بسم الله الرحمن الرحيم

PREVALENCE OF DENGUE FEVER IN SHAMBAT AREA IN KHARTOUM STATE

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DEDICATION

To My Family

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ABSTRACT

Dengue Fever (DF) is an acute febrile illness caused by a mosquito-borne flavivirus, its most severe form is known as Dengue hemorrhagic fever (DHF) which could be fatal especially among children. DF is now a disease of worldwide interest and has become a major public health problem especially in developing countries.

This study aimed to identify the prevalence of Dengue fever in Khartoum State and to know the risk factors related to Dengue fever infection among 150 individuals using both interview method by using a structured questionnaire and experimental laboratory ELISA (sandwich ELISA) method to diagnose Dengue disease in blood serum samples being collected from respondents.

The results of this study revealed that Dengue fever is a serious disease which is highly prevalent among Sudanese citizens according to the results obtained by the ELISA diagnostic method used. The percentage of infected respondents with Dengue fever was 68% (102 out of 150 respondents) and the respondents who were not infected compromised 32% (48 out of 150 respondents). Dengue fever was most common among the age group ranging between 11-20 years old, as of the respondents (35 out of 150) in this age group range were infected. Males were mostly (74%) affected than the females. This study also stated direct relations between Dengue fever infection and growing trees, living nearby the river, open drainage system and animal raising.

The findings of this study are alarming and should alert nationwide concerned parties, within the health sector including the Ministry of Health and World Health Organization in Sudan to consider DF as a major public health problem in Sudan and accordingly take immediate necessary policy actions.

ملخص الأطروحة

تشخص حمي الضنك بإنها مرض يسبب الحمي الشـديدة وتسـببه أحـد أنـواع الفيروسات التي تسمي Flavivirus وتعرف أشد أنواعه بحمي الضنك النزفيـة (DHF) والتي قد تكون قاتلة خاصة لدي الأطفال وتعتبر حمي الضنك الآن من الأمـراض التـي استحوذت على اهتمام العالم وأصبحت مشكلة صحية عامة خصوصاً في البلدان النامية.

الهدف الاساسي من هذه الدراسة التعريف بمعدل الأصابة في ولايـة الخرطـوم (منطقة شمبات) وذلك لمعرفة عوامل وخطورة عوامل العدوي لحمي الضنك، وقد تمـت الدراسة بإختيار 150 شخصاً لأجراء الاستبيان والتحليل المختبري (Sandwich ELISA) لتشخيص المرض من خلال جمع عينات مصل الدم من الاشخاص الذين استجابو لاجراء الاستبيان.

أثبتت النتائج التي استخلصت من هذه الدراسة أن حمي الضنك مـرض خطيـر وينتشر بكثافة بين المواطنين وذلك استناداً للنتائج المتحصلة. بأستعمال طرق التشـخيص لدي ELISA كانت نسبة العدوي لدي الأشخاص الذين خضعوا للاسـتبيان هـي 68% بمعدل 102 شخص من مجموع 150 شخصاً ونسبة الاشخاص غير المصابين بالفيروس 32% بمعدل 48 شخص من مجموع 150 شخصاً.

وقد تبين أن حمي الضنك أكثر شيوعاً بين الفئات العمرية التي تتراوح مـا بـين 11-20 سنة وكان عدد الأشخاص من هذه الفئة يمثل 35 شخصـاً مـن مجمـوع 150 شخص قد اصيبوا وكذلك ترتفع تنسبة الاصابة للذكور 74% عن الإناث.

وتثبت هذه الدراسة الصلة المباشرة بين الإصابة بحمي الضنك والأشجار الناميـة والعيش قرب الانهار ونظام الصرف المفتوح وكذلك مناطق تربية الحيوانات.

تنذر مستخلصات هذه الدراسة بخطورة المرض، وعليه يجب أن يتم رفع شارات الخطر بواسطة كل الأطراف ذات الصلة بالحقل الصحي من خلال أجهزة القطاع الصحي متضمناً وزارة الصحة ومنظمة الصحة العالمية في السودان لاعتبار مرض حمي الضنك مشكلة صحية عامة بالسودان وبالتالي اتخاض الاجراءات اللازمة.

CHAPTER ONE
INTRODUCTION

1.2 Background

Dengue Hemorrhagic Fever (DHF) is caused by the Dengue viruses (DENVs), members of the flaviviridae family of small enveloped viruses. The flaviviruses carry a single-stranded RNA genome of relatively simple organization. The DENV complex encompasses four closely related serotypes: DENV-1, DENV-2, DENV-3 and DENV-4. All four DENV serotype are transmitted between humans in nature by mosquitoes of the genus *Aedes*, principally *Aedes aegypti*, which is highly domesticated and has a preference for biting humans. DHF represents the severe clinical manifestation of DENV infection, which occurs in no more than 3% of infected individuals. Among symptomatic cases, the majority of subjects experience uncomplicated Dengue fever (DF), an acute febrile illness typically lasting 3-7 days, accompanied by headache, myalgias, and, less often, a maculopapular rash. The headache and myalgias may be quite debilitating, which originated the name "break-bone fever" that was recorded prior to the 1900s. Fatigue may be prolonged for months after resolution of fever, but patients eventually recover without sequelae (Grobach *et al*., 1998).

1.2 Justification for the study

Many scientists nowadays around the world have reappearance of interest in striking diseases, with the aim of protecting the global population from risks of emerging infectious disease. The new promise of effort and resources has welcome implications for the

developing world, whose population faces the greatest part of health risks. Among the biological health risks considered most serious are the viral hemorrhagic fevers. Although this term usually brings to mind the deadly outbreaks of Ebola virus, in reality over 99% of the case of viral hemorrhagic fever reported worldwide are related instead to Dengue hemorrhagic fever (DHF). It has been estimated that over 50 million DENV infections occur globally each year. Most of these infections are clinically in apparent.

The Sudan being part of the developing world is suffering from several feverish diseases usually misdiagnosed as malaria, thus this study aims to dig for one of the fatal diseases with fever being a major symptoms, which is Dengue fever.

1.3 Objective of the study

1. To identify the prevalence of Dengue fever in Khartoum State.
2. To know the risk factors related to Dengue fever infection.

CHAPTER TWO
LITERATURE REVIEW

For several centuries, the world has been a witness to various lethal and debilitating diseases such as small pox, bubonic plaque, typhoid and tuberculosis. Many of these disease were once thought to be completely eradicated and under control. But with the recent re-emergence of multiple drug resistant tuberculosis and the emergence of other potentiality deadly disease like Lume's, Ebola and Dengue fevers, it seems that history is very likely repeating itself.

2.1 History

Dengue fever (DF) was first described in the late 1770s, appearing almost simultaneously in areas of Africa, Asia and North America. Dengue fever in its manifestation can develop into Dengue hemorrhagic fever (DHF), better known as break bone fever in countries where it is most prevalent. The first real epidemic of DHF occurred in 1950, in Southeast Asia (Walsh, 2002). Dengue fever has been estimated to affect approximately 50-100 million individuals a year, the incidence of its most severe form, DHF, has been shown to affect several hundred to be 56% of those who contract the virus.

In the past, the time between major epidemics has been 10-40 years. Since World War II and especially in the last 15 years, the pandemic has intensified. In 1975 DHF had become the leading course of hospitalization and death among children in many countries of Southeast Asia. Despite its prevalence in much of the world, a majority of American had never even heard of Dengue fever.

2.2 Virus classification

Dengue virus is a vector borne disease, a member of the genus Flavi virus and family Flaviviridae. The virus generally causes a mild febrile illness, DHF much more severe form of the disease, DHF or Dengue shock syndrome (DSS) occurs less frequently. There are four antigenic distinct serotypes of Dengue fever: DEN1, DEN2, DEN3 and DEN4. These serotypes vary in their degree of virulence.

The four stereotypes are almost indistinguishable in terms of the clinical and pathological symptoms caused by them, but they could be identified by neutralization tests utilizing monoclonal antibodies and polymerase chain reaction (Cardosa, 1998).

Dengue, a flaviviridae virus, has a 10.5 kb long viral genome consisting of a single stranded positive sense RNA which is organized into a single open reading frame (ORF) with the genes encoding structural proteins C, prM and E and non-structural proteins NSI, NS2A, NS2B, NS3, NS4A, NS4B and NSS (cordosa, 1998). The structural and non-structural proteins that are mentioned were identified by the proteolytic cleavage of a polymprotein encoded by the ORF. The structural proteins are encoded by 5 one-third of the ORF and the non-structural proteins are encoded in the remainder of the ORF (Lin *et al*., 1998).

Dengue virus attaches to the host cell surface via cellular receptors in order to infect a cell. The attachment is considered to be a major determinant of viral host-rang and tissue tropism, and diverse cell surface molecules are identified as virus receptors. Dengue viral envelope (E) protein (494 amino acid protein with two glycosylation sites) is the attachment protein for the virus. It (E protein) multiple

neutralization epitopes involved in the fusion of the virus membrane and in virus binding to cellular receptor molecules (Bielefeldt-ohmann, 1988). The identification of Dengue virus receptors on target cells has not been definitive. A cell surface protein on human monocytes, as well as some glycoproteins and glycol arninoglycoms, have been speculated to play a role as virus receptors in past studies. Bielefeldt-ohmann (1998) states that the Dengue virus binding entities on the cell surface membrane vary between cell types and so it is not clear how the Dengue virus-binding molecules are related to the Dengue virus binding entities. Also the virus binding proteins have differential affinity for the four Dengue virus serotypes in the order PEN2 > PEN3 > PEN1 > PEN4. At physiological pH, E forms dimmer on the surface of the virion, but on exposure to a pH below 6.5, a conformational shift occurs which leads to a rearrangement of E to form trimers, an event allowing the fusion between the virion and the host cell membrane (Crdosa, 1998). Being a positive sense RNA virus Dengue RNA itself is directly read by the host cells ribosomes, functioning like a normal mRNA present in the cell. It stimulates the host cell to replication, transcription and translation. In order to create new viral RNA, the viral RNA creates an "anti-sense" version (negative sense RNA of itself as a template). Newly formed viruses then leave the host cell by the budding process. Dengue virus has an affinity for monocyte macrophages, B lymphyocytes and bone marrow human cells, causing an abrupt on set of high fever, abdominal flush, vomiting, and headache and in extreme case extremely low platelet counts and circulatory collapse.

2.3 Mode of transmission

Infection with dengue viruses is transmitted through the bite of infective female *Aedes sp*. Mosquitoes. *Aedes aegypti*, the principle vector, is a small black and white, highly domesticated mosquito that prefers to lay its eggs in artificial water - containers commonly found in urban areas of the tropics. Containers found in and around the home, such as those used for water storage, flowers vases, old automobile tyres, buckets and other junk items that collect rainwater are examples. The adult mosquitoes are rarely noticed, preferring to rest indoors and to feed on human during daylights hours in an unobtrusive and often undefeated way (WHO, 2002). Mosquitoes, humans and lower primates like chimpanzees, gibbons and macaques are all considered to be the natural hosts for the dengue infections (Lin *et al*., 1998).

2.4 Epidemiology

Dengue fever and *Aedes aegypti* mosquitoes have a worldwide distribution in the tropical areas of the world, with over 2.5 billion people living in dengue endemic area (Rosen, 1982). The epidemiology of dengue viruses is change with the ecological distribution in Southeast Asia during and following World War II (Halstead, 1992). During the war, existing water systems were destroyed, and water storage equipment was moved between cities and countries, and large amounts of equipment were also left behind. These materials collected rainwater and made ideal larval habitats for *Aedes aegypti* resulting in the transport of mosquitoes and their eggs to new geographical areas.

The areas that are currently affected by such emerging diseases, such as Dengue fever, have tropical climates that breed the most deadly vector-born disease known to man. These tropical climates are optimal for the aggregation and proliferation of the vectors that carry these harmful diseases such as mosquitoes and rodents. The abundance of rain, lack of freezing temperatures that limit breeding seasons, and overcrowding of urban areas are prime conditions for rapid reproduction of disease carry vectors (Henig, 1997).

The first Dengue hemorrhagic fever epidemic ever recorded as such occurred in Manila, Philippines, in 1953-1954, although retrospective analysis suggests that outbreak had occurred earlier as well before the dengue etiology was known (Hammon, 1973). During the first 20 years that epidemic dengue hemorrhagic fever was known, it was localized in several Southeast Asian countries where it had become a major cause of hospitalization and death among children by the mid-1970s. Puring the dramatic geographical expansion of epidemic DHF in Asia, it moved west into India, Pakistan, Sri Lanka and Maldives, and east into the Republic of China. There was also a resurgence of the disease in Singapore which has continued through the 1990s (Lanciotti *et al*., 1994). Just the simple movement of people and goods from one place to another increase the risk of transporting a disease to a new area via vector of infected individual (Henig, 1997).

2.5 Clinical presentation

Dengue virus infection in humans of all four virus serotypes causes a spectrum of illness ranging from unapparent or mild febrile illness to severe and fatal hemorrhagic disease. Clinical presentation in both children and adults may vary in severity, depending on the

strain and serotypes of the infecting virus, and the immune status, age and the genetic background of the patient. In dengue endemic areas, acute dengue infections are often clinically nonspecific, especially in children, with signs and symptoms of a viral syndrome (Gubler, 1993).

Classical dengue fever is primarily a disease of older children and adults, characterized by a sudden onset of fever and one and more of a number of non-specific signs and symptoms such as frontal headache, retro-orbital pain, myolgices, nausea and vomiting, weakness and rash. Clinical laboratory findings associated with dengue fever include leucopenia, and in some patients, thrombocytopenia and elevated liver enzyme. Hemorrhagic manifestations may occur, the most common being skin haemorrhages. Dengue fever is generally self-limiting and rarely fatal, the acute illness lasting 3-7 days, convalescence, however, may be prolonged for weeks with weakness and depression. No permanent sequelae are known, and immunity for the infecting virus serotype is lifelong (Gubler, 1988).

Dengue hemorrhagic fever is primarily a disease of children under 15 years of age, although it may occur in older children and adults as well. Like dengue fever, it is characterized by a sudden onset of fever and non-specific signs and symptoms, and it is difficult to distinguish from dengue fever and other illnesses during the acute stage. The critical stage in DHF occurs at the time of defervescence when the patient develops a capillary – leak syndrome, with signs of circulatory failure and hemorrhagic manifestations, primarily skin hemorrhages.

Dengue hemorrhagic fever can be a dramatic disease with the patient's condition deteriorating very rapidly with the onset of shock and resulting in death if the plasma leakage is not detected and corrected with fluid replacement therapy. Some case develop much milder symptoms, which can, when no rash is present, be mis-diagnosed as a flu or other viral infection. Thus, travelers from tropical areas may inadvertently pass on dengue in their home countries, having not being properly diagnosed at the height of their illness. Patients with dengue can only pass on the infection through mosquitoes or blood products while they are still febrile. The classic dengue fever lasts about six to seven days, with a smaller peak of fever at the trailing end of the fever (the so-called "biphasic patter"). Clinically, the platelet count will drop until the patient's temperature is normal. Cases of DHF also show higher fever, haemorrhagic phenomena, thrombocytopenia and haemoconcentration. A small proportion of cases lead to dengue shock syndrome (DSS) which has a high mortality rate (Gubler, 1997).

2.6 Diagnosis

Exposure by residence or travel in dengue-endemic areas and knowledge about the occurrence of other case in the community are important clues to the diagnosis. Other infections that clinically may be confused with dengue include influenza, rubella, malaria, scrubtyphus, leptospirosis, and a variety of other arboviral infection. Rash is a helpful differential sign, but it may be difficult to discern in dark-skinned patients. Since the hemorrhagic fevers share symptoms with many other disease relies on evidence of the viruses in the blood-stream such as detection of antigens and antibodies or isolation of the

virus from the body. Disruptions in the normal levels of blood stream components may be helpful in determining some, but not all, hemorrhagic fevers. Direct detection of dengue viral antigen in human serum has been reported by use of countercurrent immuno-electrophoresis and by monoclonal RIA (radio immunoassay).

Rapid diagnosis has been achieved by immunocytochemical staining of peripheral blood mononuclear cells obtained during the acute phase of illness, and this method is more sensitive than virus isolation. RT-PCR has been applied to the rapid diagnosis of dengue infection, using serotype specific primers and analysis of amplified sequences by agarose gel electrophoresis or hybridization with serotype-specific digoxigenin-labeled probes (Miagostovich *et al*., 2001). This technique has permitted detection of viremia levels of less than 2 dex/ml. The 1gm antibody-capture ELISA is the forward assay for serologic diagnosis (Susana *et al*., 2003).

The ratio of IgM to IgG antibodies determined by ELISA is useful for distinguishing primary and secondary infections. In recent years, several commercial kits have been designed to make dengue diagnosis simpler and more efficient. A good assay will allow dengue diagnosis without the need for a large, well developed laboratory and highly skilled personnel, resources of a premium in dengue endemic areas. The clinical usefulness of one of these tests, a capture ELISA specific for IgM and IgG antibodies indistinguishing between primary and secondary infection, was demonstrated. This distinction is important for the epidemiology of dengue hemorrhagic fever.

2.7 Treatment and prevention

Dengue virus infection poses a dangerous health threat to its patients; therefore, treatment and prevention are important facts of this virus. Presently, supportive treatment plan to follow is to take individual preventative measures. Since no specific treatment exists for dengue fever, symptomatic or supportive measures are used to treat the patients (Trofa *et al*., 1997). For patients with dengue hemorrhagic fever (HDF) or dengue shock syndrome (DSS) these can include "measures to correct hypovolemia hypoxia, shock, using non-saylicylate, antipyretics, oxygen, electrolyte, crystalloid and/or colloid fluid replacement. Incase with savers shock, plasma or plasma expanders may be required. Doctors use red blood cells transfusions, platelet transfusions, and plasma to treat savers bleeding and decreasing hematocrit values. Throughout the treatment process, mediations containing acetylasalicylic acid should not be used because of the bleeding risk (Redonda *et al*., 1997). These medical techniques only serve to combat certain symptoms of the virus; they do not combat the dengue virus, itself. Since this current treatment plan simply involves treating the symptoms, the optimal treatment of choice is prevention. Preventing the disease from ever occurring is the best way to combat dengue fever. Presently, the only technique for controlling the virus is to control its vector, *Aedes aegypti*, which breeds mainly in man-made containers such as bottles, cans, and tires. People in infected areas are directed to take specific measures to present individual contact with the vector. Although currently these simple preventive measures and symptomatic treatments are all we have to fight dengue fever, researchers are finding hopeful

information that will eventually lead to a vaccine or a cure (Yadav *et al*., 1991).

In principle, an effective vaccine against DENV is highly feasible. Viral replication is effective controlled after a short (3 to 7 day) period of viremia, and individuals who have recovered from DENV infection are immune to re-challenge. There are a number of candidate DENV vaccines currently in development. Live attenuated strains of all four DENV serotypes have been derived by the traditional approach of serial propagation in primary dog or monkey kidney cells. This effort has been slowed by the lack of an animal model or *in vitro* markers of attenuation in humans. Improved molecular virology techniques and an improved understanding of the genomic structure of DENV have permitted a more rapid approach based on engineering of attenuating mutations into infections cDNA clones of eachof the four DENV serotypes, with the added theoretical advantage of a lower possibility of reversion to virulence. Another molecular approach being utilized is the creation of four separate infectious chimeric flaviviruses, each of which contains the pre-M and E genes of one of the four DENV serotypes in a single "backbone" (Rothman, 2004).

2.8 Dengue fever in Sudan

Febrile illness in Sudan constitutes a major health problem. The first recorded dengue fever outbreak in Sudan was reported in the mid-1980s in Red Sea State, its case fatality rate was 25 percent (Hyams *et al*., 1986). In November 2004, the World Health Organization reported a recent outbreak of dengue fever responsible for more than 70 deaths in central Sudan. At least 75 people have died

and more than 300 have been infected by a dangerous strain of dengue fever in Kordofan, central Sudan (Noel King, 2005, NEWS VOA. COM from google.com). The epidemic has hit 12 communities in the eastern region of southern Kordorfan, including Habila, Dilling, Kurtala, Julud, Abu Gubeiha, Umbrembita and Alrigul towns. Cases of dengue fever also had been reported in Kassala and Blue Nile States to the east (Anon, 2006).

2.9 Dengue fever and ELISA test

Rapid diagnosis of dengue infection is essential to patient management and disease control. Studies showed that the development of a rapid commercial capture enzyme linked immunosorbent assay (ELISA) for anti-dengue IgM and IgG antibodies lead to more rapid and accurate testing in peripheral health settings and diagnostic laboratories (Lam *et al*., 1998).

Enzyme-linked immunosorbent assay (ELISA) is a useful and powerful method in estimating ng/ml to pg/ml ordered materials in the solution, such as serum, urine and culture supernatant. The enzyme-linked immunosorbent assay or ELISA is a biochemical technique used mainly in immunology to detect the presence of an antibody or an antigen in a sample. It uses two antibodies. One antibody is specific to the antigen. The other reacts to antigen-antibody complexes, and is coupled to an enzyme. This second antibody, which accounts for "enzyme-linked" in the test's name, can also cause a chromogenic or fluorogenic substrate to produce a signal. Because the ELISA can be performed to evaluate either the presence of antigen or the presence of antibody concentration (such as with the human immunodeficiency

virus, HIV test or west Nile virus) and also for detecting the presence of antigen (Vaughn *et al*., 1999).

The enzyme acts as an amplifier; even if only few enzyme-linked antibodies remain bound,the enzyme molecules will produce many signal molecules. ELISA may be run in a qualitative or quantitative format. Qualitative results provide a simple positive or negative result for a sample. The cut-off between positive and negative is determined by the analyst and may be statistical. Two or three times the standard deviation is often sued to distinguish positive and negative samples. In quantitative ELISA, the optical density or fluorescent units of the sample is interpolated into a standard curve, which is typically a serial dilution of the target (Vaughn, 1997).

2.10 Sandwich ELISA

Sandwich ELISA is used to determine the antigen concentration in unknown samples. The Sandwich ELISA measures the amount of antigen between two layers of antibodies. The antigens to be measured must contain at least two sites, capable of binding to antibody, since at least two antibodies act in the sandwich. So sandwich assays are restricted to the quantitation of multivalent antigens such as proteins or polysaccharides. Sandwich ELISAs for quantitation of antigens are especially valuable when the concentration of antigens is low and/or they are contained in high concentrations of contaminating protein.

CHAPTER THREE
MATERIALS AND METHODS

3.1 Study area

This study was carried out in Shambat area of Khartoum North in Khartoum State. The area represented different ethnic and racial groups. Khartoum State is the capital of the Sudan. Its area is 20140 Km^2. According to the year 1993 census, the population of Khartoum State had grown from 1.801.850 in 1983 to 3.413.034 in 1993, now it is 5.548.784 with an average annual growth rate of 6.29 (3.67%). Forty four percent (44%) of Khartoum populates are lifetime internal migrants. A study conducted by the National Population Council (2001) showed that approximately 1.000 people enters and settles in the state daily.

Sex ratio in Khartoum State is 410 males per 1000 population. (male : female ratio is 1:1). The working age ranged between 15-59 years which accounts for 57.9% of the total population.

Economic characteristics: 42.4% of the population is economically active. The unemployment rate in the state is 13.5%. Literacy rate for person's 6 years and over is 72% for both sexes.

Age structure: 0-14 years: 44.62% (male 8.227.011; female 7.870.783) 15-64 years: 53.92% (male 9.619.218; female 9.608.469) 65 years and over: 2.09% (male 425.898; female 328.994) (2001 est).

Ethnic groups: blacks 52%,. Arabs 39%, Beja 6%, foreigners 2%, others 1% (NPC Annual Rport, 2001).

Shambat area was selected because it is near to the River Nile and surrounded with trees and farms that has animals such as chickens and cows. Shambat area consists of different socioeconomic classes.

3.2 Study design

This study is a cross-sectional study, in order to evaluate the prevalence of dengue virus in Shambat area.

3.3 Sampling

The sampling used was simple randomized sampling. A criterion used was patients who suffer from fever and visits clinic's laboratories for blood film tests for malaria diagnosis.

3.4 Sample size

One hundred and fifty interviews were made with blood samples being taken from patients coming to the clinics for malaria test.

3.5 Sources of data

Primary data was collected using a questionnaire and laboratory diagnostic method (ELISA).

Secondary data was collected from books, journals, reports, previous studies and internet websites.

3.6 Data collection

Data was collected through interviews using a structured questionnaire. The questionnaire included questions about the socio-economic data of the respondents, habits and lifestyle.

3.6.1 Laboratory tests

One hundred and fifty serum samples (5ml) were collected for diagnosis of dengue virus using ELISA kits, which is manufactured by Human Gesellsschaft, Germany.

3.6.2 Principle of ELISA test

The dengue virus antibody (IgG) diagnosis using ELISA method is based on the sandwich ELISA technique. The micro titer strip wells as a solid phase are coated with dengue-antigens (DEN-Ag). In the first incubation step corresponding specific antibodies (DEN - IgG-Ab) present in patient specimens or control bind to the antigens at the solid phase. At the end of the incubation unbound components are washed out.

For the second incubation step anti-IgG conjugate (anti-human IgG antibodies, peroxidase conjugate) is added which binds specifically to IgG class antibodies resulting in the formation of typical immuno-complexes. After a second washing step to remove excess conjugate. TMB/substrate is added (step 3). A blue color develops changing to yellow after stopping the reaction. the intensity of the colors is directly proportional to the DEN-IgG-Ab concentration in the specimen. The absorbance of calibrators and specimen is determined by using on ELISA micro plate reader (HUMAREADER). Results for patient samples are obtained by comparison with a cut-off value or by expression in unit (u/ml), (www. human.de/data/gb/vr/el-deng.p).

Calculation of control values and cut-off mean absorbance values of wells B1 and C1 (MNC) are calculated according to the following formula:

$$MNC = A450(B1) + A450\ (C1)2$$

$$Cut\text{-}off\ value\ (COV) = MNC + 0.35$$

(information taken from dengue IgG kit leaflet manufactured by Human Gesellsschaft, Germany.

3.7 Data analysis

Questionnaires were labeled and after data collection it was finally analyzed with the Statistical Package of Social Science (SPSS) for windows version 10 and was presented in simple tables of frequencies and percentages.

CHAPTER FOUR
RESULTS

4.1 Sex and age structure of the respondents

The majority (60%) of the respondents were males and the minority 40% were females (Table 1). As shown in Table (2), one third (33%) of the respondents aged more than 30 years, and another one third (33%) aged within the age range 21-30 years old, 23% aged between the age range 11-20 years and only 10% were children whose age is less than 10 years old.

4.2 Distribution of respondents according to residence near Nile

Slightly above half (53%) of the respondents lie near the river while 47% do not live near the river (Table 3).

4.3 Percentage of respondents residence nearby the river

Over a half (53%) of the respondents living nearby the river were males and 47% were females.

4.4 Respondents habit of growing trees in their house yards

The majority (67%) grew trees in their house yards while 33% do not grow trees in their house yards (Table 4).

4.5 Water sources in the respondents houses

The majority (68%) have indoor tap and 32% use the common village tap (Table 5).

4.6 Respondents habit of raising animals

The majority (83%) of the respondents said they do raise animals in their homes, while the minority (17%) said they don't (Table 6).

4.7 Types of animals raised by the respondents who answered yes in question 5 (total is 125)

The majority of the respondents raised chicken (33%), those who raised dogs compromised (20%), (13%) raised cats, (10%) raised goats, (17%) raised cows and only 7%) raised sheep (Table 7).

4.8 Respondents habit of raising domestic animals

Two thirds (67%) of the respondents do not raise domestic animals while one third (33%) raise domestic animals (Table 8).

4.9 Respondents having rats at home

The majority (93%) of the respondents said they have rats in their homes and the minority (7%) said they don't have rats at home (Table 9).

4.10 Respondents sleeping area in their homes

Sixty (60%) of the respondents said that they sleep outdoors while (40%) said that they sleep indoors.

4.11 Respondents usage of mosquito bed nets while sleeping

Two thirds of the respondents (67%) don't use mosquito nets and one third (33%) use mosquito bed nets while sleeping (Table 11).

4.12 Respondents having wired screens for windows and doors

Two thirds (67%) of the respondents said they had wired screens in their doors and windows and one third (33%) don't have wired screens in their doors and windows (Table 12).

4.13 Respondents having insects at home

All respondents (100%) said they had insects in their homes (Table 13).

4.14 Types of insects in respondents home

More than a third (40%) of the respondents said they had mosquitoes in their homes, (27%) said they had flies, (7%) said they had ticks, the rest of the respondents had more than one answer as (20%) said they had mosquito, flies and ticks and (7%) said they had both mosquitoes and ticks (Table 14).

4.15 Respondents usage of insecticides

The majority (70%) of the respondents use insecticides and the minority (30%) don't use insecticides (Table 15).

4.16 Respondents living near a water pool

More than a half (56%) of the respondents lived near water pools and minority (44%) don't live near water pools (Table 16).

4.17 Respondents having an open drainage system

The majority (72%) of the respondents have an open drainage system while the minority (28%) don't have an open drainage system (Table 17).

4.18 Respondents health complains

Slightly above a third (34%) of the respondents had joint pain, one third (33%) had fever and fatigability, (17%) said they have fever, another (17%) had fatigability (Table 18).

4.19 Types of medications used by the respondents

Around two thirds (63%) of the respondents use analgesics, (17%) uses antipyretics, (13%) uses antibiotics, and (7%) uses both analgesics and antibiotics (Table 19).

4.20 Number of respondents with dengue fever as diagnosed with ELISA test

Those respondents who were dengue fever positive as diagnosed with the ELISA test compromised (68%) and those who were negative were slightly below one third (32%) (Table 20).

4.21 Dengue fever cross tabbed with the age of the respondents

Table (21) illustrates that the most affected age group with dengue fever are the respondents whose age ranges between 11-20 yers old, as all respondents (100%) were positive to the ELISA test. Those whom their age ranged between 21-30 years and more than 30 years old comes in the second rank as 64% and 60% of them were affected, respectively. The least affected group were the children aged less than 10 years old, as only one third of them were affected with dengue fever.

4.22 Dengue fever infection verses the sex of the respondents

The most (74%) affected sex group with dengue fever was the males and the females who were affected compromised (60%) (Table 22).

4.23 The relation between dengue fever cases and the respondents usage of mosquito bed nets

There wasn't an obvious relation between the risk of infection and the respondents usage for bed nets. As, positive cases who used bed nets were 80% and those who didn't use bed nets where 62%, while the negative cases who use a bed net were (20%), those who don't use a bed net were 38% (Table 23).

4.24 The relation between dengue fever cases and the respondents sleeping place

The percentage of infected respondents who slept indoors was higher than the percentage who slept outdoors. As, the positive cases who slept indoors were (77%) and those who slept outdoors were (62%). While the negative cases who slept indoors were (23%), those who slept outdoors were (38%) (Table 24).

4.25 The relation between dengue fever cases and the respondents growth trees habit

The percentage of infected respondents who grew trees was higher than the percentage who don't grow trees. As, the positive cases who grew trees were (72%) and those who didn't grew trees were (59%). While the negative cases who grew trees were (28%), those who didn't grew trees were (41%) (Table 25).

4.26 The relation between dengue fever cases and the respondents residence nearby the river

The percentage of infected respondents who lived nearby the river was higher than the percentage who didn't live nearby the river. As, the positive cases who lived nearby the river were (74%) and those who didn't live nearby the river were (61%). The negative cases who lived nearby the river were (26%) and those who didn't live nearby the river were (39%) (Table 26).

4.27 The relation between dengue fever cases and the respondents access to an open drainage system

The percentage of infected respondents who had an open drainage system was higher than the percentage who didn't had an open drainage system. As, the positive cases who had an open drainage system were (70%) and those who didn't have an open

drainage system were (62%). The negative cases who had an open drainage system were (30%) and those who don't have an open drainage system were (38%) (Table 27).

4.28 The relation between dengue fever cases and the types of animals raised by the respondents

Respondents with positive cases who raised chickens accounted for (80%), who raised dogs were (65%), who raised cats were also (65%), who raised goats (53%) raised sheep (60%) and those who raised cows were (35%) raised dogs, those who raised cats were (47%), those who raised sheep were (40%) and (36%) raised cows.

Table (1): Distribution of sex of respondents investigated on dengue fever in Shambat area

	Frequency (n)	Percentage (%)
Male	15	10
Female	35	23
Total	50	33

Table (2): Age groups of respondents investigated on dengue fever in Shambat area

	Frequency (n)	Percentage (%)
<10 years old	15	10
11-20 years old	35	23
21-30 years old	50	33
>30 years old	50	33
Total	150	100

Table (3): Percentage of respondents investigated on dengue fever in Shambat area, residence nearby the river

	Frequency (n)	Percentage (%)
Male	80	53
Female	70	47
Total	150	100

Table (4): Habit of growing trees in house yard among the respondents investigated on dengue fever in Shambat area

	Frequency (n)	Percentage (%)
Yes	101	67
No	49	33
Total	150	100

Table (5): Water resource in the houses of respondents investigated on dengue fever in Shamba area

	Frequency (n)	Percentage (%)
In door tap	102	68
Common village	48	32
Total	150	100

Table (6): Raising animals habit among the respondents investigated on dengue fever in Shambat area, residence nearby the river

	Frequency (n)	Percentage (%)
Yes	125	83
No	25	17
Total	150	100

Table (7): Type of animals raised in question 5 by the respondents investigated on dengue fever in Shambat area

	Frequency (n)	Percentage (%)
Chicken	49	33
Dogs	31	21
Cats	20	13
Goats	1	10
Sheep	10	7
Cows	25	17
Total	136	100

Table (8): Habit of raising domestic animal among respondents investigated on dengue fever in Shambat area

	Frequency (n)	Percentage (%)
Yes	50	33
No	100	67
Total	150	100

Table (9): Respondents having rats at home investigated on dengue fever in Shambat area

	Frequency (n)	Percentage (%)
Yes	140	93
No	10	7
Total	150	100

Table (10): Sleeping area of respondents investigated on dengue fever in Shambat area

	Frequency (n)	Percentage (%)
Indoor	60	40
Outdoor	90	60
Total	150	100

Table (11): Usage of mosquito bed nets among of respondents investigated on dengue fever in Shambat area

	Frequency (n)	Percentage (%)
Yes	50	33
No	100	67
Total	150	100

Table (12): Respondents having wired screens for windows and doors on dengue fever in Shambat area

	Frequency (n)	Percentage (%)
Yes	101	67
No	49	33
Total	150	100

Table (13): Respondents having insects at home investigated on dengue fever in Shambat area

	Frequency (n)	Percentage (%)
Yes	150	100
No	0	0
Total	150	100

Table (14): Type of insects in respondents homes investigated on dengue fever in Shambat area

	Frequency (n)	Percentage (%)
Mosquito	60	40
Flies	40	27
Mosquito + flies + ticks	50	33
Total	150	100

Table (15): Respondents usage of insecticides in investigated on dengue fever in Shambat area

	Frequency (n)	Percentage (%)
Yes	105	70
No	45	30
Total	150	100

Table (16): Respondents living near a water pool investigated on dengue fever in Shambat area

	Frequency (n)	Percentage (%)
Yes	85	56
No	65	44
Total	150	100

Table (17): Respondents having open drainage system investigated on dengue fever in Shambat area

	Frequency (n)	Percentage (%)
Yes	108	72
No	42	28
Total	150	100

Table (18): Respondents health complains investigated on dengue fever in Shambat area

	Frequency (n)	Percentage (%)
Fever	25	17
Fatigability	25	17
Fever + fatigability	49	33
Joint point	51	34
Total	150	100

Table (19): Types of medications used by the respondents investigated on dengue fever in Shambat area

	Frequency (n)	Percentage (%)
Analgesics	95	62
Antibiotics	20	13
Analgesics + antibiotics	10	7
Antipyretic	25	17
Total	150	100

Table (20): Number of respondents with dengue fever as diagnosed with ELISA test investigated on dengue fever in Shambat area

	Frequency (n)	Percentage (%)
Yes	50	33
No	100	67
Total	150	100

Table (21): Dengue fever cross tabbed with the age of the respondents investigated on dengue fever in Shambat area, residence nearby the river

	Age (years)									
	<10		11-20		31-30		>30		Total	
	(n)	%	(n)	%	(n)	%	(n)	%	(n)	%
-ve	10	67	0	0	18	36	20	40	48	32
+ve	5	33	35	100	32	64	30	60	2	68
Total	15	100	35	100	50	100	50	100	50	100

Table (22): Dengue fever infection verses the sex of the respondents investigated on dengue fever in Shambat area

	Sex					
	Male		Female		Total	
	(n)	%	(n)	%		%
-ve	24	26	24	40	48	3
+ve	66	74	36	60	102	68
Total	90	100	30	100	150	71

Table (23): Rotation between dengue fever case and the respondents usage of mosquito bed nets investigated on dengue fever in Shambat area

	Yes		No		Total	
	(n)	%	(n)	%		%
-ve	10	20	38	38	48	32
+ve	40	80	2	62	102	68
Total	50	100	40	100	150	100

Table (24): Relation between dengue fever cases and respondents sleeping place investigated on dengue fever in Shambat area

	Indoor		Outdoor		Total	
	(n)	%	(n)	%		%
-ve	14	23	34	38	48	32
+ve	46	77	56	62	102	68
Total	60	100	90	100	150	100

Table (25): Relation between dengue fever case and the respondents growing trees habit investigated on dengue fever in Shambat area

	Yes		No		Total	
	(n)	%	(n)	%		%
-ve	28	28	20	41	48	32
+ve	73	72	29	59	102	68
Total	101	100	49	100	150	100

Table (26): Relation between dengue fever case and the respondents residence nearby the river investigated on dengue fever in Shambat area

	Yes		No		Total	
	(n)	%	(n)	%		%
-ve	21	26	27	39	48	32
+ve	59	74	43	61	102	68
Total	80	100	70	100	150	100

Table (27): Relation between dengue fever case and the respondents access to an open drainage system investigated on dengue fever in Shambat area

	Yes		No		Total	
	(n)	%	(n)	%		%
-ve	32	30	16	38	48	32
+ve	76	70	26	62	102	68
Total	108	100	42	100	150	100

Table (28): Dengue fever case and the types of animals raised by the respondents

	D		Ct		G		S		Cw		Total	
	(n)	%	(n)	%	(n)	%	(n)	%	(n)	%	(n)	%
-ve	11	35	7	35	7	47	4	40	9	36	48	32
+ve	20	65	13	65	8	53	6	60	16	64	102	68
Total	31	100	20	100	15	100	10	100	25	100	150	100

Key: D: Dogs, Ct: Cat, G: Goat, S: Sheep, Cw: Cow

CHAPTER FIVE
DISCUSSION

In this study, 150 individuals were interviewed and sampled for dengue fever. Collected serum samples were tested by ELISA. The sample socio-demographic characteristics were as follows: out of the 150 respondents the majority (60%) of the respondents were males and (40%) were females. One third (33%) of the respondents aged more than 30 years, and another one third (33%) aged within the age range 21-30 years old, (23%) aged between the age range 11-20 years and only (10%) where children whose age was less than 10 years old. Slightly above half (53%) of the respondents live near the river while (47%) live away from the river. The ELIA results showed that out of 150 samples 102 were positive while 48 were negative.

It was observed from the findings of this study that human activities are the major factor that leads to emergence and spread of dengue fever. Thee activities are growing trees, raising domestic animals and sleeping outdoors nearby mosquito breeding sites. The majority of the respondents (67%) grew trees in their house yards while (33%) do not grow trees in their house yards. The majority (68%) have indoor tap and (32%) use the common village map. The majority (83%) of the respondents said that they do raise animals in their homes, while the minority (17%) said they don't.

Two third (67%) of the respondents do not raise domestic animals while one third (33%) do raise domestic animals. The majority of the respondents raised chicken (33%) those who raised dogs compromised 20%, 13% raised cats, 10% raised goats, 17%

raised cows and only 7% raised sheep. The majority (93%) of the respondents said they have rats in their homes and the minority (7%) said they don't have rats at home.

Sixty percent of the respondents said that they sleep outdoors while 40% said that they sleep indoors. Two thirds of the respondents (67%) don't use mosquito bed nets and one third (33%) use mosquito bed nets while sleeping. Two third (67%) of the respondents had wired screens in their doors and windows and one third (33%) don't have wired screens in their doors and windows. Sleeping outdoors without bed nets increases the interaction between mosquitoes and humans, and thus increase the dispersal of viruses in both mosquito and human hosts which definitely contributes (and continue to contribute) to the emergence of disease that are transmitted by mosquitoes including DHF. In addition to sleeping outdoors without using bed nets, the close contact between susceptible human hosts and close nearness to mosquitoes also facilitates mosquito transmission which in turn increase the number of infective bites per mosquito.

All respondents (100%) had insects in their homes. 40% of the respondents had mosquitoes in their homes, 27% said they had flies, 7% had ticks, 20% had mosquito, flies and ticks, and 7% had both mosquitoes and ticks. The majority (70%) of the respondents uses insecticides and 30% don't use insecticides.

More than a half (56%) of the respondents lived near water pools and 44% don't live near water pools. The majority (72%) of the respondents have an open drainage system while the minority (28%) doesn't have an open drainage system.

When the respondents were asked about any health complains, 34% of the respondents had joint pain, one third (33%) had fever and fatigability 17% have fever; another (17%) had fatigability. Around two thirds (63%) of the respondents use analgesics, 17% uses antipyretics, 13% uses antibiotics, and 7% uses both analgesics and antibiotics.

The percentage of respondents with dengue fever diagnosed using ELISA tests compromised 68% and those who were negative where slightly below one third (32%).

The most affected age group with dengue fever were the respondents whose age ranged between 11-20 years old, as all respondents within this group (35 from a total of 150), (100%) were positive to the ELISA test. This could be due to the fact that this age group of late childhood and adolescence is the most active group and with their activity they come in close contact with mosquito breeding sites indifferent places around their living places, schools and playing yards. Those whom their age ranged between 21-30 years and more than 30 years old comes in the second rank as 64% and 60% of them were affected, respectively. The least affected group were the children aged less than 10 years old, as only one third of them were affected with dengue fever. The WHO has stated that dengue fever is more common in people less than 15 years of age and in people having their second infection (WHO, 1994). In another article it was stated that dengue hemorrhagic fever is primarily a disease of children under 15 years of age, although it may occur in older children and adults as well (Gubler, 1997).

The most (74%) affected sex group with dengue fever was males and the females who were affected compromised (60%). This is may be due to that male are more exposed to the breeding areas of the mosquito because of their work in farms and near the river. Your risk of acquiring a disease depends on several factors including your age, gender, immunization status and current status of health and animal contact, fresh water as well as the local disease situation (WHO, 2002).

There wasn't an obvious relation between the risk of infection and the respondent's usage for bed nets. As positive cases who used bed nets were 80% and those who didn't use bed nets wee 62%. While the negative cases who use a bed net were 20% and those who don't use a bed net were 38%. This is because the *Aedes aegypti* mosquito bits the host at day time while people are not sleeping and during work time.

The positive cases who slept indoors were 77% and those who slept outdoors were 62%. While the negative case who slept indoors wee 23%, those who preferring to rest indoors and to feed on humans during day light hours in an unobtrusive and often undetected way (Gubler, 1997).

There was a relation between growing trees and infection; this could be due to the fact that trees are a gathering place for mosquitoes. As the percentage of infected respondents who grew trees was higher than the percentage who don't grow trees. The positive case who grew trees were 72% and those who didn't grow trees were 59%. While the negative case who grew trees were 28%, those who didn't grew trees were 41%.

There was a relation between living by the river and infection as the percentage of infected respondents who lived nearby the river was higher than the percentage that didn't live nearby the river. The positive case who lived nearby the river were 74% and those who didn't live nearby the river were 61%, while the negative case who lived nearby the river were 26%, those who didn't live nearby the river were 39%. This is mainly due to the humid moist environment near by the river area which is most favoured by many insects including mosquitoes.

The percentage of infected respondents who had an open drainage system was higher than the percentage that didn't have an open drainage system. As, the positive case who had an open drainage system were 30%, those who don't have an open drainage system were 38%. This emphasizes the findings of the previous system were 38%. This emphasizes the findings of the previous studies that inadequate sanitation and potable water supply, results in an increase in mosquito breeding places.

Respondents tested positive case who raised chicken were 80%, who raised dogs 65% who raised cats 65%, who raised goats 53% who raised sheep 60% and who raised cows were 64%, while the respondents with negative case who raised chickens were 20%, who raised dogs 35%, who raised cats 35%, who raised goats 47%, who raised sheep 40% and who raised cows wee 36%. Raising animals enhances mosquito breeding, as its wastes and food remains is a perfect mosquito as well as other insects breeding sites which could be vectors for many other vector borne diseases.

The environment, however, is not the only factor that influences the emergence of diseases in the developing regions of the world. Socioeconomic factors also play a large role (Henig, 1997). In most developing countries, people simply do not have access to disinfectants, medications, diagnostic supplies and trained personnel necessary for even moderate health care. This denied access to better health care is most often due to shortage of transportation, lack of knowledge of where to find health care, or a shortage of sufficient programs that help to supply health care.

CHAPTER SIX
CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The results of the ELISA diagnostic method used in this study to test for dengue fever antibodies revealed the following: the percentage of infected respondents with dengue fever is 68%, (102 from150) respondents) and the respondents who were not infected compromised (32%, 48 from 150 respondents). This percentage is alarming and should alert all concerned parties within the health sector including the Ministry of Health and World Health Organization in Sudan to take immediate necessary actions.

The most affected age group with dengue fever were the age group rangingbetween11-20 years old, all of the respondents (35%) in this age group range were infected, and the most (74%) affected sex group with dengue fever was the males compared to females.

This study also stated direct relation between dengue fever infection and growing trees, living nearby the river, open drainage system and animal raising.

6.2 Recommendations

1. Dengue fever surveillance/screening program should be done nationwide.

2. Dengue fever health education campaign should be formulated and implemented to aware the people with the disease, its mode of transmission, diagnosis and misdiagnosis as well as treatment and prevention.

3. Early diagnosis and treatment of infected individuals should immediately be undertaken.

4. Rapid and accurate diagnostic tests should be made available in all laboratories in Sudan.

5. Bad human activities and habits that may increase the susceptibility of the disease should be avoid.

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APPENDIX
Questionnaire

1. Sex:

Male ☐ Female ☐

2. Age:

<10 years old ☐ 11-20 years old ☐

21-30 years old ☐ >30 years old ☐

3. Do you live nearby the river:

Yes ☐ No ☐

4. Do you grow trees in your house yard?

Yes ☐ No ☐

5. What type of water source do you have in your house?

Common village tap water ☐ Indoor tap ☐

6. Do you raise animals? ☐

Yes ☐ No ☐

7. If yes, what animals do you raise?

Chicken ☐ Dog ☐

Goat ☐ Cows ☐ Sheep ☐

8. Do you raise domestic animals ?

Yes ☐ No ☐

9. Do you have rats at home?

Yes ☐ No ☐

10. Where do you sleep at night?

Indoor ☐ Outdoor ☐

11. Do you use mosquito bed nets while sleeping?

Yes ☐ No ☐

12. Do you have wired screens for windows and doors?

Yes ☐ No ☐

13. Do you have insects at home?

Yes ☐ No ☐

14. If yes, which type?

Mosquitoes ☐ Flies ☐ Ticks ☐

15. Do you use insecticides?

Yes ☐ No ☐

16. Do you live near water pools?

Yes ☐ No ☐

17. Do you have an open drainage system?

Yes ☐ No ☐

18. What health complain do you suffer from?

Fever ☐ Fatigue ☐ Joint pain ☐

19. What type of medications do you take?

Positive ☐ Negative ☐