



Sudan University for Science and Technology

College of Graduate Studies



Faculty of Medical Laboratory Sciences

**Prevalence of malaria and toxoplasmosis in donated
blood at the central blood bank , Gadaref State**

**انتشار الإصابة بالملاريا وداء القطط في الدم المتبرع به لبنك الدم
المركزي ولاية القضارف**

**A thesis submitted in partial fulfillment for the degree of MSc. in
Parasitology and Medical Entomology**

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Dedication

This work is dedicated

To my lovely family:

Parents

Brothers

My teachers

Friends who were supported me with all

ways

Anyone help me in any step from this

project

Specially Gadaref central blood bank

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I would like to express my deep appreciation and thanks to my supervisor Professor Hamid Suliman Abdalla, who gave me all the time to accomplish this work through his advice, help, and guidance and knowledge.

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ABSTRACT

This study was carried out in Central Blood Bank in Gadaref state to detect the infection rate of toxoplasmosis and malaria. Cross sectional study was carried out during period from May to September 2013. A total number of 100 studied population (age between 18-47 years old) were included in this study. Blood samples were taken from all subjects. Clinical and parasitological data was obtained and recorded. Thirty three out from 100 (33%) and 28 out of 100 (28%) Blood samples were found to be positive for *P.falciparum* when was detected by ICT and blood film respectively. On the other hand fourteen out of 100(14%) serum samples were found to be positive for toxoplasmosis by latex agglutination test. The prevalence rate of malaria / toxoplasmosis co-infection was detected 2 out 28 (7%). This study indicated that the study area is highly endemic for malaria and toxoplasmosis and the prevalence rate of malaria co-infection is refectly high. This is highly prevalence highly dangerous complications for blood recipients. There are many groups classified according to age and hemoglobin concentration. Both factors were affecting on infection especially hemoglobin concentration which is shown clearly reduction according to destruction of red blood cells by the malaria parasite and that is not shown in but the elder because of their having uncooked meat they can infect with toxoplsmosis more than the other, and the other affecting factors on distribution of both diseases contamination of the environment by cat feces in toxoplasmosis and increase the numbers of vectors of malaria in the rainy season in the State.

الخلاصة

هذه الدراسة أجريت بنك الدم المركزي ولاية القضارف، للكشف عن معدل الإصابة بداء القسطنطينية والمalaria . دراسة مقطعة عرضية أجريت في الفترة من شهر مايو- أكتوبر من العام 2013. وقد كان عدد الحالات قيد الدراسة 100 حالة تتراوح أعمارهم بين 18-47 سنة . عينات دموية أخذت من كل الحالات قيد الدراسة وأخذت بيانات سريرية وطفيلية وسجلت.

ثلاثة وثلاثون من اصل مائة 100 (33%) وثمانية وعشرون من اصل مائة 100 (%) عينة دموية كانت ايجابية لطفيل الملاриا حيث تم الكشف عنها بطريقتي المسحة الدموية وطريقة ICT على التوالى. ومن ناحية أخرى، اربعة عشر من اصل مائة 100 (14%) عينة سيرم (من المشتقات الدموية) كانت ايجابية لداء القسطنطينية وقد تم ذلك باستخدام طريقة اختبار التلازن لتشخيص داء القسطنطينية .

وقد كان معدل الارتباط بالإصابة بالملاريا/داء القسطنطينية 2 من 28 (7%) . هذا المعدل العالي بالإصابة يدل على ان المنطقة موبوءة بداء القسطنطينية والمalaria. هذا المعدل العالي في هذه الدراسة ربما ينبع من مضاعفات وخيمة لمستقبل الدم، وقد قسمت فئات المترددين حسب اعمارهم وتراكيز الهايموغلوبين في دمهم ، وقد وجد تأثير كلا العاملين على الإصابة بالملاريا كبيراً خاصة تركيز الهايموغلوبين الذي ينبع بصورة واضحة تبعاً لتكسير خلايا الدم الحمراء بواسطة طفيلي الملا리ا ، بينما وجد انه لا يوجد تأثير على تركيز الدم في حالة الإصابة بداء القسطنطينية بينما كان تأثير الإصابة بداء القسطنطينية على اكبر الفئات العمرية قيد الدراسة واضحاً ويعزى السبب الى تناولهم للحوم النيئة احياناً مما يزيد امكانية اصابتهم بالمرض دون غيرهم، وايضاً من العوامل المؤثرة في انتشار الاصابتين التلوث البيئي ببراز القسطنطينية المصابة في حالة عدوية داء القسطنطينية وزيادة عدد نوافل الملا리ا في موسم الامطار بالولاية.

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LIST OF ABBREVIATIONS

Hb	Hemoglobin
LAT	Latex agglutination test
ICT	ImmunoChromatography Technique
BF	Blood film

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

Malaria is one of the major causes of disease for people living in tropical and sub tropical areas. Despite intensive control efforts during the twentieth century, approximately 40% of the world's population still remain at risk of infection. Globally, it is estimated that there are 300-500 million new plasmodium infections and 1.5-2.7 million death annually due to malaria. Most morbidity and mortality is caused by plasmodium falciparum, and the greatest disease burden is in African children under five years in age (WHO, 1996).

1.1Classification of malaria parasites:

Malaria parasite belongs to the genus *Plasmodium*. The important species affecting human are: *Plasmodium falciparum* (laverania), which causes malignant tertian malaria, *Plasmodium vivax* and *Plasmodium ovale* causes benign tertian malaria, and *Plasmodium malariae* which causes benign quartan malaria (Zaman and Keong, 1982).

1.2 Geographical distribution:

P. falciparum is widely prevalent in many tropical areas of the world and transmission is rare or unlikely in temperate regions. *P.vivax* has wider distribution and it may be transmitted in temperate climate. *P.malariae* and *P. ovale* are much less common than *P. vivax* and *P.falciparum*. *P.ovale* is seen mainly in Africa although few cases have been reported from Asia (Zaman and Keong, 1982).

1.3 Transmission of malaria parasites:

Malaria parasites are transmitted by the bite of an infected female anopheles mosquito. Sporozoites contained in the saliva of mosquito are inoculated into the blood of a human host when the mosquito takes a blood meal. Infection can also occur by transfusion of infected donor blood or by injection through the use of needles and syringes contaminated with infected blood and very occasionally congenitally, usually when a mother is non immune (Cheesebrough, 1999).

Malaria parasite has two phases, an extrinsic phase in anopheles and an intrinsic vertebrate phase in a human (Beaver *et al.*, 1984).

1.4 Life cycle:

1.4.1 In human:

When infected female Anopheline mosquito bites human, sporozoites are injected in the blood where they circulate for about an hour, after which some of them invade the hepatocytes (liver cells) where pre erythrocytic forms develop. They develop into schizonts which normally rupture in 6-15 days producing in release thousands of merozoites. Some of the merozoites are phagocytosed while other enters erythrocytes and initiate erythrocytic phase or erythrocytic schizogony. The erythrocytic phase continues until drugs are used, death of the host, or immunity developed by the host prevent the parasite's further development. In *P. vivax* and *P. ovale*, hepatic forms called hypnozoites may persists and remain dormant in hepatocytes for considerable period before they begin to grow and undergo pre erythrocytic schizogony. Thus, liberating merozoites into the blood stream causing relapse of these infections (White, 1996) (figure1).

When the schizont ruptures, merozoites are released and reinvasion of the erythrocytes takes place. The number of parasitized red blood cells (RBCs) rarely exceeds 1% in *P.vivax*, *P.ovale*, and *P.malariae* infection while the number of parasitized RBCs is more than 2% up to 35% RBCs have been reported to be infected in *P.falciparum* infection. Some of merozoites give rise to gametocyte which develop in the RBCs of the capillaries of internal organs (gametogony). Peripheral blood reveals only the mature gametocytes. Gametogony is completed in four days time and a person carrying these gametocytes (carrier) is infective to the vector mosquito species (White, 1996).

1.4.2 In Mosquito:

There are two types of gametocytes: microgametocytes (male) and macrogametocytes (female), when are taken into the gut a susceptible mosquito, they quickly mature to gametes. Then microgametes undergo multiple nuclear divisions, mature by exflagellation process (10 to 12 munities) in which the microgametes break away from the blood cell and become motile. The macrogametocyte develops into macrogamete, its nucleus shifts to the surface, where a projection is formed. Fertilization occurs when the microgamete penetrates this projection and a zygote is formed. After about 20 minutes, the zygote puts out a pseudopodium into which its protoplasm flows to form fusiform body (the ookinete). As the ookinete grows, its male and female nuclei fuse and it penetrates the brush border and body of amid gut epithelial cell to that opposite side. There, the ookinete secretes a thin wall and grow to spherical oocyste, 50um in size or more, which extends into the insects haemocoele (Gilles and Bruce, 1996).

Depending on temperature and during a period of 4 to 15 days after the mosquito ingests the gametocytes, the oocyste mature with

multiplication of nuclei and transformation of the cytoplasm into as many as thousand separate bodies called sporozoites. The oocyte bursts out and sporozoites enter the mosquito's haemocoel from which they disperse throughout the insect's body. Those that come into contact with salivary glands bore into and through the cells and lodge in acinal ducts. Thereafter, when the mosquito injects saliva while obtaining blood meal, it also injects sporozoites, that enter their human host. Only females and not males take blood meals (Gilles and Bruce, 1996). Life cycle in human and mosquito illustrated in figure(1).

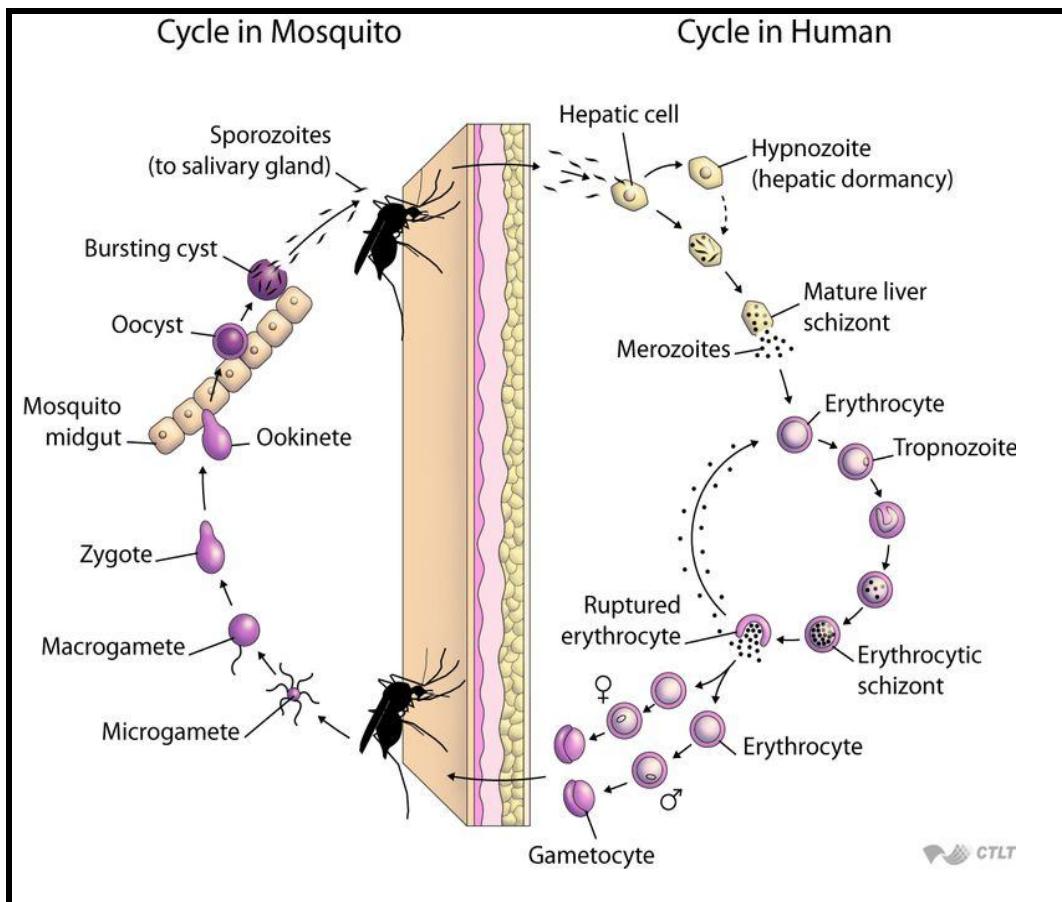


Figure1: Life cycle of malaria parasites (figure 1) (Gilles and warrell, 1996)

1.5 Pathogenesis:

The symptoms of malaria illness vary, but the majority of patients have fever. The periodicity of schizogony characteristically coincides- with paroxysms of fever and this leads to the traditional names of the different types of human malaria. Tertian malaria fever every third day, (*P. vivax* and *P. ovale*). Sub tertian malaria – fever slightly more often than every third day (*P. falciparum*). Quartan malaria fever every forth day, (*P. malariae*). *P. falciparum* malaria was sometimes called malignant tertian malaria, because of its much greater lethal potential than the other tertian malaria. Other common symptoms include headache, back pain, chills, increased sweating, myalgia, nausea, vomiting and diarrhea. The complication of the disease can rapidly progress especially in *P. falciparum* if untreated (Miller et al, 2002).

Other symptoms anaemia and black water fever (water fever is a syndrome that results from massive intravascular haemolysis and consequent haemoglobinuria), dysenteric malaria and Algid malaria which is a term used for falciparum malaria attacks characterized by rapid development of hypotension and impairment of vascular perfusion. The temperature falls rapidly, and the patient may become delirious. Symptoms of generalized vascular collapse and shock develop quickly (Markell, 1999). Pulmonary oedema, cerebral malaria, hypoglycemia, hyperparasitemia, metabolic acidosis, and tropical splenomegaly syndrome.

1.6 Laboratory diagnosis of malaria parasites:

There are many methods for diagnosis of malaria parasites. The best one depends upon detection and identification of the parasites in the red blood cells using stained blood films obtained either from capillary or venous blood.

Methods of diagnosis of malaria parasite can be divided into parasitological and serological (Hozhabri, 2002).

1.6.1 Parasitological methods:

1.6.1.1 Direct microscopy:

The gold standard for the diagnosis of malaria is microscopic examination of stained blood film, since each of the four major parasite species has distinguishing characteristics. There are two types of blood films, thick and thin that are traditionally used (Barker, 1994).

Thick blood films which concentrate layers of red cells on a small surface and allow the microscopist to screen a large volume of blood, is used for the rapid detection of malaria parasites. The appearance of the parasites in thick blood cells is much more distorted and therefore distinguishing between the different species can be identified from the thin blood film (Hozhabri, 2002, Barker, 1994).

1.6.2 Serological methods:

1.6.2.1 Fluorescence microscopy technique:

Before rapid immune-diagnostic strip test become available for the diagnosis of malaria, fluorescence techniques were developed to improve the sensitivity and speed of detecting parasites using fluorescent microscopes, after staining the blood film with fluorescent dye (Hozhabri, 2002).

1.6.2.2 Immunodiagnosis of malaria parasites:

Instead of identifying the parasite itself, immunological methods provide the mean for detecting either antigens or the antibodies directly against the parasites. The detection of antigen may be an acceptable alternative to parasite detection, particularly if the assay is robust, inexpensive, easy to use in field condition and does not require a microscope, but the detection of antibodies merely provides information on past malaria experience and it is of limited use for individual diagnosis (Forney and Wongsrichanalai, 2003).

In contrast to antibody detection, a positive antigen detection assay should detect current infection. The ideal target antigen should not persist after parasitaemia disappears, should be abundant in the blood or other body fluid, to maximize sensitivity and should be malaria specific without cross reaction (Forney and Wongsrichanalai, 2003). Therefore, a new generation of easier performed test has been developed to diagnose falciparum malaria rapidly and reliably without the need of a microscope. The most recently developed test can also diagnose vivax malaria. Blood tests are available for diagnosing falciparum malaria based on the immuno-chromographic detection of antigen, HRP2 (histidine-rich protein 2) or specific PLDH (parasite lactate dehydrogenase). HRP2 is produced mainly by *P. falciparum* and PLDH enzyme is produced by all parasite species during their growths and multiplication in red blood cells (Hozhabri, 2002).

Antibody detection tests have been used since the early 1960s, when indirect fluorescence antibody tests (IFAT) and indirect haemoagglutination assay (IHA) were described. Because such tests detect anti malaria antibodies, so it is not distinguish between current and past infection and they are, therefore, of limited value, as a guide to the

treatment or management of the disease (Forney and Wongsrichanalai, 2003, Palmer et al., 1999).

The test which is the most commonly used in the epidemiological work is indirect fluorescence antibody (IFA) because it is the most easily standardized and has high sensitivity and specificity. A thick blood film containing *P. falciparum* antigen, or obtained by growing the parasite in continuous culture. The use of Radioimmuno and Enzyme Linked Immunosorbent Assays (RIA and ELISA) may become more important than the IFA test, when the monoclonal antibodies are used to produce specific purified antigens. Such tests enable large number of samples to be tested rapidly (Hozhabri, 2002).

Indirect haemagglutination antibodies (IHA) has also been used in epidemiological work, but compared with RIA test. It lacks sensitivity and specificity (Hozhabri, 2002).

The most recent and exciting development in the field of recombinant DNA technology has been the polymerase chain reaction (PCR). PCR (and other molecular methods) is more accurate than microscopy. However, it is expensive, and requires a specialized laboratory. Moreover, levels of parasitemia are not necessarily correlative with the progression of disease, particularly when the parasite is able to adhere to blood vessel walls. Therefore more sensitive, low-tech diagnosis tools need to be developed in order to detect low levels of parasitemia in the field. In addition to that, it can be used for study of drug resistant strains, genetic type of specific strains and the identification of parasite species (Redd, et al. 2006).

1.7 Strategy of malaria control:

A strategy for malaria control has recently been formulated by the WHO to address the serious worldwide malaria situation (Gilles and Bruce, 1996).

Reduction of malaria morbidity and mortality with their consequences on human performance and socio-economic development is more modest and realistic goal. These will require an improvement in the capacity of existing health services to provide diagnosis and early treatment within the umbrella of the primary health care. A reorientation of existing malaria control programs should take into consideration of epidemiological, ecological, environmental and social factors with active participation of the community (Collier et al., 1999, Stanely, 1997).

1.8 Malaria situation in Africa:

In Africa, 550 million people are at risk of malaria. It is estimated that there are 320 million clinical cases annually and 970000 deaths. Approximately, 30% to 40% of all outpatient attendance and 10% to 20% of hospital admissions are due to malaria (Nicholas, 2004, WHO, 2002).

1.9 Malaria situation in Sudan:

Malaria is the leading cause of morbidity and mortality in Sudan. It is assumed that symptomatic malaria accounts for 20-40% of outpatient clinic visits and approximately 30% of hospital admissions. Almost 90% of all malaria cases are caused by *falciparum* malaria which causes the most severe form of the disease and deaths attributable to malaria. . High seasonal rainfall, variation in temperature and humidity which is observed during the rainy season in Sudan can favour mosquito breeding, resulting in strong seasonal transmission. (Ministry of Health, Khartoum, 1999).

1.10 Introduction to toxoplasmosis:

In 1907, at the Institute Pasteur in Tunis, while studying the infectious diseases of the desert rodent, *Ctenodactylus gundi*, Charles Nicolle observed the presence of a crescent-shaped organism in the tissues of these animals (Nicolle 1907). In 1907, Nicolle and his collaborator, Louis Manceaux, published the initial description of this discovery (Nicolle & Manceaux 1908). A year later, they provided a more complete report and named the organism *Toxoplasma gondii*, referencing the parasite's characteristic morphology and the source of the original isolate (Nicolle & Manceaux 1909). Concurrently, in Brazil, Alfonso Splendore (1908) identified the same microbe as a parasite of rabbits (Splendore 1908). At the time, the discovery of *T. gondii* perhaps seemed to be solely of academic interest; however, when Wolf A, and Cowen D (1937) discovered the parasite in infants with encephalomyelitis, *T. gondii* was recognized as a cause of congenital disease. Since *T. gondii*'s discovery, its clinical importance has influenced the research groups investigating the immunobiology of toxoplasmosis. Today, *T. gondii* is recognized as an important opportunistic pathogen of fetuses, newborns and patients with a variety of primary genetic and acquired immunodeficiencies (Dubey 2001).

1.11 Classification of *Toxoplasma gondii*:

According to Levine (1980), the classification of *Toxoplasma gondii* is as follows:

phylum	Apicomplexa
class	Sporozoa
order	Eucoccidida
Suborder	Eimerina

family	Sarcocystidae
Subfamily	Toxoplasmatinae
genus	<i>Toxoplasma</i>
species	<i>Toxoplasma gondii</i>

Toxoplasmosis, caused by intracellular protozoan parasite, *Toxoplasma gondii*, infects a wide variety of hosts, including humans. The member of the family Felidae, including domestic cat act as definitive hosts. Various warm-blooded animals including humans, birds and rodents can serve as an intermediate host (Dubey, 1998). Infection generally occurs through ingestion of either oocysts shed in cat feces, or viable tissue-cysts present in undercooked meat. In addition, primary infection during pregnancy can result in congenital toxoplasmosis. During acute infection, tachyzoites are the rapidly multiplying stage of the parasite. These can invade and proliferate in all nucleated cells by active penetration and formation of a parasitophorous vacuole. After repeated replication, host cells are disrupted and tachyzoites disseminate via the bloodstream and can invade many tissues such as the central nervous system, eye, skeletal, heart muscle and placenta. Replication leads to host cell death and rapid invasion of neighboring cells. However, the cell mediated immune response induces tachyzoites undergo stage conversion into bradyzoites and forms tissue cyst. These tissue cysts remain viable and are capable of persisting for the life of the host (Montoya and Liesenfeld, 2004). To date there is no drug which is effective against the tissue cysts. In immunocompromised hosts, it is believed that reactivation, probably due to cyst rupture, results in reversion from dormant bradyzoites into rapidly dividing tachyzoites resulting in serious neurological disease. Interestingly, there is highly incidence of toxoplasmosis infectious in the HIV patients. In this research we focus on infection in donated blood.

Furthermore, this research would provide much information for toxoplasmosis in donated blood.

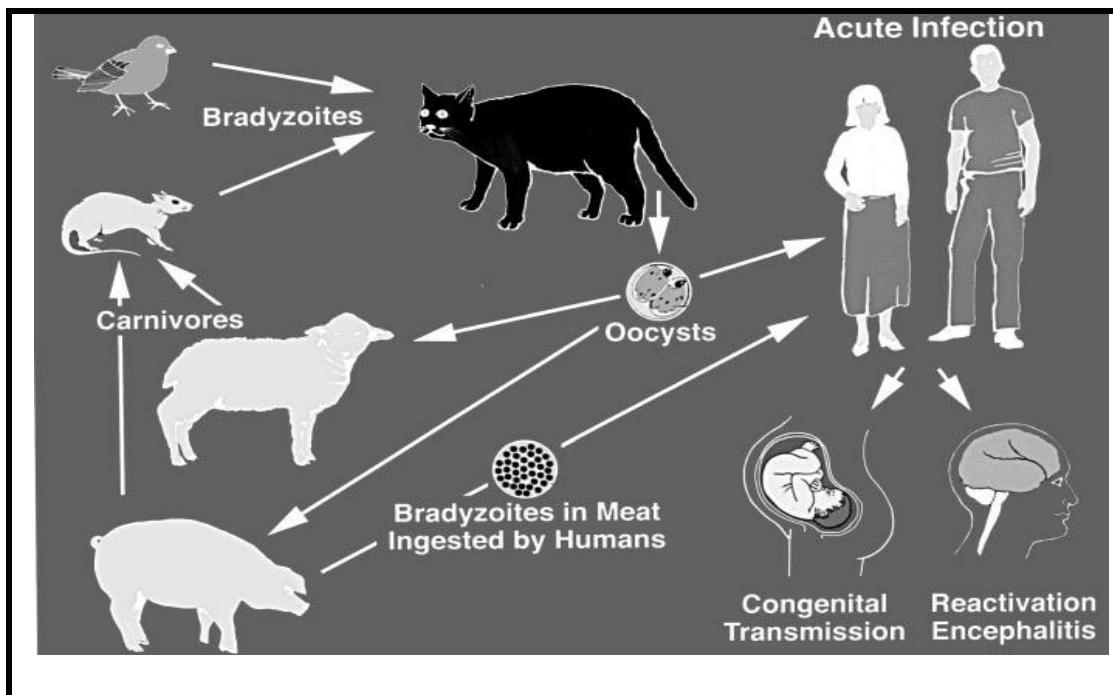


Figure (2) Life cycle of *Toxoplasma gondii* (S. Gilles and Richard D. Pearson 2001)

1.12 Pathogenesis of toxoplasmosis:

Toxoplasma infection is classified into the acute and chronic stages. The acute or early stage is mostly associated with the proliferative form (tachyzoite) while the tissue cyst is the predominant form during chronic infections, although tachyzoites have been reported outside of cysts at this stage. Tissue cysts may be formed as early as 3 days after infection but are usually not numerous until 7 weeks after infection and probably persist as viable parasites throughout the life of the host (Derouin, et al, 1989). During acute infection, the tachyzoites invade every kind of host cell except non-nucleated red blood cells. The host cell invasion is a major

step in its biological cycle and in pathogenesis. Tachyzoites enter host cells by actively penetrating through the host cells plasmalemma or by phagocytosis. The regulated release of contents from the parasites apical secretary organelles (the micronemes, the rhoptries, and the dense granules) would play the following parts: the micronemes would drive the recognition and adhesion to the target cell. Enzyme released by the rhoptries is for producing the parasitophorous vacuole, and the dense granules secrete enzyme to maturing of the vacuole into a metabolically active compartment (Carruthers, et al, 1997). They multiply intracellularly, causing host cell disruption. The liberated parasites invade and destroy adjacent cells, producing progressively larger focal lesions. If the initial infection occurs when the host is pregnant, the tachyzoites associated with the acute phase can cross the placenta and infect the fetus, which can result severe birth defects, including hydrocephaly, calcification, neurological defects and chrorioretinitis, which may be recurrent (Montoya, and Remington, 2008). However, with the onset of the host immune response, a subpopulation of tachyzoites in the brain, undergo stage conversion in bradyzoites which multiply slowly to form large tissue cysts with numerous of bradyzoites that are capable of persisting for the life of the host (Mordue, et al, 2001). Lesions or tissue necrosis may be found in many organs of the body during acute toxoplasmosis with lesions in intestines, liver, spleen, pancreas, lung and heart (Dubey, 1996). Whereas the chronic infection, lesion occurs more often in muscle eye and brain than in visceral tissue (Dubey and Beattie, 1988).

1.13 Laboratory diagnosis:

Most infections with *T. gondii* are diagnosed serologically. Serological testing, however, has only a limited value in diagnosing *Toxoplasma* encephalitis in AIDS patients. The diagnosis is usually made clinically, supported by computerized tomography or magnetic imaging scans where these facilities are available. Trophozoites may occasionally be found, e.g. in c.s.f. of AIDS patients with *Toxoplasma* encephalitis. A blood lymphocytosis with many atypical lymphocytes is usually found in acute *Toxoplasma* infections. The blood picture often resembles that seen in infectious mononucleosis (glandular fever). A low platelet count may also be found.

- 1- Sabin Feldman Dye test.
- 2- LAT.
- 3- Indirect Florescent Technique (IFT).
- 4- ELISA.
- 5- CFT.
- 6- PCR.

1.14 Serological diagnosis of toxoplasmosis:

The most reliable test for the diagnosis of acute toxoplasmosis in pregnancy, and to test neonates, is the Sabin-Feldman dye test. This highly sensitive and specific test is a complement-mediated neutralizing antigen antibody reaction which uses live trophozoites to measure *Toxoplasma* specific antibody. It shows 94.4% agreement with the dye test and is performed as a quantitative test in microtitration plates. The latex particles are coated with inactivated *T. gondii* soluble antigen. The test detects all immunoglobulin classes. The test does not require heat

inactivation of serum samples. A positive control is included in each test kit. Serodiagnosis of toxoplasmosis is carried out by several serological tests such as ELISA, Latex agglutination test, no culture approaches are readily available in Clinical Laboratories but may be available with *Toxoplasma* reference Laboratories. PCR amplification of parasite DNA from tissues, CSF, amniotic fluid or blood is a sensitive method for detection of infection, and several potential amplification targets have been described. (Grover et al, 1990).

Both vaccine on:

- **Malaria**
- **toxoplasmosis**

Toxoplasmosis in Sudan

Rationale:

In Sudan, most studies focused on diagnosis of parasitic infections in patients who have signs and symptoms of disease for treatment, but studies on parasitic infections (malaria and *Toxoplasma*) in donated blood did not receive much attention. They are not investigated routinely before blood donation, despite claims of getting infections by blood transfusion. Carrying out the studies to determine if donated blood carries *Plasmodium* species or *Toxoplasma* or not. Because highly needed.

Objectives

General objective:

To investigate the prevalence rate of infection of malaria and toxoplasmosis in donated blood in the Central Blood Bank of Gadaref state.

Specific objectives:

To relate malaria and toxoplasmosis co-infection .

To relate malaria and toxoplasmosis according to age and hemoglobin concentration.

CHAPTER TWO

MATERIAL AND METHODS

2.1 Study design:

This is a descriptive cross sectional study.

2.2 Study area:

The study was conducted in Gadaref State East – Sudan (450 km far from Khartoum).

2.3 Study population:

Samples were collected randomly from donors of Gadaref Central blood bank. All study population included in this study was males.

Study period:

The laboratory investigations were conducted in Karary Dar Al-Slam health laboratory ,Gadaref ,during the period May - October 2013.

2.4 Sample size:

One hundred (100) blood samples were collected from blood donors.

2.5 Collection of specimens:

The specimens were received in the blood bank centers. Samples of venous blood were collected for direct detection of malaria parasite and for separation of serum for *Toxoplasma* detection. In all cases 5ml of venous blood were collected in two sets of sterile tubes: one with EDTA and the other without EDTA for serum separation. Serum was obtained from the plain containers by centrifugation at 2000 rpm and stored at -20°C until tested .Blood films were prepared from EDTA blood.

2.6 Methodology and laboratory examinations:

2.6.1 Direct agglutination test:

Toxo-Latex Test is a rapid slide agglutination procedure, developed for the direct detection of antibodies anti-*Toxoplasma* in human serum.

The assay was performed by testing a suspension of latex particles coated with antigenic extract of *T gondii* against unknown samples. The presence or absence of a visible agglutination, indicates the presence or absences of anti- *Toxoplasma* antibodies in the sample tested.

2.6.2 Blood film for malaria: preparation of thick blood film:

The blood was collected preferably in a small plastic bulb pipette EDTA ant coagulated blood which collected from blood donor. A drop of blood was distributed and smeared by a corner of another slide in the center of glass slide 2x1 cm as a thick film, When the blood was spread, it was mixed to avoid the red cells forming marked rouleaux which can cause the blood to be easily washed from the slide during staining. By using a black lead pencil, the slide was labeled with the identification number. The blood films were allowed to air dry with the slide in horizontal position and placed in a safe place (where there is no risk of the blood coming into contact with the person or object).

After drying slides stained with diluted Giemsa stain 1:10 for 10 minutes (details is shown about Giemsa stain preparation in appendix (2). And buffer phosphate in appendix (3).

2.6.3 Staining of blood films for malaria:

Slides were placed back to back in a staining trough ensuring that all thick blood films were placed to one end upwards. 10% of working solution of Giemsa stain was prepared by adding 10 ml of Giemsa stock solution to 90 ml buffered water then the working solution was gently

poured into the trough, until the slides were completely covered. Slides were left in the stain for 10 minutes, and then clean water was gently poured into the trough to float the debris on the surface of the stain. The remaining stain was poured off gently and was returned again in clean water. The water was poured off. The slides were removed. The back of each slide was wiped with clean gauze, and then the slides were placed in the slides drying rack to drain and dry (thick blood films downwards).

2.6.4 Examination of thick blood films for malaria:

When the slides were completely dried, a drop of immersion oil was applied to an area of the thick film that appeared mauve colored (usually around edges). In such area, it is possible to see best staining of malaria parasite (the selected should show a clear or pale color background purple color neutrophilic nuclei and blue-colored cytoplasm). 100 microscopic fields were examined for searching malaria for malaria parasite using 100X and moving systematically from field to another and result reported.

2.6.5 Examination of blood by using ICT:

Principle of ICT

ICT test based on the detection of antigen derived from malaria parasite monoclonal antibodies react directly against target parasite antigen.

Malaria antigen are histidine rich protein (HRP II) which is soluble protein produced by trophozoite and young gametocyte, also parasite lactate dehydrogenase (PLDH) is produced by sexual stages of malaria parasite.

Practically:

The pouch was opened and the device was removed. Once opened, the device was used immediately. a large drop of blood. Touched the

sample applicator pipette to the blood and immediately blot the specimen on to the sample pad.

Dispensed four drops of the diluents' buffer into well B, by holding the plastic dropper bottle vertically. After 15 minutes, the results were read.

Negative for *P. falciparum* malaria: only one pink colored line appears in the control window (C).

Positive for *P. falciparum* malaria: in addition to control line, the district pink colored line also appears in the test window (T).

The test should be considered invalid if no line appears or if no pink line in the control line. Repeat the test with a new device. The test interpreted after 15 minutes.

2.7 Hemoglobin estimation

well-mixed EDTA anticoagulated venous blood 0.02ml or 20cmm was mixed with Drabkin solution (diluting fluid) 5 ml. the blood which was taken by calibrating pipette, was mixed gently, incubated 5 minutes the read at filter 540 nm.

2.8 Data collection:

Following written informed consent, the clinical data were collected guided by the data collection sheet (appendex1).

2.9 data analysis:

Data concerning each subject were registered in a separate row in the statistical package program for social science (SPSS for windows 11.5; Chicago, IL). Graded categorical data were given numerical code.

2.10 Ethical consideration:

The study had received an ethical approval from the ethical review committee at Sudan University of Science and Technology, Faculty of

Medical Laboratory Sciences. Written informed consents were obtained from the studied subjects after explaining the aims and protocol of the study.

CHAPTER THREE

RESULTS

3.1 Infection rate of malaria

Out of 100 blood samples were screened for malaria, 28 (28%) in Gadaref central blood bank were found to be positive for *P. Falciparum* by blood film technique and when examined by ICT, 33 (33%) were shown to be positive (table1).

Table (1) result of malaria by blood film and ICT technique

Test	NO of examined	Positive cases	Negative cases
BFFM	100 (100%)	28 (28%)	72(72%)
ICT	100 (100%)	33(33%)	67(67%)

3.2 Anti- *Toxoplasma* antibodies in serum samples :

Out of 100 serum samples screened for toxoplasmosis, 14(14%) were found to be positive (table2)

Table (2) Anti-Toxoplasma agglutination test in serum samples

Number of examined donors	Positive cases	Negative cases
100	14(14%)	86 (86%)

3.3 Co-infection between malaria and Toxoplasmosis:

Out of 28 positive cases for malaria , 2 (7%) samples were positive for toxoplasmosis by latex agglutination test(table 3).

Table (3):/ Co-infection rate of malaria and Toxoplasmosis in the study population:

		LAT		Total
		+ve	-ve	
BF	+ve	2	26	28
	-ve	12	60	72
Total		14	86	100

3.4 Age groups:

Result of toxoplasmosis examined by toxolatex agglutination test and malaria examined by BF and ICT. Among various age groups, *Toxoplasma* and *Plasmodium* were found to be higher in age group less than 25 years reaching 4 (28%),12 (42%) and 10 (30%) by LAT, BF and ICT respectively, as for the remaining age groups the percentage of 26-35 age group was 6 (42%), 5 (17%), 8 (24%)% by LAT, BF and ICT respectively, and age group 36-45 reached 4 (28%),10 (35%),14 (42%) by LAT, BF and ICT respectively, and age group >45 reached 0 (0%),1 (3%),1 (3%) (table 4).

Table (4) *Toxoplasma* and malaria among age groups

Age	LAT (+ve)	BF (+ve)	ICT (+ve)
<25	4	12	10
26-35	6	5	8
36-45	4	10	14
> 46	0	1	1

3.5 Hemoglobin group:

Result of toxoplasmosis examined by toxolatex agglutination test and malaria examined by BF and ICT among various age groups, *Toxoplasma* and *Plasmodium* were found to be higher in Hb group 61-70% reaching 5 (35%), 15 (53%) and 17(51%) by LAT, BF and ICT respectively, as for the remaining age groups the percentage of <60% group was 0 (0%), 3(10%), 3(9%)% by LAT, BF and ICT respectively, and Hb group 71-80 reached 3(10%), 9(32%), 11(33%) by LAT, BF and ICT respectively, and 81-90 Hb group reached 1(7%), (3%), 2(6%) and > 91 Hb group reached 5(35%), 0(0%), 0(%) (Table 6).

Table (5): *Toxoplasma* and malaria among Hb groups

Hb groups%	LAT (+ve)	BF (+ve)	ICT (+ve)
<than 60	0	3	3
61- 70	5	15	17
71-80	3	9	11
81-90	1	1	2
>91	5	0	0

CHAPTER FOUR

DISCUSSION

This study was an attempt to detect toxoplasmosis and malaria rates in donated blood samples.

The prevalence rate of anti- *Toxoplasma* antibodies using latex agglutination test was found to be 14%. This rate was found to be similar to result obtained by Abdel-Raouf (2001) who found it to be 13% and lower than rates reported by Abdel-Hameed (1991) and Al-Harbi (2008) who reported rates of 41.7% and 22.5% respectively.

The highest prevalence of toxoplasmosis among studied population age groups was reported 26- 35 years age group (44%). This was so far than less from the rate reported by Frenkel and Rize (1980) who reported 61.4% prevalence rate among 15-25 years age group. In this study <25 years age group reported rate 28.5% equal to 36-45 years age group and the lowest prevalence reported in >45 years age group 0%.

The highest prevalence of toxoplasmosis was reported 35.7% among >90% and 61-70% hemoglobin level group, followed by 71-80% by 21.4%, followed by 81-90%Hb level by 7.2% and finally 0% by those their Hb level less than 60%.

The prevalence rate of malaria using BF and ICT reported in this study was (28%) and (33%) by two techniques respectively. It was higher than the rate reported by Dicko and his colleagues in Mali or similar to it (23.3-33.6%) (Dicko, A, 2005). This difference in rates might probably be due to difference in transmission of malaria, and strain differences between Mali and Sudan. This rate was also found less than the rate reported in Eritrea where malaria prevalence 31.5% of outpatient

morbidity while in Ethiopia, *P.falciparum* was found in 93% of cases (Nicholas, 2004, WHO, 2002). The rate was also lower than the rate reported by Elsadig (2008) (65.12%).

It is obvious that the highest rate of malaria (33%) by ICT and (28%) by BF technique. These rates were extremely lower than the rate reported by Elsadig (2008) (65.12%). The study showed that the rate of infection with malaria in those who their age less than 25 years (42%) younger studied population and their occupy in the cultural areas in the rainy season while in those with more than 45 years old was (3%). This indicates the strong correlation between age and occurrence of malaria. This finding is consistent with the finding of Malik et al, (2004) who proved that 60% of children infected with malaria. Hassan (2006) found a very low prevalence rate among febrile patients (9.1%).

It was obvious that 3 positive cases with BF of the species *p. falciparum* were found negative with ICT. This may be attributed to low parasitaemia encountered in these 3 cases, that's similar to Yousra (2009). And 10 cases was positive with ICT and negative by BF, This may be attributed to false positive result by remaining circulating antibodies after parasite clearance or real result but sample take while parasite in the internal organ during schizogony.

Malaria among various Hb groups , the higher infection rate in those who have 61-70% Hb level (53%) and the lower infection rate in those who have more than 91% Hb level (0%).This indicate malaria affecting Hb concentration by reduction of Hb levels which due to RBC destruction..

Chapter 5

5.1 Conclusion:

The study concluded that:

1. Toxoplasmosis occurs in donated blood
2. *P.Falciparum* occurs in donated blood
3. They occur with nationality higher rates.

5.2 Recommendations:

1. Donated blood should be screened for malaria and toxoplasmosis before transfused to recipient.
2. Further studies is needed to confirm our results and increase the sample size.
- 3.
- 4.

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Appendix 1

ID No
Age
Hb
BFFM
ICT
LAT

Appendix 2

Preparation of Giemsa stain:

Giemsa stain (stock solution):

Giemsa powder 3.8 gm. (azur II eosin 3.0+ azur II 0.8 gm)

Methanol 250 ml

Glycerol 250 ml

Giemsa powder was weighed and transferred to a dry bottle of 500 ml capacity which contains about 50 glass beads using a clean and dry measuring cylinder. 250 ml of methanol was added to the stain and mixed well. Using the same cylinder, 250 ml of glycerol was added to the solution and mixed thoroughly. The bottle with stain was placed in the water path at 50-60 °C for one hour. Then, it was stored in a dark brown bottle at room temperature in a dark place. For better dissolving of the stain, bottle stain was mixed four times every day for five days. The stock solution was kept in room temperature in well stoppered bottle for a year or more.

Appendix 3

Preparation of phosphorus buffer water:

For staining blood films for malaria, it is important to have buffer water with PH =7.2 to dilute the stock solution of Giemsa stain. The buffer solution was prepared by dissolving buffer tablet in 1 liter of distilled water. Each buffer tablet contains 0.7 KH₂PO₄ and 1 gm Na₂HPO₄.