8)	26-46
TOTAL	460	191 (41.5)	37-46

KEY: AKTH= Aminu Kano Teaching Hospital; χ^2 =1.513,df=1,P=0.2
HBPH= Hasiya Bayero Paediatric Hospital

Table 4.4: Some Sociodemographic Factors Associated with HPVB19 IgG Among Patients in Selected Hospitals in Kano Metropolis

Variable	No. Screened	No. Positive (%)	p-value
Age (Years)			
<1	4	1 (25.0)	
1-10	91	24 (26.3)	
11-20	59	23 (38.9)	0.04
21-30	103	47 (45.6)	
31-40	97	44 (45.4)	
41-50	59	28 (47.5)	
≥51	47	24 (51.0)	
Gender			
Male	230	93 (40.4)	0.3
Female	230	98 (42.6)	
Marital Status			
Married	253	115 (45.4)	0.06
Single	207	76 (36.7)	
Formal educational level			
None	145	64 (44.0)	
Primary	93	40 (43.0)	0.03
Secondary	144	61 (42.0)	
Higher	78	26 (33.0)	
Occupation			
Civil Servant	58	19 (32.8)	
Unemployed	94	32 (34.0)	
Self employed	50	27 (54.0)	
Farmer	28	18 (64.2)	0.02

Full- time Housewife	82	41 (50.0)	
Skilled labourer	31	12 (38.7)	
Student	63	22 (34.9)	
Retired	11	3 (27.2)	
Others	43	17 (39.5)	
Type of residence			
Single room	8	3 (37.5)	
Room and parlour	47	21 (44.7)	0.5
1-3Bedroom	156	63 (40.3)	
Family compound	200	90 (45.0)	
Duplex	49	14 (28.5)	
No of persons living in a house			
≤5	269	92 (34.2)	0.00
>5	191	99 (51.8)	

Key: **Mean age** = 28.8;**S.D** = 1.6; Others= Unskilled labourers, bike riders.

Other possible risk factors associated with transmission of HPVB19 infection among the population were also analysed (Table 4.5). Patients who have been transfused were two times more likely to be infected with HPVB19 than those who had never been transfused with blood (χ^2=2.917,df=1,P=0.08,O.R=2.0) the results show that although there was no statistically significant difference between the prevalence among those who had been transfused and those had not. Pregnancy, Sickle cell anaemia and organ transplantation were not statistically significant association with HPVB19 infection in this study with prevalence (χ^2 =000, df=1, P=0.9), (χ^2 =1.16, df=1, P=0.2) (χ^2 =1.41, df=1, P=0.2) respectively.

Table 4.5: Possible Risk Factors Associated with HPVB19 among Patients in Selected Hospitals in Kano Metropolis

Variable	No. Screened	No. Positive (%)	p-value	OR	95%CI
Blood transfusion					
No	430	183 (39.7)	0.08	1	0.8-4.6
Yes	30	8 (26.6)		2.0	
Pregnancy					
No	419	174 (41.5)	0.9	1	0.5-1.9
Yes	41	17 (41.5)		0.9	
Organ transplant					
No	459	190 (41.4)	0.2	1	0.9-1.0
Yes	1	1 (100)		0.9	
Sickle cell Disease					
No	438	179 (38.9)	0.2	1	0.7-4.1
Yes	22	12 (54.5)		1.7	

KEY: OR= Odds Ratio

4.3 Analysis of Hepatitis B Surface Antigen

The result shows that 8.5% (39/460) of the study population were seropositive for HBsAg and there was no statistical significance in the prevalence of the HBsAg between the two hospitals with AKTH having the highest prevalence (9.5%:35/368) (χ^2=2.528, df=1, P=0.1) (Table 4.6). Analysis of some demographic characteristics (Table 4.7) showed that among those positive for HBsAg 19 (8.2%) were male patients while 20 (8.7%) were female with prevalence higher in female patients (χ^2=0.028, df=1, P=0.8). The distribution of HBsAg was also analysed according to age. Individuals in age group 21-30 years old had the highest prevalence of 15.5% (16/103) while HBsAg was not detected among those <1year old (0%: (0/4) (P=0.01).

According to level of education, the highest prevalence (11.7%: 17/145) of HBsAg was detected among patients with no formal education and with the lowest prevalence (5.5%: 8/144) among those with secondary education (χ^2=8.867, df= 4, P=0.06). Occupation was not significantly associated with HBV infection. However, the highest prevalence of 13.8% was observed among bike riders and unskilled labourers while the virus was not detected among the retirees. Looking at the number of persons living in a house and type of residence there was no significant association between these parameters with HBV infection (P=0.9; P=0.5) respectively.

Some possible risk factors that might be associated with acquiring HBV infections were analysed (Table 4.8). There was no significant association between HBV and the risk factors studied (P>0.05). The results showed that 2 (6.6%) patients with a history of transfusion had HBV, the only woman who had an organ transplant was not positive for HBV, only 1(10%) patient who had jaundice had HBV infection and pregnancy was not significantly associated with HBV infection (χ^2=0.095, df=1, P=0.7).

Table 4.6: Seroprevalence of HBsAg among Patients 0-70 years old in Selected Hospitals in Kano Metropolis

Hospital	No. Screened	No. Positive (%)	95%CI
AKTH	368	35 (9.5)	7-13
HBPH	92	4 (4.3)	1.7-10.6
TOTAL	460	39 (8.5)	6.2-11.3

KEY: AKTH= Aminu Kano Teaching Hospital; χ^2 =2.528, df=1, P=0.1

HBPH= Hasiya Bayero Paediatric Hospital

Table 4.7: Some Sociodemographic Factors Associated with HBsAg Prevalence Among Patients in Selected Hospitals in Kano Metropolis

Variable	No of Patient	No. Positive (%)	p-value
Age(Years)			
<1	4	0 (0)	
1-10	91	1 (1.1)	
11-20	59	3 (5.1)	
21-30	103	16 (15.5)	0.01
31-40	97	12 (12.3)	
41-50	59	4 (6.7)	
≥51	47	3 (6.4)	
Gender			
Male	230	19 (8.2)	0.8
Female	230	20 (8.7)	
Marital Status			
Married	253	25 (9.0)	0.2
Single	207	14 (6.8)	
Formal educational level			
None	145	17 (11.7)	
Primary	93	6 (6.5)	0.06
Secondary	144	8 (5.5)	
Higher	78	8 (10.2)	
Occupation			
Civil Servant	58	8 (13.7)	
Unemployed	94	6 (6.4)	
Self employed	50	6 (12.0)	0.3
Farmer	28	2 (7.1)	

Full time Housewife	82	7 (8.5)	
Skilled labourer	31	2 (6.5)	
Student	63	2 (3.2)	
Retired	11	0 (0)	
Others	43	6 (13.9)	
Type of residence			
Single room	8	1 (12.5)	
Room and parlour	47	6 (12.7)	
1-3Bedroom	156	12 (7.7)	
Family compound	200	13 (6.5)	0.5
Duplex	49	7 (14.2)	
No of persons living in a house			
≤5	269	23 (8.5)	
>5	191	16 (8.3)	0.9

Key: Mean age = 28.8; **S.D** = 1.6; Others = Unskilled labourers, bike riders

Table 4.8: Some Possible Risk Factors Associated with HBsAg Prevalence Among Patients in Selected Hospitals in Kano Metropolis

Variable	No. Screened	No. Positive (%)	p-value	OR	95%CI
Blood Transfusion					
No	430	37 (8.6)	0.7	1	1.7-3.3
Yes	30	2 (6.6)		0.7	
Pregnancy					
No	419	35 (8.3)	0.7	1	0.4-3.5
Yes	41	4 (9.8)		1.1	
Organ Transplant					
No	459	39 (8.5)	0.9	1	1.7-3.3
Yes	1	0 (0)		0.7	
History of Jaundice					
No	450	38 (8.4)		1	0.1-9.7
Yes	10	1 (10)	0.8	1.2	

KEY: OR=Odds Ratio

4.4 Analysis of HPVB19 Infection among Women of Child Bearing Age

Further analysis of the population based on child bearing age showed that 170 (73.1%) of the female patients studied were of child bearing age and HPVB19 IgG was detected among 48.2% (82/170). Prevalence of HPVB19 according to age was analysed and the result showed that those within 31-40 years age group had the highest prevalence of HPVB19 IgG (55% : 27/49) and lowest prevalence was among age group 10-20 years (38%: 13/34) (Table 4.9). Among the female patients 41(24%) were pregnant and only 17/41 (41.5%) of them had antibodies to HPVB19 which leaves about 58.5% of those pregnant still susceptible to HPVB19 infection.

Table 4.9: Distribution of Human Parvovirus B19 Antibody among Women of Childbearing Age Attending Two Selected Hospitals in Kano Metropolis

Age group (years)	No. Screened	No. Positive	Percentage (%)	95% CI
10-20	34	13	38	23-54
21-30	57	28	49	36-61
31-40	49	27	55	41-68
41-50	30	14	46.7	30-63
Total	**170**	**82**	**48.2**	

CHAPTER FIVE

5.0 DISCUSSION

The prevalence of HPVB19 IgG antibody obtained in this study was 41.5% which suggests that HPVB19 infection is endemic in Kano metropolis. This result is almost similar to the prevalence of 39.5% reported in Jos (Girei *et al*., 2010) and 39.9% in India but lower than the prevalence of 85.4% reported in Zaria (Ujo *et al.,* 2012). Prevalence ranges of 60-70% have been reported from developed countries such as England (Cohen and Buckley, 1988) and lower prevalence of 16.2% has been reported in Singapore (Matsunag *et al*., 1994). These discrepancies could be due to differences in the specificity and sensitivity of the assays used. Human parvovirus B19 infection has been reported in many countries around the world and the seropositive rate varies by location (Salimi *et al*., 2008). However the seroprevalence obtained in this study is within the range of 35%-70% reported from different serosurveys among the general healthy population in different regions of the world (Kelly *et al*., 2000).

Several studies in different regions of the world have identified different socio-demographic variables like age, gender, socio-economic status and environmental conditions as risk factors for acquisition of parvovirus B19 infection (Alao *et al*., 2010). For example, age has been consistently shown to be a major predictor of anti parvovirus B19 IgG seropositivity (Ooi *et al*., 2002; Mazyar and Abdolvahab, 2007; Alao *et al*., 2010).

This study showed that seropositivity increased with age. This increasing seropositivity with increasing age is consistent with the work of Girei in Jos (Girei *et al*., 2010) and those reported in other countries (Ooi *et al*., 2002; Salimi *et al*., 2008; Kishore *et al*., 2010). For Parvovirus B19, seropositivity implies immunity, because

increase in seroprevalence with age means that the proportion of individuals susceptible to parvovirus B19 decreases with age (Gilbert *et al*., 2005; Salimi *et al*., 2008).

However, the finding contrasts the result of Ujo and colleagues (2012) who reported that seropostivity did not increase with age. This could be due to the fact their study was carried out in children with sickle cell disease whereas the present study was carried out among the general population aged from 0-70 years.

The prevalence obtained among patients age group >51 years in this study is similar to 50% found in India (Kishore *et al*., 2010) and slightly lower than 55% reported in Malaysia (Ooi *et al.,* 2002) for the same age group. This difference could be due to dwindling immune response in the elderly, as a result of natural waning of antibodies with time as the antibodies could have decreased to a level beneath the detection limit of the test used here.

This study however did not demonstrate any association between gender and seropositivity as the prevalence rates were not significantly different between male and female patients. However a higher seropositivity was observed in female patients than in male patients. This is in agreement with the findings previously reported in Malaysia (Ooi *et al*., 2002), Iran (Salimi *et al*., 2008) and Nigeria (Girei *et al*., 2010). The reason for this finding could be because women are always in contact with children.

Marital status did not affect the prevalence of the HPVB19 in this study. No statistically significant difference in the positivity was evident among the married patients and the unmarried individuals. This could be due to the fact that transmission of parvovirus infection is not through sexual route. A study on blood

donors in India did not find any association between HPVB19 and marital status (Kishore *et al*., 2010).

This study determined the effect of occupation, type of residence as well as number of people living in house on seropositivity of the viruses. This study observed a higher positivity among farmers, bike riders and unskilled labourers. This finding conforms to work of Kishore *et al*. (2010) among blood donors in India where high positivity was observed among unskilled labourers, could be explained by their poor living conditions such as poor housing and low educational levels. Outdoor activities could lead to more exposure and thus contact with infectious agents and could be a reason for higher prevalence among these occupational groups. High positivity observed in this study among full time housewives could also be due to the fact that women are always in contact with children both at home and outside the home. This finding does not conform to the work of Ujo *et al.*(2012) who did not find any association between occupation and HPVB19 among parents of children in their study (Ujo *et al*., 2012).

The relationship between socio-economic status and health outcome is well known (Alao *et al*., 2010). World Health Organization (1989) proposed a definition of standards of living "Income, occupation, standard of housing, sanitation, nutrition and the level of provision of health, educational, recreational, and other services may all be used individually as measures of socioeconomic status and collectively as an index of the standard of living."(Kishore *et al*., 2010).

Education is a major factor influencing health status. The world map of literacy closely coincides with the maps of poverty, malnutrition, ill health, and high mortality rates. Studies have indicated that education, to some extent compensates the effects of poverty on health, irrespective of the availability of health facilities

(Kishore *et al.*, 2010). In this study the highest prevalence was observed among those without formal education and lowest prevalence was among the highly educated. The finding is consistent with findings of Kishore and colleagues conducted among blood donors and HPVB19 in India who also reported higher prevalence among illiterates, low educated group and lowest among the highly educated group (Kishore *et al*., 2010).

In this study it was observed that low educational level and number of persons living in a house predisposed patients to Parvovirus infection. The seroprevalence was higher in patients who lived in houses with more than five people than among those with five or less people. This difference was statistically significant. This finding is in agreement with reports of Alao and colleagues who reported higher parvovirus B19 prevalence rate in Jos among children who lived in crowded areas as compared with those living in less crowded areas (Alao *et al*., 2010) which was attributed to overcrowding in urban areas. This occurrence is common in a populous state like Kano where there is influx of people from the rural area to the urban areas and also because most people live in highly compacted areas with little or no ventilation. Overcrowding leads to a higher and faster spread of infection through the respiratory route. Human Parvovirus B19 is transmitted primarily through respiratory secretions and saliva. Therefore, in crowded environments such as daycare centers, kindergartens and schools, transmission of the virus from infected to non-infected individuals is probable (Heegaard and Brown, 2002, Miron *et al*., 2010).

In this study, a seroprevalence of 48.2% for HPVB19 IgG was obtained women of reproductive age, defined as women from 10-50 years old (Al-Khan *et al*., 2003) this result is similar to 47% observed in a study in Brazil (DeOliveira *et al*., 2009). This finding shows that about 52% of women in this age group are still

susceptible to parvovirus B19 infection which poses a public health problem. Parvovirus infection during pregnancy can cause severe foetal anaemia as a result of foetal erythroid progenitor cell infection with a shortened half-life of erythrocytes, causing high output cardiac failure and subsequently non-immune hydrops foetalis (Heegaard and Brown, 2002; Miron *et al*., 2010). It is important that the role of Parvovirus B19 during pregnancy be studied, it is certain that it is responsible for cases of foetal hydrops reported in Campania (Liguori *et al*., 1998).

Since Human Parvovirus B19 transmission is mainly through respiratory droplets. Similar trend have been documented in the epidemiology of communicable diseases spread through respiratory route such as diphtheria, rubella, influenza, pertussis, meningococcal meningitis, and tuberculosis (Kishore *et al*., 2010). The risk of exposure is greater in lower social classes, living in overcrowded and poor housing conditions. Lack of education, large families, and poor qualities of life are the predisposing factors for such infections. Blood-borne infections like hepatitis B are also influenced by the socioeconomic status (Kishore *et al*., 2010).

However, when one considers the low prevalence rate of HPVB19 in this study, it may be possible that the timing of this study corresponds to a period of low transmission in our environment, since in many regions of the world Parvovirus B19 transmission follows a seasonal pattern with outbreaks every three to six years (Nicolay and Cotter, 2009).

The prevalence of 8.5% for Hepatitis B Surface Antigen obtained in this study, makes this study area fall in the category of high endemicity as defined by Uneke (2005) (prevalence greater than 7% in an adult population). This prevalence is lower than the of 23.1 % reported previously in Kano (Nwokedi *et al*., 2006), 28.8% in Biu (Bello *et al*., 2011) and 12.5% in Zaria (Aminu *et al.,* 2013) but slightly higher than

6% in Kano (Taura *et al*., 2008), 6% in Abeokuta (Okonkwo *et al*., 2010), 6% reported in Nassarawa (Adoga *et al*., 2010), 2.4% in Yola (Olokoba *et al*., 2009) and 2.6% in Iran (Merat *et al*., 2009).

In all epidemiological studies, age has always been the most important factor (Agbede *et al*., 2007). The age of acquiring infection is the major determinant of the incidence and prevalence rates. In this study, the significant differences in prevalence of HBsAg in the various age groups indicate that this factor played an important role in the prevalence obtained and was associated with HBV transmission with younger patients 21-30 years were more susceptible to HBV. This result is similar to findings of Taura *et al*. (2008) and Adoga *et al*. (2010) who also reported the highest prevalence among this age group. The reason could be due to high sexual activity within these age brackets. Hepatitis B surface Antigen was not detected in patients <1 year old which could be explained by the very low number of patients screened (four) in that age group.

This study did not find any significant association between gender and HBV infection, though higher prevalence was found in female patients, similar findings have been reported in Nigeria (Nwokedi, *et al*., 2006, Agbede *et al*., 2007, Okonkwo *et al*., 2010) and in Iran (Merat *et al*., 2009). Similar studies in Nigeria (Adoga *et al*., 2010; Bello *et al*., 2011; Aminu *et al.,* 2013) and in Greece (Gogos *et al*., 2003) reported higher prevalence in male than female patients. This could be due to chance. Likewise, no association was found between marital status and HBsAg although a higher prevalence was found among married than single individuals in this study. In a similar study (Bello *et al.,* 2011) the highest prevalence was reported among married and the lowest among divorcees.

With respect to the educational background of the patients, the highest prevalence was found among those without formal education and the lowest among those with secondary education. This is in disagreement with Bello *et al.* (2008) who found higher prevalence among stark illiterates and least prevalence among those with higher education but in agreement with a study in Ethiopia (Negero *et al*., 2011) which found no association between level of education and HBsAg positivity.

According to occupation, bike riders and unskilled labourers had the highest prevalence which is in agreement with findings in Ethiopia (Negero *et al*., 2011) who reported no association with occupation while among retirees the virus was not detected which could be due to spontaneous clearance among the older age group. A study also found association between HBV transmission and occupation in China (Zhang *et al*., 2011).

In relation to other sociodemographic factors such as type of residence and number of persons living in a house, the prevalence was highest among persons living in duplexes and lowest among those living in family compound. Highest prevalence was also found among patients living in houses with less than five persons and lowest among those living in houses with more than five persons. This finding is in agreement with a study in China (Zhang *et al*., 2011) who found no association between HBV and number of persons living in a house.

This study considered the history of transfusion and found that there was no significant association between blood transfusion and HBV. The prevalence among those with transfusion history was lower than among those with no history of transfusion. This is consistent with findings of Aganga–williams *et al.* (1999) who found no association between HBV and history of transfusion but contrast the reports of Agbede *et al.* (2007) who found association between history of transfusion and

HBV in children. The finding in the present study may suggest that transfusion may not be a significant mode of transmission for HBV in Kano metropolis which means infection could be through other means other than blood transfusion.

Other risk factors such as organ transplantation, history of jaundice and pregnancy were not statistically associated with transmission of HBV among the population. This could be because most HBV infections in underdeveloped countries like Nigeria result from perinatal infection or household contacts, while sexual contact, shared needles in intravenous drug abusers, occupational exposure and contaminated blood or organ transplants are responsible for HBV and HCV transmission in the developed world (Gogos *et al*., 2003).

The high rate of infection for HBV observed among patients may not be unconnected with lack of awareness, since most of the patients in the study area from low socio-economic class where campaign against these diseases are limited and factors that predisposes to the disease prevails.

5.1 CONCLUSION

The findings in this study confirmed the presence of Human Parvovirus B19 infection in Kano State with sero-prevalence comparable to the rates found in various countries in the world. Sero-prevalence of HPVB19 among patients was low leaving a large proportion (58%) of the population in Kano still susceptible to B19 infection. The study also showed that about 52% of women of childbearing age were still susceptible to HPVB19. This study also showed the effect of formal education, occupation and number of persons living in a house on seropositivity of individuals to HPVB19.

The prevalence of 8.5% obtained for the HBV showed that HBV infection is endemic in Kano and still poses as a public health problem which must be addressed.

It is very important, especially for health providers and policy makers, to recognize the health implication of these viruses in this area and design effective preventive programs.

5.2 RECOMMENDATIONS

In view of low prevalence of HPVB19, obtained in this study hence high susceptibility among population coupled with lack of data regarding seroprevalence of HPV B19 among childbearing women in Kano, there is need for awareness on the virus to educate the public on the risk especially when pregnant.

In view of low prevalence of HPVB19 among pregnant women a prospective study is therefore recommended to investigate the prevalence of B19 parvovirus associated non immune hydrops and stillbirths in order to plan a national preventive approach.

There is also need to investigate the presence of HPVB19 among blood donors for probable inclusion as routine screening since Parvovirus B19 can be transmitted through blood products.

There is need for further investigation on the main risk factors associated with HBV infection in Kano state.

This study was hospital based, therefore there is need for a community based study, to detect the presence of current infection by detecting IgM and if possible molecular epidemiology researches as well as in the population in order to find out the persistence of HPVB19 DNA in tissues.

REFERENCES

Adoga, M.P., Gyar, S.D., Pechulano, S., Bashayi, O.D., Emiasegen, S.E., Zungwe, T., Iperepolu, O.H., Agupugo, C. and Agwale, S.M. (2010). Hepatitis B virus infections in apparently healthy urban Nigerians: data from pre-vaccination tests. *Journal of Infections for Developing Countries,* 4(6):397-400.

Aganga-williams, O.M., Akanmu, A.S., Akinete, I. and Njoku, O.S. (1999). Prevalence of hepatitis B surface antigen among women of child bearing age in Lagos, Nigeria. *African Journal of Reproductive Health,* 3(1).45-50.

Agbede, O.O., Iseniyi, J.O., Kolawole, M.O. and Ojuawo, A. (2007). Risk factors and seroprevalence of hepatitis B surface antigenaemia in mothers and their pre-school age children in Ilorin, Nigeria. *Therapy*, 4(1): 67-72.

Anderson, M. J., Lewis, E., Kidd, I.M., Hall, S.M. and Cohen, B.J. (1984). An outbreak of *Erythema infectiosum* associated with human Parvovirus infection. *Journal of Hygiene,* 93(1):85–93.

Anderson, L.J., Tsou, C., Parker, R.A., Chorba, T.L., Wulff, H., Tattersall, P.and Mortimer, P.P. (1986). Detection of antibodies and antigens of human Parvovirus B19 by enzyme linked immunosorbent assay. *Journal of Clinical Microbiology,* 24:522-526.

Anderson, L.J. (1987). Role of parvovirus B19 in human disease. *Paediatric Infectious Disease Journal,* 6: 711-781.

Alao, O.O., Girei, A.I., Joseph, D.E., Banwat, E.B., Araoye, M.O., Orkuma J., Jombo, G.T.A. and Damulak, D.O.(2010). Effect of Socio-demographic Variables on Anti-Parvovirus B19 Antibody Seropositivity among Children with Sickle cell Anaemia in Jos, North Central Nigeria. *The Internet Journal of Epidemiology*. 8: 2.

Al-Khan, A., Caligiur, I.A. and Apuzzio, J.(2003). Parvovirus B-19 infection during pregnancy. *Infectious Disease Obstetrics and Gynaecology,*11: 175-179.

Al-Shamahy, H. (2000). Prevalence of Hepatitis B surface antigen and risk factors of HBV infection in a sample of healthy mothers and their infants in Sana'a, Yemen. *Annals of Saudi Medicine,*20,464-467.

Aminu M, Okachi, E.E., Abubakar, S.M. and Yahaya, A. (2013). Prevalence of Hepatitis B Virus Surface Antigen among Healthy Asymptomatic Students in a Nigerian University. *Annals of African Medicine,* 12(1): 57-58.

Barah, F., Vallely, P.J., Chiswick, M.L., Cleator, G.M and Kerr, J.R. (2001). Association of Human Parvovirus B19 infection with acute meningoencephalitis. *Lancet*, 358(9283):729–730.

Baddour, M.M. (2006). Are plasma derivatives sources for parvovirus B19 transmission? *Egyptian Journal of Medical Microbiology*, 15(2):419-426.

Bello, R. H., Obot, E. and Olabode, H. O. K. (2011). Sero-prevalence and risk factors associated with hepatitis B surface antigen (HBsAG) amongst patients in Biu, Borno State, Nigeria. *Journal of Public Health and Epidemiology*, 3(10), 448-453.

Bernau, J., Durand, F. and Valla, D. (1999). Parvovirus B19 infection and fulminant hepatitis. *Lancet*, 353: 754.

Berns, K.1. (1996). *Parvoviridae*: The Viruses and Their Replication. In: Fields, B.M., Knipe, D.M., Howley, P.M., Chanock, R. M., Melnick, J.L., Monath, T.P., Roizman, B and Straus, S.E *(ed)*. Fields Virology. 3rd edition. Philadelphia. Lippincott-Raven publishers, 2173-2197.

Bowyer, S.M., Sim, J.G.M. and Webber, L.M. (2011). Current laboratory diagnosis of hepatitis B virus infection including 8 years of retrospective laboratory data. *Continuing Medical Education,* 29(5), 210-213.

Blumel, J., Schmidt, I., Effenberger, W., Seitz, H., Willkommen, H., Brackmann, H.H., Lower, J. and Eis-Hubinger, A. M. (2002). Parvovirus B19 transmission by heat-treated clotting factor concentrates. *Transfusion*, 42 (11), 1473-1481.

Boctor, F.N. and Schreiber, Z. (2002). Transfusion medicine illustrated.Giant pronormoblasts due to Parvovirus B19 infection. *Transfusion*, 42(1):1.

Bousquet, F., Segondy, M., Faure, J.M., Deschamps, F. and Boulot, P. (2000). B19 Parvovirus-induced fetal hydrops: good outcome after intrauterine blood transfusion at 18 weeks of gestation. *Fetal Diagnosis and Therapy*, 15: 132–133.

Bűltmann, B.D., Klingel, K., Nabauer, M., Wallwiener, D. and Kandolf, R. (2005). High prevalence of viral genomes and inflammation in peripartum cardiomyopathy. *American Journal of Obstetrics and Gynaecology*, 193: 363–365.

Broliden, K., Tolfvenstam,T. and Norbeck, O.(2006). Clinical aspects of Parvovirus B19 infection. *Journal of Internal Medicine*, 260:285-304.

Brown, K.E., Anderson, S.M. and Young, N.S. (1993). Erythrocyte P antigen: cellular receptor for B19 Parvovirus. *Science*, 262(5130):114–117.

Brown, K. E., Young, N. S., Alving, B. M. and Barbosa, L. H. (2001). Parvovirus B19: Implications for transfusion medicine. Summary of a workshop. *Transfusion, 41* (1), 130-135.

Brown, K. E. (2004). Detection and quantitation of parvovirus B19. *Journal of Clinical Virology,* 31 (1), 1-4.

Brown, K.E. (2010). The expanding range of parvoviruses which infect humans. *Reviews in Medical Virology,* 20 (1), 231-244.

Cassinotti P.and Siegl G. (2000). Quantitative evidence for persistence of human Parvovirus B19 DNA in an immunocompetent individual. *European Journal of Clinical Microbiology of Infectious Disease*, 19:886-895.

Candotti, D., Etiz, N., Parsyan, A. and Allain, J.P. (2004). Identification and characterization of persistent human erythrovirus infection in blood donor samples. *Journal of Virology*, 78: 12169–12178.

Cavinta, L., Sun, J.and May, A. (2009). A new isolate of hepatitis B virus from the Philippines possibly representing a new subgenotypes of C6. *Journal Medical Virology,*81(6): 983-987.

Centers for Disease Control and Prevention. Testing and Public Health Management of Persons with Chronic Hepatitis B Virus Infection. Available at: http://www.cdc.gov/hepatitis/HBV/TestingChronic.htm. Accessed September 28,2009.

Chen, M.Y., Hung, C.C., Fang, C.T. and Hsieh, S.M. (2001). Reconstituted immunity against persistent Parvovirus B19 infection in a patient with acquired immunodeficiency syndrome after highly active antiretroviral therapy. *Clinical Infectious Diseases*, 32 (9):1361–1365.

Cheesbrough, M. (2005). Haematology Test: District Laboratory Practice in Tropical Countries (Part two).pp 125.

Chin, J. (2000). Control of communicable diseases manual, 17th Ed. Washington DC, USA: American Public Health Association.

Chisaka H, Morita, E., Yaegashi, N. and Sugamura, K. (2003). Parvovirus B19 and the pathogenesis of anaemia. *Reviews of Medical Virology*, 13(6):347–359.

Cioc, A.M., Sedmak, D.D., Nuovo, G.J., Dawood, M.R., Smart, G. and Magro, C.M.(2002). Parvovirus B19 associated adult Henoch Schonlein Purpura. *Journal of Cutaneous Pathology*, 29(10):602–607.

Cohen, B.J. and Buckley, M.M. (1988). The prevalence of antibody to human parvovirus B 19 in England and Wales. *Journal of Medical Microbiology*, 25, 151-153.

Cohen, B.J. and Brown, K.E. (1992). Laboratory infection with human parvovirus B19. *Journal of Infection, 24* (1):113-114.

Cohen, B.J.(1995). Parvovirus B19: An expanding spectrum of disease. *British Medical Journal*, 311: 1549-1552.

Cossart, V.E., Field, A.M., Cant,B. and Widdows, D.(1975). Parvovirus-Like particles in human sera. *Lancet*,1:72-73.

Corcoran, A., Doyle, S., Waldron, D., Nicholson, A. and Mahon, B.P. (2000). Impaired gamma interferon responses against parvovirus B19 by recently infected children. *Journal of Virology,* 74:9903–9910.

Corcoran, A. and Doyle, S.(2004). Advances in the biology, diagnosis and host-pathogen interactions of Parvovirus B19. *Journal of Medical Microbiology,* 53 (6): 459–475.

Corcoran, A., Mahon, B.P. and Doyle, S. (2004). B cell memory is directed toward conformational epitopes of Parvovirus B19 capsid proteins and the unique region of VP1. *Journal of Infectious Disease*, 189(10):1873–1880.

Corcoran, A., Doyle, S., Allain, J.P., Candotti, D. and Parsyan, A. (2005). Evidence of serological cross reactivity between genotype 1 and genotype 3 erythrovirus infections. *Journal of Virology*, 79: 5238–5239.

Corcoran, A. and Doyle, S. (2006). Parvovirus B19: Molecular Virology, Clinical Features, Prevalence, Diagnosis and Control. In Unspecified Elsevier, pp.173-209.

Corcioli, F., Zakrzewska, K. and Rinieri, A. (2008). Tissue persistence of Parvovirus B19 genotypes in asymptomatic persons. *Journal of Medical Virology*, 80: 2005–2011.

Crook, T.W., Rogers, B.B. and McFarland, R.D. (2000). Unusual bone marrow manifestations of Parvovirus B19 infection in immunocompromised patients. *Human Pathology,* 31(2):161–168.

Deiss, V., Tratschin, J.D., Weitz, M. and Siegl, G. (1990). Cloning of the human Parvovirus B 19 genome and structural analysis of its palindromic termini. *Virology,* 175:247–254.

DeOliveira, M.I., Curti, S.P., Afonso, A.M.S., Figueiredo, C.A., Azevedo, R.S., Passos, S.D., Stewien, K.E. and Durigon, E.L.(2009). Parvovirus B19 frequency in exanthematic cases initially suspected as measles or rubella infections in São Paulo, Brazil. *Virus Reviews and Research* 14, 1-12.

D'Eufemia, P., Nigro, G., Celli, M., Finocchiaro, R., Iannetti, P. and Giardini, O. (2000). Low-dosage immunoglobulins for an infant with hypogammaglobulinemia, maple syrup urine disease and Parvovirus B19-associated aplastic crisis. *Journal of Paediatrics, Haematology and Oncology*, 22(5):485–487.

Dienstag, J.L.(2008). Hepatitis B Infection. *New England Journal of Medicine,* 359:148-150.

Ekman, A., Hokynar, K., Kakkola, L., Kantola,K., Hedman, L., Bondén, H., Gessner, M., Aberham, C., Norja, P., Miettinen, S.,Hedman, K. and Söderlund-Venermo, M. (2007). Biological and immunological relations among Human Parvovirus B19 genotypes1 to 3. *Journal of Virology,* 81:6927–6935.

Eis-Hübinger, A. M., Reber, U., Abdul-Nour, T., Glatzel, U., Lauschke, H. and Pütz, U. (2001). Evidence for persistence of Parvovirus B19 DNA in livers of adults. *Journal of Medical Virology*, 65:395-401.

Enders, M., Weidner, A., Zoellner, I., Searle, K. and Enders, G. (2004). Foetal morbidity and mortality after acute human parvovirus B19 infection in pregnancy: prospective evaluation of 1018 cases. *Prenatal Diagnosis*, 24: 513–518.

Erdman, D.D., Usher, M.J. and Tsou, C. (1991). Human Parvovirus B19 specific IgG, IgA, and IgM antibodies and DNA in serum specimens from persons with Erythema Infectiosum. *Journal of Medical Virology*, 35(2):110–115.

Ergaz, Z. and Ornoy, A. (2006). Parvovirus B19 in Pregnancy. *Reproductive Toxocology*, 21:421-435.

Franssila, R., Hokynar, K. and Hedman, K. (2001). T helper cell-mediated in vitro responses of recently and remotely infected subjects to a candidate recombinant vaccine for human parvovirus B19. *Journal of Infectious Disease,* 183:805–809.

Frickhofen, N., Abkowitz, J.L., Safford, M., Berry, J.M., AntunezdeMayolo, J., Astrow, A., Cohen, R., Halperin, I., King, L. and Mintzer, D. (1990). Persistent B19 parvovirus infection in patients infected with human immunodeficiency virus Type 1(HIV-1): a treatable cause of anaemia in AIDS. *Annuals of Internal Medicine,* 113, 926-933.

Gaboulaud, V., Parquet, A., Tahiri, C., Claeyssens, S., Potard, V., Faradji, A., Peynet, J. and Costagliola, D.(2002). Prevalence of IgG antibodies to Human Parvovirus B19 in haemophilia Children treated with recombinant factor (F) VIII or with at least one plasma derived FVIII or FIX concentrates: results from the French haemophilia Cohort. *British Journal of Haematology*.116. 383-389.

Gilbert, N.L., Gyorkos, T.W., Beliveau, C., Rahme, E., Muecke, C. and Soto, J.C. (2005). Seroprevalence of Parvovirus B19 infection in daycare educators. *Epidemiology of Infection*, 133: 299-304.

Girei, A.I., Alao, O.O., Joseph, D.E., Damulak, D.O., Banwat, E.B., Nwadioha, S.I. and Jombo, G.T.A. (2010). Haematological profile of sickle cell anaemia with human Parvovirus B19 in Jos, North central Nigeria. *Journal of Clinical Medicine and Research*. 2:9.

Gogos, C.A., Fouka, K.P., Nikiforidis, G., Avgeridis, K., Sakellaropoulos, G., Bassaris, H., Maniatis, A. and. Skoutelis, A. (2003). Prevalence of hepatitis B and C virus infection in the general population and selected groups in South-Western Greece. *European Journal of Epidemiology,* 18: 551–557.

Guan, W., Wong, S. and Zhi, N.(2009). The genome of human Parvovirus b19 can replicate in nonpermissive cells with the help of adenovirus genes and produces infectious virus. *Journal of Virology*, 83: 9541–9553.

Heegaard, E.D., Peterslund, N.A. and Hornsleth, A. (1995). Parvovirus B19 infection associated with encephalitis in a patient suffering from malignant lymphoma. *Scandinavian Journal of Infectious Disease,* 27(6): 631–633.

Heegaard, E.D. and Brown, K.E. (2002). Human Parvovirus B19. *Clinical Microbiology. Review,* 15: 485–505.

Heegaard, E.D., Petersen, B.L., Heilmann, C.J. and Hornsleth, A. (2002). Prevalence of parvovirus B19 and parvovirus V9 DNA and antibodies in paired bone marrow

and serum samples from healthy adults. *Journal of Clinical Microbiology,* 40:933-936.

Heymann, D. L. (2004). In American Public Health Association (Ed.).Control of Communicable Diseases Manual (18th ed., pp. 196-199). Washington, D.C: American Public Health Association.

Hoang, M.P., Dawson, D.B., Rogers, Z.R., Scheuermann, R.H. and Rogers, B.B. (1998). Polymerase chain reaction amplification of archival material for Epstein-Barr virus, cytomegalovirus, human herpes virus 6, and Parvovirus B19 in children with bone marrow hemophagocytosis. *Human Pathology*, 29 (10):1074–1077.

Ho, J.K., Tha, S,P., Coupland, R., Dalal, B.I., Bowie, W.R. and Sreenivasan, G.M.(2005). Parvovirus B19 in an immunocompetent adult patient with acute liver failure: an underdiagnosed cause of acute non-A-E viral hepatitis**.** *Canadian Journal of Gastroenterol*ogy, 19:161-162.

Hou, J., Liu, Z. and Gu, F. (2005). Epidemiology and Prevention of Hepatitis B virus Infection. *International Journal of Medical Science*, (2) (1):50-57.

Howard, C.R. (1986). The Biology of Hepadnaviruses. *Journal of General Virology*. 67(7):1215-1235.

Hsu, T.C., Chen, T.Y., Lin, M.C., Tzang, B.S. and Tsay, G.J. (2005). Human parvovirus B19 infection in patients with chronic hepatitis B or hepatitis C infection. *Journal of Gastroenterology and Hepatology,* 20:733–738.

Hsu, T.C., Tzang, B.S., Huang, C.N., Lee, Y.J., Liu, G.Y., Chen, M.C. and Tsay, G.J. (2006). Increased expression and secretion of interleukin-6 in human parvovirus B19 nonstructural protein (NS1) transfected COS-7 epithelial cells. *Clinical and Experimental Immunology,* 144: 152–157.

Innovative Biotech Limited. (2012). Treatment of Hepatitis B.www.innovativebiotechng.com. Assessed 5 November, 2012.

Jawetz, Melnick and Adelberg's (2007). Medical Microbiology. In: Geo, F.B., Karen,C.C., Janet, S.B and Stephen, A.M.Pp 413-417,24th (ed) Mc Graw Hill International Edition.

Jawetz, Melnick and Adelberg's (2007). Medical Microbiology. In: Geo,F.B., Karen,C.C., Janet, S.B and Stephen, A.M.Pp 466-484,24th (ed) Mc Graw Hill International Edition.

Jordan, J. (2001). Diagnosing Human Parvovirus B19 infection: Guidelines for test selection. *Molecular Diagnosis*,6:307-312.

Jordan, J., Tiangco, B., Kiss, J., Koch W. and Jordan. (1998). Human Parvovirus B19: prevalence of viral DNA in volunteer blood donors and clinical outcomes of transfusion recipients. *Vox Sanguinis,* 75: 97–102.

Kahn, J.S., Kesebir, D. and Cotmore, S.F. (2008). Seroepidemiology of Human Bocavirus defined using recombinant virus-like particles. *Journal of Infectious Disease,* 198 (1): 41–50.

Karetnyi, Y.V., Beck, P.R., Markin, R.S., Langnas, A.N. and Naides, S.J. (1999). Human Parvovirus B19 infection in acute fulminant liver failure. *Archive of Virology*,144(9):1713–1724.

Kaufmann, B., Alan, A. S. and Rossmann, G.M. (2004). The structure of human parvovirus B19. *Proceedings of National Academy of Sciences,* 101(32):11628-11633.

Kelly, H.A., Siebert, D., Hammond, R., Leydon, J., Kiely, P. and Maskill, W. (2000). The age specific prevalence of human parvovirus immunity in victoria Australia compared with other parts of the world. *Epidemiology and Infection*, 124:449-457.

Kerr, J. R. (2000). The *Parvoviridae*; an emerging virus family. *Infectious Disease Review*. 2:99–109.

Kerr, J.R. and Tyrrell, D.A. (2003). Cytokines in Parvovirus B19 infection as an aid to understanding chronic fatigue syndrome. *Current Pain Headache Report*; 7(5):333–341.

Kishore, J., Srivastava, M. and Choudhary, N.(2010). Standardization of B19 IgG ELISA to study the seroepidemiology of parvovirus B19 in North Indian voluntary blood donors. *Asian Journal of Transfusion Science*, 4:86-90.

Klein, R.M., Jiang, H. and Niederacher, D. (2004). Frequency and quantity of the Parvovirus B19 genome in endomyocardial biopsies from patients with suspected myocarditis or idiopathic left ventricular dysfunction. *Kardiology*; 93(4):300–309.

Kleinman,S.H., Glynn,S.A., Busch,M.P., Wright,D.J., McMullen,Q. and Schreibe, G.B. (2002). Declining incidence rates and risks of transfusion–transmitted viral infections in US blood donors. *Vox Sanguinis*, 83:106.

Krajden, M., McNabb G. and Petric, M. (2005). The laboratory diagnosis of hepatitis B virus. *Canadian Journal of Infectious Disease and Medical Microbiology*, 16(2): 65–72.

Kramvis, A., Kew, M. and Francois, G. (2005). Hepatitis B virus genotypes.*Vaccine*, 23(19): 2409-2423.

Kurtzman, G. J., Cohen, B.J., Field, A.M., Oseas, R., Blaese, R.M. and Young, N.S**.** **(**1989). Immune response to B19 parvovirus and an antibody defect in persistent viral infection. *Journal of Clinical Investigation*, 84**:**1114–1123.

Kurbanvo, F., Tanaka, Y.M., Kramvis, A., Simmonds, P. and Mizokami, M. (2008). When should i consider a new Hepatitis B virus genotype? *Journal of Virology*,82(16):8241-8422.

LaMonte, A. C., Paul, M. E. and Read, J.S. (2003). Persistent Parvovirus B19 infection without the development of chronic anaemia in HIV infected and uninfected children: the women and infant transmission study. *Journal of Infectious Disease,*189: 847-851.

Labau, E., Bonnet, E., Alric, L. and Massip, P. (1996). Infection par le parvovirus B19. Nouvelles approches physiopathologiques. *La Presse Medicale*, 25: 162–166.

Lefrere, J. J., Servant-Delmas, A., Candotti, D., Mariotti, M., Thomas, I., Brossard, Y., Lefrere, F., Girot, R., Allain, J. P. and Laperche, S. **(**2005). *Persistent B19 infection in immunocompetent individuals: implications for transfusion safety. Blood,* 106*,* 2890*–2895.*

Liguori, G., Esposito,C., Fossi E. and Guastafierro, S. (1998). Parvovirus B19 infection in Campania: serum-epidemiological study on healthy subjects and patients affected by blood transmitted viruses. *Journal of Preventive Medicine and Hygiene,*39: 18-20.

Lui, S.L., Luk, W.K., Cheung, C.Y., Chan, T.M., Lai, K.N. and Peiris, J.S. (2001). Nosocomial outbreak of parvovirus B19 infection in a renal transplant unit. *Transplantation,* 71: 59–64.

Lusida, M.I., Nugrahuputra, V.E., Soetjipto Handajani, R., Nagano- Fujii,M., Sasayama, M., Utsumi, T., Hotta,H.(2008).Novel Subgenotypes of Hepatitis B virus genotypes C and D in Papua, Indonesia. *Journal of Clinical Microbiology,* 46(7): 2160-2166.

MacLean, B., Hess, R.F., Bonvillian, E., Kamate, J., Doada, D., Cosimano, A. and Shannon, H. (2012). Seroprevalence of Hepatitis B surface antigen among pregnant women attending the Hospital for women and Children in Koutiala,Mali. *South African Medical Journal*,102,47-49.

Matsunaga, Y., Goh, K.T. and Utagawa, E.(1994). Low prevalence of antibody to parvovirus B19 in Singapore. *Epidemiology of Infection*, 113:537-540.

Mazyar, Z., R. and Abdolvahab, A.(2007). The seroprevalence of Parvovirus B19 among Preschool age/young adults in Shiraz, Iran. Pakistan. *Journal of Biological Sciences,*10 (10):1763-1765.

McNall, R.Y., Head, D.R., Pui, C.H. and Razzouk, B.I. (2001). Parvovirus B19 infection in a child with acute lymphoblastic leukemia during induction therapy. *Journal of Pediatric Hematology and Oncology,* 23(5):309–311.

Merat,S., Rezvan, H., Nouraie, M., Jamali, A., Assan, S., Abolaghasemi, H., Radmard,A.R., Zaer-Rezai, H., ZeidAbadi-Nejhad, M., Hosseini, M.R., Amini, Kafiabad,S., Maghsudlu,M., Pourshams,A.and Malekzadeh,R. (2009). The prevalence of hepatitis B surface antigen and anti-hepatitis B core antibody in Iran: A population-based study. *Archives of Iran Medical Journal*, 12(3):225-231.

Miron, D., Horovitz, Y., Luder, A., Ohnona F.S. and Schelisinger, Y.(2010). Age-Related Immunoglobulin G Seroprevalence of Human Parvovirus B-19 in Israeli Children. *International Medical Journal,* 12:277-279.

Modrow, S., Gigler, A. and Hemauer, A. (1997). Parvovirus B19 Infection in Humans. *Biomedical Progress*, 10:45-50.

Moffatt, S., Yaegashi, N., Tada, K., Tanaka, N. and Sugamura, K.(1998). Human parvovirus B19 nonstructural (NS1) protein induces apoptosis in erythroid lineage cells. *Journal of Virology,* 72:3018–3028.

Momoeda, M., Kawase, M., Jane, S.M., Miyamura, K., Young, N.S. and Kajigaya, S. (1994a). The transcriptional regulator YY1 binds to the 50-terminal region of B19 parvovirus and regulates P6 promoter activity. *Journal of Virology,* 68: 7159–7168.

Momoeda, M., Wong, S., Kawase, M., Young, N.S. and Kajigaya, S. (1994b). A putative nucleoside triphosphate-binding domain in the nonstructural protein of B19 parvovirus is required for cytotoxicity. *Journal of Virology*, 68:8443–8446.

Mossong, J., Hens, N., Friederichs, V., Davidkin, I., Broman, M. and Litwinska, B. (2008). Parvovirus B19 infection in five European countries: Seroepidemiology, force of infection and maternal risk of infection. *Epidemiology of Infection*, 136:1059-1068.

Muzyczka, N. and Berns, K.I. (2001). *Parvoviridae*: the viruses and their replication. In Griffin, D.M., Lamb, R.A., Martin, M.A., Broizman, S.E Straus (eds), *Fields Virology*, Vol. 2. Lippincott Williams & Wilkins, Philadelphia, Pp. 2327-2359.

National Population Commission. (NPC). Federal Republic of Nigeria and ORC Macro. (2006). Nigeria Demographic and Health Survey.

Negero, A., Sisay, Z. and Medhin, G.(2011). Prevalence of Hepatitis B surface antigen (HBsAg) among visitors of Shashemene General Hospital voluntary counselling and testing centre. *BioMedCentral Research Notes,* 4:35; 1-5.

Nicolay, N and Cotter, S. (2009). Clinical and epidemiological aspects of parvovirus B19 infections in Ireland, January1996-June 2008. *Eurosurveillance*: 14 (25): pii=19249.Availableonline:http//www.eurosurvelliance.org/ViewArticle.aspx?Articleld=19249 (updated 2009 June 25, accessed 2010 September.7).

Nguyen, Q.T., Sifer, C., Schneider, V., Bernaudin, F., Auguste, V. and Garbarg-Chenon, A. (1998). Detection of an erythrovirus sequence distinct from B19 in a child with acute anaemia. *Lancet,* 352: 1524.

Nguyen, Q.T., Sifer, C., Schneider, V., Allaume, X., Servant, A., Bernaudin, F., Auguste, V. and Garbarg-Chenon, A. (1999). Novel human erythrovirus associated with transient aplastic anaemia. *Journal of Clinical Microbiology,*37(8),2483–2487.

Nguyen, Q.T., Wong, S., Heegaard, E.D. and Brown, K.E. (2002). Identification and characterization of a second novel human erythrovirus variant, A6. *Virology,* 301: 374–380.

Norja, P., Hokynar, K., Aaltonen, L.M., Chen, R., Ranki, A., Partio, E.K., Kiviluoto, O., Davidkin, I., Leivo, T., Eis-Hübinger, A.M., Schneider, B., Fischer, H.P., Tolba, R., Vapalahti, O., Vaheri, A., Söderlund-Venermo, M. and Hedman, K.(2006). Bioportfolio: lifelong persistence of variant and prototypic erythrovirus DNA genomes in human tissue. *Proceedings of National Academy of Sciences*,103**:**7450-745.

Norja, P., Eis-Hübinger, A.M., Söderlund-Venermo, M., Hedman, K. and Simmonds, P. (2008). Rapid sequence change and geographical spread of human parvovirus B19: comparison of B19 virus evolution in acute and persistent infections. *Journal of Virology,* 82:6427-6433.

Nwokedi, E.E., Emokpae, M. A., Taura, A.A. and Dutse, A. I. (2006). The trend of hepatitis B surface antigenemia among teaching hospital patients in Kano. *African Journal of Clinical and Experimental Microbiology*, 7(3):43-47.

Okonkwo, I.O., Soleye, F.A., Amusan, T.A., Udeze, A.O., Alli, J.A., Ojezele, M.O., Nwanze, J.C. and Fadeyi, A. (2010). Seroprevalence of Hbsag Among Patients in Abeokuta, South Western Nigeria. *Global Journal of Medical Research,* 10: (2).40-43.

Olokoba, A.B., Salawu, F.K., Danburam, A., Desalu, O.O., Olokoba, L.B., Wahab, K.W., Badung, L.H., Tidi, S.K., Midala, J., Aderibigbe, S., Abdulrahman, M.B., Babalola, O.M. and Abdukkarim, A.(2009). Viral Hepatitides in Voluntary Blood Donors in Yola, Nigeria. *European Journal of Scientific Research, 31(3)*:329-334.

Oliveira, S.A., Camacho, L.A., Pereira, A.C., Faillace, T.F., Setubal, S. and Nascimento, J.P. (2002).Clinical and Epidemiological Aspects of Human Parvovirus 19 Infection in an Urban Area in Brazil (Niterói City Area, State of Rio de Janeiro, Brazil). *Mem Inst Oswaldo Cruz Rio de Janeiro*, 97(7): 965-970.

Olinger, C.M., Jutavijittum, P. and Hubschen, J.M.(2008). Possible new Hepatitis B genotype, Southeast Asia. *Emerging Infectious Disease,* 14(11):1777-1780.

Ooi, S.L., Hooi, P.S., Chua, B.H., Lam, S.K. and Chua, K.B.(2002). Seroprevalence of Human Parvovirus B19 Infection in an Urban Population in Malaysia. *Medical Journal of Malaysia*, 57 (1):97-103.

Opaleye, O.O., Fagbami, A.H., Lalremruata, A. and Kun, J.F.J. (2011). Prevalence and Association of Human Parvovirus B19 with Hepatitis B and C Viruses in Nigeria. *Journal of Medical Virology,* 83:710–716.

Ozawa, K., Ayub,J., Hao, Y.S., Kurtzman,G., Shimada,T. and Young.N.(1987). Novel transcription map for the B19 (human) pathogenic parvovirus. *Journal of Virology*,61:2395–2406.

Ozawa, K. and Young, N. (1987). Characterization of capsid and noncapsid proteins of B19 parvovirus propagated in human erythroid bone marrow cell cultures. *Journal of Virology,* 61:2627–2630.

Ozawa, K., Ayub, J., Kajigaya, T., Shimada, T. and Young, N.S.(1988). The gene encoding the non-structuralprotein of B19 (human) parvovirus may be lethal in transfected cells. *Journal of Virology,* 62: 2884–2889.

Palumbo, E. (2007). Hepatitis B genotypes and response to antiviral therapy: a review. *American Journal on Therapy*, 14(3):306-309.

Rodis, J.F., Borgida, A.F., Wilson, M., Egan, J.F., Leo, M.V., Odibo, A.O. and Campbell, W.A. (1998). Management of parvovirus infection in pregnancy and outcomes of hydrops: a survey of members of the Society of Perinatal Obstetricians. *American Journal of Obstetrics and Gynaecology*, 179:985–988.

Rohrer, C., Gartner, B., Sauerbrei, A., Bohn, S., Hotentrager, B., Raab, U., Thierfelder, W., Wutzler, P. and Modrow, S.(2008). Seroprevalence of Parvovirus B19 in the German Population. *Epidemiology and Infection,* 136(11):1564-1575.

Salimi, V., Gouya, M.M., Esteghamati, A.R., Safaie, A., Heshmat,R., Saadatmand, Z. and Mokhtari-Azad, T. (2008). Seroepidemiology of Human Parvovirus B19 in 5-25 Year Old Age People in Iran. *Iranian Journal of Public Health*, 37 (4):19-25.

Sarmukaddam, S.B. and Garad, S.G. (2006). On Validity of Assumptions while Determining Sample Size. *Indian Journal of Community Medicine*, 29(2):2004-2006.

Servant, A., Laperche, S., Lallemand, F., Marinho, V., De Saint Maur, G., Meritet, J.F. and Garbarg-Chenon, A. (2002). Genetic diversity within human erythroviruses: identification of three genotypes. *Journal of Virology,* 76: 9124–9134.Servey, J.T., Reamy, B.V. and Hodge, J. (2007). Clinical presentations of parvovirus B19 infection. *American Family Physician,* 75 (3):373–376.

Scheurlen, W., Ramasubbu, K., Wachowski, O., Hemauer, A. and Modrow, S. (2001). Chronic autoimmune thrombopenia/neutropenia in a boy with persistent Parvovirus B19 infection. *Journal of Clinical Virology*, 20(3):173–178.

Schowengerdt, K.O., Ni, J. and Denfield, S.W. (1997). Association of Parvovirus B19 genome in children with myocarditis and cardiac allograft rejection: diagnosis using the polymerase chain reaction.*Circulation*, 96 (10):3549–3554.

Shade, R. O., Blundell, M. C., Cotmore, S. F., Tattersall, P. and. Astell. C. R. (1986). Nucleotide sequence and genome organization of human parvovirus B19 isolated from the serum of a child during aplastic crisis. *Journal of Virology*, 58:921–936.

Shashwat, S.and Suman, K. (2005). Emerging Human Infections: An Overview on Parvovirus B19. *Journal of Indian Academy Clinical Medicine*, 6:4:319-326.

Siegl, G., Bates, R.C., Berns, K.I., Carter, B.J., Kelly, D.C., Kurstak, E. and Tattersall, P. (1985). Characteristics and taxonomy of *Parvoviridae. Intervirology,* 23:61–73.

Skjoldebrand-Sparre, L., Tolfvenstam, T., Papadogiannakis, N., Wahren, B., Broliden, K. and Nyman, M.(2000). Parvovirus B19 infection: association with third-trimester intrauterine foetal death. *British Journal of Obstetrics and Gynaecology*, 107: 476–480.

Soderlund-Venermo, M., Hokynar, K., Nieminen, J., Rautakorpi, H.and Hedman, K. (2002). Persistence of human Parvovirus B19 in human tissues. *Pathology Biology,* 50: 307–316.

Taura, D.W., Oyeyi, T.I. and Hafsat, M.N. (2008). Prevalence of Hepatitis B surface antigen (HbsAg) among HIV positive patients attending Aminu Kano Teaching Hospital, Kano, Nigeria. *International Journal of Biomedical and Health Sciences*, 4(1)33-39.

Toan, N.L., Song, L.H., Kremsner, P.G., Duy, D.N., Binh, V.Q., Duechting, A., Kaiser, H., Torresi, J., Kandolf, R. and Bock, C.T. (2006). Co-infection of human parvovirus B19 in Vietnamese patients with hepatitis B virus infection. *Journal of Hepatology*, 45:361-369.

Tolfvenstam,T. (2001). Human Parvovirus B19: Studies on the pathogenesis of infection. Karolinska University Press Dissertation.

Tovari, E., Mezey, I., Hedman, K. and Czirjak, L. (2002). Self-limiting lupus-like symptoms in patients with Parvovirus B19 infection. *Annuals of Rheumatic Disease*, 61(7):662–663.

Ujo, H., Mamman, A. I., Aliyu, A. and Ogunrinde, G.O. (2012). The seroprevalence of Parvovirus antibodies among children with sickle cell anaemia in Zaria. *African Journal of Clinical and Experimental Microbiology,* 13, 2:74-78**.**

Uneke, C.J., Ogbu, O., Inyama, P.U., Anyanwie, G.I., Njoku, M.O and Idoko, J.H. (2005). Prevalence of hepatitis B surface antigen among blood donors and Human Immunodeficiency Virus - infected patients in Jos, Nigeria. *Memorias do Instituto Oswaldo Cruz, Rio de Janeiro*, 100(1): 13-16.

Ugwuja,E.I.(2010). Seroprevalence of Hepatitis B surface Antigen and Liver function tests among adolescents in Abakaliki, South Eastern. *Internet Journal of Tropical Medicine,* 6(2):1-6.

Vafaie, J. and Schwartz, R. (2004). Parvovirus B19 infections. *Internal Journal of Dermatology,* 43: 747-749.

Valeur-Jensen, A.K., Pedersen, C.B., Westergaard, T., Jensen, I.P., Lebech, M., Andersen, P.K., Aaby, P., Pedersen, B.N. and Melbye, M. (1999). Risk factors for parvovirus B19 infection in pregnancy. *Journal of American Medical Association*, 281: 1099–1105.

van Elsacker-Niele, A.M and Kroes, A.C.(1999). Human parvovirus B19: relevance in internal medicine. *Netherland Journal of Medicine,* 54: 221–230.

van Hemert, F.J., Van de Klundert, M.A., Lukashov,V.V., Kootstra, N.A., Berkhout, B. and Zaaijer, H.L.(2011). Protein X of hepatitis B virus: Origin and structure similarity with the central domain of DNA glycosylase. *Public Library of Science.* 6(8): 23392.

Wattre, P., Dewilde, A., Subtil, D., Andreoletti, L. and Thirion, V. (1998). A clinical and epidemiological study of human Parvovirus B19 infection in fetal hydrops using PCR Southern blot hybridization and chemiluminescence detection. *Journal of Medical Virology*, 54(2):140–144.

World Health Organization.(1989). Health principles of Housing: 1-15.

Wong, S., Young, N.S. and Brown, K.E. (2003). Prevalence of parvovirus B19 in liver tissue: no association with fulminant hepatitis or hepatitis-associated aplastic anaemia. *Journal of Infectious Diseases,* 187: 1581–1586.

Yaegashi, N., Niinuma, T. and Chisaka H. (1998). The incidence and factors leading to, Parvovirus B19-related hydrops fetalis following maternal infection; report of 10 cases and meta-analysis. *Journal of Infection*, 37(1):28–35.

Yaegashi, N. (2000). Pathogenesis of nonimmune hydrops fetalis caused by intrauterine B19 infection. *Tohoku Journal of Experimental Medicine*,190(2):65–82.

Young, N., Harrison, M., Moore, J., Mortimer, P. and Humphries, R.K. (1984). Direct demonstration of the human Parvovirus in erythroid progenitor cells infected in vitro. *Journal of Clinical Investigation*, 74(6): 2024–2032.

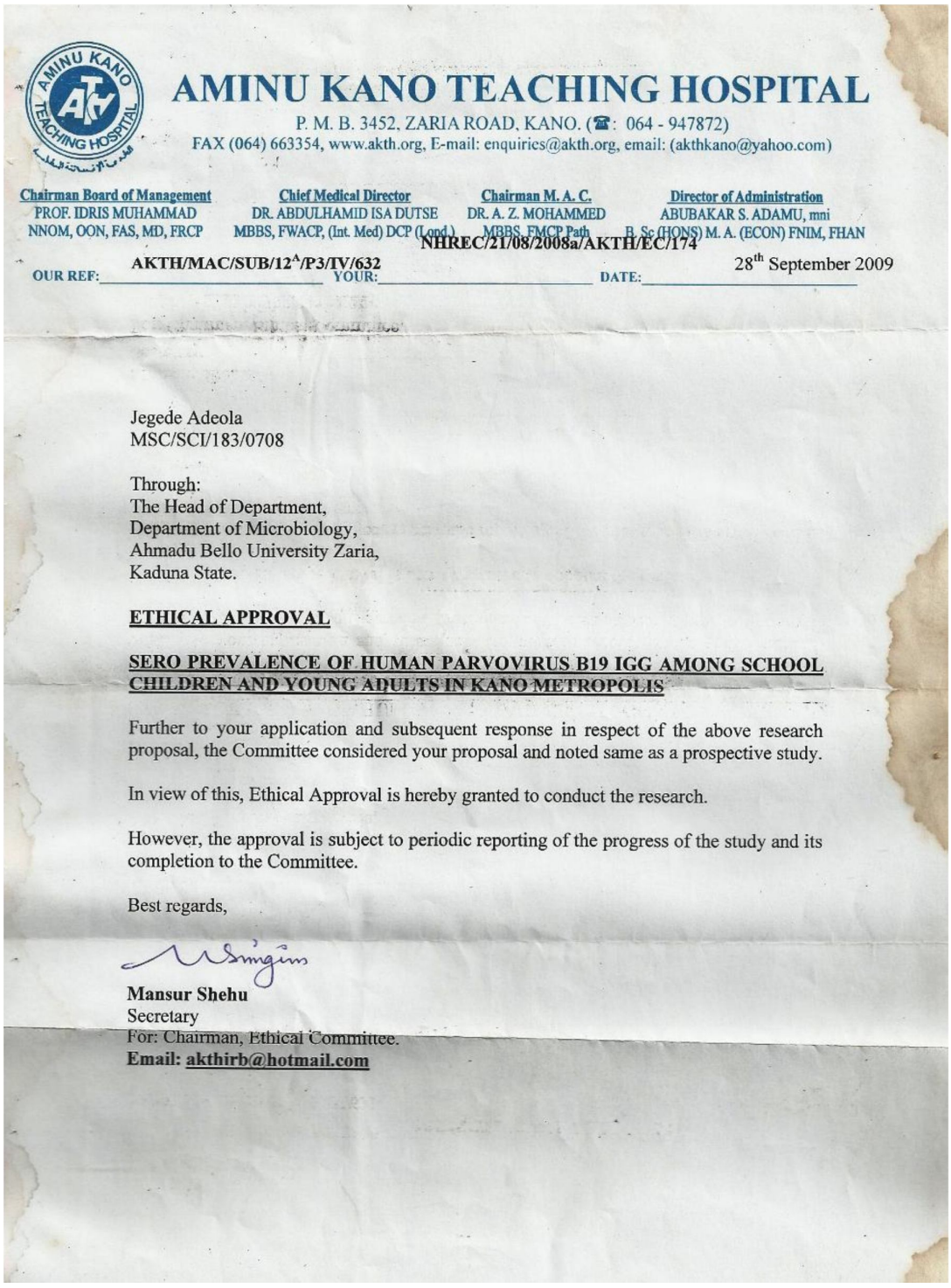
Young, N.S. and Brown, K.E. (2004). Parvovirus B19. *New England Journal of Medicine*, 350 (6):586-597.

Yoto, Y., Kudoh, T., Haseyama, K., Suzuki, N. and Chiba, S. (1996). Human Parvovirus B19 infection associated with acute hepatitis. *Lancet,* 347 (9005):868–869.

Zhang, H., Li, Q., Sun, J., Wang, C., Gu, Q., Feng, X., Du, B., Wang, W., Shi X, Zhang, S., Li, W., Jiang, Y., Feng, J., He, S. and Niu, J. (2011). Seroprevalence and Risk Factors for Hepatitis B Infection in an Adult Population in Northeast China. *International Journal of Medical Science,* 8(4):321-331.

APPENDICES

APPENDIX I



AMINU KANO TEACHING HOSPITAL

P. M. B. 3452, ZARIA ROAD, KANO. (☎: 064 - 947872)
FAX (064) 663354, www.akth.org, E-mail: enquiries@akth.org, email: (akthkano@yahoo.com)

Chairman Board of Management	Chief Medical Director	Chairman M. A. C.	Director of Administration
PROF. IDRIS MUHAMMAD	DR. ABDULHAMID ISA DUTSE	DR. A. Z. MOHAMMED	ABUBAKAR S. ADAMU, mni
NNOM, OON, FAS, MD, FRCP	MBBS, FWACP, (Int. Med) DCP (Lond.)	MBBS, FMCP Path	B. Sc (HONS) M. A. (ECON) FNIM, FHAN

NHREC/21/08/2008a/AKTH/EC/174

OUR REF: AKTH/MAC/SUB/12^A/P3/IV/632 YOUR: _____ DATE: 28th September 2009

Jegede Adeola
MSC/SCI/183/0708

Through:
The Head of Department,
Department of Microbiology,
Ahmadu Bello University Zaria,
Kaduna State.

ETHICAL APPROVAL

SERO PREVALENCE OF HUMAN PARVOVIRUS B19 IGG AMONG SCHOOL CHILDREN AND YOUNG ADULTS IN KANO METROPOLIS

Further to your application and subsequent response in respect of the above research proposal, the Committee considered your proposal and noted same as a prospective study.

In view of this, Ethical Approval is hereby granted to conduct the research.

However, the approval is subject to periodic reporting of the progress of the study and its completion to the Committee.

Best regards,

Mansur Shehu
Secretary
For: Chairman, Ethical Committee.
Email: akthirb@hotmail.com

APPENDIX II

APPENDIX III

CONSENT FORM

DEPARTMENT OF MICROBIOLOGY,

AHMADU BELLO UNIVERSITY, ZARIA

CONSENT FORM

I.....the Parent/guardian do hereby agree to enrol my child /ward.....in a study titled "**Seroprevalence of Human Parvovirus B19 Antibodies and Hepatitis B Surface Antigen Among Patients attending selected Hospitals in Kano Metropolis**"

I have been made to understand that:

1. The interview is strictly for the purpose of research and confidentiality is assured.
2. The blood sample taken will be used to detect IgG antibodies to human Parvovirus B19

....

Signature or thumb print of client/Date Signature of Witness/Date

APPENDIX IV:

RESEARCH QUESTIONAIRE

SOCIO ECONOMIC VARIBLE SERIAL NO.....

SEX: M { } F { }

AGE: 0-9 10-19 20-29 30-39 40-49 50-59 60-69 70-79 above 80

{ } { } { } { } { } { } { } { } { }

MARITAL STATUS: Single { } Married { } Divorced { }

OCCUPATION:

Civil Servant { } Artisan { } Unemployed { } Full time house wife { } Retired { } self employed { } Others { } Farmer { } Student { }

LEVEL OF EDUCATION:

None { } Primary { } Secondary { } Higher { }

TYPE OF RESIDENCE

Family house { } Duplex { } 1-3 bedroom flat { } room& parlour { } Single room { }

How many people live in the house.....

B. MEDICAL INFORMATION

1. Do you have sickle cell anaemia: Yes { } No { }

2. Have you ever had Jaundice? Yes { } No { }

3. Have you ever had a blood transfused? Yes { } No { }

4. Are you pregnant? Yes { } No { }

5. Have you had organ transplant? Yes { } No { }

C. LAB RESULT.....