Determination of seroprevalence of *Marburgvirus* among humans in Isiro, Oriental Province, Democratic Republic of Congo

By

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A dissertation submitted to the University of Zambia in partial fulfilment of the requirements for the degree of Masters of Science in One Health Analytical Epidemiology

THE UNIVERSITY OF ZAMBIA

LUSAKA

DECLARATION

I, **NUNDU SABITI Sabin**, hereby declare that the content of this dissertation is my own work and has not been submitted to another University or institution for any award or degree.

Signature…… Date……

CERTIFICATE OF APPROVAL

This dissertation of **NUNDU SABITI Sabin** has been approved in partial fulfilment of the requirements for the degree of Masters of Science in One Health Analytical Epidemiology at the University of Zambia

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ABSTRACT

Marburgvirus and *Ebolavirus* are among the most virulent pathogens for humans and non-human primates (NHPs) causing outbreaks of fulminant hemorrhagic fever. In the Democratic Republic of Congo (DRC), outbreaks caused by *Marburgvirus* have occurred exclusively in Oriental Province. This region borders Uganda where three outbreaks of Marburg virus disease (MVD) occurred in 2007, 2012 and recently in 2014. In this study, a sero-survey was conducted to generate up-to-date data on the circulation of *Marburgvirus* in humans in Isiro, an area of unconfirmed MVD outbreak in Oriental province in order to better understand the epidemiology of the disease in Isiro/DRC. Blood samples were collected during July 2013 from 400 apparently healthy humans in Isiro. Of these, a total of 172 serum samples were analyzed using Enzyme-Linked Immunosorbent Assay to detect *Marburgvirus*-specific IgG. Of the 172 individuals, the proportion of males was 52.9 percent. The median age was 36 years. The prevalence of *Marburgvirus*-specific IgG was 4.7 percent overall. This study shows the presence of *Marburgvirus*-specific IgG antibodies in humans in Isiro and indicates that the *Marburgvirus* IgG seropositive individuals may have been exposed to *Marburgvirus* possibly through contact with exposed populations or some unidentified animal reservoir host (e.g. fruit bats). Furthermore, these findings highlight the need for a country-wide surveillance of MVD in humans for mitigation purposes.

DEDICATION

To my wife BAHATI MUTINGWA Parfaite who authorized me to leave my family for a long period for this MSc program.

To my child ZABIBU SABITI Gift, for missing my presence during many months.

I feel proud of you!!!

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS AND ACRONYMS

AIDS	Acquired immunodeficiency syndrome
BDBV	Bundibugyo virus
BSL-4	Biosecurity level four laboratory
CDC	Centers for Disease Control and Prevention
CI	Confidence interval
DRC	Democratic Republic of Congo
EBOV	Ebola virus
EVD	Ebola virus disease
GP	Glycoprotein
GIS	Geographical information system
HIV	Human immunodeficiency virus
INTRA-ACP	Intra-African Caribbean Pacific mobility scheme
NHPs	Non-human Primates
MARV	Marburg virus
MVD	Marburg virus disease
OHAE	One Health Analytical Epidemiology

OR	Odds ratio
P	Probability (P-value)
RAVV	Ravn virus
SUDV	Sudan virus
TAVF	Tai Forest virus
VP	Viral protein
WHO	World Health Organisation

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Marburgvirus (along with *Ebolavirus)* causes a fulminant hemorrhagic fever in humans and nonhuman primates (NPHs) (Geisbert and Feldmann, 2011; Nakayama and Takada, 2011). These viruses belong to the family *Filoviridae,* order *Mononegavirales*. *Marburgvirus* includes only one species, *Marburg marburgvirus* with two virus members; Marburg virus (MARV) and Ravn virus (RAVV). In contrast, *Ebolavirus* is subdivided into five species: *Zaire ebolavirus*, *Tai Forest ebolavirus*, *Sudan ebolavirus*, *Reston ebolavirus* and *Bundibugyo ebolavirus* (Bukreyev *et al*., 2014). *Marburgvirus* was the first filovirus to be identified in 1967 (Martini, 1973; WHO, 2012a); while *Ebolavirus* was discovered nine years later (Bowen *et al*., 1977). Since the first outbreak of Marburg virus disease (MVD) in West Germany and Yugoslavia (now Serbia) in 1967 (Martini, 1973; WHO, 2012a), it took eight years to find the first case of MVD in Africa (Conrad *et al*., 1978; WHO, 2012a). To-date, at least thirteen MVD outbreaks have been recorded worldwide with case fatality rate ranging from 0 percent to 100 percent (Brauburger *et al*., 2012; CDC, 2014). For a long time *Marburgvirus* was considered to be less threatening compared to *Ebolavirus*. However, since the two largest outbreaks of MVD in Democratic Republic of Congo (DRC) in 1998-2000 in which the case fatality rate reached up to 83 percent (Borchert *et al.*, 2002; Bausch *et al*., 2006) and in Angola in 2004-2005, with case fatality rate reached up to 90 percent (CDC, 2005; WHO, 2005; Towner *et al.*, 2006), *Marburgvirus* infection is now considered to be a major public health problem comparable to *Ebolavirus* infection (Geisbert and

Feldmann, 2011; Nakayama and Takada, 2011; WHO, 2014a). Given the lack of licensed chemotherapeutics and vaccines for MVD, together with the high case fatality rate, *Marburgvirus* is considered to be potential biological threat pathogens (Borio *et al.,* 2002; Rotz *et al.,* 2002; Geisbert and Feldmann, 2011). Thus, a possible MVD outbreak would present a serious threat to human health and would pose lots of questions to policy makers in the management of an outbreak. As such, a deeper knowledge of circulation of *Marburgvirus* among humans or animals in high risk areas is crucial for planning of effective control measures.

1.2 Statement of the problem and justification

The DRC is one of the largest and also poorest countries of sub-Saharan Africa affected by several endemic and epidemic tropical diseases whose epidemiology is poorly described (Hotez *et al*., 2009). At present, many health and research structures in DRC are in total disarray due to the on-going civil war. The civil war that started in 1996 has led to the displacement of populations across the country and can influence the emergence/re-emergence and spread of diseases such as MVD throughout the country and possibly in the sub-region. During 1998-2000, DRC recorded the largest outbreaks of MVD which occurred in the north-eastern region in Oriental Province. These outbreaks affected 154 people and caused 128 (83 percent) deaths (Bausch *et al*., 2006). Since these outbreaks, no other outbreak has occurred in DRC. However, outbreaks of MVD occurred in Uganda in 2007 and 2012 and recently in 2014 (Adjemian *et al*., 2011; WHO, 2012b; WHO, 2014b). Although Isiro zone shares borders with areas that have previously reported MVD outbreaks (i.e. Durba and Watsa), to-date, no MVD has been documented in this area. However, Ebola virus disease (EVD) was reported in Isiro in 2012 (Kratz *et al*., 2015). Antibody surveys

conducted in 1998-1999 and 2000 in Durba/Watsa have shown that 2 percent and 0 percent of the participants were positive for *Marburgvirus*-specific IgG antibody, respectively (Bausch *et al*., 2003; Borchert *et al.*, 2005). Since then, no other study has evaluated the circulation of *Marburgvirus* antibodies in humans in this province. Therefore, this study was conducted to generate up-to-date data on the circulation of *Marburgvirus*-specific IgG antibodies in humans in Isiro, in order to better understand the epidemiology of MVD in Isiro/DRC.

1.3 Objectives

1.3.1 Main objective

To generate reliable up-to-date data on the seroprevalence of *Marburgvirus* in humans in Isiro, Oriental Province**,** DRC.

1.3.2 Specific objectives

To determine the seroprevalence of *Marburgvirus* among humans in Isiro.

To establish whether there is an association between gender/age and *Marburgvirus*-specific IgG seropositivity.

CHAPTER II

2.0 LITERATURE REVIEW

2.1. *Marburgvirus* classification

The *Marburgvirus* genus belongs to the order *Mononegavirales,* family *Filoviridae* and contains only one known species namely *Marburg marburgvirus* with two virus members, MARV and RAVV (Bukreyev *et al*., 2014). MARV strains include Angola, Ci67, Musoke, Ozolins, and Popp (Ursic-Bedoya *et al.*, 2014). These strains exhibit genomic sequence differences of 0 percent to 7.4 percent. On the other hand, RAVV strain shows greater sequence disparity of 21 percent when compared to MARV (Johnson *et al*., 1996; Peterson and Holder, 2012).

2.2 *Marburgvirus* structure and genome organization

Marburgvirus particles are filamentous and enveloped, measuring 80 nm in diameter and about 790 nm in length (Kiley *et al*., 1982; Dag *et al.,* 2015). Generally, *Marburgvirus* particles are shorter considering the mean unit length than *Ebolavirus* (Geisbert and Jahrling, 1995; Dag *et al.*, 2015). The genome of *Marburg Marburgvirus* is about 19 kb in size, single-strand, negative-sense RNA (Regnery *et al*., 1980). The genome encodes seven proteins arranged sequentially in the order 3'-untranslated region (UTR), NP (nucleoprotein), VP35 (polymerase cofactor), VP40 (matrix protein), GP (glycoprotein), VP30 (transcription activator), VP24 (secondary matrix protein), an RNA-dependent RNA polymerase (L polymerase) and 5'-UTR (Figure 2-1**)** (Feldmann *et al*., 1992; Rougeron *et al*., 2015). From these seven structural proteins of *Marburgvirus*, four proteins (NP, VP35, VP30, and L) make up the helical nucleocapsid, which is encircled by the matrix protein that is composed

of the viral proteins VP40 and VP24. The *Marburgvirus* virions surface is covered with spikes which is GP structure (Dadario-DiCaprio *et al.,* 2006). NP, VP35, VP40 and L genes are mostly stable, while GP, VP30 and VP24 are variable (Feldmann and Klenk, 1996). The surface membrane protein GP mediates entry into susceptible cells through receptor binding. It also plays an important role of inducing neutralizing antibodies, antiviral and pro-inflammatory responses (de Wit *et al*., 2011; Bale *et al*., 2012). Furthermore, GP plays a role in virus pathogenesis and is useful to major target for vaccine candidates (Licata *et al*., 2004; Dadario-DiCaprio *et al.,* 2006; de Wit *et al*., 2011).

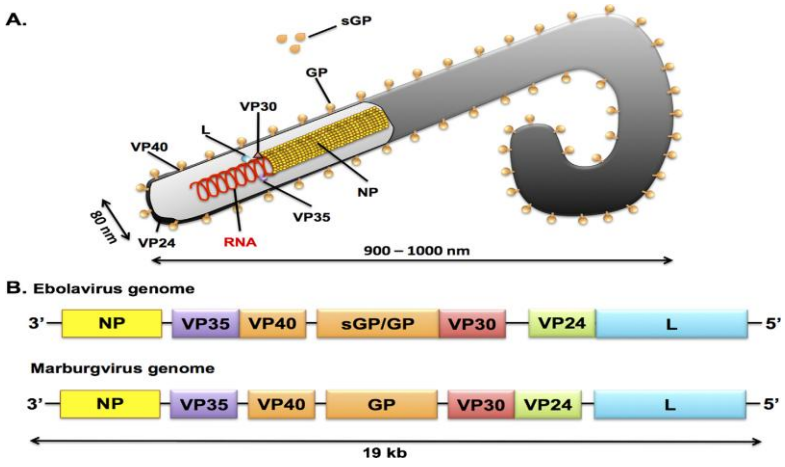

Figure 2-1. (A) A schematic illustration of a filovirus particle and (B) schematic representation of *Ebolavirus* and *Marburgvirus* . Four proteins are involved in the formation of the ribonucleoprotein complex: L, NP, VP30, and VP35. The GP is a type I trans membrane protein and is anchored with the carboxy-terminal part in the virion membrane. The soluble GP (sGP) is a non-structural glycoprotein secreted from infected cells and is only secreted by *Ebolaviruses*. VP40 and VP24 are membrane-associated proteins (Rougeron *et al*., 2015).

2.3 *Marburgvirus* host range

Marburg virus disease is a zoonotic disease that persists in a healthy reservoir host in endemic areas in sub-Saharan Africa. Humans and NHPs are susceptible spillover hosts of MVD (Brauburger *et al*., 2012). Some species of bats were suspected to be associated with

MVD outbreaks in Kenya (Smith *et al*., 1982; Johnson *et al.*, 1996; Monath, 1999). The cave-dwelling Egyptian fruit bat (*Rousettus aegyptiacus*) seemed to be the primary host for *Marburgvirus* (Towner *et al*., 2007; Swanepoel *et al.*, 2007; Towner *et al*., 2009; Amman *et al*., 2012). Since MVD outbreaks have been associated to entry of humans into caves inhabited by bats, the evidence of bats to be the reservoir host of *Marburgvirus* was found after isolation of *Marburgvirus* from Egyptian fruit bats (Towner *et al*., 2007; Swanepoel *et al*., 2007; Towner *et al*., 2009; Amman *et al*., 2012). Recently, *Marburgvirus*-specific nucleic acids and IgG antibodies were found in *R. aegyptiacus* during surveys in Gabonese bat populations (Pourrut *et al*., 2009; Maganga *et al*., 2011). It has been suggested that *Marburgvirus* is enzootic and can spread into countries, possibly through bat migrations (Maganga *et al*., 2011). Figure 2-2 **s**hows the locations of MVD outbreaks in humans and reported animal infections across Africa.

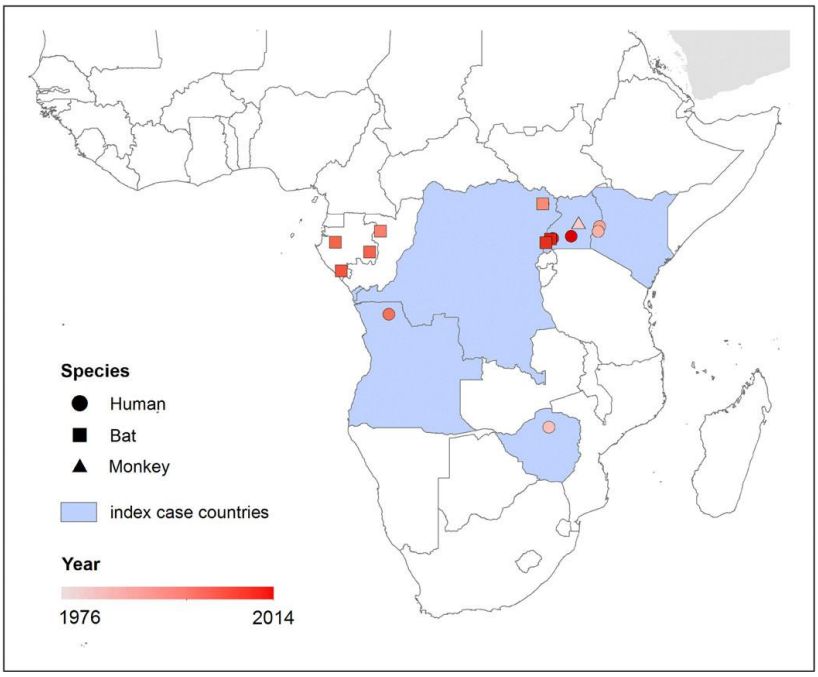

Figure 2-2. The locations of MVD in humans and reported animal infections across Africa. (Pigott *et al*., 2015).

2.4 Marburg virus disease

2.4.1 Historical perspectives of MVD

Historically, MVD was first observed in laboratory workers in Germany and Serbia in 1967 (Martini, 1973; WHO, 2012a). The origin of the etiological agent of this first documented MVD outbreak which affected 31 patients and caused seven deaths was African green monkeys imported from Uganda. Since 1967, no MVD cases were recorded until 1975 when a young tourist Australian from Zimbabwe fell sick in South Africa, died in Johannesburg and infected two others persons, his companion and a nurse (Gear *et al*., 1975; Conrad *et al.*, 1978). In 1980, an MVD outbreak occurred in which a 56-year-old Frenchman became acutely ill after his travels in Western Kenya and the patient's attending physician became the secondary case (Smith *et al*., 1982). Furthermore, an MVD outbreak occurred in 1987, when a 15-year old Danish boy who had traveled to Kenya, died after few days of illness (Johnson *et al*., 1996). Between 1988 and 1990, two sporadic MVD outbreaks occurred outside Africa in Russia (Brauburger *et al*., 2012; CDC, 2014), however, eight years later, the African continent recorded the first largest MVD outbreak which occurred in Durba/Watsa, DRC from 1998 to 2000 (Bausch *et al*., 2006). During this outbreak, there were 154 cases with case fatality rate of 83 percent. This was followed by a large MVD outbreak that occurred in Angola in 2004-2005 with 252 cases and case fatality rate of 90 percent (CDC, 2005; Ligon, 2005; WHO, 2005; Towner *et al.*, 2006). Since then sporadic outbreaks have occurred in Uganda, USA and Netherlands (CDC, 2009; Timen *et al.*, 2009; Adjemian *et al*., 2011; WHO, 2012b; WHO, 2014b). Table 2-1 below, shows the history of laboratory confirmed cases of MVD worldwide.

Table 2-1. History of laboratory confirmed cases of Marburg virus disease

Year	City	Country	Death/Case (%)
1967	Marburg	Germany	5/24 (21)
1967	Frankfurt	Germany	2/6 (33)
1967	Belgrade	Sebia	0/2 (0)
1975	Johannesburg	South Afica	1/3 (33)
1980	Nairobi	Kenya	1/2 (50)
1987	Nairobi	Kenya	1/1 (100)
1988	Koltsovo	Russia	1/1 (100)
1990	Koltsovo	Russia	0/1 (0)
1998-2000	Durba/Watsa	DR Congo	128/154 (83)
2004-2005	Uige	Angola	227/252 (90)
2007	Kamwenge	Uganda	1/4 (25)
2008	Colorado City	USA	0/1 (0)
2008	Leiden	Netherlands	1/1 (100)
2012[b]	Kabale	Uganda	9/20 (45)
2014[c]	Mpigi/Kampala	Uganda	1/1 (100)
Total			**378/470 (80)**

Table adapted from Brauburger *et al*., 2012. [a] World Health Organization (2012b)

[b] World Health Organization (2014b)

2.4.2 Geographical distribution of MVD

The geographical distribution of filovirus diseases generally spans the Afrotropics (see details in Figure 2-3). Marburg virus disease is predicted to be absent in the humid areas, but generally occurs in the arid woodlands in drier and less forested central and eastern Africa, while EVD appears to occur in the central and western African rain forests (Peterson *et al*., 2004; Rougeron *et al*., 2015).

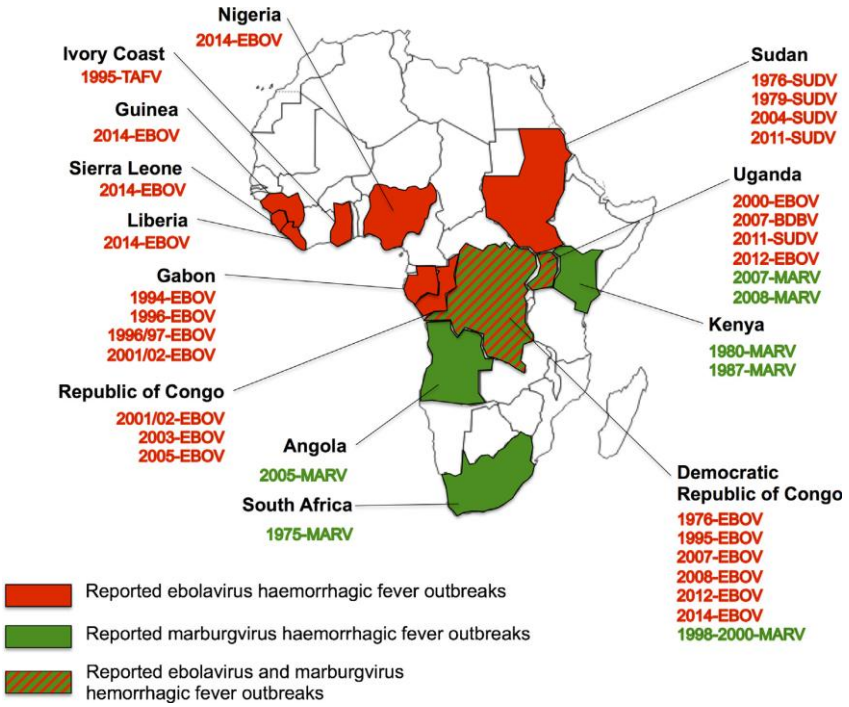

Figure 2-3. Reported outbreaks or isolated cases of haemorrhagic fever caused by *Marburgviruses* (*Marburg marburgvirus*, represented in green) and *Ebolaviruses* (EBOV, TAFV, SUDV, and BDBV, represented in red) in Africa (Rougeron *et al*., 2015).

Thus the occurrence sites of MVD are quite distinct from those of EVD with minimal overlap and this seems to correspond with *Marburgvirus*' distant position in the phylogeny of the *Filoviridae*. It has been suggested that the distribution pattern of MVD and EVD may be associated with the distribution of their natural hosts which may have markedly different

ecological requirements (Peterson *et al*., 2004). Except European and American MVD outbreaks, to-date, MVD has occurred exclusively in five countries in Africa (Uganda, Kenya, DRC, South Africa/Zimbabwe and Angola) (Pigott *et al*., 2015). It has been predicted that MVD may occur in some African countries, including Western African countries, due to displacement/migration of reservoir hosts (Peterson *et al.*, 2006; Pigott *et al*., 2015). The summary of this prediction is shown in Figure 2-4 below.

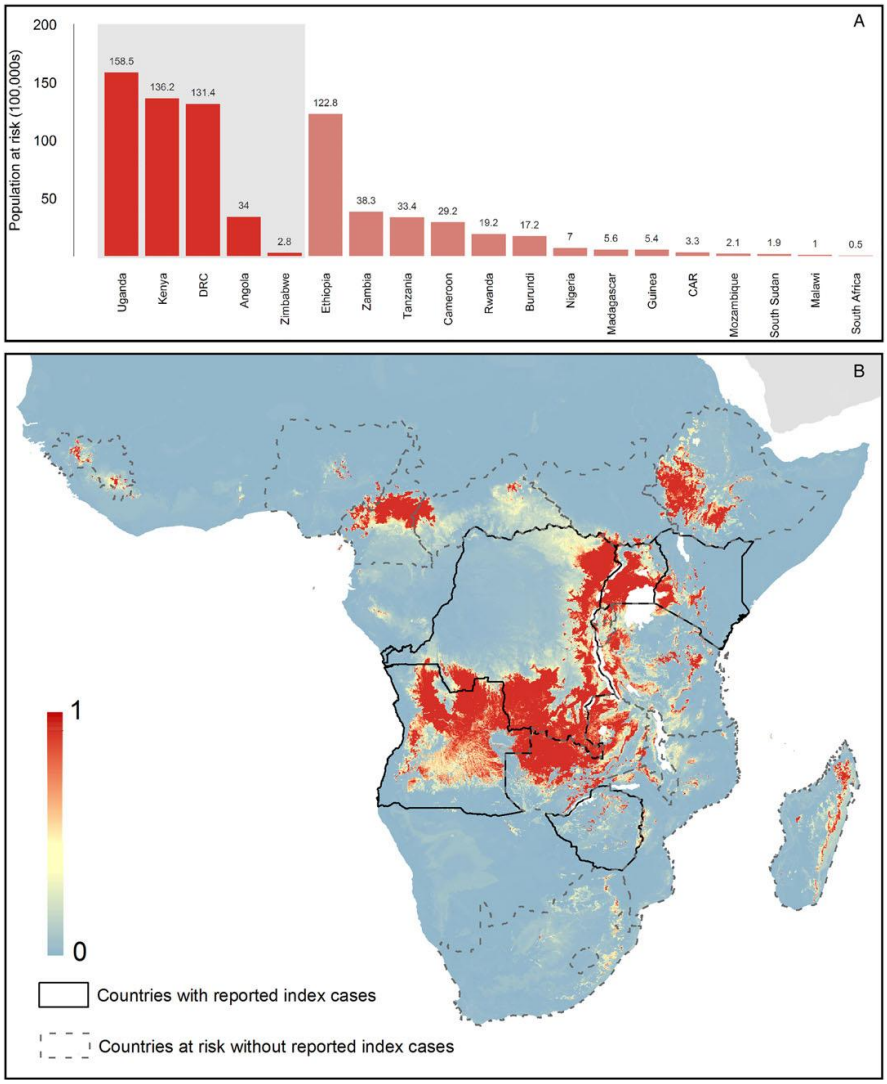

Figure 2-4. Predicted geographical distribution of the zoonotic niche for *Marburgviruses* using model 1 – human index cases only. Panel A shows the total populations living in areas of risk of zoonotic transmission for each at-risk country. The grey rectangle highlights countries in which index cases of disease have been reported (set 1); the remainder are countries in which risk of zoonotic transmission is predicted, but in which index cases of MVD have not been reported and have more than hundred at-risk pixels (set 2). These countries are ranked by population-at-risk within each set. The population-at-risk figure in 100 000s is given above each bar. Panel B shows the predicted distribution of zoonotic *Marburgviruses*. The scale reflects the relative probability that zoonotic transmission of *Marburgviruses* could occur at these locations; areas closer to one (red) are more likely to harbor zoonotic transmission than those closer to zero (blue). Countries with borders outlined are those which are predicted to contain at-risk areas for zoonotic transmission based on a threshold approach (Pigott *et al*., 2015).

2.4.3 Transmission of MVD

2.4.3.1 Primary transmission

Human and NHPs may contract the *Marburgvirus* via direct exposure to the natural reservoir host (*Rousettus aegyptiacus*) or through direct contact with their excrement (Bausch *et al*., 2006; Swanepoel *et al*., 2007; Towner *et al*., 2009). Although there has been no evidence of direct contact of humans and infected healthy bats, during the 1998-2000 DRC outbreak, it was found that the mine from which humans got infected was heavily contaminated with human and bat excrement, and the miners worked without proper tools and protective equipment (Bausch *et al*., 2003; Bausch *et al*., 2006). To-date, no evidence of transmission between humans and NHPs has been reported other than the 1967 MVD outbreak which was associated with contact with infected monkeys (Martini, 1973; WHO, 2012a).

2.4.3.2 Secondary transmissions of MVD

Transmission by human-to-human generally occurs in the patient's family members or in hospital settings due to close contact with infected individuals or contact with patient's body fluids or other contaminated objects (CDC, 1995; Bausch *et al*., 2003).

Secondary transmissions of MVD often occur as a result of inadequate or lack of disease control measures. Consequently, person-to-person transmission of *Marburgvirus* can spread among family members, Health-care workers and in the communities (Bausch *et al*., 2006, WHO, 2012a). The transmission is greatest during the latter stages of disease. Actually, transmission during incubation period has not been reported, but the patients may become infectious during the few days after onset of fever (CDC, 1995). *Filoviruses* have been isolated from semen and ocular fluid of recovered patients and thus there is a possibility of

transmission of this virus through sexual transmission or infected semen from survivors of MVD and EVD to partners after clinical recovery (Kuming and Kokoris, 1977; Bausch *et al*., 2007; Christie *et al*., 2015; Yasri and Wiwanitkit, 2015).

2.4.4 Pathogenesis of MVD

The pathogenesis of *Marburgvirus* infections is similar both in humans and NHPs (Glaze *et al*., 2015). Following virus entry through small skin lesions or mucosal membranes, there is an activation of mononuclear phagocyte system (i.e. monocytes, macrophages and dendritic cells) which generally has been identified as the initial targets of *Marburgvirus* infection. (Alves *et al*., 2010; Hensley *et al*., 2011). These cells are found in lymph nodes, liver and spleen. Infection and replication of virus in these cells lead to severe lesions of these organs (Giesbert *et al*., 2008; Alves *et al*., 2010; Hensley *et al*., 2011). Migration of these infected cells into surrounding tissues and/or transport of free virus through the circulatory system leads to the dissemination to multiple organs (Schnittler and Feldmann, 1998; Schnittler and Feldmann, 2003). Also, *Marburgvirus* infection leads to upregulation of the proinflammatory cytokines (particularly IL6, IL8 or IL1β mRNA), nitric oxide and hypoalbumine (Baize *et al*., 2002; Geisbert *et al*., 2003; Geisbert *et al*., 2007; Fritz *et al.*, 2008; Alves *et al.*, 2010; Cross *et al*., 2015). Activation of monocytes and macrophages induces the secretion of cytokines and the production of proinflammatory cytokines TNF-α and high levels of chemokines which have been suggested to be associated with the death of patients infected by filoviruses (Baize *et al*., 1999; Villinger *et al.*, 1999). *Marburgvirus* infection also may induce severe coagulopathy including increased prothrombin and protein C activity, decreased thrombin times, marked fibrinogen degradation, increased prostacyclin, thromboxane, circulating thrombin-thrombomodulin complex levels and deposition of fibrin

in tissues and decreased C3 Complement (Zhang *et al*., 2006; Geisbert *et al.,* 2007; Reis *et al*., 2008; Hensley *et al*., 2011) leading to disseminated intravascular coagulation (Hensley *et al*., 2011).

2.4.5 Disease manifestation of MVD

The incubation period ranges between three to 21 days (WHO, 2012a). The onset of MVD is abrupt, with symptoms resembling those of other illnesses such as malaria or typhoid fever and other tropical diseases. Most patients will present with fever, chills, severe frontal headache, myalgia and weakness (Gear, 1989; Bausch *et al*., 2006; WHO, 2012a). Few days after the onset, typically after three to five days, maculopapular rashes, mostly on the trunk (chest, back, stomach), may occur. These may be followed by nausea, vomiting, diarrhea, abdominal pain, chest pain and sore throat. Hemorrhagic symptoms such as epistaxis, hemoptysis, hematemesis, melaena or bleeding gums may also occur (Gear, 1989; Borchert *et al.,* 2002; WHO, 2012a). End-stage manifestations of the disease include shock, coma, with hypotension and multiple organ failure; sometimes cerebral edema and encephalopathy can be noted (CDC, 2005; van Paassen *et al*., 2012).

2.4.6 Diagnosis of MVD

2.4.6.1 Clinical diagnosis

Clinically, identification of MVD may be difficult because majority of the signs and symptoms of this disease are similar to many infectious tropical diseases. It is difficult to distinguish MVD from EVD, and many other tropical diseases, such as other viral hemorrhagic fevers, Falciparum malaria, trypanosomiasis, visceral leishmaniasis, typhoid fever, shigellosis, typhus, cholera, gram-negative septicemia, borreliosis, leptospirosis,

plague, Q fever, candidiasis, histoplasmosis, hemorrhagic smallpox, measles, fulminant viral hepatitis and other infectious and non infectious diseases (Gear, 1989; Bausch *et al.,* 2006; WHO, 2012a). Medical history of the patient, exposure to wildlife, travel and occupational activities are important to differentiate MVD and others tropical diseases (Mahendra *et al*., 2014).

2.4.6.2 Laboratory diagnosis

Diagnosis of MVD becomes a challenge due to limited laboratory facilities in endemic areas and difficulties in transportation of samples, which can cause substantial delays in laboratory response in many developing countries (Borchert *et al*., 2002). Also, manipulations of *filoviruses* including *Marburgvirus*, requires a biosafety level 4 (BSL-4) laboratory (Masayuki *et al*., 2006; Bannister, 2010). The World Health Organization (WHO) recommends for certitude diagnosis of filoviruses, which includes virus isolation by cell culture, reverse transcriptase polymerase chain reaction (RT-PCR), serum neutralization test, antigen-capture enzyme-linked immunosorbent assay (ELISA) and electron microscopy (WHO, 2015). The IgM-capture ELISA can be used to confirm a case of MVD within a few days of symptomatic onset. The IgG-capture ELISA is appropriate for testing persons later in the course of MVD or after recovery and it is useful for epidemiological survey (Nakayama *et al*., 2010). In deceased patients, immunohistochemistry, virus isolation, or PCR on tissue specimens may be used to diagnose MVD, retrospectively. To improve field diagnosis of *Marburgvirus* infections by eliminating the need for PCR machines, which requires expensive equipment and sophisticated techniques, RT loop-mediated isothermal amplification (LAMP) was developed for the rapid and simple detection of *Marburgvirus* (Kurosaki *et al*., 2010). The RT-LAMP assay does not require the use of sophisticated

equipment or highly skilled personnel and can provide accurate results within a short time frame. These characteristics make this assay potentially useful for the clinical diagnosis of *Marburgviru*s infection in a field laboratory.

2.4.7 Treatment of MVD

To-date, there is no specific treatment and vaccine approved available for filovirus infections, including MVD (Brauburger *et al*., 2012; Mire *et al*., 2014). During MVD outbreaks, supportive care (fluids, anti-microbials, and blood transfusions) has been used for treatment of infected persons (Brauburger *et al*., 2012). Supportive therapy aims at balancing the patient's fluids and electrolytes, maintaining oxygen status and blood pressure, replacing lost blood and clotting factors, and treatment for any complicating infections.

After immediate exposure, phosphorodiamidate morpholino oligomers (PMO*plus*), VSV-based vaccine expressing MARV GP, has also been demonstrated to be effective as a post-exposure treatment of infected NHPs (Geisbert *et al*., 2008; Warren *et al*., 2010; Mire *et al*., 2014). It has been found to be totally protective if administered 20 to 30 minutes after MARV infection (Geisbert *et al*., 2010; Warren *et al*., 2010; Mire *et al*., 2014). However, the use of this candidate vaccine is difficult in MVD outbreaks in humans because of non-specificity of early symptoms of the disease. Many other candidate vaccines tested in animal models were unsuccessful in humans (Warfield *et al*., 2004; Daddario-DiCaprio *et al*., 2006; Swenson *et al*., 2008; Warfield and Aman, 2011).

2.4.8 Control measures of MVD

Preventive measures against Marburg virus infection are not well defined, as transmission from wildlife to humans remains an area of ongoing research. Major control measures

include avoidance of contact with reservoir host fruit bats, infected animals and patients (Roberts and Andrews, 2008; Timen *et al*., 2009; Adjemian *et al*., 2011). During MVD outbreak, people at highest risk (family members of patients and hospital staff who care for patients, technicians and veterinarians) must be given more attention to avoid spread of outbreak. Some measures of controlling MVD outbreaks are: strict quarantine procedures, measures to minimize contact with infected animals, strict isolation of infected individual, use of personal protective equipments (PPEs), strict barrier nursing techniques, safe and early laboratory diagnosis of suspected cases (Gear *et al*., 1975; Adjemian *et al*., 2011) and education of health care workers and populations (Jeffs *et al.,* 2007; Roddy *et al.,* 2007).

CHAPTER III

3.0 MATERIALS AND METHODS

3.1 Study area

The study was conducted in Isiro, Oriental Province, in north-eastern part of DRC (Figure 3-1). The province is divided into four districts (Bas-Uele, Haut-Uele, Tshopo and Ituri) and twenty six territories. Isiro is the district seat of Haut-Uele including six territories (Watsa, Dungu, Faradje, Niagara, Wumba and Rangu). It is located at 02°.77 latitude north and 27°.62 longitude east and it is situated at elevation 761 meters above sea level. It lies between the equatorial forest and the savannah and its main resource is coffee. In 2012, its population was estimated at approximately 182,900. The local language is Lingala, but Swahili is not uncommon. Isiro is served by Kinshasa, capital city, through Matari airport however the isolated narrow gauge to the river port of Bumba on the Congo River is not currently operational. Dirt roads allow its commercial trade with Uganda and South Sudan (Sources: https://fr.wikipedia.org/wiki/Isiro; http://www.worldatlas.com/af/cd/5/where-is-isiro.html)

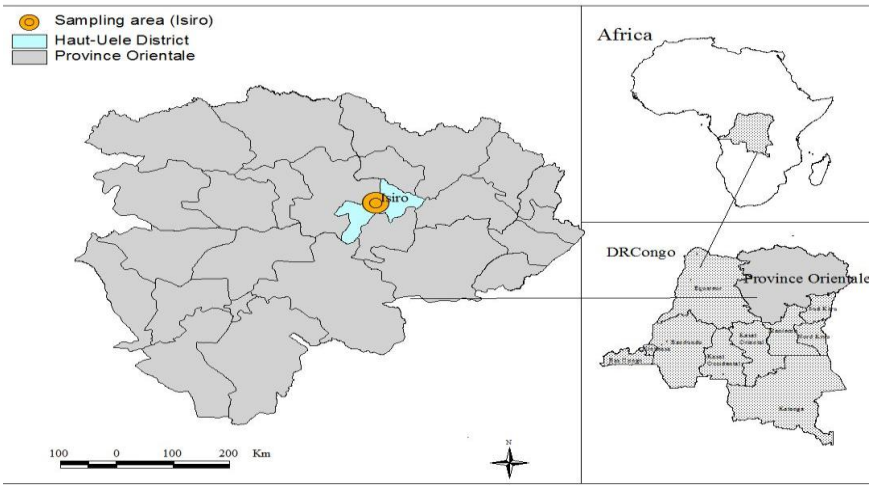

Figure 3-1. The map showing DRC, Oriental Province and Isiro (Source: ArcView GIS® 3.2 software)

3.2 Study design

A cross-sectional study was carried out to generate reliable up-to-date data on the seroprevalence of *Marburgvirus* among humans in Isiro for a period of one month (July, 2013).

3.3 Study population

Volunteers, inhabitants of Isiro, apparently healthy, aged at least 16 years who were willing to have their blood samples collected were included in the study.

3.4 Sample size

Sample size estimation was calculated using the simple Radom/systematic sampling of proportion formula:

$$n = \frac{Z^2 \times P \times Q}{e^2}$$

n = required number of individuals to be examined,

Z = Z score for given confidence level, $z_{(1-a/2)}$ = 1.96 = value of the standard distribution corresponding to a significance level of a (1.96 for a 2-sided test at the 0.05 level)

p = expected proportion in the population = 0.50 (survey for Marburg antibodies in the province is unknown), Q = (1-P) and e = relative precision (0.1).

$n = 1.96^2 \times 0.50 \times 0.50/0.1^2 = 96$

To minimize the risk of selection bias, the sample size was multiplied by 2. The minimal sample size was 96 x 2 = 192 individuals.

3.5 Data source and sample collection

The sample data of this study were obtained from Ministry of Health through the National Program against HIV/AIDS and National Institute of Biomedical Research (INRB), during a serosurvey of HIV/AIDS other viral diseases in July 2013. The teams were composed by national and local teams, which were comprised of medical doctors, nurses and technicians. Laboratory facilities were established inside the general hospital where blood samples were collected. From each person, 5 mL of venous blood were collected in a plain tube as well as in a vacutainer containing an anticoagulant (EDTA). The tubes were stored in the dark at 4°C until arrival at the laboratory. After centrifugation (1500 rpm), 2 mL of serum were aliquoted into cryotubes, placed in cryoboxes and stored at -20°C. At the end of the field mission, the aliquots were transferred to the INRB in Kinshasa where the samples were stored at -80°C until laboratory analysis. A total of 400 sera samples were collected, due to

limited availability of ELISA test kits, 172 samples were selected randomly by Excel Software using ALEA command.

3.6 Laboratory analyses

Serum samples were tested for the presence of *Marburgvirus*-specific IgG antibodies using ELISA. The antigens and other reagents for the IgG ELISA used in this study were kindly provided by Prof. Ayato Takada, Research Center for Zoonosis Control, Hokkaido University, JAPAN. The IgG ELISA method was performed as previously reported by Nakayama *et al*. (2010). Briefly, ELISA plates (Nunc Maxisorp) were coated with *Marburgvirus* GP antigens or negative control antigens (other proteins) (100 ng of GP/50 µL/well) in phosphate-buffered saline (PBS) overnight at 4°C and washed three times with 200 µL/well of PBST (PBS containing 0.05 percent Tween 20). Serum samples were diluted 1:1000 with β-propriolactone (1 percent) and were stored in cryotubes overnight at 4°C. Nonspecific binding of the antibodies was avoided by blocking with 3 percent skim milk (150 µL/well) for 2 hours at room temperature and washed three times with 400 µL/well of PBST. After washing three times with PBST, 50 µL/well of appropriately diluted serum samples (1/100 and 1/1000) in PBST containing 1 percent skim milk was added and incubated for 1 hour at room temperature. The bound antibodies (horseradish peroxidase - antibodies human IgG) were detected by using the secondary antibodies conjugated (1/4000 dilution) with human antibodies diluted in 1 percent skim milk in PBST. After incubation for 1 hour at room temperature followed by three times washing with PBST, 50 µL of 3,3',5,5'-tetramethylbenzidine (TMBO) was added to each well, and the mixture was incubated for 15 min at room temperature. The reaction was stopped by adding 1 N sulfuric acid to the mixture, and the optical density (OD) at 450 nm was measured with an ELISA plate reader

(Thermo Scientific-NUNC A/S, Denmark). For each sample, we calculated the corrected OD as the OD of the antigen-coated well minus the OD of the corresponding control well.

3.7 Data analysis

Grubbs-Smirnov test was used to establish the positivity of each OD value at 0.05 and 0.01 of significance level. Data was double-entered and checked into Excel Software and Stata software (version 11) was used for all analyses. Presence of *Marburgvirus* IgG was estimated by frequencies and percentages. Chi square test (or Fisher's exact test when appropriate) was used to assess associations between independent variables and *Marburgvirus*-specific IgG prevalence. Odds ratios (ORs) and exact 95% confidence intervals (CIs) were derived to assess the measures of association. The p-value of < 0.05 was considered to be statistically significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Descriptive characteristics of study population

Among the 172 individuals whose serum was tested, the median age was 36 years (range: 18–58). Participants were categorized in three groups based on age to assess the level of risk of exposition to the virus in young, middle age and old people, according to previous studies in DRC (Bausch *et al*., 2003). The first group consisted of participants aged between 16 and 30 years while the second group consisted of those aged between 31 and 45 and the third group being those aged between 46 and 60. The number (proportion) of individuals that formed each age category was 58 (33.7 percent) for those aged 15-30 years; 69 (40.1 percent) for those aged 31-45 years and 45 (26.2 percent) for those aged 46-60 years. The number (proportion) of males and females was 91 (47.1 percent) and 81 (52.9 percent), respectively.

The distribution of sex by age category was such that the proportion of females was higher than that of males only in the middle aged (i.e. the 31-46 years age category) (Figure 4-1).

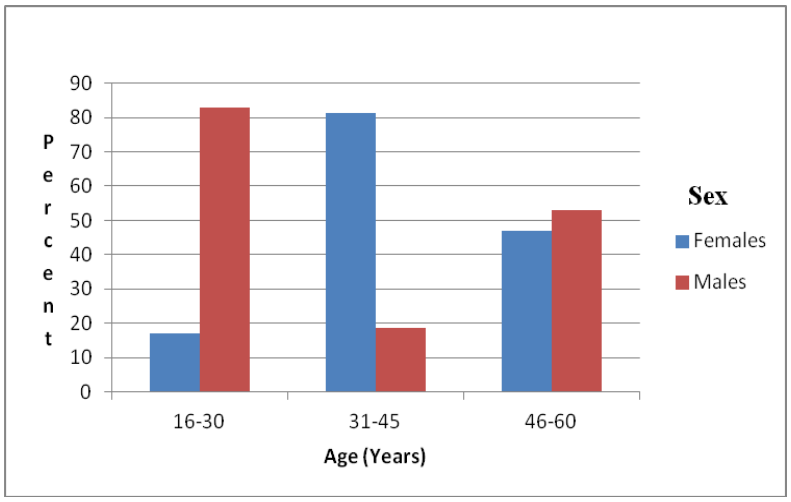

Figure 4-1. Distribution of sex by age in the study population

4.2 Determination of *Marburgvirus*-specific IgG seroprevalence

The overall prevalence of *Marburgvirus*-specific IgG was 4.7% (95% IC: 1.5-7.8). The prevalence of *Marburgvirus*-specific IgG was insignificantly higher in males than in females (5.5% versus 3.7%; OR = 1.5; 95% CI: 0.3-6.5; p = 0.58) and in individuals aged 46-60 years than to those aged 16-30 years (6.7% versus 1.7%; OR = 1.7; 95% CI: 0.4-40.5; p = 0.23) (Table 4-1).

Table 4-1. Prevalence and measure of association of *Marburgvirus*-specific IgG by sex and age (N = 172)

Variable	Number	Positive	%	OR	95% CI	p-value
Sex						**0.58**
Female	81	3	3.7	1		
Male	91	5	5.5	1.5	0.3-6.5	0.58
Age (Years)						**0.47**
16-30	58	1	1.7	1		
31-45	69	4	5.8	3.5	0.4-32.3	0.27
46-60	45	3	6.7	4.5	0.4-40.5	0.23

After stratification of age by sex (Table 4-2), the proportion of *Marburgvirus*-specific IgG was insignificantly higher in females aged 46-60 years (6.7%, p = 0.69) and in males aged 31-45 years (15.4%, p = 0.17) than in other age groups, respectively.

Table 4-2. Prevalence of *Marburgvirus*-specific IgG by age, stratified by sex (N = 172)

Variable	Number	Positive	%	p-value
Female (n = 81)				**0.69**
16-30	10	0	0.0	
31-45	56	2	3.6	
46-60	15	1	6.7	
Male (n = 91)				**0.17**
16-30	48	2	2.1	
31-45	13	2	15.4	
46-60	30	2	6.7	

CHAPTER FIVE

5.0 DISCUSSION

This study was undertaken to determine the seroprevalence of *Marburgvirus*-specific IgG among humans in Isiro, Oriental Province**,** DRC. In this study, it was expected that at least 192 samples would be tested. But due to limited antigen availability of for the ELISA test, only 172 samples were actually tested. Although this represented 89.6 percent of the subjects examined, it was within the allowable 15 percent error of sampling.

The detection of *Marburgvirus*-specific IgG antibodies among some residents of Isiro indicates possible exposure of these individuals to *Marburgvirus* probably through contact with exposed human populations or the natural reservoir host of this virus. The possibility of contact with exposed human populations is supported by the fact that Isiro zone shares borders with areas that have experienced MVD outbreaks, particularly Durba/Watsa where MVD outbreaks occurred in 1998-2000 (Bausch *et al*., 2006). It is also worth noting that Oriental Province, where Isiro zone lies, shares boundaries with Uganda which has experienced three MVD outbreaks (i.e. in 2007, 2012 and 2014) (Adjemian *et al*., 2011; WHO, 2012b; WHO, 2014b). The mass movements of people across borders of neighboring countries as a result of the on-going civil war in DRC (including Oriental Province) may lead to exposure of some individuals to new pathogens. It is thus feasible that the presence of *Marburgvirus* antibodies in Isiro populations could be attributable to exposure to *Marburgvirus* from Ugandan MVD outbreaks and not only Durba/Watsa outbreaks. Moreover, the high case-fatality rate (83 percent) associated with the Durba/Watsa MVD outbreak left few survivors who could have developed *Marburgvirus* antibodies (Bausch *et*

al., 2003; Bausch *et al*., 2006). In contrast, Uganda MVD outbreaks had moderate case-fatality ratio (45 percent) with small number (20 cases) of confirmed cases (WHO, 2012b) thus leaving survivors who may have transmitted the virus to other individuals with no obvious signs of disease (Borchert *et al*., 2002).

The possibility of exposure to *Marburgvirus* from an animal reservoir host cannot be dismissed entirely. Various previous findings support the idea of an association between MVD and exposure in mines and caves (Smith *et al.,* 1982; Johnson *et al.,* 1996; Towner *et al*., 2009; Adjemian *et al*., 2011). In fact, in the Durba/Watsa outbreaks of 1998-2000, the most affected were male miners suggesting that the local mines were a site of primary infection with *Marburgvirus*, particularly from bats inhabiting the mines (Bausch *et al*., 2003; Bausch *et al*., 2006). Also, in another survey carried out to verify whether pygmies living in Watsa area constitute another population at risk for primary infection and transmission of *Marburgvirus*, it was found that pygmies who were significantly more exposed to wild animals than the non-mining general population showed a 0.0 percent seroprevalence of *Marburgvirus* IgG antibodies compared to non-mining population (0.35 percent) (Borchert *et al.,* 2005). However, earlier studies found that pygmies were seropositive for ELISA IgG antibodies for *Ebolaviruses* and *Marburgviruses* (Gonzalez *et al*., 2000). It has been suggested that lack of anti-*Marburgvirus* IgG in the pygmy populations, living in Watsa, may be attributed to the lack of the reservoir host(s) in the pygmies' environment, the absence of exposure to the reservoir, or a combination of these (Borchert *et al*., 2005).

The prevalence (4.7 percent) of *Marburgvirus*-specific IgG determined in this study was surprisingly higher compared to a serosurvey conducted in Durba/Watsa (2.0 percent)

(Bausch *et al.*, 2003) and to some studies conducted in Republic of Congo and in Central African Republic, which found low prevalence (less than 2 percent) of *Marburgvirus*-specific IgG (Johnson *et al*., 1993a; Moyen *et al.*, 2015). Similar results (5.2 percent of seroprevalence *of Marburgvirus* IgG antibodies) were found in the study of Gonzalez *et al.* (2000) in pygmy populations in Central African Republic. The reason for this difference with Durba/Watsa serosurvey is unclear. However, as earlier indicated, in the case of the Durba/Watsa MVD outbreaks, the high case-fatality ratio (83 percent) may have left few survivors with *Marburgvirus*-specific antibodies and hence the observed low prevalence. The higher seroprevalence found in this study may be linked to exposure to exposed populations.

In this study, sex and age did not play any significant role for influencing the seropositivity of *Marburgvirus*-specific IgG in Isiro ($p > 0.05$); similar results to the study of Johnson *et al.* (1993b), which did not find a significant difference between young and old human populations in Central African Republic. In contrast, previous studies have shown a higher prevalence of *Marburgvirus*-specific IgG in pygmy males (Gonzalez *et al*., 2000) and in young miner males (Bausch *et al*., 2003).

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

These findings indicate the presence of *Marburgvirus*-specific IgG antibodies in humans in Isiro and suggest possible close contact with exposed populations or exposure to natural reservoirs of the virus (e.g. fruit bats). Furthermore, these findings highlight the need for a country-wide surveillance of MVD for mitigations purposes.

6.2 Recommendations

1. Further studies to identify risk factors of MVD in Isiro and other zones of Oriental Province should be conducted.
2. The government and other stakeholders should consider the possibility of occurrence of mild or asymptomatic infections in the formulation of strategies for the development of measures for the control and prevention of MVD.
3. Educational campaigns and surveillance programs should be implemented country-wide. Such programs should provide training for all health care workers to facilitate early outbreak recognition and patient treatment.

REFERENCES

Adjemian, J., Farnon, E.C., Tschioko, F., Wamala, J.F., Byaruhanga, E., Bwire, G.S., Kansiime, E., Kagirita, A., Ahimbisibwe, S., Katunguka, F., Jeffs, B., Lutwama, J.J., Downing, R., Tappero, J.W., Formenty, P., Amman, B., Manning, C., Towner, J., Nichol, S.T and Rollin, P.E (2011). Outbreak of Marburg hemorrhagic fever among miners in Kamwenge and Ibanda Districts, Uganda, 2007. J. Infect. Dis. 204: S796-S799.

Alves, D.A., Glynn, A.R., Steele, K.E., Lackemeyer, M.G., Garza, N.L., Buck, J.G., Mech, C and Reed, D.S (2010). Aerosol exposure to the Angola strain of Marburg virus causes lethal viral hemorrhagic fever in cynomolgus macaques. Vet. Pathol. 47: 831–851.

Amman, B. R., Carroll, S.A., Reed, Z.D., Sealy, T.K., Balinandi, S., Swanepoel, R., Kemp, A., Erickson, B.R., Comer, J.A., Campbell, S., Cannon, D.L., Khristova, M.L., Atimnedi, P, Paddock, C.D., Crockett, R.J., Flietstra, T.D., Warfield, K.L., Unfer, R., Katongole-Mbidde, E., Downing, R., Tappero, J.W., Zaki, S.R, Rollin, P.E., Ksiazek, T.G., Nichol, S.T and Towner, J.S (2012). Seasonal pulses of Marburg virus circulation in juvenile Rousettus aegyptiacus bats coincide with periods of increased risk of human infection. PLoS. Pathog. 8: e1002877.

Baize, S., Leroy, E.M., Georges-Courbot, M-C., Capron, M., Lansoud-Soukate, J., Debre P., Fisher-Hoch, S.P., McCormick, J.B and Georges, A.J (1999). Defective humoral responses and extensive intravascular apoptosis are associated with fatal outcome in Ebola virus-infected patients. Nat. Med. 5: 423–426.

Baize, S., Leroy, E.M., Georges, A.J., Georges-Courbot, M-C., Capron, M., Bedjabaga, I., Lansoud-Soukate, J and Mavoungou, E (2002). Inflammatory responses in Ebola virus-infected patients. Clin. Exp. Immunol. 128: 163–168.

Bale, S., Dias, J.M., Fusco, M.L., Hashiguchi, T., Wong, A.C., Liu, T., Keuhne, A.I., Li, S., Woods, V.L., Jr., Chandran, K., Dye, J.M and Saphire, E.O (2012). Structural basis for differential neutralization of ebolaviruses. Viruses 4: 447-470.

Bannister, B. (2010). Viral haemorrhagic fevers imported into non-endemic countries: risk assessment and management. Br. Med. Bull. 95: 193-225.

Bausch, D.G.; Borchert, M., Grein, T., Roth, C., Swanepoel, R., Libande, M.L., Talarmin, A., Bertherat, E., Muyembe-Tamfum, J.J., Tugume, Colebunders, B.R., Konde, K.M., Pirad, P., Olinda, L.L., Rodier, G.R., Campbell, P., Tomori, O., Ksiazek, T.G and Rollin, P.E (2003). Risk factors for Marburg hemorrhagic fever, Democratic Republic of the Congo. Emerg. Infect. Dis. 9: 1531–1537.

Bausch, D.G., Nichol, S.T., Muyembe-Tamfum, J.J., Borchert, M., Rollin, P.E., Sleurs, H., Campbell, P., Tshioko, F.K., Roth, C., Colebunders, R., Pirard, P., Mardel, S., Olinda, L.A., Zeller, H., Tshomba, A., Kulidri, A., Libande, M.L., Mulangu, S., Formenty, P., Grein, T., Leirs, H., Braack, L., Ksiazek, T., Zaki, S., Bowen, M.D., Smit, S.B., Leman, P.A., Burt,

F.J., Kemp, A and Swanepoel, R (2006). Marburg hemorrhagic fever associated with multiple genetic lineages of virus. N. Engl. J. Med. 355: 909–919.

Bausch, D.G., Towner, J.S., Dowell, S.F., Kaducu, F., Lukwiya, M., Sanchez, A., Nichol, S.T., Ksiazek, T.G and Rollin, P.E (2007). Assessment of the risk of Ebola virus transmission from bodily fluids and fomites. J. Infect. Dis. 196: S142-S147.

Borchert, M., Muyembe-Tamfum, J.J., Colebunders, R., Libande, M., Sabue, M and Van der Stuyft, P (2002). Short communication: A cluster of Marburg virus disease involving an infant. Trop. Med. Int. Health 7: 902–906.

Borchert, M., Mulangu, S., Swanepoel, R., Tshomba, A., Afounde, A., Kulidri, A., Muyembe-Tamfum, J.J and Van der Stuyft, P (2005). Pygmy populations seronegative for Marburg virus. Emerg. Infect. Dis. 11:174-177.

Borio, L., Inglesby, T., Peters, C.J., Schmaljohn, A.L., Hughes, J.M., Jahrling, P.B., Ksiazek, T., Johnson, K.M., Meyerhoff, A., O'Toole, T., Ascher, M.S., Bartlett, J., Breman, J.G., Eitzen, E.M., Hamburg, Jr.M., Hauer, J., Henderson, D.A., Johnson, R.T., G. Kwik, Layton, M., Lillibridge, S., Nabel, G.J., Osterholm, M.T., Perl, M.T., Russell, P and Tonat, K (2002). Hemorrhagic fever viruses as biological weapons: medical and public health management. JAMA. 287: 2391-2405.

Bowen, E.T., Lloyd, G., Harris, W.J., Platt, G.S., Baskerville, A and Vella, E.E (1977). Viral haemorrhagic fever in southern Sudan and northern Zaire. Preliminary studies on the aetiological agent. Lancet 1: 571–573.

Brauburger, K., Hume, A.J., Mühlberger, E and Olejnik, J (2012). Forty-Five Years of Marburg Virus Research. Viruses 4: 1878-1927.

Bukreyev, A.A, Chandran, K., Dolnik, O., Dye, J.M., Ebihara, H., Leroy, E.M., Mühlberger, E., Netesov, S.V., Patterson, J.L., Paweska, J.T., Saphire, E.O., Smither, S.J., Takada, A., Towner, J.S., Volchkov, V.E., Warren, T.K and Kuhn, J.H (2014). Discussions and decisions of the 2012–2014 International Committee on Taxonomy of Viruses (ICTV) Filoviridae Study Group, January 2012–June 2013. Arch. Virol. 159: 821–830.

Centers for Disease Control and Prevention (1995). Notice to readers update: management of patients with suspected viral hemorrhagic fever—United States. Morb. Mortal. Wkly. Rep. 44: 475-9.

Centers for Disease Control and Prevention (2005). Outbreak of Marburg virus hemorrhagic fever–Angola, October 1, 2004–March 29, 2005. Morb. Mortal .Wkly. Rep.54:308-309.

Centers for Disease Control and Prevention (2009). Imported Case of Marburg Hemorrhagic Fever – Colorado, 2008. Morb. Mortal. Wkly. Rep. 58: 1377–1381.

Centers for Disease Control and Prevention (2014). Chronology of Marburg Hemorrhagic Fever outbreaks. Known cases and outbreaks of Marburg Hemorrhagic Fever, in chronological order. http://www.cdc.gov/vhf/marburg/resources/outbreak-table.html#six Accessed: 11 November 2015.

Christie, A., Davies-Wayne, G.J., Cordier-Lasalle, T., Blackley, D.J., Laney, A.S., Williams, D.E., Shinde, S.A., Badio, M., Lo, T., Mate, S.E., Ladner, J.T., Wiley, M.R., Kugelman, J.R., Palacios G, Holbrook MR, Janosko KB, de Wit E, van Doremalen N, Munster VJ, Pettitt J, Schoepp, R.J., Verhenne, L., Evlampidou, I., Kollie, K.K., Sieh, S.B., Gasasira, A., Bolay, F., Kateh, F.N., Nyenswah, T.G and De Cock, K.M (2015). Possible sexual transmission of Ebola virus - Liberia, 2015. Morb. Mortal. Wkly. Rep. 64: 479-481.

Conrad, J. L., Isaacson, M., Smith, E.B, Wulff, H., Crees, M., Geldenhuys, P and Johnston, J (1978). Epidemiologic investigation of Marburg virus disease, Southern Africa, 1975. Am. J. Trop. Med. Hyg. 27: 1210-1215.

Cross, R.W., Fenton, K.A., Geisbert, J.B., Ebihara, H., Mire, C.E and Geisbert, T.W (2015). Comparison of the Pathogenesis of the Angola and Ravn Strains of Marburg Virus in the Outbred Guinea Pig Model. J. Infect. Dis. 212: S258-S270.

Daddario-DiCaprio, K.M., Geisbert, T.W., Geisbert, J.B., Stroher, U., Hensley, L.E., Grolla, A., Fritz, E.A., Feldmann, F., Feldmann, H and Jones, S.M (2006). Cross-protection against

Marburg virus strains by using a live, attenuated recombinant vaccine. J. Virol. 80: 9659–9666.

Dag, A., Zhao, J and Stenzel, M.H (2015). Origami with ABC Triblock Terpolymers Based on Glycopolymers: Creation of virus-like morphologies ACS. Macro. Lett. 4: 579−583.

de Wit, E., Feldmann, H and Munster V.J (2011). Tackling Ebola: new insights into prophylactic and therapeutic intervention strategies. Genome Med. 3: 5.

Feldmann, H., Muhlberger, E., Randolf, A., Will, C., Kiley, M.P., Sanchez, A and Klenk, H.D (1992). Marburg virus a filovirus: messenger RNA, gene order, and regulatory elements of the replication cycle. Virus Res. 24:1-19.

Feldmann, H and Klenk, H.D (1996). Marburg and Ebola viruses. Adv. Virus Res. 47: 1-52.

Fritz, E.A., Geisbert, J.B., Geisbert, T.W., Hensley, L.E and Reed, D.S (2008). Cellular immune response to Marburg virus infection in cynomolgus macaques. Viral. Immunol. 21:355–363.

Gear, J.S., Cassel, G.A., Gear, A.J., Trappler, B., Clausen, L., Meyers, A.M., Kew, M.C., Bothwell, T.H., Sher, R., Miller, G.B., Schneider, J., Koornhof, H.J., Gomperts, E.D., Isaäcson, M and Gear, J. H (1975). Outbreak of Marburg virus disease in Johannesburg. Br. Med. J. 4: 489–493.

Gear, J. H (1989). "Clinical aspects of African viral hemorrhagic fevers". Rev. Infect. Dis. 11: S777–S782.

Geisbert, T.W and Jahrling, P.B (1995). Differentiation of filoviruses by electron microscopy. Virus Res. 39: 129–150.

Geisbert, T.W., Hensley, L.E., Larsen, T., Young, H.A., Reed, D.S., Geisbert, J.B., Scott, D.P., Kagan, E., Jahrling, P.B and Davis, K.J (2003). Pathogenesis of Ebola hemorrhagic fever in cynomolgus macaques: evidence that dendritic cells are early and sustained targets of infection. Am. J. Pathol. 163: 2347–2370.

Geisbert, T.W., Daddario-DiCaprio, K.M., Geisbert, J.B., Young, H.A., Formenty, P., Fritz, E.A., Larsen, T and Hensley, L.E (2007). Marburg virus Angola infection of rhesus macaques: pathogenesis and treatment with recombinant nematode anticoagulant protein c2. J. Infect. Dis. 196: S372–S381.

Geisbert, T.W., Daddario-DiCapriod, K.M., Geisberta, J.B., Reedf, D.S., Feldmann, F., Grollag, A., Ströherg,h, U., Fritz, E.A., Hensleye, L.E., Jonesg, S.M and Feldmann, H (2008). Vesicular stomatitis virus-based vaccines protect nonhuman primates against aerosol challenge with Ebola and Marburg viruses. Vaccine 26: 6894–6900.

Geisbert, T.W., Hensley, L.E., Geisbert, J.B., Leung, A., Johnson, J.C., Grolla, A and Feldmann, H (2010). Postexposure treatment of Marburg virus infection. Emerg. Infect. Dis. 16: 1119–1122.

Geisbert, T. W and Feldmann, H (2011). Recombinant vesicular stomatitis virus-based vaccines against Ebola and Marburg virus infections. J. Infect. Dis. 204: S1075-S1081.

Glaze, E.R., Roy, M.J., Dalrymple, L.W and Lanning, L.L (2015). A Comparison of the Pathogenesis of Marburg Virus Disease in Humans and Nonhuman Primates and Evaluation of the Suitability of These Animal Models for Predicting Clinical Efficacy under the 'Animal Rule'. Comp. Med. 65: 241-259.

Gonzalez, J.P., Nakoune, E., Slenczka, W., Vidal, P and Morvan, J.M (2000). Ebola and Marburg virus antibody prevalence in selected populations of the Central African Republic. Microbes Infect. 2:39–44.

Hensley, L.E., Alves, D.A., Geisbert, J.B., Fritz, E.A., Reed, C., Larsen, T., and Geisbert, T.W. (2011). Pathogenesis of Marburg hemorrhagic fever in cynomolgus macaques. J. Infect. Dis. 204: S1021–S1031.

Hotez, P.J., Fenwick, A., Savioli, L and Molyneux, D.H (2009). Rescuing the bottom billion through control of neglected tropical diseases. Lancet 373: 1570-1575.

Jeffs, B., Roddy, P., Weatherill, D., de la Rosa, O., Dorion, C., Iscla, M., Grovas,., Palma, P.P., Villa, L., Bernal, O., Rodriguez-Martinez, J., Barcelo, B., Pou, D and Borchert, M (2007). The Medecins Sans Frontieres intervention in the Marburg hemorrhagic fever epidemic, Uige, Angola, 2005. In Lessons learned in the hospital. J. Infect. Dis. 196: S154–S161.

Johnson, E.D., Gonzalez, J.P and Georges, A (1993a). Filovirus activity among selected ethnic groups inhabiting the tropical forest of equatorial Africa. Trans. R. Soc. Trop. Med. Hyg. 87: 536–538.

Johnson, E.D., Gonzalez, J.P and Georges, A (1993b). Haemorrhagic fever virus activity in equatorial Africa: distribution and prevalence of filovirus reactive antibody in the Central African Republic. Trans. R. Soc. Trop. Med. Hyg. 87: 530-535.

Johnson, E.D., Johnson, B.K., Silverstein, D., Tukei, P., Geisbert, T.W., Sanchez, A.N and Jahrling, P.B (1996). Characterization of a new Marburg virus isolated from a 1987 fatal case in Kenya. Arch. Virol. Suppl. 11: 101–114.

Kiley, M.P., Bowen, E.T., Eddy, G.A., Isaäcson, M., Johnson, K.M., McCormick, J.B., Murphy, F.A., Pattyn, S.R., Peters, D., Prozesky, O.W., Regnery, R.L., Simpson, D.I., Slenczka, W., Sureau, P., Van Der Groen, G., Webb, P. A and Wulff, H (1982). "Filoviridae: A taxonomic home for Marburg and Ebola viruses?" Intervirology 18: 24–32.

Kratz, T., Roddy, P., Tshomba Oloma, A., Jeffs, B., Pou Ciruelo, D., de la Rosa O and Borchert, M (2015). Ebola Virus Disease Outbreak in Isiro, Democratic Republic of the Congo, 2012: Signs and Symptoms, Management and Outcomes. PLoS One 10: e0129333.

Kuming, B.S and Kokoris, N (1977). Uveal involvement in Marburg virus disease. Brit. J. Ophthalmol. 61: 265–266.

Kurosaki, Y., Grolla, A., Fukuma, A., Feldmann, H and Yasuda, J (2010). Development and evaluation of a simple assay for Marburg virus detection using a reverse transcription-loop-mediated isothermal amplification method. J. Clin. Microbiol. 48: 2330–2336.

Licata, J.M., Johnson, R.F., Han, Z and Harty, R.N (2004). Contribution of ebola virus glycoprotein, nucleoprotein, and VP24 to budding of VP40 virus-like particles. J. Virol. 78: 7344-7351.

Ligon, B.L (2005). Outbreak of Marburg hemorrhagic fever in Angola: A review of the history of the disease and its biological aspects. Semin. Pediatr. Infect. Dis. 16: 219–224.

Maganga, G.D., Bourgarel, M., Ella, G.E., Drexler, J.F., Gonzalez, J P., Drosten, C and Leroy, E. M (2011). Is Marburg virus enzootic in Gabon? J. Infect. Dis. 204: S800–S803.

Mahendra, P., Pratibha, D and Raj, M (2014). Ebola hemorrhagic fever: An emerging highly contagious and fatal viral zoonosis. Ethiopian Int. J. Multidiscip. Res. 2: 1-2.

Martini, G.A (1973). Marburg virus disease. Postgrad. Med. J. 49: 542-546.

Masayuki, S., Masahiro, N., Tetsuro, I., Ichiro, K., Takeshi, K and Shigeru, M (2006). Laboratory Diagnostic Systems for Ebola and Marburg Hemorrhagic Fevers developed with Recombinant Proteins. Clin. Vaccine Immunol. 13: 444-451.

Mire, C. E., Geisbert, J.B., Agans, K.N., Satterfield, B.A., Versteeg, K.M., Fritz, E.A., Feldmann, H., Hensley, L.E and Geisbert, T.W (2014). Durability of a vesicular stomatitis virus-based marburg virus vaccine in nonhuman primates. PLoS One 9: e94355.

Monath, T.P (1999). Ecology of Marburg and Ebola viruses: speculations and directions for future research. J. Infect. Dis. 179: S127-S138.

Moyen, M., Thirion, L., Emmerich, P., Dzia-Lepfoundzou, A., Richet, H., Boehmann, Y., Dimi, Y., Gallian, P., Gould, E.A., Günther, S and de Lamballerie, X (2015). Risk factors associated with Ebola and Marburg viruses seroprevalence in blood donors in the Republic of Congo PLoS Negl. Trop. Dis. 9: e0003833.

Nakayama, E., Yokoyama, A., Miyamoto, H., Igarashi, M., Kishida, N., Matsuno, K., Marzi, A., Feldmann, H., Ito, K., Saijo, M and Takada, A (2010). Enzyme-linked immunosorbent assay for detection of filovirus species-specific antibodies. Clin. Vaccine Immunol. 17: 1723–1728.

Nakayama, E and Takada, A (2011). Ebola and Marburg viruses. JDR. 4: 381-382.

Peterson, A.T., Bauer, J.T and Mills J.N (2004). Ecologic and geographic distribution of filovirus disease. Emerg. Infect. Dis. 10: 40–47.

Peterson, A.T., Lash, R.R., Carroll, D.S and Johnson, K.M (2006). Geographic potential for outbreaks of Marburg hemorrhagic fever. Am. J. Trop. Med. Hyg. 75: 9–15.

Peterson, A.T and Holder, M.T (2012). Phylogenetic assessment of filoviruses: how many lineages of Marburg virus? Ecol. Evol. 2: 1826-1833.

Pigott, D.M., Golding, N., Mylne, A., Huang, Z., Weiss, D.J., Brady, O.J., Kraemer, M.U.G and Hay, S.I (2015). Mapping the zoonotic niche of Marburg virus disease. Trans. R. Soc. Trop. Med. Hyg. 109: 366–378.

Pourrut, X., Souris, M., Towner, J.S., Rollin, P.E., Nichol, S.T., Gonzalez, J.P and Leroy, E (2009). Large serological survey showing cocirculation of Ebola and Marburg viruses in Gabonese bat populations, and a high seroprevalence of both viruses in Rousettus aegyptiacus. BMC Infect. Dis. 9: 159-168.

Roberts, J.A and Andrews, K (2008). Nonhuman primate quarantine: its evolution and practice. ILAR J. 49: 145–156.

Roddy, P., Weatherill, D., Jeffs, B., Abaakouk, Z., Dorion, C., Rodriguez-Martinez, J., Palma, P.P., de la Rosa, O., Villa, L., Grovas, I and Borchert, M (2007). The Medecins Sans Frontieres intervention in the Marburg hemorrhagic fever epidemic, Uige, Angola, 2005. II. Lessons learned in the community. J. Infect. Dis. 196: S162–S167.

Regnery, R.L., Johnson, K.M and Kiley, M.P (1980). Virion nucleic acid of Ebola virus. J. Virol. 36: 465–469.

Reis, E.S., Barbuto, J.A., Kohl, J and Isaac, L (2008). Impaired dendritic cell differentiation and maturation in the absence of C3. Mol. Immunol. 45: 1952–1962.

Rotz, L.D., Khan, A.S., Lillibridge, S.R., Ostroff, S.M and Hughes, J.M (2002). Public health assessment of potential biological terrorism agents. Emerg. Infect. Dis. 8: 225–230.

Rougeron, V., Feldmann, H., Grard, G., Becker, S and Leroy, E.M (2015). Ebola and Marburg haemorrhagic fever. J. Clinic. Virol. 64: 111–119.

Schnittler, H.J and Feldmann, H (1998). Marburg and Ebola hemorrhagic fevers: does the primary course of infection depend on the accessibility of organ-specific macrophages? Clin. Infect. Dis. 27: 404–406.

Schnittler, H.J, and Feldmann, H (2003). Viral hemorrhagic fever--a vascular disease? Thromb. Haemost. 89: 967-972.

Smith, D.H., Isaacson, M., Johnson, K.M and Bagshawe, A (1982). Marburg virus disease in Kenya. Lancet 319: 816–820.

Swanepoel, R., Smit, S.B., Rollin, P.E., Formenty, P., Leman, P.A., Kemp, A., Burt, F.J., Grobbelaar, A.A., Croft, J., Bausch, D.G., Zeller, H., Leirs, H., Braack, E., Libande, M.L., Zaki, S., Nichol, S.T., Ksiazek, T.G and Paweska, J.T (2007). Studies of reservoir hosts for Marburg virus. Emerg. Infect. Dis. 13: 1847–1851.

Swenson, D. L., Wang, D., Luo, M., Warfield, K.L., Woraratanadharm, J., Holman, D.H., Dong, J.Y and Pratt, D.W (2008). Vaccine to confer to nonhuman primates complete protection against multi-strain Ebola and Marburg virus infections. Clin. Vaccine Immunol. 15: 460-467.

Timen, A., Koopmans, M.P.G., Vossen, A.C.T.M., van Doornum, G.J.J., Gu¨nther, S., van den Berkmortel, F., Verduin, K.M., Dittrich, S., Emmerich, P., Osterhaus, A.D.M.E, van Dissel, J.P and Coutinho, R.A (2009). Response to imported case of Marburg hemorrhagic fever, the Netherlands. Emerg. Infect. Dis. 15: 1171–1175.

Towner, J.S., Khristova, M.L., Sealy, T.K., Vincent, M.J., Erickson, B.R., Bawiec, D.A., Hartman, A.L., Comer, J.A., Zaki, S.R., Ströher, U., Gomes da S.F., del C.F., Rollin P.E., Ksiazek T.G and Nichol, S.T (2006). Marburgvirus genomics and association with a large hemorrhagic fever outbreak in Angola. J. Virol. 80: 6497–6516.

Towner, J.S. Pourrut, X. Albarino, C.G. Nkogue, C.N. Bird, B.H. Grard, G. Ksiazek, T.G. Gonzalez, J.P. Nichol, S.T and Leroy, E.M (2007). Marburg virus infection detected in a common African bat. PLoS One 2: e764.

Towner, J.S., Amman, B.R., Sealy, T.K., Carroll, S.A., Comer, J.A., Kemp, A., Swanepoel, R., Paddock, C.D., Balinandi, S., Khristova, M.L., Formenty, P.B, Albarino, C.G., Miller, D.M., Reed, Z.D., Kayiwa, J.T., Mills, J.N., Cannon, D.L., Greer, P.W., Byaruhanga, E., Farnon, E.C., Atimnedi, P., Okware, S., Katongole-Mbidde, E., Downing, R., Tappero, J.W., Zaki, S.R., Ksiazek, T.G., Nichol, S.T and Rollin, P.E (2009). Isolation of genetically diverse Marburg viruses from Egyptian fruit bats. PLoS Pathog. 5: e1000536.

Ursic-Bedoya, R., Mire, C.E., Robbins, M., Geisbert, J.B., Judge, A., MacLachlan, I and Geisbert, T.W (2014). Protection against lethal Marburg virus infection mediated by lipid encapsulated small interferon RNA. JID. 209: 562-570.

van Paassen, J., Bauer, M.P., Arbous, M.S., Visser, L.G., Schmidt-Chanasit, J., Schilling, S., Olschlager, S., Rieger, T., Emmerich, P., Schmetz, C., van de Berkmortel, F., van Hoek, B., van Burgel, N.D., Osterhaus, A.D., Vossen, A.C., Günther, S and van Dissel, J.T (2012). Acute liver failure, multiorgan failure, cerebral oedema, and activation of proangiogenic and antiangiogenic factors in a case of Marburg haemorrhagic fever. Lancet Infect. Dis. 12: 635–642.

Villinger, F., Rollin, P.E., Brar, S.S., Chikkala, N.F., Winter, J., Sundstrom, J.B., Zaki, S.R., Swanepoel, R., Ansari, A.A and Peters, C.J (1999). Markedly elevated levels of interferon (IFN)-α, IFN-γ, interleukin (IL)-2, IL-10, and tumor necrosis factor-α associated with fatal Ebola virus infection. J. Infect. Dis. 179: S188–S191.

Warfield, K.L., Swenson, D.L., Negley, D.L., Schmaljohn, A.L., Aman, M.J and Bavari, S (2004). Marburg virus-like particles protect guinea pigs from lethal Marburg virus infection. Vaccine 22: 3495–3502.

Warfield, K.L and Aman, M.J (2011). Advances in virus-like particle vaccines for filoviruses. J. Infect. Dis. 204: S1053–S1059.

Warren, T.K., Warfield, K.L., Wells, J., Swenson, D.L., Donner, K.S., Van Tongeren, S.A., Garza, N.L., Dong, L., Mourich, D.V., Crumley, S., Nichols D.K., Iversen, P.L Bavari, S (2010). Advanced antisense therapies for postexposure protection against lethal filovirus infections. Nat. Med. 16: 991–994.

Wikipedia, the free encyclopedia (2012). Isiro.

https://en.wikipedia.org/wiki/Isiro Accessed: 05 November 2015.

World atlas, Where is Isiro, The Democratic Republic Of The Congo?

http://www.worldatlas.com/af/cd/5/where-is-isiro.html/ last modified on October 19, 2015 Accessed: 12 November 2015.

World Health Organization (2005). Marburg haemorrhagic fever in Angola - update 3. http://www.who.int/csr/don/2005_03_29a/en/ Accessed: 08 November 2015.

World Health Organization (2012a). Marburg haemorrhagic fever. http://www.who.int/mediacentre/factsheets/fs_marburg/en/Accessed: 08 November 2015.

World Health Organization (2012b). Marburg haemorrhagic fever-update. http://www.who.int/csr/don/2012_11_23_update/en/ Accessed: 25 October 2015.

World Health Organisation (2014a). Ebola and Marburg virus disease epidemics: preparedness, alert, control, and evaluation. http://www.who.int/csr/disease/ebola/PACE_outbreaks_ebola_marburg_en/ Accessed: 11 November 2015.

World Health Organization. (2014b). Marburg virus disease-Uganda. http://www.who.int/csr/don/13-november-2014-marburg/en/ Accessed: 25 October 2015.

World Health Organization (2015). Ebola virus disease. http://www.who.int/mediacentre/factsheets/fs103/en/ Accessed: 05 November 2015.

Yasri, S and Wiwanitkit, V (2015). Spermatogenic transmission of Marburg and Ebola virus. APJR. 4: 83-84.

Zhang, R., Becnel, L., Li, M., Chen, C and Yao, Q (2006). C-reactive protein impairs human CD141 monocyte-derived dendritic cell differentiation, maturation and function. Eur. J. Immunol. 36: 2993–3006.