Sudan University of Science and Technology
College of Graduate Studies

Sero- Prevalence and Risk Factors Associated with *Toxoplasma gondii* in Cattle in Sharg Elnil and Omdurman Localities, Khartoum State, Sudan

الانتشار المصلي وعوامل الخطر المرتبطة بالتوكسوبلازما جوندي في الابقار في محليتي شرق النيل و ام درمان، ولاية الخرطوم ، السودان

A thesis submitted in partial fulfillment of the requirements the degree of Master in Preventive of Veterinary Medicine (M.P.V.M.)

By

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Dedication

To my beloved ones

My mother

My father

My sister and brothers

Sheryhan

Acknowledgement

In the name of Allah, the Most Gracious and the Most Merciful.

All praises to Allah and his blessing for the completion of this thesis. I thank God for all the opportunities, trials and strength that have been showered on me to finish this thesis.

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Abstract

Toxoplasmosis is an important zoonosis caused by an obligate intracellular parasitic protozoan, *Toxoplasma gondii*. The disease is distributed worldwide and can affect all warm-blooded vertebrates, including humans.

To determine the prevalence and associated risk factors of *Toxoplasma gondii* infection in cattle, a cross-sectional study was conducted between June and September 2019 from Sharqelnile and Omdurman localities in Khartoum state, Sudan. Serum samples were collected from cattle and tested by Latex agglutination test (LAT). Data, about sources of water, hygienic status at the farm, presence of other animal species, size of the herd, and presence of cats in the vicinity of the farm were obtained using a questionnaire and face to face interviews with cattle owners or cattle keepers were carried out to fill the questionnaires.

The overall prevalence of *Toxoplasma gondii* and the infection was 14.8%. Seroprevalence was found to be associated with: sex (P=0.040), size of herd (P=0.026) and presence of cats (P=0.030). No difference of seroprevalence of *T.gondii* was observed with: age (P=0.247), breed (P=0.100), localities (P=0.100), presence of other animals (P=0.509), sours of water (P=0.574) and hygiene condition (P=0.125).

The present study found that *T. gondii* is prevalent in cattle in Khartoum State, Sudan, and this infection may have important implications for the livestock industry and public health.

ملخص البحث

داء المُقَوَّسَات هو مرض حيواني مهم ناجم عن طفيلي مُلزم داخل الخلايا ، وهو التوكسوبلازما جوندي. ينتشر المرض في جميع أنحاء العالم ويمكن أن يصيب جميع الفقاريات ذوات الدم الحار ، بما في ذلك البشر.

لتحديد مدى انتشار عدوى التوكسوبلازما جوندي في الأبقار وعوامل الخطر المرتبطة بها ، أجريت دراسة مقطعية بين شهري يونيو وسبتمبر 2019 من محليتي شرق النيل وأم درمان في ولاية الخرطوم ، السودان. تم جمع عينات المصل من الماشية واختبارها باختبار تراص اللاتكس (LAT). تم الحصول على بيانات حول مصادر المياه ، والحالة الصحية في المزرعة ، ووجود أنواع حيوانية أخرى ، وحجم القطيع ، ووجود القطط بالقرب من المزرعة باستخدام استبيان ومقابلات وجهاً لوجه مع أصحاب الماشية أو مربي الماشية لملء الاستبيانات.

كان معدل انتشار العدوى الكلي (14.8٪). تم العثور على الانتشار المصلي في : الجنس (P = 0.040) ، وحجم القطيع (P = 0.026) ووجود القطط (P = 0.030). لم يلاحظ أي اختلاف في الانتشار المصلي مع: العمر (P = 0.247) ، السلالة (P = 0.100) ، المحليات (P = 0.100) وجود حيوانات أخرى (P = 0.509) ، مصدر المياه (P = 0.574) وحالة النظافة (P = 0.125). وجدت الدراسة الحالية أن *T. gondii* منتشر في الابقار في ولاية الخرطوم، السودان ، وقد يكون له آثار مهمة على صناعة الثروة الحيوانية والصحة العامة.

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Introduction

Toxoplasmosis is an anthropozoonic disease caused by the infection with the obligate intracellular parasite *Toxoplasma gondii* (Gharekhani, 2014; Elhassan *et al*., 2015). *Toxoplasma gondii* is a ubiquitous parasite that occurs in most areas of the world. It is capable of infecting an unusually wide range of hosts and many different host cells (Tenter, 2009). Infection with *T. gondii* is one of the most common parasitic infections of man and other warm-blooded animals. Nearly one-third of humanity has been exposed to this parasite; serologic surveys indicate that *T. gondii* infections are common in wild carnivores, including pigs, bears, felids, fox, raccoons, and skunks. Clinical and subclinical toxoplasmosis has been reported from wild *cervids, ungulates, marsupials, monkeys, and marine mammals* (Hill *et al*., 2005). The life cycle of *T. gondii* includes asexual multiplication in various tissues of intermediate hosts and sexual reproduction in the intestine of definitive hosts (Tenter, 2009). In its host, the parasite multiplies rapidly and forms tissue cysts. In wild felids and domestic cats, the rapid multiplication stage is followed by oocyst formation in the intestines. Millions of oocysts are then shed in the feces and spread in the environment (Woudt, 1990).

All mammals and birds that are consumed by humans may serve as intermediate hosts for *T. gondii* and, thus, may be a potential source of infection for humans (Tenter, 2009).

In livestock, *T. gondii* tissue cysts are most frequently observed in various tissues of infected pigs, sheep and goats, and less frequently in infected poultry, rabbits, dogs and horses. By contrast, tissue cysts are found only rarely in skeletal muscles of cattle or buffaloes (Tenter *et al*., 2000). In cattle, according to numerous sources (Dubey, 1983; Dubey and Jones, 2008; Jones and Dubey, 2012), cattle have been considered to be a poor

host for the parasite, they can get infected with *T. gondii* and carry tissue cysts (Dubey, 1986; Dubey 1992; Esteban-Redondo *et al*., 1999).

Objectives:

The main objectives of this study were:

- To estimate the overall prevalence of antibodies against *T. gondii* in Sharqelnile and Omdurman localities in Khartoum state, Sudan in cattle.
- To investigate the potential risk factors related with occurrence of *T.gondii* in the study area.

Chapter One

Literature Review

1.1. Etiology of Toxoplasmosis:

Toxoplasma gondii belongs to the phylum Apicomplexa, subclass Coccidiasina, order Eimeriorina, and there is only one known species in the genus *Toxoplasma* (Dubey, 2010). The parasite is a protozoan with a heteroxenous life cycle that is able to infect all homoeothermic animal species, including mammals, birds and humans (Dubey, 1996; Lopes *et al*., 2013). It is one of the most prevalent parasitic diseases that have medical and veterinary importance.

After the discovery of its life cycle in 1970, this small parasite changed the definition of coccidia from being only single-host-specific parasites to include multi-host pathogens with important public health and biological significance (Dubey, 2009).

Inside the host, it is an obligatory intracellular organism, however, outside the host, in the environment the oocyst form is able to survive and remain infective for a long time (Dubey, 2010).

1.2. History:

Toxoplasma gondii was first described in Tunisia in 1908 by Nicolle and Manceaux and by Splendore in Brazil independently. In 1909 Nicolle and Manceaux differentiated the protozoan from *Leishmania*, then they named it *Toxoplasma gondii* after the curved shape of its infectious stage (Greek root 'toxon'= bow) (Ferguson, 2009).

First recorded case of congenital toxoplasmosis in 1923, but it was not identified as caused by *T. gondii*. In 1923 Janků described in detail the

autopsy results of an 11-month-old boy who had presented to hospital with hydrocephalus. The boy had classic marks of toxoplasmosis including chorioretinitis (inflammation of the choroid and retina of the eye). Histology revealed a number of "sporocytes", though Janků did not identify these as *T. gondii* (Weiss and Dubey, 2009).

Until 1937 the first detailed of scientific analysis of *T. gondii* took place using techniques previously developed for analyzing viruses. Sabin and Olitsky analyzed *T. gondii* in laboratory monkeys and mice in the same year. Sabin and Olitsky showed that *T. gondii* was an obligate intracellular parasite and that mice fed *T. gondii*-contaminated tissue also contracted the infection.Thus Sabin and Olitsky demonstrated *T. gondii* as a pathogen transmissible between animals (Ferguson, 2009).

The parasite was first described as a human pathogen in 1939 in Babies Hospital in New York City (Ferguson, 2009). Wolf, Cowen and Paige identified *T. gondii* infection in an infant girl delivered full-term by Caesarean section. The infant developed seizures and had chorioretinitis in both eyes for three days. The infant then developed encephalomyelitis and died at one month of age, they isolated *T. gondii* from brain tissue lesions, also they reviewed additional cases and concluded that *T. gondii* produced recognizable symptoms and could be transmitted from mother to child.The first adult case of toxoplasmosis was reported in 1940 with no neurological signs. Pinkerton and Weinman reported the presence of *Toxoplasma* in a 22-year-old man from Peru who died from a subsequent bacterial infection and fever (Weiss and Dubey, 2009).

1.3. Morphology and life cycle of the parasite:

Toxoplasma gondii, discovered in 1908 by Nicolle and Manceaux (2009) in tissues of a hamster-like rodent called the gundi (*Ctenodactylus gundi*).

Toxoplasma gondii is a protozoan parasite, named after its morphology *Toxoplasma*: mod. L. toxo = arc or bow, plasma = life, and the host gundi (Dubey, 2010). Felids are the only definitive hosts of *T gondii*; both wild and domestic cats therefore serve as the main reservoir of infection.The parasite presents itself in three infectious stages (tachyzoites (in groups) (Fig1.1A), the bradyzoites (in tissue cysts) (Fig1.1 B,C), and the sporozoites (in oocysts) (Fig1.1G)) that form a complex life cycle with sexual or asexual reproduction phases (Gilot-Fromont *et al*., 2012).

1.3.1. Sexual life cycle:

In this cycle all three forms of the parasite can be described so that it starts and finishes in a cat (Jokelainen, 2013). Oocysts is the outcome of the sexual cycle of *T. gondii*, release of sporozoites in another hosts' digestive tract this is considered a successful cycle. Cats can become infected after the ingestion of any infectious form of the parasite (tachyzoites, bradyzoites or oocycsts). The optimal way of infection for forming oocysts is infection with bradyzoites of tissue cysts. According to Dubey (2010), nearly all cats shed oocysts after ingesting tissue cysts. The parasites are released by proteolytic enzymes dissolving the wall of the cyst in the digestive system of a cat. The sexual replication phase is the main part of a sexual life cycle, begins two days after ingestion of tissue cysts in the epithelial cells of small intestines of cats. During the sexual replication, the formation of male (microgamonts) and female (macrogamonts) gamonts takes place in enterocytes. Male gamonts (fig1.1E) undergo microgametogenesis, where they leave behind a large residual body and become microgametes. After fertilization between mature macrogamonts and microgametes, the oocyst wall is formed. The completed forms have to survive outside a cell as the oocysts are released in the cat faeces. The time

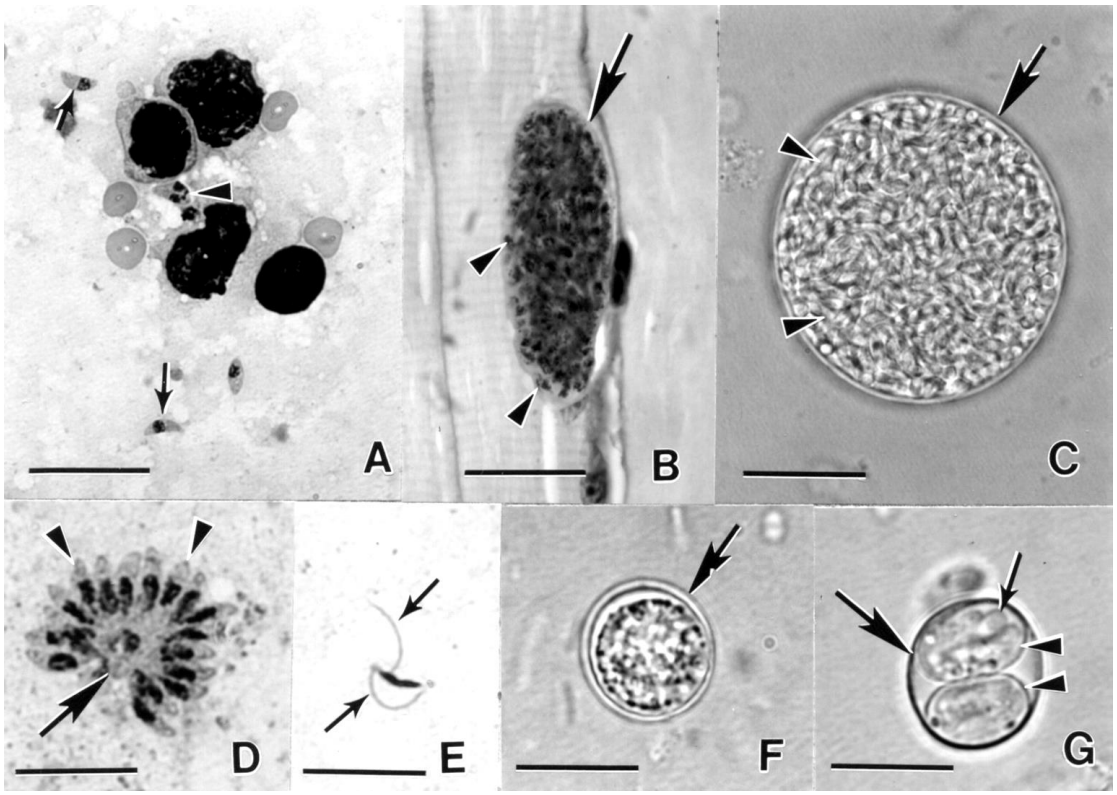

Fig1.1. Stages of *T. gondii*. Scale bar in (A)–(D) =20 mm, in (E)–(G)=10 mm. (A). Tachyzoites in impression smear of lung. Note crescent-shaped individual tachyzoites (arrows) and dividing tachyzoites (arrowheads) compared with size of host red blood cells and leukocytes. Giemsa stain. (B). Tissue cysts in a section of muscle. tissue cyst wall (arrow) and encloses many tiny bradyzoites (arrowheads). H & E stain. (C). Tissue cyst separated infected brain. Note tissue cyst wall (arrow) and hundreds of bradyzoites (arrowheads). Unstained. (D). Schizont (arrow) with several merozoites (arrowheads). Impression smears of infected cat intestine. Giemsa stain. (E). A male gamete with two flagella (arrows).. Giemsa stain. (F). Unsporulated oocyst in fecal float of cat feces. Unstained. Note double layered oocyst wall (arrow). (G). Sporulated oocyst with a thin oocyst wall (large arrow), two sporocysts (arrowheads). Each sporocyst has four sporozoites (small arrow) that are not in complete focus. Unstained (Dubey, 2010).

of shedding oocysts after the initial infection (prepatent period) in cat vary between 3-10 days after ingesting tissue cysts and is over 18 days after ingestion of oocysts or tachyzoites (Dubey, 2010). At the time of shedding, oocysts are unsporulated and not infectious to hosts. The sporulation occurs in the environment on exposure to various climatic conditions (air, humidity, temperature) within 1-5 days. During sporulation, the unsporulated oocyst (10-12 μm in diameter) becomes a sporulated oocyst (11-13 μm in Diameter) with two sporocysts both containing four sporozoites. Thus each sporulated oocyst contains eight single-celled sporozoites (Dubey, 2010).

1.3.2. Asexual life cycle:

This cycle is induced either by oocysts, bradyzoites in tissue cysts or tachyzoites. The asexual cycle occurs in all hosts and ends usually with formation of tissue cysts (Dubey, 2010). After the sporulated oocysts ingestion, sporozoites excyst and penetrate cells of the intestinal epithelium. Sporozoites move quickly to various cells, mostly the vascular endothelium, fibroblasts, mononuclear cells and segmented leukocytes in the lamina propria. Sporozoites divide into two tachyzoites. Tachyzoites are responsible of the primary infection, and undergo rapid asexual multiplication by repeated endodyogeny. Tachyzoites are the forms predominating in acute toxoplasmosis. Tachyzoites are single-celled, crescent shaped, pointed headed, with a rounded posterior end, and sized approximately 2 x 6 μm. Having no visible means of locomotion with cilia, flagella or pseudopodia, they are still able to move by gliding, flexing, rotating or undulating. Tachyzoites become widely disseminated inside an organism during the parasitemia phase. They start to transform into bradyzoites with the increase of the hosts' immune response. Bradyzoites are structurally similar to tachyzoites, single-celled, crescent-shaped, 5.0-8.5 μm long, but have a different nucleus position (nucleus in tachyzoites is

centrally located and in bradyzoites in the posterior end). Bradyzoites multiply asexually by repeated endodyogeny, intracellularly within tissue cysts in a variety of tissues (Dubey, 2010).

Tissue cysts have a high affinity for neural and muscular tissues. They are located predominantly in the central nervous system, the eye, as well as skeletal and cardiac muscles. Tissue cysts of *T. gondii* contained in meat, meat-derived products or offal may be important sources of infection for humans. However, for public health purposes it is important to note that the organotropism of *T. gondii* and the number of tissue cysts produced in a certain organ vary with the intermediate host species (Tenter, 2009).

1.4. Transmission and distribution in the environment:

Toxoplasma gondii has developed many transmission patterns. It has adapted to three main cycles (Figure 2.1) in relation to its three forms: oocyst-oral cycle in herbivores, omnivores and carnivores; tissue cyst-oral cycle in carnivores; and tachyzoite cycle in all hosts (Dubey, 2004). Transmission can be classifed as horizontal or vertical. The horizontal transmission between different host species or from the environmental reservoirs can be foodborne, waterborne, milk-borne, soil-transmitted, cat-litter-box-derived, occupational, or iatrogenic. Consumption of tissue cysts in meat is one of the primary means of *T. gondii* infection, both for humans and for meat-eating, warm-blooded animals (Weiss and Kim, 2011*).* The consumption of raw or undercooked meat containing *T. gondii* tissue cysts and the consumption of raw vegetables or water contaminated with *T. gondii* oocysts from cat feces is associated with human illness. The risk of acquiring *Toxoplasma* infection via food varies with cultural and eating habits in different human populations (Hussain *et al.,* 2017). The three infectious forms can be zoonotic, tissue cysts in undercooked meat from

infected animals are considered to be the main risk factor for humans (Cook *et al*., 2000; Tenter *et al*., 2000).

Pigs, sheep, goats and cattle are epidemiologically important as a source of infection to human (Tenter *et al*., 2000).The vertical transmission of the parasite is blood-borne and can result in congenital toxoplasmosis (Dubey, 2004; 2010; Jones and Dubey, 2012).

Tenter *et al*. (2000) detected tachyzoites of *T. gondii* in body fluids, including saliva, sputum, urine, tears, semen and milk of several intermediate hosts, including sheep, goats, cows and camels. An early study reported that *T. gondii* tachyzoites may be isolated from raw chicken eggs laid by hens with experimentally induced infection (Jacobs and Melton, 1966). Professional groups such as slaughterhouse workers, butchers and hunters may also become infected during evisceration and handling of meat (Tenter, 2009). Bradyzoites of *T. gondii* are more resistant to digestive enzymes (i.e. pepsin and trypsin) than tachyzoites (Jacobs *et al*., 1960, Dubey, 1998).

Tissue cysts of *T. gondii* are relatively resistant to changes in temperature and remain infectious in refrigerated (1-4°C) carcasses or minced meat for up to three weeks (Dubey and Kirkbride, 1989, Dubey *et al*., 1990).

The safe way to kill tissue cysts by heating to 67°C or higher (Dubey and Foreyt 2000). Survival of tissue cysts at lower temperatures depends on the duration of cooking. For example, tissue cysts remained viable at 60°C for about 4 min and at 50°C for about 10 min under laboratory conditions (Dubey *et al*., 1990). It is important to note that cooking for a prolonged period of time may be necessary under household conditions to achieve the temperatures that are required to kill all tissue cysts of *T. gondii* in all parts of the meat. Some tissue cysts will remain infectious if cooking procedures

are used in which the meat is heated unevenly, for example, microwave cooking (Lundén and Uggla, 1992).

After primary infection with tissue cysts or oocysts of *T. gondii*, a single cat may shed more than 100 million oocysts into the environment. Under environmental conditions with sufficient aeration, humidity and warm temperature, oocysts can sporulate and become infectious within one day, while sporulation may be delayed under microaerophilic conditions (Tenter, 2009).

Unsporaluted oocysts are less environmentally resistant, and their lifespan and infectivity are affected by climate conditions, particularly by extremes of temperature and decreased relative humidity (Meerburg and Kijlstra, 2009). Unsporulated oocysts exposed to 37°C for 24 hours were killed. After the sporulation and depending on the surrounding climatic conditions oocysts may remain infective for several years, (Dubey, 2010). Moist soil, sand, or surface water offer favorable conditions for *T. gondii* oocyst, while dryness and temperatures over 50°C or less than 4°C will inhibit the sporulation process, and heating will kill the oocyst (Dubey, 2010; Lélu *et al*., 2012). Freezing and most of the disinfectants do not kill *T. gondii* oocysts, but ultraviolet rays and heating to 55-60 °C are deleterious to them (Dubey, 2010).

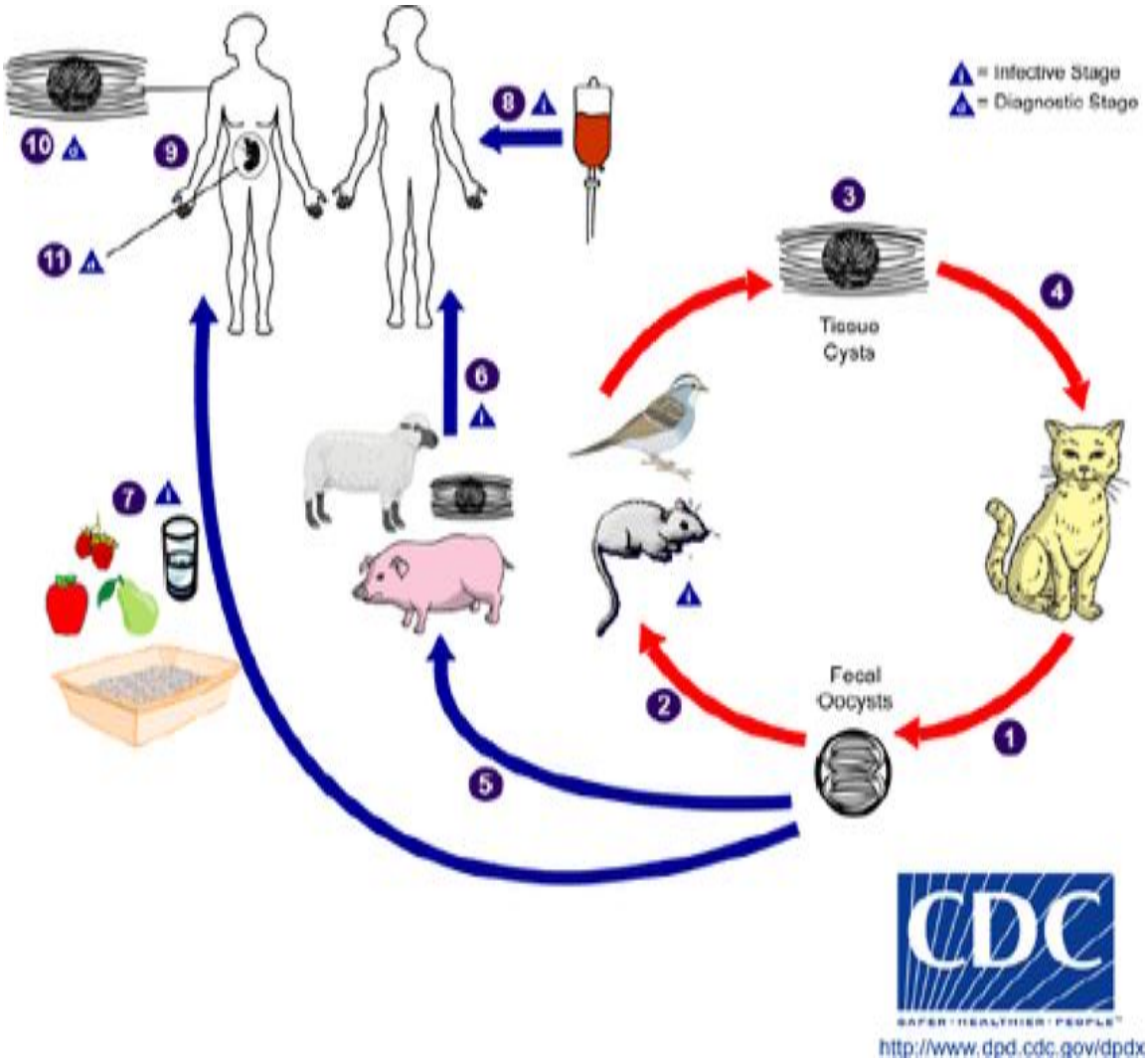

Figure1.2. Life cycle and mode of transmission of *Toxoplasma gondii* in different species of animals. Source: Keith, 2010.

1.5. The infection and clinical disease in cattle:

1.5.1. Epidemiology:

Toxoplasmosis occurs worldwide, the infection rates differ significantly by country (Pappas *et al*., 2009). The difference depending on the climatic conditions those are relevant for the sporulation and survival of oocysts (Dubey, 2010). Places with higher humidity and warmer climate, that is, better oocysts survival conditions have higher prevalence (Klun *et al*., 2006). Splendore in Brazil, reported the protozoan in a rabbit, while Nicolle and Manceaux identified it in a North African rodent, the gundi *(Ctenodactylus gundi)* (Weiss and Dubey, 2009). Toxoplasmosis is common in sheep, goats, pigs, and chickens as intermediate hosts; cattle and horses are notably resistant to the disease. In sheep, congenital infection is a leading cause of stillbirth and preterm lamb loss. Lambs that are born infected and survive usually exhibit normal growth, but they still represent a public health risk if their infected meat is consumed (Dubey, 2009).

When cows being herbivorous animals (> 18 months of age), can get the infection from the environmental oocyst reservoir, for example by ingesting feed or water contaminated with *T. gondii* oocysts. They might also get the infection by accidentally eating a rodent or other host carrying tissue cysts. The infection is possible also via semen from infected hosts (Scarpelli *et al*., 2009). Calves may acquire the infection prenatally, via placenta (Canada *et al*., 2002; Costa *et al*., 2011), or postnatally via milk (Dubey, 1986; EFSA, 2015) or from other feed, water or the environment. Heifers, from the age 7 to 18 months, most likely get the infection from the same sources, feed, water or the environment, as adult cows.

1.5.2. Clinical infections:

There have been few studies focusing on the clinical signs of cattle with toxoplasmosis (Esteban- Redondo *et al*., 1999). In general, the clinical signs in cattle are often nonspecific and considered mild (Canada *et al*., 2002).

Sanger *et al*. (1953) reported the first natural infection in cattle, with major losses and even death in younger animals. Esteban-Redondo *et al*. (1999) detected clinical signs in 10 calves aged 6-7 months during the first week after infection with *T. gondii*. All animals showed an increased respiratory rate and a febrile response. Temperature rose on day 5 and returned to reference values on day 9.

In livestock, reproductive disorders cause problems to the livestock industry and economy. Before the discovery of *Neospora caninum*, which is now considered to be a major cause of cattle coccidian abortions, it is probable that the cause for reproductive problems in cattle was sometimes misdiagnosed as *T. gondii* (Thilsted and Dubey, 1989).

Even though *T. gondii* is not a major cause in bovine abortions, there are studies reporting reproductive disorders, such as abortions due to the infection and congenital toxoplasmosis in calves (Gottstein *et al*., 1998; Canada *et al*., 2002; Ortega-Mora *et al*., 2007; Gharekhani, 2014). While studying *T. gondii* infections in experimentally infected gestating cows, the group isolated *T. gondii* from the brain and liver of four cows, from the placenta of two cows and from the gastric contents of two fetuses, a study of Gottstein *et al*. (1998) detected *T. gondii* DNA in 5% of bovine fetuses, and one fetus was positive for both *N. caninum* and *T. gondii* antibodies. Costa *et al*. (2011) examined 50 gestating cows and nine tested seropositive for *T. gondii*. Of these nine animals, one fetus also had antibodies against

T. gondii, and the parasite was isolated by bioassay in the cerebral and retinal tissue samples from fetuses of three seropositive cows.

1.6. Zoonotic implication of Toxoplasmosis:

In humans, toxoplasmosis causes a variety of disease syndromes ranging from flu-like symptoms in immunocompetent adults, to severe disseminated disease in immuno-suppressed individuals and birth defects in infants when women get exposed during pregnancy (Radostits *et al*., 2006; Dubey, 2010; Innes, 2010).

Toxoplasmoses in humans are present globally, but the prevalence varies geographically and between populations (Dubey, 2010). It is the most prevalent infection in humans (estimated to be 30–50% of the world population) more than latent tuberculosis which infects about one-third of the human population (WHO, 2013). In USA toxoplasmosis is the second foodborne illness cause death (Scallan *et al*., 2011; Gao *et al*., 2016). The prevalence varies due to different cooking habits, the level of hygiene, the environment and implemented preventive measures. People who are in contact with soil and animals show higher prevalences (Dubey, 2010). For women of childbearing age, a survey of 99 studies within 44 countries found the areas of highest prevalence are within Latin America (about 50–80%), parts of Eastern and Central Europe (about 20–60%), the Middle East (about 30–50%), parts of Southeast Asia (about 20–60%), and parts of Africa (about 20–55%) (Pappas *et al*., 2009).

There has been human toxoplasmosis outbreaks linked to consuming raw or undercooked beef (Dubey, 2010; FAO/WHO, 2014).

1.6.2. Clinical infections:

Infection in humans can range from asymptomatic to severe, depending on the parasite strain and the immune status of the host. The majority of cases

in human are asymptomatic and infection rates in some areas are as high as 70% (Pappas *et al*., 2009). *Toxoplasma gondii* is a well-adapted parasite and once inside a host, it may persist dormant in tissues for a long time, even a lifetime – or cause a severe disease. Symptoms of human toxoplasmosis depend on the parasite infectious stage, virulence of the parasite strain, age and physical condition of the infected human. During pregnancy acquired infection may cause severe damage to the fetus. In immunocompromised patients, reactivation of latent disease can cause life-threatening encephalitis (Montoya and Liesenfeld, 2004).
The *Toxoplasma* parasites multiply within cells that line the human digestive tract and can spread to almost any organ in the body, including the brain, skeletal muscles, heart muscle, eyes, lungs and lymph nodes. In healthy people, the body's immune system eventually stops the spread of *Toxoplasma* parasites, although some remaining parasites can lie dormant in the brain or retina. Up to 90% of cases of toxoplasmosis do not cause any symptoms, few cases develop: painless swelling of the lymph nodes headache, malaise (a general sick feeling), fatigue and low-grade fever. In rare cases, patients also have experienced muscle aches, sore throat, abdominal pain, rash or neurological symptoms (Harvard, 2019). Individuals with compromised immune systems, including those treated with corticosteroids, cytotoxic medicines, and antibody to tumor necrosis factor alpha ,l atent or primary toxoplasmosis can be particularly dangerous (Lykins *et al*., 2016; Wang *et al*., 2017). Approximately one third of human immunodeficiency viruses (HIV), infected individuals with *T. gondii* infection develop encephalitis (Walker and Zunt, 2005). Initial infection acquired by pregnant women may cross the placenta and reach the fetus (McLeod *et al*., 2014). Woman develops toxoplasmosis during pregnancy or within six weeks before becoming pregnant, her child may be born with congenital toxoplasmosis (Harvard, 2019). This congenital infection may

be systemic and result in fetal death, premature birth, intrauterine growth retardation, fever, pneumonia, hepatosplenomegaly, thrombocytopenia, or involve the eyes and brain (McAuley *et al*., 1994; Peyron *et al*., 2016).

1.7. Diagnosis:

As the clinical signs of toxoplasmosis are usually nonspecific, and in farm animals a detailed clinical history is often lacking, laboratory tests are needed for final diagnosis. In laboratory *T. gondii* infectin can either be diagnosed directly or indirectly. The direct detection of the parasite can be done by microscopy (histopathology, cytology), bioassays of tissues in laboratory mice or cats, or by detection of *T. gondii* DNA. For indirect methods there are several procedures for detecting antibodies that indicate a previous infection (Dubey, 2010).

1.7.1. Direct detection methods:

Tachyzoites may be detected by microscopic examination during the acute phase of toxoplasmosis from body fluids and tissues. This method shows insufficient reliability if the parasite load is low (Luptakova *et al*., 2012). Dubey (1986) noted that in cattle, *T. gondii* were usually microscopically seen only when their number was > 10 000/g of tissue.

Bioassays of the tissues in mice and cats have been used for the isolation of *T. gondii* from infected animals (Dubey, 2010), and cell cultures can be used as well (Jokelainen, 2013). Samples for inoculation and bioassay depend upon circumstances. The samples taken from cattle for bioassay should be from places where the organism is recovered more likely, such as the mesenteric lymph nodes, intestines, liver, heart, skeletal muscles, kidneys and diaphragm (Dubey, 2010). Dubey (1983) recovered ten to 10 000-fold more *T. gondii* from mesenteric lymph nodes and intestines than from liver and lungs of calves. By the time toxoplasmosis is suspected in

cattle, they have probably formed tissue cysts and are chronically infected. This means the number of *T. gondii* in the tissues is low and it may be necessary to concentrate *T. gondii* in the inoculum. Thus a pepsin (which destroys tachyzoites) or trypsin digestion method is used for destroying the tissue cysts wall and releasing bradyzoites into the suspension (Dubey, 2010). The suspension is then inoculated by subcutaneous or intraperitoneal route into mice. For larger volumes of tissues (such as cattle), *T. gondii* free cats have been used, by feeding them the tissues (Dubey, 2010). For the diagnosis, mice brain is examined for *T. gondii* tissue cysts and cat feces are examined for oocysts (Esteban-Redondo *et al.*, 1999; Dubey, 2010).

Since bioassay in mice and cats are time consuming and ethically questionable, molecular methods, such as detection of parasitic DNA with polymerase chain reaction (PCR) is recommended (Esteban-Redondo *et al*., 1999). PCR is known to be highly sensitive, specific and fast for the diagnosis of *T. gondii* infection. Moreover, the results resemble those from 20 mouse inoculation (Esteban-Redondo *et al*., 1999; Dubey, 2010). For PCR, amniotic fluid, blood, samples of tissues and cerebrospinal fluid can be used as specimens. PCR-methods can also be used for genotyping (Luptakova *et al*., 2012, Jokelainen, 2013). Since the distribution of tissue cysts and the density of the parasite in bovine tissues can be low, the sensitivity of PCR may be limited due to small amount of samples tested (Esteban-Redondo *et al*., 1999).

1.7.2. Indirect detection methods:

There are several serological tests available for the detection of *T. gondii* antibodies . In one type of tests the observer judges the given colour of tachyzoites under a microscope, such as with the dye test (DT) and indirect fluorescent antibody (IFA) test. Another test depend on the principle of agglutination of *Toxoplasma* tachyzoites, red blood cells or latex particles,

as with the direct agglutination test (DAT), modified agglutination test (MAT) and indirect haemagglutination test (IHA) and latex agglutination (LA) test, respectively. In enzyme-linked immunosorbent assay (ELISA), the degree of colour change defines the quantity of specific antibody in a given solution (OIE, 2017).

The dye test (DT) described first by Sabin and Feldman in 1948 is the so-called ‘gold standard’ serological test for *Toxoplasma* antibody in humans. Live *Toxoplasma* tachyzoites are incubated with a complement-like accessory factor and the test serum at 37°C for 1 hour before methylene blue is added. Specific antibody induces membrane permeability in the parasite so that the cytoplasm is able to leak out and the tachyzoite does not incorporate the dye and so appears colourless. Tachyzoites not exposed to specific antibody (i.e. a negative serum sample) take up the dye and appear blue. The dye test (DT) is both specific and sensitive in humans, but may be unreliable in other species. In addition, it is potentially hazardous as live parasite is used. It is expensive and requires a high degree of technical expertise. It should be noted that on animal welfare grounds, tachyzoites should be grown in tissue culture rather than in mouse peritoneum whenever possible (OIE, 2017).

The indirect fluorescent antibody (IFA) test described first by Munday and Corbould in 1971 is a simple and widely used method. Whole, killed *Toxoplasma* tachyzoites are incubated with diluted test serum, the appropriate fluorescent antispecies serum is added, and the result is then viewed with a fluorescence microscope. Fluorescent-labelled antibodies are available commercially for a variety of animal species, the method is relatively inexpensive and kits are commercially available. However, the method requires a fluorescence microscope and the results are read by eye, so individual variation may occur. It may be difficult to find some species-

specific conjugates and there is a risk of possible cross-reactivity with rheumatoid factor and anti-nuclear antibodies (OIE, 2017).

The direct agglutination test (DAT) first described by Desmonts and Remington in 1980 is both sensitive and specific. Formalinised *Toxoplasma* tachyzoites are added to U-shaped well microtitre plates and dilutions of test sera are then applied. Positive samples will produce agglutination that can be graded, while negative samples will produce a 'button' of precipitated tachyzoites at the bottom of the well. The test is simple and easy to perform although relatively large amounts of antigen are required. Kits are commercially available. It is important to treat sera with mercaptoethanol to avoid false positives due to non-specific IgM. The procedure was modified by Dubey and Desmonts (1987), who called it the modified agglutination, test (MAT). The MAT has been used extensively for the detection of *T. gondii* animals in sera of all species of animals(OIE, 2017).

In latex agglutination test (LAT), the soluble antigen is coated on latex particles, and in indirect hemagglutination test, the soluble antigen is coated on tanned red blood cells. Positive results are observed when the agglutination with antibody occurs. Sensitivity and specificity of the latex agglutination kit were 100 and 96 %, respectively (Golchin *etal.*, 2016). While indirect hemagglutination test detects only IgG antibodies, LAT does not differentiate between immunoglobulin classes. Furthermore, IHA may be nonspecific for animals with titers lower than 1:128 and as well the sensitivity in LAT for livestock could be improved (Dubey, 2010).

The original ELISA uses a soluble antigen preparation made from *Toxoplasma* RH strain tachyzoites and layered into wells in a microtitre plate (Voller *et al*., 1976). The assay is simple, can readily test a large number of samples and is easy to perform with the chosen anti-species

conjugate. Defined anti-species conjugates, substrates and whole kits are commercially available. However, the assay does require a spectrophotometer. The ELISA is well suited to laboratories required to analyse large numbers of samples (OIE, 2017).

1.8. Prevention and Control:

In farm animals, the most important source of *T. gondii* are the oocysts. Oocysts may be found in all places where cats that have the infection have been allowed to defecate. Epidemiological studies have concluded that pastures may be the most common source of infection (Ortega-Mora *et al*., 2007) due to field treatments with manure and beddings from farms where infected cats have lived. To prevent *T. gondii* infection in farm animals, the most important is to avoid feline fecal contamination of feed, bedding, water and pastures. Moreover, appropriate rodent control helps to prevent the infection of the farm animals (and cats) via ingestion of infected rodents (Jones and Dubey, 2012).

Work has been under way to develop vaccines against toxoplasmosis for cats, sheep, and swine. So far, the only successfully effort has been a modified live parasite vaccine for sheep, which is administered before impregnation to prevent congenital infections. This live vaccine consisting of tachyzoites attenuated by repeated passage in mice (Urquhart *et al*., 2001). Available vaccine only for sheep is a commercially produced live preparation vaccine, use in the UK, Ireland, France, Portugal and Spain and New Zealand. It consists of tissue culture grown *T. gondii* tachyzoites attenuated by over 3000 passages in mice (OIE, 2017).

1.8.2. Treatment:

Sulfadiazine and pyrimethamine (Daraprim) are two drugs widely used for treatment of toxoplasmosis (Guerina *et al*., 1994; Chirgwin *et al*., 2002).

While these drugs have a beneficial action when given in the acute stage of the disease process when there is active multiplication of the parasite, they will not usually eradicate infection. It is believed that these drugs have little effect on subclinical infections, but the growth of tissue cysts in mice has been restrained with sulfonamides. Certain other drugs, like diaminodiphenylsulfone, atovaquone, spiramycin, and clindamycin are also used to treat toxoplasmosis in difficult cases (Hill *et al*., 2005).

A combination of pyrimethamine and sulfadimidine has been effective in treatment of toxoplasmosis in sheep (Buxton and Losson 2007).

Chapter Two

Materials and Methods

2.1. Study Area:

Khartoum State, the capital of the Sudan is one of the 18 States of the Sudan and it is the smallest State by area which is about 22,142 km^2. (Sudan Geohive 2008). In 2008 population census estimates the population of Khartoum state to be about 5,274,321 (Sudan census, 2008) .The State is located in the central region of the country, between 15 and 16 degrees latitude north, and between 31 and 32 degrees longitude east(Khartoum Elevation, 2018). The River Nile, Blue Nile and White Nile divide the State into three districts (towns) namely, Khartoum, Khartoum north and Omdurman. The State has seven localities, two in Khartoum (Khartoum and Jabal Aolia), two of them in Khartoum North (Bahri and Sharg elnile) and three localities in Omdurman (Omdurman, Karary and Ombadda).

This study was conducted in two localities in Khartoum State (Sharg elnile and Omdurman)**,** in the period between June 2019 to September 2019, and included dairy farms in Eastern Nile and beef farms in Omdurman.

2.2. Study animals and study desigen:

To estimate the prevalence bovine toxoplasmosis in Sharg elnile and Omdurman localities, across-sectional study was conducted in the period between June-September 2019. All information regarding the potential risk factors associated with the occurrence of bovine toxoplasmosis were collected through a pre-designeel questionnaire. The questionnaire was divided into sections. The first section included the host characteristicslike: age (young animals range from 6-24 months, old animals > 25 months) breed (local and cross) and sex (male or female). The second section included factors that related to the environment such as: location (Sharg

elnile and Omdurman) herd size (small herd 0-100 animals and large herd 150-1000) presence of cats in the farms (presence and absent) water sourse (well or Tap water) , present or absent of other species in the farms and hygienic conditions at the farm (high, moderate, and low). High referred to farms that was cleaned every two days , moderate referred to farms that was cleaned every week or two time at week and low referred to farms cleaned one time every two weeks or more. The questionnaires were filled for each examinal cattle through face to face interviews with cattle owners.

All animals were kept in intensive management systems with large confinements, zero grazing and large amounts of input in the form of feeding and labor.

2.3. Sampling method and sample size determination:

Samples were selected by using random sampling methods. Selected animals randomly due to the difficulty of controlling the animal in one line, lack of some farms on the narrow lane system and the limited number of workers on the farm.

The sample size was determined by using the formula of Thrusfield (2007) and 95% confidence interval as following:

$n=1.96^2.P_{exp}(1-P_{exp})/d^2$

n: sample size

P_{exp}: expected prevalence

1.96: the value of 95% confidence interval

D: desired accuracy level at 95% confidence interval

A previous prevalence of cattle toxoplasmosis (12.7%) was reported by Elfahal *et al.*

Referring to that the sample was calculated as following:

$n=1.96^2(0.12)\ (1-0.12)/0.0025=162$ samples.

2.5. Collection of blood samples:

A total of 162 serum samples were collected from jugular vein of each examinal cattle using disposable Syringe (5ml) after applying disinfection. Blood samples were kept in thermo flask and transported to Diagnostic Laboratory, Department of Clinical Studies, College of Veterinary medicine. Sera were harvested following centrifugation of clotted blood, labeled, and stored at −20°C until tested.

2.6. Diagnosis of toxoplasmosis by Latex test:

Latex agglutination test Toxo-Latex® (SPINRER EACT, S. A. Ctra. Santa Coloma, Spain) was used to screen the sera basically.

The TOXO-Latex reagent is suspension of latex particles coated with soluble *T.gondii* antigen. Latex particles allow visual observation of the antigen-antibody reaction. If the reaction occurs, latex suspension changes and aclear agglutination becomes evident, due to the presence of toxoplama antibody.

After the thawing of the frozen sera, samples were analyzed with Latex agglutination test for the presence of specific anti-*T. gondii* antibodies.

After the reagents (latex, control + and control -) and samples reach room temperature, then measured 50 µL of the test serum, a drop of (50 µL) the positive control serum and the negative control serum were separately placed on the slide and a drop of the antigen (25µL) was added. After mixing the antigen and the serum samples (test and controls) by special sticks, and mixed thoroughly by shaking for ≤ 4 min. After that interpretation was done by examined the slide for the presence or absence

of visible agglutination. The serology was carried out at Sudan University of science and technology.

2.7. Statistical analysis:

All data were entered in excel spread sheet and then transferred to the statistical package for social sciences (SPSS) version 16.0.

Data were analyzed by simple descriptive statistic and expressed frequencies and cross tabulation. A univariate analysis using chi-square test (x^2) was then used to assess the association between risk factor and the disease. A p-value less than 0.05 was considered statistically significant. Then multivariate logistic regression was used for all variables showing a statistical significance ($p < 0.05$and < 0.25) in the univariate analysis, to determine the strength of factor in occurrence of disease.

Chapter Three

Results

3.1. Overall prevalence:

From a total of 162 animals 24 were found positive (14.8%) for toxoplasma. The overall prevalence of cattle toxoplasmosis in Khartoum State, Sudan was 14.8% (Table3. 1).

Table (3.1): The overall prevalence of cattle toxoplasmosis in Khartoum State, Sudan:

Result	No. of animals	frequency%
ve+	24	**14.8**
ve-	138	**85.2**
Total	162	**100**

3.2. Risk factor analysis:

Regarding the age of examined cattle 9 out of 48 young animals and 15 out of 114 adult animals were found infected with *T.gondii* (Table 3. 2). Higher prevalence was detected in younger animals (18.8%) than in old animals (13.2%). There was no significant association observed between Toxoplasmosis infection and age factor (X^2=0.837; *P* =0.247) (Table 3. 2).

Out of 55 males 4 of them were positive, while 20 females were positive from 107 animals (Table3.2). Prevalence was higher in female animals (18.7%) than in male (7.3%), and there was significant differences between two different sexes (X^2=3.753, *P*=0.040) (Table 3.2).

Considering the breed, blood sample were taken from 48 local breed and 114 from cross one, and the parasite was detected in 4 and 20 animals respectively (Table3.2). Local animals shown less prevalence (8.3%) than cross animals (17.5%) and there was no significant association between Toxoplasmosis infection and different breeds (X^2=2.271; *P*=0.100) (Table 3.2).

According to location, 114 animals tested in Sharge elnile and 20 animals were positive, as 48 animals tested from Omdurman and 4 animals where as positive (Table3.2). cattle that located in Sharge elnile had a higher prevalence (17.5%) than that located in Omdurman (8.3%) and the chi-square test of Toxoplasmosis shown that there was no significant association between location of examinal cattle and T.gondii infection (X^2=2.271; *P*=0.100) (Table2.3).

Regarding herd size, from small herd 82 animals were tested and 17 were positive but from large herd 80 animals were tested and 7 of them were positive (Table3.2). The prevalence proportion of Toxoplasmosis was higher in small herd (20.7%) than that reported in cattle kept in large herd size (8.8%). There was a significant association between Toxoplasmosis and herd size (X^2=4.606; *P*=0.026) (Table3.2).

Concerning presence of other animals species, farm that included sheep and goats were recorded lower prevalence (14.3%) than those had only cattle (15.4%) and there was no significant association observed between T.gondii and presence of other animals species within the herd (X^2=0.039; *P*=0.509) (Table3.2),

Higher prevalence was shown in farms where cats were present (19.0%) compared to farms with no cats (7.0%). This result indicated that presence

of cats within a herd is considered a risk factor (X^2=4.237, *P*=0.030) (Table3.2).

There was no significant difference in the number of *T. gondii* positive animals in the farms that used wells system for drinking (14.8%) than that used tap water (15.0%) (X^2=.001, *P*=0.574) (Table3.2).

high prevalence of Toplasmosis was detected in farms with high hygienic condition (26.1%), followed by farms with low hygiene (15.3%) and the low prevalence was recorded in farms with moderate condition (7.3%). There was no significant association observed between T.gondii infection and different hygienic condition (X^2=4.161; *P*=0 .125) (Table3.2).

Table (3.2): Summary of univairate analysis of the risk factors associated with Toxoplasma infection in cattle in Khartoum,State, Sudan:

Risk factor	**No. of tested**	**No. of positive**	**% of positive**	**Chi-squar**	***p*.value**
Age groups					
Young	48	9	18.8	0.837	**0.247**
Adult	114	15	13.2		
Sex					
Male	55	4	7.3	3.753	**0.040**
Female	107	20	18.7		
Breeds					
Local	48	4	8.3	2.271	**0.100**
Cross	114	20	17.5		

Localities					
Eastern Nile	114	20	17.5	2.271	**0.100**
Omdurman	48	4	8.3		
Size of herd					
Large	80	7	8.8	4.606	**0.026**
Small	82	17	20.7		
Other animal					
Present	84	12	14.3	0.039	**0.509**
Absent	78	12	15.4		
Cats in farm					
Yes	105	20	19.0	4.237	**0.030**
No	57	4	7.0		
Sours of water					
Well	122	18	14.8	0.001	**0.574**
Tap water	40	6	15.0		
Hygienic condition					
High	23	6	26.1	4.161	**0.125**
Moderate	41	3	7.3		
Low	98	15	15.3		

3.3. Multivariate analysis:

Multivariate logistic regression was done for all variables showing statistical significance in the univariate analysis (P<0.05) include: sex, herd size and present of cats. Also p<0.25 included age, breed, localities and hygiene condition. The result show there was no significant association observed (Table3.3).

Table (3.3): Multivariate association of anti-toxoplasma antibodies positive status of Toxo-latex agglutination test in Khartoum State, Sudan:

Risk factors	**No. of tested**	**No. of positive**	**% of positive**	**Exp(B)**	***P* .value**
Sex					
Male	55	4	7.3	1.303	**0.999**
Female	107	20	18.7		
Age groups					
Young	48	9	18.8	0.567	**0.372**
Adult	114	15	13.2		
Breeds					
Local	48	4	8.3	3.33	**0.998**
Cross	114	20	17.5		
Localities					
Eastern Nile	114	20	17.5	3.33	**0.998**
Omdurman	48	4	8.3		

Cats in farm					
Yes	105	20	19.0	000	**0.999**
No	57	4	7.0		
Size of herd					
Large	80	7	8.8	0.556	**0.462**
Small	82	17	20.7		
Hygienic condition					
High	23	6	26.1	0.561	**0.433**
Moderate	41	3	7.3		
Low	98	15	15.3		

Chapter Four

Discussion

Insufficient data until now are available on cattle toxoplasmosis in the world, and there have been limited number of reports on cattle toxoplasmosis from Sudan. A serological investigation for Toxoplasma antibodies in 4 animal species from Kordofan and central regions of the Sudan was carried out using indirect haemagglutination test detected that positive sera, 63 p. 100 for goats, 54 p. 100 for camels, 40 p. 100 for cattle, and 34 p. 100 for sheep(Zain eldin etal., 1985). Khalil and Elrayah, (2011) found that seroprevalence of toxoplasmosis was 32% in Khartoum state and that was higher than the present study. Other survey was carried out to study the prevalence of toxoplasmosis in cattle in Khartoum and Gazira States, were assayed for antibodies to *T. gondii* by ELISA, 12.7% and 14.9 was detected in Khartoum and Gazira State respectively (Elfahal *et al*., 2013). In El-Gadarif state, Sudan, the overall sero-prevalence was 52.0% using Toxo-latex agglutination test and 45.7% and 27.2% using IELISA in sheep and goats, respectively (Atail *etal*.,2017). In the present study on overall seroprevalence of toxoplasmosis was reported among cattle in Sharg elnile and Omdurman localities using LAT test (14.8%) this result higher than that reported for cattle in Brazil (2.68%) (Fajardo *et al.*, 2013), France (7.8%) (Gilot-Fromont *et al*., 2009), Portugal (7.5%) (Lopes *et al*., 2013), West Indies (8.4%) (Chikweto *et al*., 2011), and Iran (2.3%) (Gharekhani, 2014).This result was lower than that (76.3 %) recorded in Serbia (Klun *et al*, 2006) and Algeria (28.7%) (Abdallah *et al*., 2019). The differences in prevalence proportion among countries may be due to the samples size of different studies, the wide geographic area concerned or

covered, management practices (traditional, semi-intensive, and extensive); and also geographical within and among different countries.

In the present study high prevelance was reported in young animals (18.8%) and low prevalence was in adult (13.2%) and there was no significant association between age of animals and toxoplasmosis infection and that may be due to sample size, the number of adult animals(114 animals) that tested was bigger than young animals (48 animals). These findings are differ to those of Jittapalapong *et al*. (2005), Teshale *et al*. (2007), Tilahun *et al*. (2019) and Abdallah *et al*, (2019) who reported high prevalence in adults animals and low in young. This could be due to a longer exposure of the adults to *T. gondii* infection (Tenter *et al*., 2000).

With regard to the sex risk factor, the study showed that the seroprevalence of anti-*T. gondii* antibody is higher in cattle females (18.7%) than in cattle males (7.3%) and there was asignifecant difrrence between two sexes (p=0.040). This is in agreement with that reported by Ahmad and Qayyum (2014), and Abdallah *et al*. (2019) and in disagreement with result reported by Fajardo *et al.* (2013) and Elfahal *et al* (2013) who confirmed that toxoplasmosis infection was higher in males than females.

There was no significant difference of toxoplasmosis seroprevalence in examinal cattle breeds and within different localities of the study area, similar findings have also been reported previously in cattle in Pakistan (Ahmad, and Qayyum, 2014). Also this was consistent with the findings of Elfahal *et al*. (2013) who found no significant relationship between antibody prevalence and location and that the prevalence was 12.7% in Khartoum state and 14.9% in Al-Gazira state and also localities (12.9%, 14.0%, 10.3%) in Khartoum, Khartoum North and Omdurman respectively, and 25.0% in Alkamleen and 12.8% in Wad Madani localities. This was in agreement with the results of Elfahal *et al*. (2013) who found no significant relationship between antibody prevalence and

breed. This suggests an equal susceptibility to the infection in breeds and localities.

The size of the herd, specialy when it is small, is considered as the major risk factor in the present study and suggests horizontal transmission between animals, also the livestock's aliment is widely accessible to cats, this result similer to that founded in Algeria (abdallah *et al*., 2019). Gilot-Fromont *et al*. (2009) found a positive correlation between herd size and seroprevalence. Similar correlation was detected also by Klun *et al* (2006).

There was no relationship between the presence of other animal species in the farms and the seropositive cattle. This result in disagreement with Fajardo *et al* (2013) that found there were significant differences in the number of positive animals in relation to the variable amount of different species ($p = 0.002$, $\chi 2 = 16.465$). The explanation that the presence of other animal species in farm is increased the chance of finding one seropositive bovine.

This study also showed a higher number of positive animals in farms where cats were present in the vicinity (19.0%) compared with farms free of cats (7.0%). This result confirmed significant association between presence of cats within farms and toxoplasmosis infection ($x^2=4.237$, $p=.030$). Similar result observed that the presence of cats in the vicinity of the farm also stood out as a significant contributing factor for the spread of the infection (Ahmad and Qayyum, 2014). This result is due to the fact that the presence of, and close contact with cats is a very important factor in the epidemiology of toxoplasmosis. Cats shed millions of oocysts in the environment, which could be ingested by animals along with food and water. Direct contact with cats is less important than the presence of cats in the vicinity because cats' shedding of oocysts is the source of infection, rather than direct contact with cats (Dubey and Beattie, 1988).

In the present study no significant difference was detected in the number of *T. gondii* positive animals in the farms that depended wells or taps water in drinking system. This result agreed with Fajardo *et al*. (2013) who found there was no significant difference in the number of *T. gondii* positive animals in the farms that have, or do not have at least one water source.

In this study, it was found that farms with a high level of hygiene contain a greater number of positive animals followed by farms with a lower level of hygiene then the moderate one, and there was no significant association observed ($x2=4.161$, $p= .125$). It is possible that the information obtained from the owners of farms is not accurate in terms of disposal of animal droppings and periodic hygiene. Other study found that the farms with poor hygienic conditions showed a significantly higher number of seropositive animals (Ahmad and Qayyum, 2014).

Conclusions and Recommendations

Conclusions:

The present study confirmed that toxoplasmosis is prevalent (14.8%) among cattle in Sharge elnile and Omdurman and risk factors like sex, herd size and presence of cats in the farms are potential factors contributing in the occurrence of cattle toxoplasmosis infection.

Recommendations:

1. Set the control and prophylactic measures and strategies to reduce *T. gondii* infection in domestic animals.

2. The animal owners should be sensitized through education on the modes of transmission and prevention of *T. gondii* infection among livestock.

3. Further study should be conducted to explore the impact of the disease on food animal production.

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Appendices

Appendix 1

Questionnaire

Owner name:

Name of the farm:

No. animal:

Section one

Sex:

Male ( ) Female ( )

Age:

6-24 months ( ) animals > 25 months ( )

Breed:

Local ( ) Cross ( )

Section two

Location:

Sharg elnile ( ) Omdurman ( )

Herd size:

0-100 animals ( ) 150-1000 animals ( )

Presence of cats in the farms:

Presence ( ) absent ( )

Water sourse:

Well ( ) Tap water ( )

Other species in the farms:

Present ( ) absent ( )

Hygienic conditions at the farm:

Cleaned every two days ( ) cleaned every week or two time at week ( ) cleaned one time every two weeks or more ( )

Appendix 2

Figure.3. latex agglutination test, control positive and control negative:

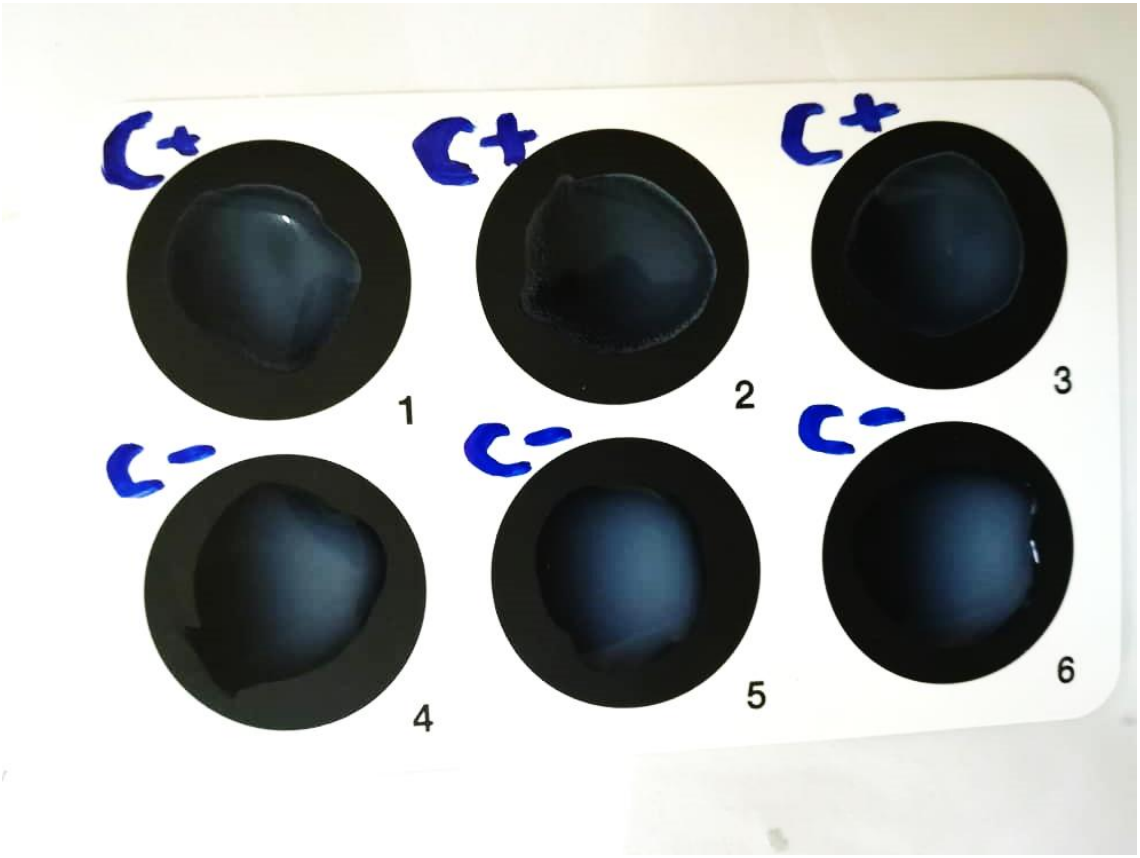

Figure.4. latex agglutination test results: