SEROPREVALENCE OF PARVOVIRUS B19 IN BLOOD DONORS AT THE KITWE CENTRAL HOSPITAL, BLOOD BANK, KITWE, ZAMBIA

By

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A Dissertation Submitted to the University of Zambia, in Partial Fulfilment of the Requirements for the Master of Science Degree in Pathology (Haematology)

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DECLARATION

This work/dissertation in its present form has not been submitted or accepted previously for the award of a degree or diploma at the University of Zambia or any other tertiary institution. I **Memory Chirambo-Kalolekesha** declare that this Dissertation contains my own work and where other authors have been cited due acknowledgement has been given. I further declare that I followed all the applicable ethical guidelines in the conduct of the research. This dissertation has been prepared in accordance with the Master of Science in Pathology (Haematology), University of Zambia guidelines.

..... **Date.....**

Memory Chirambo Kalolekesha

SUPERVISOR

I have read this dissertation and approved it for final submission.

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Signature.....

Date.....

CERTIFICATE OF APPROVAL

This dissertation of **Memory Chirambo Kalolekesha** has been approved in partial fulfilment of the requirements for the degree of Master of Science in Pathology (Haematology) at the University of Zambia.

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ABSTRACT

Human Parvovirus (B19V) is a small, single-stranded, non-enveloped DNA virus which is pathogenic to humans causing a wide array of clinical complications which include erythema infectiosum, aplastic crisis and hydrops foetalis. It is generally harmless in healthy individuals but may be life threatening in immunocompromised individuals such as patients with sickle cell disease and pregnant women. It has been shown to be transmissible by blood transfusion but donor screening for the virus is not yet mandatory in most sub-saharan African countries including Zambia.

This was a cross sectional study which aimed to determine the seroprevalence of Parvovirus B19 infections among healthy blood donors at the Kitwe Central Hospital, Blood Bank. The specific objectives were to detect Parvovirus B19 IgM antibodies in donor blood using serology and to analyse the age and sex distribution of parvovirus among blood donors.

The net prevalence of parvovirus B19 IgM in this study was 15.6%. The majority of the positive cases were in the age group 15-22 years(17.8%) but there was no statistical significance between occurrence of parvovirus and age (p value=0.756). Prevalence in males was higher than in females i.e. 16.4% and 13.8%, respectively. The relationship between gender and parvovirus B19 occurrence was however not significant either (p value=0.646).

DEDICATION

This dissertation is dedicated to my very supportive husband Hickey, and my son Tumpale who inspire me to work hard always**.**

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Firstly, I would love to give thanks to God who has seen me through to the end of this research process.

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LIST OF ABBREVIATIONS

UTH	University Teaching Hospital
PCR	Polymerase Chain Reaction
SCD	Sickle Cell Disease
DNA	Deoxyribonucleic Acid
VP 1 or 2	Viral Protein 1or 2
Kb	Kilobyte
Nm	Nanometre
kDa	KiloDalton
TAC	Transient Aplastic Crisis
LOD	Limit Of Detection
NAT	Nucleic Acid Test
ELISA	Enzyme Linked Immunosorbent Assay
SPSS	Statistical Package for Social Sciences
EDTA	Ethylene Diamine Tetra-acetic Acid
HIV	Human Immunodeficiency Virus
IU	International Units
B19V	Human parvovirus B19

USA	United States of America
SS	Sample Size
TDRC	Tropical Diseases Research Centre
KCH	Kitwe Central Hospital
IgM	Immunoglobulin M

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CHAPTER 1: INTRODUCTION

1.1.Background

Parvovirus B19 is a small, single-stranded, non-enveloped DNA virus which is pathogenic to humans and can result in a wide array of clinical complications. The virus targets rapidly growing erythroid progenitor cells, which are found in human bone marrow, fetal liver, human umbilical cord, and peripheral blood (Brown *et al*., 1991; Srivastava et al., 1992). It is generally harmless in healthy individuals but may have a serious clinical outcome in susceptible recipients such as patients with shortened red cell survival (e.g. Sickle Cell Disease patients,thalassemia major etc.), immunocompromised patients and pregnant women (Marano *et al*., 2014). Parvovirus B19 has been shown to be transmissible by blood transfusion (Koppelman *et al*., 2007) but donor screening for the virus is not yet mandatory in most sub-Saharan African countries including Zambia. There is need therefore to establish the occurrence of this virus in the donor population and establish the possible implications on the recipient population which includes the high risk SCD patients and pregnant women.

Parvovirus B19 is resistant to heat, cold, and solvents. It is inactivated by formalin and β-propiolactone. Approximately 5600 nucleotides in the genome of parvovirus B19 (Cotmore *et al*., 1984) show remarkably few differences among isolates, with the exception of the sequences of two variants, V9 and A6 (Nguyen *et al*., 2002) which are of uncertain clinical

significance. Replication of a parvovirus entails double-stranded intermediate forms, which can be detected in tissue culture and clinical specimens by simple methods of DNA hybridization. The genome of the virus codes for three proteins; a non-structural protein named NS1 and two structural proteins termed VP1 and VP2. The NS1 protein is cytotoxic to human target cells and is also responsible for packaging viral progenies while VP1 and VP2 are the minor and major components of the viral capsid with VP1 playing a major role in target cell attachment and cell entry (Cotmore *et al*., 1986). The developing erythroblast is the main cell affected by parvovirus B19 as the receptor which binds to the virus, identified as P blood group or Globoside, is found in abundance on erythroblasts and cells of the erythroid lineage (Brown *et al*., 1993).

Parvovirus B19 has a specific tropism for erythroid progenitor cells and thus can cause a temporary infection of the bone marrow eventually leading to a transient arrest in erythropoiesis (Gillespie *et al*., 1990). Patients with haematological disorders are at risk of severe clinical illness due to parvovirus and this is especially common in chronic haemolytic anaemia such as sickle cell disease, thalassaemias and hereditary spherocytosis. In these diseases, erythroid progenitor cell formation is increased to compensate for red blood cell lysis and B19 infection can suppress erythropoiesis and induce acute erythroblastopenia, which is often referred to as transient aplastic crisis (Xu *et al*., 2003).

Human Parvovirus B19 infection has been recognized as a cause of cytopaenia in immunocompromised patients, including organ transplant recipients, patients with congenital and acquired immunodeficiency, and

cancer patients (Florea *et al*., 2007). There are however two patient groups at risk for a more severe and more threatening clinical picture of the parvovirus B19 infection and these are; patients with increased red blood cell destruction resulting in high erythrocyte turnover, and pregnant women due to transplacental infection of the foetus. In the former patient group, B19V infection may result in a transient aplastic crisis and in the latter group in severe foetal anaemia with consecutive hydrops foetalis and foetal death (Gillespie *et al*., 1990). Parvovirus B19 has been implicated as a causative agent in fulminant hepatitis even in healthy individuals (Langnas *et al*., 1995). Transmission of parvovirus B19 is through respiratory secretions, transplacentally and by transfusion of blood or blood products.

1.2. Statement of the problem

Anti-Human Parvovirus antibodies are found in 40% to 60% of adults. Studies have shown that by 15 years of age, about 50% of the general population are positive for parvovirus B19 IgG antibodies (Corcoran *et al*., 2007) and more than 70% adults have measurable levels of B19-specific IgG antibodies (Kerr *et al*., 1999). This shows high levels of exposure to Human Parvovirus B19 in the general population.

Parvovirus B19 infection may result in severe clinical manifestations in certain high risk groups such as pregnant women, patients with haemophilia, persons with chronic or acquired immunodeficiency, and persons with elevated red cell production (Harald *et al*., 2002). These

high risk groups are constantly transfused and hence have increased chances of exposure to Human parvovirus B19 but still screening for this virus is not yet mandatory.

1.3. Justification of the study

In Europe it has been proposed that 15% of immunocompromised patients may succumb to parvovirus B19 infection per year (Schleuning et al., 1999). The rate of viral transmission is nearly 50% in household contacts and varies from 10% to 60% in school and daycare exposure (Gillepsie *et al*., 1990). Studies show that nosocomial, transplacental, tissue transplantation, and blood product transmission can also occur (Katta *et al*., 2002). About 30% to 60% of blood donors have antibodies to parvovirus B19 (Kaur *et al*., 2005). Patients with haematological disorders are at risk of severe clinical illness especially in chronic haemolytic anaemia such as sickle cell anaemia (Harms *et al*., 1999; Abuhammour *et al*., 1999). Incidence and prevalence of parvovirus B19 infection in blood donors has been documented in western literature. In Zambia, there is no reliable data or recorded studies on the occurrence of parvovirus B19 in blood donors. Thus, there is a need to explore the prevalence of parvovirus B19 in blood donors, and thereby prevent and/or minimize its transmission in various clinical settings as a result of transfusion. This study aimed to assess parvovirus B19 infection among blood donors at the Kitwe Central Hospital using established methods of detection. This study investigated human parvovirus B19 infection in

healthy blood donors using ELISA to detect parvovirus B19 IgM antibodies.

1.4. Research Question

What is the prevalence of parvovirus B19 infections among blood donors at Kitwe Central Hospital Blood Bank?

1.5. Objectives

1.5.1 General Objective

To determine the seroprevalence of parvovirus B19 infections among healthy blood donors at the Kitwe Central Hospital Blood Bank.

1.5.2 Specific Objectives

1.5.2.1 To detect parvovirus B19 IgM antibodies in donor blood using serology.

1.5.2.2 To analyse the age and sex distribution of parvovirus among blood donors.

CHAPTER 2: LITERATURE REVIEW

Parvovirus B19 infection is a very common infection in humans. Seroprevalence of this virus has been shown to increase with age, with more than 70% of the adult population being seropositive (Kerr *et al*., 1996). The clinical disorders associated with parvovirus B19 infection are influenced mainly by age and haematological and immunological status of the host (Brown *et al*., 1993). Infection with the virus can occur either through respiratory secretions or contaminated blood or blood products (Prowse *et al*., 1997).

The pathogenesis of disease in parvovirus B19 infections may be attributed to direct infection or effects on particular cell types, and also effects as a result of the specific acquired immune response (Kerr *et al*., 2005). B19V virus targets erythroid progenitor cells in the bone marrow. This may result in apoptosis of the cells and as such in persons with shortened red cell survival, aplastic crisis may occur in non-immune individuals and may be fatal if not treated. In immmunocompromised individuals, chronic bone marrow failure occurs due to failure of immune response and also on going viral replication in the bone marrow precursor cells (Pallier *et al*., 1997) several of the clinical manifestations of B19V infection appear to be mediated by the immune response. Specific IgM and IgG antibodies are produced following infection. Volunteer studies demonstrated that occurrence of symptoms of Erythema infectiosum and arthritis coincided with appearance of specific IgG antibodies to the virus (Anderson *et al*., 1985). Infection leads to a biphasic clinical course: One

week after intranasal inoculation with B19 in healthy adult volunteers viraemia 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. Recovery involves production of IgM antibody 10 to 12 days post infection, 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). IgG antibody is detectable in volunteers about 2 weeks after inoculation and presumably persists for life and protects against secondary infections. During viraemia reticulocyte numbers fall to undetectable levels, recovering 7 to 10 days later, resulting in a temporary drop in haemoglobin of 1 g/dl (0.6 mmol/liter) in a healthy person. 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 pre-incubation levels. Viral replication in neutrophils has been proposed by one group (Kurtzman *et al*., 1988), but these results have not been confirmed by others.

This may provide an explanation for the neutropenia sometimes observed, although B19 is apparently not linked to the development of clinically significant neutropenia (Hartman *et al*., 1994). The infrequently reported fulminant thrombocytopenia associated with B19 infection may consist of two types. In one type, thrombocytopenia precedes the onset of

rash due to bone marrow suppression, while the other type is probably mediated by immunologic mechanisms (Heegard *et al*., 2002).

Erythema infectiosum is the most common presentation of parvovirus B19 infection and it affects mostly children 4 to 10 years of age (Plummer *et al*., 1985). Approximately 8 percent of children infected with the virus have arthralgia. However, arthralgia is more common in adolescents and adults with parvovirus B19 infection, affecting up to 60 percent of these persons.

Arthropathy affects women twice as often as men (Nesher *et al*., 1997). In immunocompromied persons, rashes and arthropathy do not develop because they occur secondary to antibody complex deposition in the skin and joints. (Posfay *et al*., 2003).Gloves and sock syndrome typically occurs in young adults and presents as symmetric, painful erythema and oedema of the feet and hands. The condition gradually progresses to petechiae and purpura and may develop into vesicles and bullae with skin sloughing (Alfadely *et al*., 2003). In women, pregnancy does not alter parvovirus B19 infection in the mother although the foetal liver and heart may become infected (Alger *et al*., 1997). The infant may develop severe anaemia, caused by an already shortened red cell lifespan, or may develop myocarditis from direct infection of the heart. The combination of severe anaemia and myocarditis can cause congestive heart failure and hydrops foetalis (Morey *et al*., 1992).

2.1. Prevalence of parvovirus in general population

A study done in Lagos, Nigeria to determine prevalence of parvovirus B19 antibodies in a population of sickle cell anaemia patients and non-sickle cell anaemia patients found an overall parvovirus B19 infection rate of 13.3% (12.5% in sickle cell anaemia patients; 14.6% among control volunteers) (Bamidele *et al*., 2013). This observed rate of 13.3% is comparable to the 13.2% reported for a study aimed at determining parvovirus infection prevalence among pregnant women in Jos, Nigeria (Emiasegen *et al*., 2011) and the 11.3% found among SCD patients in the USA (Smith *et al*., 2013). The prevalence found in these few studies illustrate the magnitude of the threat presented by parvovirus B19 infection in susceptible blood transfusion recipients.

In Saudi Arabia, a study by Obeid et al in 2011 estimates the prevalence of anti B19 Ig G antibodies among sickle cell patients and the control group (37.6 and 39.3%) is considerably higher in their study than that in previous reports of B19 antibodies in the general Saudi population which showed a general prevalence of 19.0% (Al-Frayh *et al*., 1993). This difference could be alluded to differences in the specificity and sensitivity of the assays used.

Anti B19 IgG antibodies reflect previous exposure to the virus. B19 virus infection is common, and 40% to 60% of adults have antibodies against the virus due to previous exposure (Florea *et al*., 2007). Epidemiologically, infection rates increase every three to four years, but there can be longer cycles, with viral activity increasing every four to seven years. The overall prevalence of IgG antibodies to parvovirus B19

in healthy adults is 50% in the United States and Japan (Foto *et al*., 1993; Anderson *et al*., 1986) 60%-70% in England and Wales(Gay *et al*., 1994); 50% in India (Abraham *et al*., 2002) and 53.2% in Spain (Guerra *et al*., 2000).

Several researchers from different countries which include Germany, Belgium, Netherlands and the United kingdom,, have been able to detect parvovirus B19 DNA in 1 percent of all blood cell preparations and blood products applied to the patients on a haematology ward these include 0.9 percent of standard blood components (in 2.0 percent of pooled plasma products and in 0.7 percent of single donor products), (Plentz *et al*., 2005) in 0.006 percent of blood donations, in 0.14 percent of single-donor blood products, (Koppelman *et al*., 2004) in 0.16 percent of plasma samples,(Thomas *et al*., 2003) and in 0.6 to1.3 percent of blood donors.(Candotti *et al*., 2004).

Although the prevalence and seasonality of parvovirus B19 appear to vary between different donor populations and geographic locations, the fact remains that parvovirus B19 is found consistently within blood donor population, throughout the year, and throughout the world.

Two detection methods are currently utilized for the identification of parvovirus B19 infection, nucleic acid testing techniques and antibody detection methods (Corcoran *et al*., 2004). The main nucleic acid testing (NAT) technique used for B19 detection is polymerase chain reaction (PCR) and both qualitative and quantitative PCR strategies exist for the detection of all three B19 genotypes (Daly *et al*., 2002). Apart from total genotype detection, the balance between PCR assay sensitivity and

specificity to facilitate detection of acute infection only is important (Daly *et al*., 2002).

The second method for detection of parvovirus B19 infection is the antibody detection method. And two types are utilized in the clinical setting namely Enzyme- linked immunosorbent assay (ELISA) and immunochromatographic techniques (Corcoran *et al*., 2004; Daly *et al*., 2002). The sensitivity of ELISA was compared to the nucleic acid technique by Sato et al and was found to be less sensitive and specific. However, ELISA methods remain a very popular and valid method for the detection of Parvovirus B19 (Doyle *et al*., 2011).

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 *et al*., 2001). The latter has proven to be particularly useful for diagnosing materno-foetal B19 transmission (Baschat *et al*., 2003). However, B19 viraemia may not be associated with symptoms of infection, so in a blood donor context, an infected person could potentially donate blood within this time window, with obvious implications for blood or blood product recipient exposure to the virus. Consequently, many blood collection organisations and blood product manufacturers have implemented screening protocols to detect B19 DNA by PCR using minipool screening (i.e. making pools of aliquots of blood serum/plasma and screening the same for B19 DNA) (Schimdt *et al*., 2007). Minipool, as opposed to individual specimen screening, is performed to reduce the cost associated with detection of B19 presence.

The disease transmission by transfusion of blood components is rare. However, extremely high levels of parvovirus B19, up to 10^{12} IU/ mL, in plasma of acutely infected but asymptomatic donors may present a greater risk in plasma derivatives due to pooling of large numbers of plasma units in manufacture of these products (Department of Health and Human Services, Food and Drug Administration, 2009). Screening of source plasma for parvovirus DNA is currently recommended in North America and Europe as an in-process control to ensure that levels of parvovirus B19 DNA do not exceed 10,000 IU per ml in the manufacturing pool (Koppelman *et al*., 2004; Tabor *et al*., 2002). It has also been recommended that adding parvovirus B19 testing of the donor and recipient to the routine pretransplant evaluation should be established (Subtirelu *et al*., 2005).

2.2. Parvovirus in pregnant women

Parvovirus B19 is a widespread infection that may affect between 1-5% of pregnant women, mainly with normal pregnancy outcome (Feldman *et al*., 2010; Crane *et al*., 2002). The prevalence of infection is higher during epidemics - between 3 and 20% with seroconversion rate of 3-34% (Tolfvenstam *et al*., 2009; Ergaz *et al*., 2006). Pregnancy does not affect the course of the infection but infection may affect the pregnancy (Alger *et al*., 1997). Presentation of parvovirus B19 infection in pregnancy may be maternal (rash, arthropathy or myocarditis) or foetal (foetal loss or hydrops). Maternal symptoms may be unspecific and may delay early diagnosis (de Haan *et al*., 2008). The spontaneous loss rate of

foetuses affected with parvovirus B19 before 20 weeks gestation is 14.8% and after 20 weeks gestation is 2.3% (Miller *et al*., 1998). Possible mechanisms for hydrops foetalis due to B19 infection in pregnancy are foetal anaemia (due to the virus crossing the placenta) combined with the shorter half-life of foetal red blood cells, leading to the severe anaemia, hypoxia and high output cardiac failure. Other possible causes are foetal viral myocarditis leading to cardiac failure, and impaired hepatic function. This is caused by direct damage of hepatocytes and indirect damage due to hemosiderin deposits (Miller *et al*., 1998; Gratacos *et al*., 1995). There is a higher foetal loss rate when the infection is acquired before 19 to 20 weeks gestation (14.8%), compared to that after 20 weeks (2.3%) (Rodis *et al*., 1995). Ultrasound signs in a foetus with hydrops, include ascites, skin oedema, pleural and pericardial effusions and placental oedema (Levy *et al*., 1997).

2.3. Parvovirus in sickle cell patients

Serological studies indicate that more than 50% of people are infected with parvovirus B19 during childhood but higher prevalence rates are observed among children with SCD in some tropical regions: Brazil (80% among children of 5–15 years) (Amaku *et al*., 2009) and Australia (55% of the same year range) (Kelly *et al*., 2000).

Manifestations of parvovirus B19 infection in SCD patients can range from transient and isolated anaemia to a life-threatening drop in haemoglobin levels (Biesma *et al*., 1997; Fartoukh *et al*., 2006). In children with SCD the parvovirus B19 anaemia is characterized by

profound reticulocytopenia and frequent splenic sequestration (Yates *et al*., 2009). Moreover, in patients with SCD, B19V can be a causative agent of massive virally induced bone marrow necrosis, complicated by systemic fat embolism, fungal super infections (Fartoukh *et al*., 2006), and even fatal bone marrow embolism. Bone marrow necrosis is a common complication of sickle-cell vaso-occlusion and is frequently found at autopsy in patients dying from such episodes. Parvovirus B19 infection can provoke bone marrow necrosis, triggering embolic syndrome (Godeau *et al*., 1991; Rayburg *et al*., 2010). Parvovirus B19 can also trigger acute cessation of erythrocyte production, causing transient aplastic crisis (TAC) in patients with hereditary anaemia's including SCD that already have a shortened red blood cell lifespan (Servey *et al*., 2007).

2.4. Donor Screening for Parvovirus B19

The general introduction of screening tests for hepatitis B virus, hepatitis C virus and HIV, has illustrated a sharp fall in the risk of these viruses being transmitted via blood products (Kerr *et al*., 1999). Hence determination of the presence of Parvovirus in blood and its products would be the first step in the reduction of the risk of parvovirus transmission. The introduction of screening tests for all blood products for the presence of emerging pathogens though possible would be expensive. Since, the transmission of parvovirus is a risk to only a part of those receiving these blood products; a less costly option is the risk-group approach, in which only selected groups of patients receive tested blood

products. In this way, patients for whom infection with parvovirus B19 could cause problems will be given maximum safety blood products. This approach is in keeping with measures previously used in blood transfusion medicine with respect to cytomegalovirus transmission (Kerr *et al*., 1999). Safe cellular blood products are to be administered to pregnant women (except in the case of transfusions given during birth), patients with congenital or acquired haemolytic anaemia who have no detectable antibodies to B19V and patients with cellular immunodeficiency who have no detectable antibodies to B19V.

Incidence and prevalence of parvovirus B19 infection in blood donors has been documented in western literature but till date there is no reliable data or recorded studies on occurrence of parvovirus B19 in blood donors in Zambia. The need to explore the prevalence of parvovirus B19 in blood donors in Zambia cannot be overemphasized.

CHAPTER 3: METHODOLOGY

3.1. Study design

The study was a cross sectional type of study involving serological assessment of parvovirus B19 infection in blood donors.

3.2. Study site

The study was conducted at Kitwe Central Hospital Blood Bank

3.3. Target population

All blood donor samples collected for screening at the KCH Blood Bank.

3.4. Study population

Samples to be analysed were obtained from the blood samples which had been found to be negative for routinely screened infections at the blood bank.

3.5. Sample size

A total of 192 samples required were calculated using the formula for sample size estimation of a prevalence study and the finite population correction factor.

There is no known prevalence of parvovirus B19 in Zambia therefore an estimated 50% prevalence was assumed. The formula for sample size estimation for a prevalence study was used as shown below;

$$\mathbf{n = \frac{Z^2\ P\ (1 - P)}{d^2}}$$

Where;

n = sample size

Z = z-statistic for a level of confidence

P = expected prevalence

d = precision

Therefore $n = \frac{1.96^2 \times 0.5(1-0.5)}{0.005^2}$

$n = 384$

For the level of confidence of 95%, which is conventional, Z value is 1.96. P is the proportion (prevalence to be estimated) by the study. As there is no known prevalence in Zambia an estimated prevalence of 50% (expressed as a proportion of 1 i.e. 0.5) is used to get the largest possible sample size. Assuming that the prevalence of the disease will lie between 10% and 90% a precision of 5% (expressed as a proportion of 1 i.e. 0.005) is used. This precision will give the width of 95% CI as 10%. (Naing *et al*., 2006)

Finite population correction factor

When the sample represents a significant (e.g. > 5%) proportion of the population, a finite correction factor can be applied. This reduced the sample size required to 192.

$$n' = \frac{NZ^2P(1-P)}{d^2(N-1)+Z^2P(1-P)}$$

where

n' = sample size with finite population correction,

N = Population size,

Z = Z statistic for a level of confidence,

P = Expected proportion (in proportion of one), and

d = Precision (in proportion of one).

3.6. Sampling methods

Systematic random sampling was used to collect samples. A random sample was obtained by selecting one unit on a random basis and choosing additional elementary units at evenly spaced intervals until the desired number of units was obtained. Whole blood was collected in plain red top containers and spun in a centrifuge to separate serum, the serum was collected using micropipette and transferred into storage vials for future analysis. At least 500ul of serum was stored in each vial and stored at -20^0C.

3.6.1 Inclusion criteria

All blood donor samples from healthy voluntary blood donors at KCH Blood Bank were included in the study.

Healthy blood donors were considered as those found asymptomatic at donor interview screening stage and those that tested seronegative for all transfusion transmissible infections screened at the blood bank such as hepatitis and HIV.

3.6.2. Exclusion criteria

Blood donor samples found to have other transfusion transmissible infections such as Hepatitis and HIV.

3.7. Material and methods

3.7.1. Specimen preparation and storage

In the laboratory, each blood donor specimen serial number was recorded on both the plain tube used for collection and the containers to be used for storage. The blood was left to clot in the tube and thereafter the blood specimen was centrifuged at 3000 revolutions per minute (3000 rpm) in order to separate the serum (supernatant) from the blood cellular components (sediment). Only supernatant (serum) was then carefully collected from tube using pipettes and transferred to plastic cryovial containers with sealable screw caps which was stored in a freezer at -20^{o}C until the specimens were required for analysis.

3.7.2. Quality Control

Standard laboratory operating procedures were upheld at all times. All the equipment used during specimen analysis were checked for calibration according to the quality control guidelines. Analytical control runs were performed prior to each test analysis to ensure accuracy and reliability of results.

3.8. Specimen analysis

3.8.1. Parvovirus B19 Ig M ELISA test protocol

Human Parvovirus B19 IgM levels in blood serum were determined using a competitive human parvovirus B19 IgM immunoassay from mybiosource, USA. Samples were diluted with sample diluents and additionally incubated with IgG-RF-sorbent, containing hyper immune anti-human IgG-class antibody to eliminate competitive inhibition from specific IgG and to remove rheumatoid factors. This pre-treatment avoided false negative or false positive results. Microtiter wells as a solid phase are coated with Parvovirus B19 antigen. Pre-treated samples and ready-for-use controls were pipetted into these wells. During incubation Parvovirus B19-specific antibodies were bound to the immobilized antigens. After a washing step to remove unbound sample and control material horseradish peroxidase conjugated anti-human IgM antibodies were dispensed into the wells. During a second incubation, the anti-IgM conjugate bound specifically to IgM antibodies resulting in the formation of enzyme-linked immune complexes. After a second washing step to remove unbound conjugate the immune complexes formed (in case of positive results) were detected by incubation with TMB substrate and development of a blue colour. The blue colour turned into yellow by stopping the enzymatic indicator reaction with acidic solution. The intensity of this colour was directly proportional to the amount of Parvovirus B19-specific IgM antibody in the sample. Absorbance at 450 nm was read using an ELISA microtiter plate reader.

3.8.1.1. Reagent Preparation

All kit components including the microtiter plates and samples were brought to room temperature prior to use. A 1 in 19 dilution of 20X wash solution concentrate was performed with germ free distilled water. 30ml of wash solution was diluted with 570ml of distilled water.

3.8.1.2. Assay Procedure

All reagents and samples were thoroughly mixed prior to assaying. 10 ul of each sample was first diluted with 0.5ml of the sample diluent and mixed well with a vortex mixer. For the absorption of rheumatoid factor 60 ul of each prediluted sample was diluted with 60ul of the IgG-RF-Sorbent and thoroughly mixed. This mixture was left to stand for 15 minutes at room temperature. A plate map was established carefully for the distribution and identification of the samples and controls. 100ul of the negative, positive and cut off controls was added to the appropriate wells in duplicate. The first two wells were left empty for substrate blank. 100ul of the pretreated sample was then added into the appropriate wells with new diposable pipette tips for each sample. The wells were then covered with foil and left to incubate for 60 minutes at 37^{o}C. After incubation, the contents of the wells were briskly shaken out by carefully tapping wells onto absorbent paper. The wells were then rinsed 5 times in an ELISA washer with 300 ul of the diluted wash solution per well. After the 5 wash cycles were complete, the wells were struck sharply onto absorbent paper to remove any residual droplets. 100 ul of the enzyme conjugate was then added to all the wells except the first two reserved for the blank (i.e. well A1 and B1). The wells were then covered with foil

and placed in a cupboard to incubate in the dark for 30 minutes. After this incubation, the contents of the wells were briskly shaken out and the wells were washed 5 times with 300ul of wash solution per well. After the 5th wash, the wells were struck sharply onto absorbent paper to remove any residual droplets. 100ul of substrate solution was then added into all the wells. The wells were covered and left to incubate in the dark for 15 minutes at room temperature. The enzymatic reaction was then stopped by adding 100 ul of the stop solution to each well and the optical densities were read at 450nm using gen5 reader and Maggellan microtitre plate reader.

3.8.1.3. Sensitivity and Specificity

The diagnostic specificity of the assay is 100%. This is defined as the probability of the assay scoring negative in the absence of a specific analyte. The diagnostic sensitivity is also 100%. This is the probability of the assay of scoring positive in the presence of the specific analyte.

3.9. Ethical considerations and permissions

3.9.1. Ethical considerations

Ethical clearance was obtained from the University of Zambia Biomedical Research Ethics Committee (UNZABREC) before the commencement of the study. The research proposal was submitted to UNZA-BREC for approval and was approved Assurance No. FWA00000338 IRB00001131 of IORG0000774, Ref. No.006-10-15 obtained on 19th January 2016 and renewed on 20th January, 2017. There was no direct contact with the patient as only routine samples were used

for the study. Demographic data such as age and sex were obtained from the donor interview forms. The specimen container or any other material for the patients was assigned a serial number hence they were identified by a unique study identifier. In no way was the participant's name or file number linked to the specimen or research results. Permission to obtain blood samples for the study from the Blood Bank was sought from the Medical Superintendent at the Kitwe Central Hospital Blood Bank and also from the director of the Blood Bank at Kitwe Central Hospital. Permission to use equipment and facilities at the Tropical Diseases Research Centre Immunology department was obtained from the head of department and other relevant offices**.**

3.9.2. Data analysis

Analysis of the data was performed using IBM SPSS Statistical version 20 for Microsoft and Microsoft Excel 2011. The results of the analysis were expressed in tables as graphs expressing normally distributed variables as mean ± SEM or median (interquartile range) for non-normally distributed variables. Normality was assessed using the Shapiro and Wilk statistic and the normality plots.

All statistical tests were performed at 5% significance level or 95% confidence interval with p-value of <0.05 to determine statistical significance. The chi square test was used to ascertain the association of age and gender with parvovirus B19 infection.

The seroprevalence of parvovirus B19 infections was determined using appropriate formula.

CHAPTER 4: RESULTS

4.1. Sex and Age distribution of the blood donors

The mean age of the randomly selected participant donors was 22 years (ranging from 15 to 53 years), of which the highest percentage were of age group 15-22 years (67.2%). (Figure 1) There was a predominance of male donors observed in this study with a percentage of 69.2% compared to 30.8% of the female donors (Figure 2).

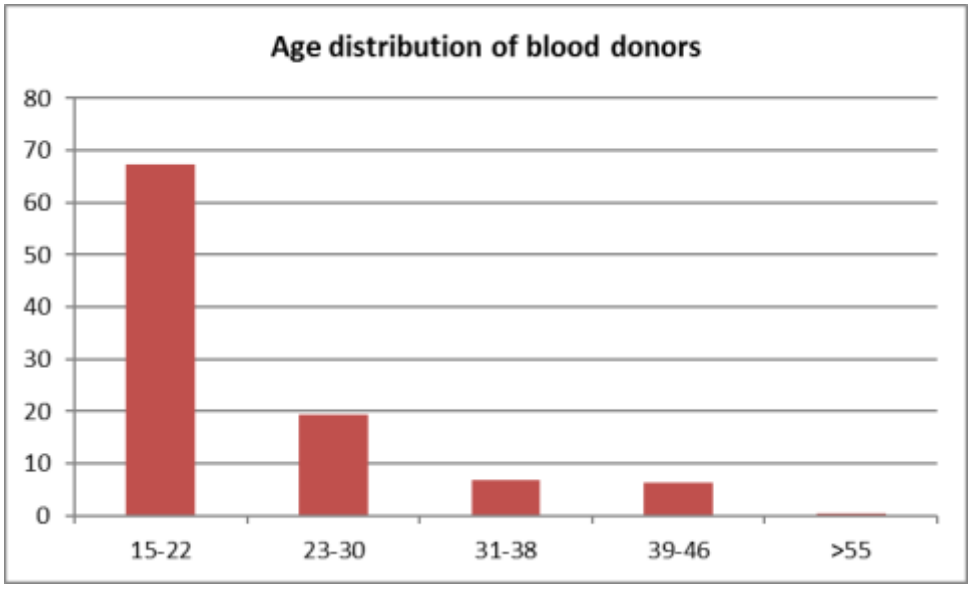

Figure 1: Age distribution of blood donors. The largest number of donors was aged between 15-22 years, less than 1% of the donors were over 46 years.

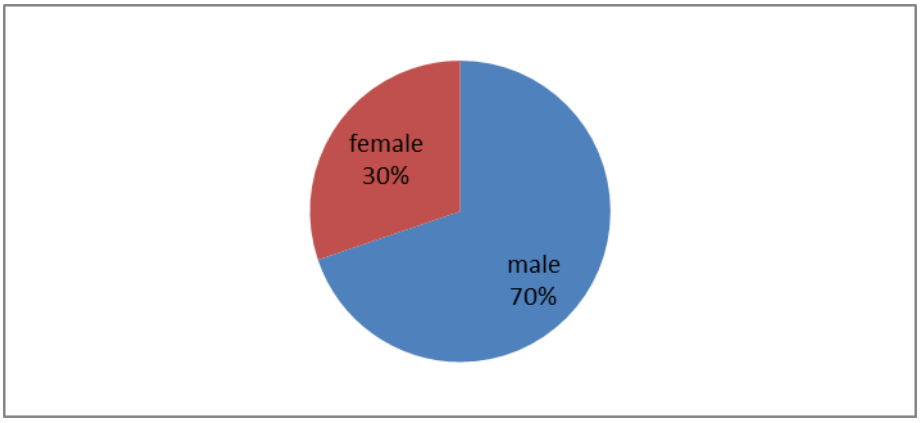

Figure 2: Gender distribution of blood donors. There were more male donors than female donors in the study. Out of the 192 donors, 134 were male and 58 were female, representing 70% and 30% of the donors, respectively**.**

4.2 Human parvovirus B19 Seroprevalence among donors

Table 1: Human Parvovirus B19 Ig M Serology

B19V Ig M serology	Number of donors	Percentage
Negative	162	84.4
Positive	30	15.6
TOTAL	**192**	**100**

Table 1: The net parvovirus B19 IgM serology in blood donors was found to be 15.6 %.

4.2.1 Human Parvovirus B19 and Age

Table 2: Parvovirus B19 IgM in different age groups

Age range	Number of donors B19V +	Number of donors B19V -	Percentage of B19V +	P-value
15-22	23	106	17.8	0.370
23-30	5	32	13.5	0.589
31-38	1	12	7.7	0.5609
39-46	1	11	8.3	0.217
>47	0	1	0	N/A
TOTAL	30	162	15.6	0.756

Table 2: Majority of parvovirus B19 IgM positive donors were in the age group 15-22 years. However no statistically significant association was found between age group and B19V IgM positivity. P value= 0.756

4.2.2. Human parvovirus B19 IgM gender distribution

The study observed a seroprevalence of 16.4% in males, compared to a lower 13.8% in females. Out of a total of 134 males, 22 were positive for parvovirus IgM whereas 8 females out of the total 58 tested positive for the virus (figure 3) .P value=0.646.

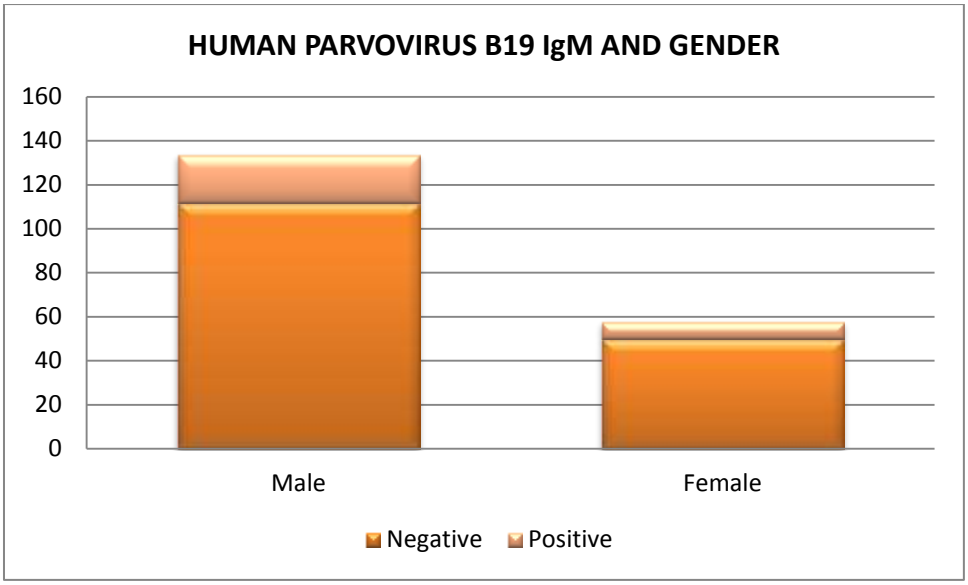

Figure 3: Human Parvovirus B19 IgM and gender

4.2.3. Gender distribution of Parvovirus B19 infections in different age groups

Table 3: Prevalence of anti-B19 Ig M antibodies according to age and sex

Age	Sex distribution		B19-Ig M antibodies positive	
	male	female	male	female
15-22years	91	38	18	5
23-30	26	11	3	2
31-38	10	3	1	0
39-46	7	5	0	1
>55	0	1	0	0
Total	134	58	22	8

Table 3: Parvovirus B19 Ig M prevalence was highest in males aged 15-22years with 19.8% compared to 13.2% of the females in the same age group.

CHAPTER 5: DISCUSSION

The results of this study confirm a relatively high presence of Parvovirus B19 IgM antibodies in otherwise healthy blood donors. Our study also revealed no statistically significant association between Parvovirus B19 infection and gender or age.

The prevalence of IgM to B19V in blood donors or in other healthy populations is usually below 2%, but it can be higher depending on the time of study in relation to the epidemic cycle. In temperate climates most infections occur in early spring or late winter (Kumar *et al*., 2013). In this present study evidence for parvovirus B19 Ig M antibodies was found in 30 (15.6%) of the 192 donors screened. The samples analysed in our study were collected between February and March, this might explain the high prevalence record. These months are predominantly associated with cold weather and rain, both of these factors have been said to influence B19V transmission (Hager *et al*., 1998). Several studies published between 1995 and 2014 show that the prevalence of human Parvovirus B19 in blood donor populations ranges from 6% to 79.1% for IgG (Brayn *et al*., 2010; Manaresi *et al*., 2004), from 0.72% to 7.53% for IgM (Ihara *et al*., 2013; Kumar *et al*., 2013), from 0.01% to 15.3% for IgM+ IgG (Sakata *et al*., 2013; Wasfy *et al*., 1996) and from 0% to 1.3% for human parvovirus B19 DNA (Slavok *et al*., 2012; Obeid *et al*., 2011). This shows that the prevalence of IgM antibodies in the present study was higher than the studies done by other researchers. Doyle and his co-worker found seroprevalence of 1% prevalence among American blood

donors (Doyle *et al*., 2000) while Munoza reported 0% in Spanish blood donors (Munoz *et al*., 1998). In addition to geographical and seasonal variations, differences in sampling methods, population size and assay methods are likely causes of the differences in seroprevalence rates observed. A study done in Nigeria using parvovirus IgM ELISA showed a prevalence rate of 14.8% which is comparable to the one observed in this study (Musa *et al*., 2013).

Our study showed no significant statistical association between gender and parvovirus B19 infection (P value=0.646). In most studies, the prevalence of antibodies to B19 in sera obtained from men and women is similar (Nascamento et al., 1990). A study done in Nigeria by Iheanacho et al also showed no significant association with sex. This seems to agree also with the generalisation of Cennimo (2010) that parvovirus B19 infection affects males and females in equal numbers. However, a few studies have reported that women have a higher rate of Parvovirus B19 infection than men (Alder et al.,1993; *Koch et al*., 1989, Schwartz *et al*., 1992). In one study of adult blood donors, the proportion of women who were seropositive, 47.5% was 1.5 times higher than for men (Alder *et al*., 1993). In Taiwan, the prevalence of IgG antibodies to B19V among females was significantly higher than among males (36.4% versus 29.4%, $P < .001$) (Lin *et al*., 1999). The most likely explanation for the higher rates of parvovirus B19 infection among women as seen is some of the studies, compared with men is that women are likely to have more frequent contact with children, especially school aged children, who are the major sources of the transmission because of school attendance. For

adults, contact with school aged children is the major risk factor for infection with B19V.

More than 50% of the donors in this study were aged between 15-22 years. The mean age of the donors was 22 years and the oldest donor was 53 years old. A study conducted in Nigeria to establish blood donor practices showed a similar age group pattern (Pondei *et al*., 2013). There was no significant statistical association between age and Parvovirus B19 infection in this study (P value=0.756). Numerous studies of B19 infection based on serologic testing show that the seroprevalence of B19 infection increases with age (Cossart *et al*., 1975; Serjeant GR *et al*., 1993; Cohen *et al*., 1988; Yaegashi *et al*., 1990). A study by Emiasegen *et al*. (2011) reported increase in seroprevalence with age as well as the reviews of Heegard and Brown (2002) and Kaur and Basu (2005). The greatest increase in seroprevalence and B19 infection occurs between 5 and 20 years of age. By age 20 years, the seroprevalence of B19 infection rises from about 5% to almost 40%**.** Afterward, without regard to risk factors, B19 seroprevalence increases slowly. In adult blood donors, the seroprevalence of IgG antibodies to B19 ranges from 29% to 79% with a median of 45%.76- In our study however, there was decrease in seroprevalence with increase in age. (Brown *et al*., 1990; Salimans *et al*., 1992) The difference in sampling populations may be a factor of this difference. The study by Emiasegen *et al*. (2011) for example used pregnant women as opposed to the blood donors used in this study. The findings of this study however, agree with that of Emiasegen *et al.*

(2011), and most other studies in the sense that age had no statistically significant effect on Human Parvovirus infection.

CHAPTER 6: CONCLUSION

The seroprevalence of human parvovirus B19 among blood donor population in our study is high, and poses an adverse transfusion risk especially in high-risk group of patients. This also suggests that there is an active transmission of the virus in the community. The prevalence rate was highest in males than in females, and also in individuals aged between 15-22 years. Statistical tests however show there is no association with sex or gender and parvovirus B19 infection. Patients other than those in the high-risk groups should continue to receive blood products that have been produced in accordance with current safety criteria. Units should be screened for parvovirus B19 using IgM ELISA, and may be supplemented through sensitive nucleic acid testing methods such as real time PCR.

6.1. Recommendations

A lack of data on the burden of parvovirus B19 in our population is a major concern. The findings from this study suggest the need to conduct a national level seroprevalence of human parvovirus B19 among blood donors as a matter of public health concern. Secondly, the Zambia National Blood Transfusion Service (ZNBTS) should consider routine screening of blood donors for B19V to avoid contaminated transfusion, particularly for non-immune women in the child bearing age and polytransfused individuals. An option of choice recommended by this study to improvise blood safety is to use a high risk-group approach. In this way, groups of individuals which include pregnant women, Rh

isoimmunised pregnancies requiring intrauterine transfusion, patients with congenital or acquired haemolytic anaemia and patients with cellular immunodeficiency who have no detectable antibodies to B19, would receive tested blood products. Patients other than those in the high-risk groups would continue to receive blood products that have been produced in accordance with current safety criteria.

Screening of the units could be achieved using parvovirus B19 using IgM ELISA, and may be supplemented through sensitive nucleic acid testing methods such as real time PCR.

6.2. Limitations

The findings of this study may not be generalized to the larger population due to sample size used. Also, there was a lack of prior research studies on the topic in Zambia and the majority of sub-Saharan African to be used as a reference point for our study.

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