1. INTRODUCTION

1.1. Background:

Over the last few decades, an intense genetic selection has significantly increased milk yields of dairy animals and the production efficiency of the dairy industry. In order to maintain optimal reproductive performance in a dairy herd, cows should produce one calf each year. Embryonic mortality has a major impact on reproductive efficiency in farm animals (Ortega-Mora *et al.,* 2007). Embryonic mortality has been estimated to be about 20–40% and fetal death about 5–10% (Lopez-Gatius *et al.,* 1996). Pre-natal losses can be caused by infectious and by non-infectious factors. Infection of the embryonic environment can be caused by specific and non-specific uterine pathogens. Specific pathogens (viruses, bacteria and protozoa) enter the uterus by the haematogenous route (e.g. *Toxoplasma gondii*, *Neospora caninum*). The farming of Cattle, sheep, goats as well as camel forms the core of livestock agriculture in many parts of the world (Ortega-Mora *et al.,* 2007; Seri *et al.,* 2003). These farms ruminant industry suffers major economic losses from reproductive failure result in not only loss of offspring and an increase in calving or (lambing/kidding) intervals, but in other factors such as increased culling, reduced milk production as well as reduced value of breeding stock. Routine healthy production is very important to sustain the economy of dairy farms. Many studies on diary

animal reproduction stated that, reproductive disturbances are the main cause of infertility (Borsberry and Dobson, 1989; Ferry 1997). *Neospora caninum* (*N. caninum*) and *Toxoplasma gondii* (*T. gondii*) are two of the most important causes of protozoal parasitic infectious abortion in livestock worldwide (Malik *et al.,* 1990; Dubey and Lindsay, 1996; Huong *et al.,* 1998). They are genetically very closely related (Bjerkas *et al.,* 1984; Dubey *et al.,* 1988; Dubey and Lindsay, 1996) although there are distinct differences in their biology and respective host-pathogen relationship (Dubey and Lindsay, 1996; Innes, 2011). There are no accurate assessments of *N. caninum* and *T. gondii*-induced losses in cattle because of the difficulty of monitoring when small foetuses expelled in the first trimester (Dubey and Schares, 2011). The international Embryo Transfer Society has recommended searching these protozoa agents (together with other viral and bacterial abortifacients) for the safety of animal husbandry, reproduction and production (Ataseven *et al.,* 2006).

N. caninum is one of the most important causes of abortions in ruminants (Dubey and Lindsay, 1996). *N. caninum* is a heteroxenous cyst forming coccidian of animals closely related to *T. gondii* (Dubey and Lindsay, 1996; Dubey *et al.,* 2002). It was first recognized in 1984 in dogs (Dubey *et al.,* 1988; Dubey *et al.,* 2002). Until 1988 (Dubey *et al.,* 1988), *N. caninum* was misdiagnosed as *T. gondii* (Dubey and

Lindsay, 1996; Dubey and Schares, 2011). *N. caninum* is an important cause of abortion and stillbirth in cattle worldwide (Ortega-Mora *et al.,* 2007). The latter reference stated that, Serological surveys suggested that 5-60% cattle are seroposistive worldwide. Infection with *N. caninum* has been strongly associated with bovine abortion in both dairy and beef cattle worldwide. In many countries and regions with an intensive dairy industry it is cited as the most commonly diagnosed cause of abortion (Anderson *et al.,* 1991, 1995). The majority of *Neospora*-associated abortions occur between 4-6 months of gestation (Taylor *et al.,* 2007; Ortega-Mora *et al.,* 2007). Cattle may acquire infection through the ingestion of oocysts that are shed in the faeces of infected dogs, shown to be a definitive host of the parasite, or by congenital infection where the parasite passes from mother to foetus via the placenta (Dubey and Lindsay, 1996; Figliuolo *et al.,* 2004; Taylor *et al.,* 2007; Ortega-Mora *et al.,* 2007; Dubey and Schares, 2011). The earliest stage of pregnancy, the foetus acquires *N. caninum* is unknown; embryos from seroposistive cows found to be not infected with *N. caninum* (Moskwa *et al.,* 2008). The absence of correlation between age and seropositivity for *N. caninum* indicates the homogenous presence of the agent in herds. In this case, the probable transmission would be vertical (Figliuolo *et al.,* 2004). There are very few evidences of the economic, clinical and epidemiological importance of *N.*

caninum in camels, sheep and goats (Masala *et al.,* 2007; Dubey and Schares, 2011). Recent serological surveys indicate 0.6% to 30.8% in sheep and 2% to 23% in goats (Dubey and Schares, 2011).

T. gondii the obligate, intracellular protozoa, produces a disease generates severe economic losses in sheep, where infection becomes a major cause of abortion, foetal malformation, pre-term deliveries and still births (Weiss and Kim,, 2007; Taylor, 2007; Malik *et al.,* 1990). Abortion in ruminants may also involve a very considerable public health risk as many of the pathogens such as *T. gondii* and *Brucella abortus* (*B. abortus*) can pose a significant danger to humans. Thus rapid, accurate diagnosis is vital in order to be able to assess the degree of risk caused by potential ruminant abortifacients with zoonotic potential like *T. gondii*. Because of the zoonotic importance of *T. gondii*, public health organizations, such as the European Food Safety Authority (EFSA), have advised the introduction of monitoring and surveillance programmes for *T. gondii* infections in animals used for human consumption (EFSA 2007; Tenter, 2009). The decline of the incidence of human toxoplasmosis is directly related to the reduction of the infection in animals, which may be achieved through suitable control strategies (Hossein *et al.,* 2008). With exception of only one report (Mbiye *et al.,* 2013), *N. caninum* is not currently considered to be zoonotic. Even though, there is no

strong evidence to date that *Neospora* is a zoonotic pathogen. Contrastingly, all warm blooded animals including humans can be infected with *T. gondii* (Dubey, *et al.,* 2007). Human toxoplasmosis is caused by infection with *T. gondii* and in the immunocompromised individual; in particular one with AIDS, frequently develops toxoplasmic encephalitis which leads to high mortality rate (Weiss and Kim,, 2007). Transmission of *T. gondii* to man is commonly associated with exposure to the oocyst stage in cat faeces, but increasing body of knowledge has shown that meat and milk containing tissue cysts are important source of human infection (Weiss and Kim,, 2007; Taylor *et al.,* 2007). Infection of *T. gondii* may be transmitted to people through the consumption of food or water contaminated with oocysts or through eating undercooked meat containing tissue cysts of *T. gondii* (Weiss and Kim,, 2007; Tenter *et al.,* 2000). Therefore there is a need to look at the disease from control point of view and look at the persistent infections in food animals to help protect public health (Innes, 2010). The diagnosis of *T. gondii* and *N. caninum* abortion are based on the detection of specific antibodies in the adult population by serological tests and the visualization of the agent or its lesion molecularly or histopathologically in the placenta and brain of the aborted foetus. Since direct observation of cysts in tissues is not a suitable diagnostic method to be carried out on live animals, the serological techniques appear to be

the method of choice (Hossein *et al.,* 2008; Dubey and Schares, 2011). Epidemiological assessment of these agents in animals and human beings is based mainly in sero-diagnosis by different antibody detection methods and using different cut-off points. Dairy farm animals play an important role in individual and national income and they are essential source of meat and milk. Farm animals have multifaceted economical impacts. Both a live or slaughtered animals are mainstay of Sudan trade with neighbouring countries. In addition to racing breed camel value. It worth mentioning that local market absorbed the meat of all unproductive farm animals. Cattle are the major component of the Sudanese diet (Naema and Angara, 2014). Angara and Elfadel, (2014) reported 17.31% infertility among examined dairy cattle in Eastern Nile locality due to direct (87.5%) and indirect (12.5%) causes. However, *Brucella* was the only organism discussed as the infectious agent affecting cow's infertility in the investigated area (Elfadel, 2014). About 65,875 SDG, 36,000 SDG and 856,800 SDG was reported as economic losses due to calve loss, culling and milk loss respectively (Elfadel, 2014). For the latter author, veterinary intervention cost, consisted of diagnosis (1,455 SDG), treatment (3,062 SDG). The slaughter house survey revealed that 56.3% of the culled dairy cows are infertile. Most of them were ranked due to indirect causes (54.69%). Metritis and pyometra are the main signs observed (Elfadel, 2014). The available

literature in the Sudan, revealed that meager data on animal toxoplasmosis (El Badawi *et al.,* 1984; Zein Eldin *et al.,* 1985; Abbas *et al.,* 1987; Khalil and El Rayah, 2011; Seri *et al.,* 2003 and Abdel Hafez, 2013) and cattle neosporosis (Amira *et al.,* 2012). Unfortunately, consistent data on human toxoplasmosis in the Sudan have not been reached (Anon 2010; Siddig, 2010). The project of veterinary extension and technology transfer (2012-2016) of the Ministry of Live stock, Fisheries and Range land (MLFR), was based on the development of small producer capacity building in dairy animal production (MLFR, 2012). One of the promising work-shop conducted was producers skill-building in goat breeding for dairy production. In their workshop they addressed some infectious diseases such as brucellosis; however, toxoplasmosis and neosporosis were neglected. Generally, Toxoplasmosis and neosporosis are neglected diseases in the Sudan. Epidemiological information of protozoal agents causing abortion and reproductive failure in farm animals has not been available in the Sudan. Yet these animals have important role in local economy. Risk factors associated with the prevalence of toxoplasmosis in Sudanese and their animals are unknown. Consumption of raw or undercooked meat or/and milk, which are among the main risk factors for acquiring human infection, are popular tradition in the Sudan (Seri *et al.,* 2003). Studies on toxoplasmosis in food animals used for human consumption in the Sudan are very scarce.

No nation-wide survey in the prevalence of *T. gondii* and *N. caninum* infection has ever been conducted simultaneously in the Sudan, neither in animals nor in individuals. This prompted us to conduct the present large scale cross-sectional survey in dairy cattle, and the co-herded camels, sheep and goats from all regions of the Khartoum State as the highest area that rearing and consuming food producing animals and food animal's product respectively. Therefore, the aim of the present study was to determine the seroprevalence of *T. gondii* infection using different serological techniques in dairy animal species mostly used for human consumption in the Sudan culture and to determine the main potential risk factors associated with the infection. Moreover, the economical and public health value of this abortifacient parasite was also discussed. On view of the importance of *T. gondii* as a possible cause of reproductive failure and in worldwide public health, farm animals breeding, the lack of recent data on its large scale epidemiological data in the Sudan, and the lack of data on *N. caninum* large scale epidemiological information in the country, this work aimed to estimate the *T. gondii* and *N. caninum* seroprevalence in dairy cows, sheep, goats and camels by specific antibody search using Latex agglutination test (LAT), indirect enzyme linked immunosorbent assay (ELISA) and competitive enzyme linked immunosorbent assay (cELISA). The socio-economic consequences and

contribution to reproductive failure of these two related protozoa was also discussed using questionnaire. It was also designed to assess possible association between different farm systems and the prevalence of each protozoon. Additionally, the association between farms with seropositive sera to *T. gondii* and *N. caninum* and the presence of cats and dogs was to be evaluated respectively. At last, comparison between the occurrence and serological co-existence of *N. caninum*, *T. gondii* and *B. abortus* in dairy farms was to be available, since the present study takes advantage of our concurrent ongoing national research project on brucellosis conducted by Angara *et al.,* (ongoing).

1.2. Statement of the Research Problem:

Food security is one of the major global challenges we face. Efforts aimed at reducing the impact of disease in livestock will be vital to improve production efficiency. However, some diseases such as early abortion which passed unrecognized due to lack of awareness and lack of means of diagnosis in live animals. Neosporosis and toxoplasmosis are important causes of reproductive failure in farm animals resulting in significant economic losses to producers worldwide (Innes, 2011). However, to date no large scale data regarding the prevalence of these diseases in Sudanese farm animals are available, particularly dairy animals. The incidence of abortion and reproductive failure in dairy animals were found to be very high in the Sudan (Elfahal *et al.,* 2013; Elfadel,

2014) and the complaining of herders is increasing with inadequate diagnostic data and prophylaxis. The economic losses due to neonatal mortalities and missed lactation are probably very high. The very few scientific reports on animal toxoplasmosis available in farm animals in the Sudan (Zein Eldin *et al.,* 1985; Khalil and Elrayah, 2011; Abdelhafez, 2013) were from slaughter houses (table 1.1). Moreover, data concerning neosporosis in the Sudan is scanty (Table 1.2). There are only three published preliminary reports on its occurrence in the Sudan (Amira *et al.,* 2012; Hussein *et al.,* 2012; Manal *et al.,* 2013), only one of them include the Khartoum State (Amira *et al.,* 2012). The number of cattle tested in their studies was very few, and they both recommended large scale investigation on neosporosis in the Sudan. Abortion diagnosis in dairy farms in the Sudan is relatively limited to brucellosis. Data on the dynamics of toxoplasmosis and other abortifacients in domestic herds is needed (Gilot-Fromont *et al.,* 2009). However, little information is available on the factors that determine the level of infection in cattle herds. Therefore, the present study is meant to avail data on a large scale prevalence (individual and herd level) of these abortifacient pathogens (*Neospora caninum* and *Toxoplasma gondii*) using different serological techniques. Possible potential risk factors and the socio-economic consequences of their occurrence were to be analyzed using questionnaire. Generally, there is a little

information on the prevalence of the *T. gondii* and *N. caninum* infection in the Sudan. Though few, there is no information on the risk factors and the economic and public health importance of these abortifacient protozoa. Thus, -in order to reduce the economical losses in dairy industry- the aim of this study was to carry out a cross-sectional survey of antibody against *T. gondii* and *N. caninum* in adult female dairy farm animals including Cows, ewes, Does and she-camel and subsequently, to discuss the possible role of these parasites in the reproduction failure in dairy farm industry. The serological co-existence of these parasites with *B. abortus* in the investigated dairy farms was also determined in this study. Additionally, the study investigated the level of agreement and correlation between the two different commercially available diagnostic kits (ELISA and LAT) used for detection of *T. gondii* IgG antibodies in man and animal worldwide including the Sudan.

Table 1.1: Previous data on Serological surveys for *T. gondii* infection in animals in the Sudan.

Location	Animals	Source	No Examined	P+ve %	Test	Reference
Red sea State	Sheep	Abattoir	700	45.4	LAT	Abdel-Hafez, (2013)
Khartoum	cattle	Farms	134	12.7	ELISA	Elfahal *et al.,* (2013)
Tumbool	Camel	Abattoir	100	44	LAT	Husna *et al.,* 2012
	Cattle		50	32		
Kadaro	Camel Sheep	Abattoir	70 80	20 57.5	LAT	Khalil and Elrayah, (2011)
Camel zone	Calf-camel	field	306	51.7	LAT	Manal and Majid, (2008)
Camel	Camels	field		61.7	LAT	Manal, (2003)

zone						
Butana plains	Camel	field	482	67	LAT	Elamin *et al.,* (1992)
Butana	Camel	Abattoir		12		Abbas *et al.,* (1987)
Butana	Camel	Abattoir		22.5	IHAT	Bornstein and Musa, (1987)
	Cattle		175	40		
Western and Central Sudan	Camel		204	54	HAT, CFT	Zein Eldin *et al.,* (1985)
	Sheep	Abattoir	576	34		
	Goats		134	63		

Table 1.2: Previous Serological surveys for *N. caninum* infection in cattle from the Sudan.

Location	Animals	Source	N Examined	P+ve %	Test	Reference
Sudan	Cattle	Herds	276	15.9%	ELISA	Hussein *et al.,* (2012)
Khartoum	D. Cattle	Farms	200	9.0%	ELISA	Amira et al., (2012)

1.3. OBJECTIVES

1.3.1. The Overall Objective:

To control the economical losses in dairy industry by understanding the prevalence of *T. gondii* and *N. caninum* as possible protozoal causes of abortion and reproductive failure in farm animals and to improve the concept of diagnosis of abortifacient agents other than *Brucella* in the Sudan.

1.3.2. Specific Objectives:

1. To determine the seroprevalence of *T. gondii* and *N. caninum* in dairy farms (at herd and individual levels) from the different localities of the Khartoum State.

2. To determine the differences in seroprevalence of these biologically related parasites in the different animal species (cattle, camels, sheep and goats) co-herded in dairy farms.

3. To assess the distribution of the level of antibody titration and/or percent inhibition in the seropositive sera to *T. gondii* and/or *N. caninum* in different animal species, in order to assess their susceptibility to these parasites.

4. To compare between the seroprevalence of *T. gondii*, *N. caninum* and the concurrently recorded results of *B. abortus* in the sera of these dairy animals (cattle, camels, sheep and goats) elsewhere.

5. To assess the serological co-existence and the possible association between *T. gondii, N. caninum* and *B. abortus* in dairy animals.

6. To improve the diagnostic concept on abortifacient agent's diagnosis in the Sudan.

7. To determine the level of agreement between LAT and ELISA, and to determine the sensitivity and specificity of ELISA against LAT (screening test) in the detection of the specific IgG antibody to *T. gondii* in dairy cows.

8. To assess the potential risk factors associated with *T. gondii* and *N. caninum* in dairy animals.

9. To evaluate the opinion, perception and awareness of dairy farm personnel (owners, workers and farm managers) on causes of reproductive failure (Abortion) in the country.

2. LITERATURE REVIEW

2.1. *Toxoplasma gondii (T. gondii)*:

Toxoplasma, the genus of tissue cyst forming coccidia, includes a single (*T. gondii*) species (Wolf and Cowan, 1937). *T. gondii* is an obligate intracellular apicomplexan protozoan parasite. According to Zia-Ali *et al.,* (2007) and Owen and Trees, (1999), the single species *T. gondii* is grouped into three strains (type I, II and III). Type II is the most predominant and pathogenic strain in humans and animals (Dubey *et al.,* 2008; Sibley, 2003). The parasite is considered as one of the most important parasite in the group of two hosts coccidia (Soulsby, 1986; Urquhart *et al.,* 1996; Dubey, 2004; Taylor *et al.,* 2007; Weiss and Kim,, 2007). The parasite has a wide range of hosts and the parasite is capable to infect different types of host cells (Dubey *et al.,* 1988; Taylor *et al.,* 2007; Weiss and Kim,, 2007).

2.1.1. History of *T. gondii*:

The first recognition of an organism similar to *T. gondii* was made by Laveran in 1900 in a section of the spleen and bone marrow of Java sparrows (Laveran, 1900). *T. gondii* was first recognized in African rodent (*Ctenodactylus gondii*) by Nicolle and Manceeaux, (1908). The first human case was recorded in the same year in a young (20 years) person in Panama (Darling, 1908). However, the first case of

congenital toxoplasmosis was discovered in a child (3 months old) suffering from hydrocephalus (Janku, 1923). There was only a single species causing human infection (toxoplasmosis) and capable of infecting a wide range of hosts (Wolf and Cowan, 1937). The importance of animal toxoplasmosis was first reported in New Zealand (Hartley *et al.,* 1954; Hartley and Marshall, 1957). In the late 1960's cat was incriminated as the animal that could shed the environmentally stable form (oocyst) of the parasite in their faeces (Hutchison, 1965) which led to consider cats as the definitive host of the parasite (Hutchison *et al.,* 1970) and the Sporulated oocyst as the major source of infection for animals and humans (Soulsby, 1986; Urquhart *et al.,* 1996; Dubey, 2004; Taylor *et al.,* 2007).

2.1.2. Biology of *T. gondii*:

Toxoplasma gondii is a facultative heteroxenous, polyxenous protozoon that has developed several potential routes of transmission within and between different host species (Tenter, 2000). The life cycle of *T. gondii* includes several routs of infection between the definitive host (domestic cats and wild felids) (Taylor *et al.,* 2007; Black and Boothroyd, 2000) and the intermediate host (all mammals as well as the cat itself) or between the intermediate hosts themselves (Sukthana, 2006) where the parasite shows its public health importance as food borne parasite. Generally, all developmental stages of Toxoplasma gondii could be

considered as infective stage (Taylor *et al.,* 2007); including, Sporulated oocyst, tachyzoites and the tissue cyst (Bradyzoite). This give the parasite it's characteristic life cycle diagram (routs of infection).

The prepatent period varies depending on the stage of *T. gondii* ingested (Dubey, 2004). It is 3-10 days after ingesting tissue cyst, 19 days or longer for tachyzoites and 20 days or longer after ingestion of Sporulated oocyst.

2.1.2.1. Transmission of *T. gondii*:

T. gondii can be transmitted horizontally and vertically (Taylor *et al.,* 2007; Tenter, 2009). Transmission occurs following ingestion of food, feedstuff and water contaminated with Sporulated oocyst or/and tachyzoites and bradyzoites (Tenter *et al.,* 2000; Dubey, 2004; Weiss and Kim, 2007; Taylor *et al.,* 2007; Tenter, 2009).

Tachyzoites of *T. gondii* have been detected in body fluids, including saliva, sputum, urine, tears, semen and milk of several intermediate hosts, such as sheep, goats, cows and she-camel (Tenter *et al.,* 2000; Tenter, 2009; Manal 2003; Fusco, 2007). Consumption of unpasteurized goat's milk has been associated with acquired clinical toxoplasmosis in humans (Tenter *et al.,* 2000; Tenter, 2009). Though intake of milk or milk by-products containing tachyzoites may cause the infection in animals and humans (Dubey and Beattie, 1988; Ataseven *et al.,* 2006). Tachyzoites can penetrate the

mucus membrane; thereby gain access to the circulatory and lymphatic system of the host (Johnson, 1997).

Tissue cyst (bradyzoites) contained in meat, meat products or offal (visceral organs) is an important source of infection for cats (the final host), other carnivores and humans. About 50% of human toxoplasmosis cases were related to food borne infection (Slifko *et al.,* 2000; Tenter *et al.,* 2000) during consumption of raw or undercooked meat or other edible parts of meat producing animals. The risk of acquiring *T. gondii* infection via food varies greatly with cultural and feeding habits in different human societies. Frequent consumption of raw or undercooked meat has been associated with seropositivity for *T. gondii*. The consumption of undercooked lamb was stronger risk factor (Baril *et al.,* 1999) in some part of Europe. Consumption of beef had been also identified as a risk factor in some European countries (Cook *et al.,* 2000).

Blood transfusion and organs implantation (Schaffner, 2001) also have a role in human Toxoplasmosis. Limited and accidental infections by needles or splashing infective material into eyes during laboratory work or postmortem examination of infected host are also considered as means of transmission (Marquardt and Demaree, 1985).

In case of congenital toxoplasmosis the transmission is through placenta (Dubey, 1996; Sukthana, 2006). Transmission of *T. Gondii* through semen was also reported in

experimentally infected rams (Lopez *et al.,* 2009), buck (Dubey and Sharma,, 1980), swine (Moura *et al.,* 2007), bulls (Scarpelli *et al.,* 2009) and male dogs (Arnates *et al.,* 2009).

Contaminating soil, water and food (Aramini *et al.,* 1999) with oocyst of *T. gondii* were also considered as a source of Infection. Oocyst can also be transmitted mechanically by flies and earth worms as well as dog (Lindsay *et al.,* 1997).

2.1.2.2. Source of infection of *T. gondii*:

Cat is borne free of Toxoplasma infection and start to excrete oocysts following a primary infection (Dubey and Jones,, 2008) after weaning. Its faeces can create a large potent, long-lasting source of infection for animals and humans. Oocyst contamination of dairy farm feeds and bedding, as well as pasture, is a threat to susceptible animals based on the number and distribution of cats in the environment (Dubey, 2008; Dubey and Jones, 2008). It is estimated that at any time given, about 1% of cats shed oocyst (Dubey and Bettie,, 1988). Persistently infected mice, rats, voles, shrews, rabbits and small birds are the most important source of infection for cat (Jackson and Hutchison, 1989).

Cats defecating in farm feeds, such as hay and stored grain, will pose a risk for animals (Plant *et al.,* 1974; McColang *et al.,* 1988; Lopez *et al.,* 2008). Ingestion of contaminated feed and pasture with faeces of infected cat is the most important source of infection for animals (Dubey and Bettie,, 1988; Dubey, 2004; Dubey and Jones, 2008). Contaminated water

represents a real threat to both animal and man (Bowie *et al.,* 1997). Feeding goat whey was also identified as an important risk of infection for pigs (Meerburg *et al.,* 2006).

2.1.2.2.1. Transmission and source of *T. gondii* Infection for Humans:

The oral rout is the natural portal of entry of *T. gondii* (Slifko *et al.,* 2000; Tenter *et al.,* 2000; Ogendi *et al.,* 2013; Weiss and Kim, 2007; Tenter, 2009). Humans become infected with *T. gondii* post-natally by ingestion of oocysts in soil, water and food contaminated with cat faeces including unwashed fruits and vegetables (Bout *et al.,* 2002; Aramini *et al.,* 1999). In some populations of vegetarians, seropositivity to *T. gondii* reached 24-47% (Hall *et al.,* 1999; Roghmann *et al.,* 1999). Ingestion of tissue cyst in raw or undercooked meat or/and meat products of wide range of meat-producing animals (El-Hassan *et al.,* 1991; Cook *et al.,* 2000; Dubey *et al.,* 2003; Dubey, 2004; Dubey *et al.,* 2005; Moura *et al.,* 2007; Bahrieni *et al.,* 2008; Ragozo, 2009) is the main source of human toxoplasmosis. Professional groups, such as abattoir workers, butchers and hunters may be infected during evisceration and handling of infected meat (Buzby and Roberts, 1997; Swai and Schoonman 2009). Blood transfusion or organ-transplantation from infected donor can also act as a source of infection (Schaffner, 2001).

Congenital toxoplasmosis is generally occurs when a woman is newly infected with *T. gondii* during pregnancy (Remington *et al.,* 2006) or just before pregnancy (Vogel *et al.,* 1996). In

immunosuppressed woman, reactivation of an infection acquired before pregnancy can lead to congenital toxoplasmosis (Minkoff *et al.,* 1997).

Milk, when consumed unpasteurized, was also incriminated as a source of human toxoplasmosis (Dubey and Beattie, 1988; Ataseven *et al.,* 2006; Bonyadian *et al.,* 2007). In particular, Goat's milk was reported to be a source of infection for human (Riemann *et al.,* 1975; Sacks *et al.,* 1982; Skinner *et al.,* 1990; Jones *et al.,* 2009). In order to reduce the danger of a primary infection in pregnant women, screening before pregnancy is recommendable (Fiedler *et al.,* 1999).

2.1.2.3. Life cycle of *T. gondii*:

2.1.2.3. 1. Development of *T. gondii* in the Final Host:

Oocyst stage of *T. gondii* is found only in cat including both domestic and wild felids (Weiss and Kim, 2007; Taylor *et al.,* 2007). Following primary infection, cats pass oocysts in their faeces (Dubey and Bettie, 1988) for only 2 to 3 weeks. Most feline infection occur soon after weaning through ingestion of tissue cyst in infected intermediate hosts, or rarely oocysts although congenital infection can take place (Dubey and Jones, 2008). However, cats are more likely to shed oocysts following ingestion of tissue cysts rather than tachyzoites or oocysts (Weiss and Kim, 2007; Dubey, 2001). The latter author stated that, ingestion of one bradyzoite is enough to establish the infection in cat; however, it must

ingest thousands of oocysts to develop an infection. When cat feed on infected meat with tissue cyst, the wall of the cyst is digested and bradyzoites released in the gastro-intestinal tract where they begin the sexual development to form the oocyst stage. Non sporulated oocysts are excreted into environment with the cat faeces (Soulsby, 1986; Urquhart *et al.,* 1996; Dubey, 2004; Taylor *et al.,* 2007). Sporulation takes place outside the cat within 3-5 or 10 days depending on the environment condition (temperature and relative humidity) and become infective to the intermediate hosts (Gajadhar *et al.,* 2004). Infected cats shed about 13 millions of oocyst in the environment for two to three weeks (Schares *et al.,* 2008). In laboratory condition, cat can shed as many as 500 million oocysts after ingesting one infected mouse or fed few bradyzoites (Dubey 2001). Oocyst in cat faeces can survive for 12-18 months in the environment depending on the environmental conditions; and therefore, they are important source of infection for the grazing animals (Tenter *et al.,* 2000; Innes *et al.,* 2009). Shedding of oocysts is more extensive among young cats than old ones (Buxton and Rodger, 2008). Oocysts are distributed in the environment through wind, rain and surface water or harvested food stuffs and feeds. Additionally, oocysts may be spread via earthworm, coprophagous invertebrates and manure (Hiepe and Bushwalker 1991).

2.1.2.3.2. Development of *T. gondii* in the Intermediate Hosts:

All mammals including man are intermediate hosts of *T. gondii* (Soulsby, 1986; Urquhart *et al.,* 1996; Dubey, 2004; Taylor *et al.,* 2007; Weiss and Kim, 2007). They are mainly been infected by eating food contaminated with Sporulated oocysts. Consumption of undercooked meat was also identified as the principle risk factor in several case control studies on *Toxoplasma* infection in human (Cook *et al.,* 2000). After ingestion of oocyst, the sporozoites released and undergo asexual development in the epithelial cells of the gut (Soulsby, 1986; Urquhart *et al.,* 1996; Dubey, 2004; Taylor *et al.,* 2007). Then, tachyzoites are found in the mesenteric lymph nodes (Dubey, 2004), and the circulatory system and other tissues (Wastling *et al.,* 1993) including uterus. That is the acute phase of *T. gondii* infection, which is very short (Dubey, 1994; Dubey and Sharma, 1980). In pregnant animal or woman, the tachyzoites multiply in placenta and invade the foetus (Weiss and Kim, 2007). Second generation tachyzoites initiate the second phase which results in formation of the tissue cyst containing bradyzoites or cystozoites which multiply slowly in tissues (the meat). They are located predominantly in brain, eyes, skeletal and cardiac muscles as well as visceral organs (Dubey, 1998). Bradyzoites, which may persist for life, are the terminal stage of toxoplasma life cycle in the intermediate hosts. However, many authors (Frenkel, 2000)

believe that tissue cysts break down periodically and transform into tachyzoites which re-invade new host cells and again transforming to bradyzoites. These are infective to the final host and any carnivores including humans during eating raw or/and undercooked meat. Tachyzoites could be infective through unpasteurized milk and milk product (Dubey and Beattie 1988; Ataseven *et al.,* 2006).

2.1.2. 4. Pathogenesis of *T. gondii* Infection:

Abortion and neonatal mortality occur when intermediate host (particularly, woman, sheep and goats) suffer a primary infection during pregnancy (Weiss and Kim, 2007; Dubey and Bettie, 1988). Following oocyst ingestion, excystation takes place in the gut releasing sporozoites. These can actively invade the gut cells and multiply to produce tachyzoites. By day four post infection (p.i) tachyzoites may be spread throughout the host body through blood circulation causing parasitaemia which may last until day 12 p.i (Dubey, 1994; Dubey and Sharma, 1980). Antibodies start to appear at this time and the innate and adaptive immune responses are activated to limit tachyzoites multiplication (Lopez *et al.,* 2009; Innes and Vermeulen 2006; Innes and Wastling, 1995). Thus animal, seropositive for *T. gondii* are immune to the parasite although infection persists as bradyzoites within tissue cyst in brain and muscles. Darcy *et al.,* (1988) stated that antibody against *T. gondii* play an important role in the

immune response of the host and their presence is an indication of both an exposure to infection and a vital diagnostic aid. In a pregnant animal, the parasite (Tachyzoites) circulating in the blood is established in the placenta before invading foetus (Buxton and Finlayson, 1986). The parasite makes the placenta and the foetus susceptible to other pathogens (Yildiz *et al.,* 2009). In addition to the virulence of the strain and acquired immunity, animal species is one of the most important factors affecting the pathogenicity of *T. gondii* (Seri *et al.,* 2003; Weiss and Kim, 2007; Taylor *et al.,* 2007; Ortega-Mora *et al.,* 2007; Innes, 2011). Cattle and equines are more resistant than sheep and goats (Munday, 1978; Dubey *et al.,* 1999) and rats are genetically highly resistant (Jacobs, 1956). The inflammatory reactions induced in the placenta by *T. gondii* stimulate synthesis of prostaglandin F which decreases progesterone level and subsequent abortion (Engeland *et al.,* 1996). The basic aspect was that *T. gondii* can cause abortion only once in infected animals. However, recent references (Morely *et al.,* 2005; Ortega-Mora *et al.,* 2007; Buxton *et al.,* 2007; Morely *et al.,* 2008) indicated that repeated transplacental transmission may be more common occur than previously believed.

2.1.2.4.1. Pathology of *T. gondii* infection:

The main pathological changes in infected animal are small white foci and necrosis in cotyledons and focal necrosis in

foetal brain, liver and lungs (Buxton and Finlayson, 1986). Tachyzoites can be demonstrated in association with necrosis and inflammation (Dubey, 2008). Neuro-pathological changes (Giadinis *et al.,* 2009) were reported in both animals and humans toxoplasmosis. Pathological changes in man are more severe than in animals including necrotizing retinochoriodititis, calcification and hydrocephalus (Beverley, 1971b). Myocarditis, interstitial nephritis, encephalitis, hepatitis and pneumonia were the predominant histopathological changes reported in aborted foetuses due to experimental or naturally infected animals with *T. gondii* (Freyre *et al.,* 1997; Ahmed *et al.,* 2008).

2.1.3. Toxoplasmosis or (*T. gondii* infection):

Toxoplasmosis is a worldwide zoonotic disease caused by *T. gondii* (Weiss and Kim, 2007; Tenter, 2009). Toxoplasmosis is one of the major causes of infectious reproductive failure (Freyre *et al.,* 1999; Weiss and Kim, 2007). It causes economic losses in livestock and serious public health implications, due to severe neurological and reproductive symptoms (Tenter *et al.,* 2000). There is a wide spectrum of the diseases associated with *T. gondii* infection which is dependent on: the host species; the immune status of the host and the virulence of the particular strain of the parasite (Innes, 2010). *T. gondii* infection is a common congenital disease in humans and domestic animals. The prevalence of

the disease showed considerable geographical variation (Tenter *et al.,* 2000; Weiss and Kim, 2007; Ortega-Mora *et al.,* 2007; Tenter, 2009).

2.1.3.1. Animal Toxoplasmosis:

Toxoplasmosis is recognized as a cause of abortion, stillbirth, infertility and neonatal mortality in sheep and other domestic animals (Wiss and Kim 2007; Ortega-Mora *et al.,* 2007; Innes, 2011). Among farm animals, sheep are more widely infected with *T. gondii* than other animals (Soulsby, 1986; Taylor *et al.,* 2007). Congenital infection in domestic animals is common, which may results in abortion and neonatal mortality (Hossein *et al.,* 2008; Buxton, 1998). *T. gondii* is a major cause of infectious abortion in sheep in many temperate areas (Innes, 2011). The disease in sheep mainly occurs following primary infection in a pregnant ewe which can result in abortion, mummified foetus, stillbirth or birth of a congenitally infected lamb (Buxton, 1998). Unlike the situation with *N. caninum* following an abortion due to *T. gondii*, sheep develop good immunity against the parasite in subsequent pregnancy (Innes and Vermeulen, 2006). Animals are infected by eating contaminated food, by ingestion of faeces of a cats containing infective (sporulated) oocyst, or by transmission congenitally from mother to fetus. Cats have been shown as the major host for this parasite.

2.1.3.2. Human Toxoplasmosis:

Toxoplasmosis is one of the most common infections in humans worldwide (Tenter *et al.,* 2000; Tenter, 2009). About 3-80% of healthy adults have been exposed to the parasite (Weiss and Kim, 2007). Other serological studies worldwide (Ira *et al.,* 2009) showed that over one third of the human population had antibody against *T. gondii.* This lends to support the importance of the zoonotic view of toxoplasmosis, particularly in pregnant women and immunocompromised patients (Tenter *et al.,* 2000). The disease is one of the most prevalent zoonotic parasitic infections. About two billion people throughout the world are infected, with considerable geographical variation. Human toxoplasmosis infection attributed to man use of animals as pets or for food (Tenter *et al.,* 2000; Tenter, 2009). Its transmission to humans is usually attributed to ingestion of undercooked or raw meat or primary offal (viscera) from infected livestock (Tenter, 2009; Ciamak-Ghazaoi, 2005; Tenter *et al.,* 2000; El Hassan *et al.,* 1991). The fore author stated that, the infection rate in livestock is an important predictor of human toxoplasmosis risk. Since contaminated meat is a significant source of infection in humans, it is particularly important to ensure continuous surveillance of *T. gondii* prevalence in animal species destined for human consumption (Ciamak-Ghazaoi, 2005; Tenter *et al.,* 2000). Unlike neosporosis, toxoplasmosis is a zoonotic disease and infection in people may result in severe disease in the

developing foetus and in immune-compromised individual as well as eye disease in immuno-competent individuals following infection with *T. gondii* (Glanser *et al.,* 1992). Applying methods to estimate disease impact such as Disability Adjusted Life Years (DALYs) has shown toxoplasmosis to be one of the most significant food borne pathogens across the world (Kortbeek *et al.,* 2009).

2.1.3.3. Clinical Manifestation of Toxoplasmosis:

There is a wide spectrum of clinical signs of the diseases associated with *T. gondii* infection which is dependent on: the host species; the immune status of the host and the virulence of the particular strain of Toxoplasma (Innes 2010). Cattle and equines are more resistant to clinical *T. gondii* infection than sheep (Munday, 1978; Dubey *et al.,* 1999; Dubey *et al.,* 2003). The effect of Iodine deficiency on clinical manifestation of toxoplasmosis in Angora goats was discussed by (Slosarkova *et al.,* 1999).

In the Sudan diarrhoea and lymph nodes enlargement were reported as clinical signs of calf-camel delivered by experimentally infected she-camels (Ishag *et al.,* 2006). They also reported different histopathological lesions from the organs of these calf-camels, particularly, haemorrhages, infiltration of lymphoid cells, congestion and focal necrosis. Toxoplasma tachyzoites and cysts were detected in the brain of suckling calf-camels and mice inoculated with milk of

three experimentally infected she-camels (Manal 2003). Congenital toxoplasmosis can cause abortion and neonatal death of calf-camel (Manal and Majid, 2008; Ishag *et al.,* 2006; Hagemoser *et al.,* 1990).

The relationship between congenital toxoplasmosis and diarrhoea in calf-camels was discussed by Manal and Majid, (2008) and Manal, (2003). The role of toxoplasmosis in cattle abortion has been still under discussion (Dubey *et al.,* 2003; Ortega-Mora *et al.,* 2007).

2.1.3.3.1. Clinical Manifestation in Sheep:

Although transplacental transmission has been reported (Lopes *et al.,* 2009; Scarpelli *et al.,* 2009), most of sheep acquire *T. gondii* infection after birth. About only 2% of sheep become congenitally infected and less than 4% of persistently infected sheep transmit *T. gondii* to the next generation (Higa *et al.,* 2010). According to Borde *et al.,* (2006) in animals, particularly in small ruminants (sheep and goats), the economic implication of toxoplasmosis are reflected through abortion (29.2%), stillbirth (19.4%) and infertility (11%) Clinical signs of sheep toxoplasmosis include early embryonic death and resorption, mummification, stillbirths, neonatal death or birth of a live but weak offspring (Buxton and Rodger, 2008). They also recorded pyrexia, dyspnoea and abortion at any stage of pregnancy as well as mild nervous signs manifested by unsteady gaits among aborted ewes (Ahmed *et al.,* 2008). Malformation in the

foetus has also been reported (Woods and Anderson, 1992). Clinical signs of toxoplasmosis are more often seen when ewe in mid pregnancy and become infected for the first time (Ahmed *et al.,* 2008).

2.1.3.3.2. Clinical Manifestation in Cats:

Feline infections are typically subclinical. Ninety percent of *T. gondii* infections in cats has symptomless course even if the current prevalence of serologically positive animals is rather high. Congenitally infected Kittens may have clinical signs (Dubey and Jones, 2008). Common signs include lethargy, pyrexia and anorexia as well as dyspnoea and other signs of pneumonia (Dubey and Jones, 2008). Ocular toxoplasmosis can cause blindness in some infected cats and infection of CNS revealed several nervous signs which can affect feeding of the infected cat (Alison, 2009).

2.1.3.3.3. Clinical Signs of Human Toxoplasmosis:

Human toxoplasmosis is usually being, but may occasionally lead to severe or lethal damages when combined with immunosuppressive states including people with HIV/AIDS or those undergoing cancer therapies (Tenter *et al.,* 2000), organs transplantation, or when transmitted to foetus during pregnancy (Bout *et al.,* 2002; Qublan *et al.,* 2002). *T. gondii* infections during pregnancy can result in abortion or

congenital defects. The most severe signs appear when occur in the first trimester (Remington *et al.,* 2006). These signs in foetus include spontaneous abortion, stillbirth, and a live infant with classic signs of congenital toxoplasmosis such as hydrocephalus or microcephalus, cerebral calcification and retinochorioditis or infant who fails to thrive or has CNS involvement. In addition to apparently normal infant who develops retinochorioditis, learning disability as well as blindness later during his life (Guerina *et al.,* 1994; McLeod *et al.,* 2006; Remington *et al.,* 2006). Various mild symptoms of which lymphadenopathy are the most significant (Remington *et al.,* 2006) may be observed. Lacks of energy or tiredness in the last 12 months were the only clinical features associated with positive titer (Taylor *et al.,* 1997). Severe signs, such as encephalitis, shock, myocarditis, or hepatitis as well as pulmonary and multivisceral involvement may occur, but very rare in immunocompetent humans (Demare *et al.,* 2007).

In people suffering from acquired immune deficiency syndrome (AIDS), and in other immunocompromized states, reactivation of chronic toxoplasmosis results in severe cell destruction, often leading to severe morbidity and mortality. Neurologic disease is the most common sign in those immunosuppressed patients, in addition to encephalitis, disorientation, drowsiness, reflex changes and convulsions which may lead to coma and death (CDC, 2005).

2.1.3.4. Economic Importance of Toxoplasmosis:

Toxoplasmosis has an economic and clinical significance in sheep and goats producing countries (Negash *et al.,* 2004). Therefore, most of toxoplasmosis economical data was reported from sheep toxoplasmosis as well as humans.

Although incidence of ovine toxoplasmosis varies between countries and regions, a conservative estimate by Blwett & Trees (1987) suggested losses of around 1.5 million lambs lost in Europe. In Uruguay Losses due to toxoplasmosis during pregnancy were estimated to be 1.4 to 3.9% of the total number of ewes investigated, amounting to approximately US$1.4–4.7 million for the whole country (Freyre *et al.,* 1999). The incidence of abortion and reproductive failure are very high in the Sudan with inadequate diagnostic data and prophylaxis. The economic losses due to neonatal mortalities and missed lactation are probably very high in the Sudan.

2.1.3.4.1. Socioeconomic Importance of Human Toxoplasmosis:

Seropositivity for Toxoplasma in healthy individuals in the United States varies between 10% to 40%, while in parts of Western Europe and Central America can be as high as 70% to 90% (Tenter *et al.,* 2000). Toxoplasmosis accounts for $3.3

to $7.8 billion per year in economic costs in America. The USA government ranks it as one of the most expensive forms of food poisoning. *T. gondii* infection sicken about 112,500 and killed about 375 Americans due to eating contaminated meat and unwashed fruits and vegetables (Bout *et al.,* 2002; Tenter *et al.,* 200). In addition congenital toxoplasmosis is estimated to cause mental retardation and blindness in as many as 400-6000 children and may kill another 80 foetuses and newborns each year in USA. In Europe congenital toxoplasmosis affect between 1 and 10 in 10,000 newborn babies. *T. gondii* has re-emerged as a threat to human health due to the advent of human immunodeficiency virus (HIV) and advances in medical techniques and the treatment of malignancies using procedures such as transplantation and chemotherapy (Bout *et al.,* 2002; Qublan *et al.,* 2002). The mechanism of disease onset in most of the patients involves the reactivation of persistent and latent toxoplasma infection via tissue cysts. Toxoplasmic encephalitis is the most common cause of focal central nervous system infection in AIDS patients unable to afford multitherapy for HIV (Bout *et al.,* 2002). The importance of human toxoplasmosis is dramatically increasing as the number of AIDS cases rises. In many of AIDS cases, toxoplasmosis is the first indication of human immunodeficiency virus infection (Fauci *et al.,* 1985).

2.1.3.5. Epidemiology of Toxoplasmosis:

T. gondii infection occurs all over the world and the main reason of its widespread is the lack of host specificity (Tenter *et al.,* 2000). It is the common parasitic zoonoses worldwide. The cat plays a central role in the epidemiology of toxoplasmosis. Epidemiological investigations in USA and elsewhere indicated that 60% of cats are serologically positive to Toxoplasma antigen. About three to ten days following infection, cats start to shed oocysts for 2-3 weeks with peak output of ten Millions of oocysts at 6-8 days p.i (Dubey and Frenkel, 1972; Dubey and Bettie, 1988). Less than 200 sporulated oocysts can cause congenital infection in animal like sheep (McColgan *et al.,* 1988). Fifty grams of infected cat faeces may contain millions of oocysts, which can survive after sporulation and remain infective for more than one year (Dubey, 1977; Hutchison *et al.,* 1996). Long term immunity in cat is not effective when it can be re-infected with *T. gondii* later on and shed lower number of oocysts again (Suzan 2008). The mechanical transmission by earthworm, flies and cockroaches as well as dogs also participates indirectly in the epidemiology of the disease in humans and food producing animals (Lindsay *et al.,* 1997; Dubey and Bettie, 1988). Santos *et al.,* (2009) considered dog as a common source of *T. gondii* infection for both, humans and animals. Infection in animals is associated with feed or grazing range land contaminated with sporulated

oocysts (Plant *et al.,* 1974; Innes *et al.,* 2009) and transplacentally (Dubey, 1994). Additionally, seroprevalence of toxoplasmosis in free-ranging chickens is a good indicator of general prevalence of *T. gondii* oocysts in the soil (Dubey *et al.,* 2005). Contact with cats has no influence on the probability of having anti-toxoplasma immunity (assessed by the toxoplasma skin test) while the consumption of raw meat increased this probability (Flegr *et al.,* 1998). According to Alexander and Stimson (1988) and Van der Puije *et al.,* (2000), female animals are generally more susceptible to protozoan infection than male. Oral application of tachyzoites might have cause an infection (Dubey, 1998c; Sacks *et al.,* 1982). *T. gondii* has been detected in semen of experimentally infected rams (Blewett *et al.,* 1982), goats (Dubey and Sharma, 1980) and bulls (Scarpelli *et al.,* 2001).

The role of *T. gondii* infection in cattle in the epidemiology of human toxoplasmosis is an important issue (Dubey 1995). Because one beef carcass infected with *T. gondii* may produce hundreds of infectious meals for humans. In Europe, between 30% and 67% of *T. gondii* infection in different countries were attributed to consumption of undercooked or crude meat products (Cook *et al.,* 2000), suggesting that meat is the major source of infection. Consumption of cattle and sheep meat appeared as risk factor for contamination of pregnant women in France (Baril *et al.,* 1999). A direct measure of the environmental contamination by oocysts

counting is unfeasible for technical reasons. An interesting alternative for measuring *T. gondii* environmental spreading is the seroprevalence in animal reservoirs (Papini *et al.,* 2006) that can be used as a good indicator in urban areas. Serological based tests can be useful aids to diagnosis in sheep flocks and to provide epidemiological information (Innes, 2011). Other authors (Dubey and Frenkel 1972) also believed that, serological surveys even in cat are more useful for epidemiological data than the results of faecal examination because cat with antibodies have already shed oocysts and are indicators of environmental contamination (Dubey and Frenkel, 1972). *T. gondii* seroprevalence is ecologically varies, higher prevalence have been observed in warm and moist areas than in cold or hot dry areas (Abu Samra *et al.,* 2007; Deconinck *et al.,* 1996).

2.1.3.5.1. Risk factors Associated with Toxoplasmosis:

A few studies have been aimed at identifying risk factors that may be associated with acquiring *T. gondii* infection postnatally (Gilot-Fromont *et al.,* 2009; Clun *et al.,* 2006; Figliuolo *et al.,* 2004). The source of infection is of epidemiological and public health importance (2003; Seri 2003; Bout *et al.,* 2002; Aramini *et al.,* 1999). These sources of infection vary greatly among different ethnic and occupational groups and geographical areas (El Hassan *et al.,* 1991; Cook *et al.,* 2000; Dubey, 2004; Dubey *et al.,*

2005; Moura *et al.,* 2007; Bahrieni *et al.,* 2008; Ragozo 2009; Swai and Schoonman, 2009). Cats are important animal in the life cycle of *T. gondii* and epidemiology of toxoplasmosis as they are important source of infection for grazing animals (Tenter *et al.,* 2000). Farm animals in close contact with cats and rodents have higher exposure risk to *T. gondii* (Dubey *et al.,* 1995). Field investigations showed that the prevalence of toxoplasmosis in farm animals is higher in areas where cats are present (Dubey 1980; Dubey and Bettie, 1988; Mainar *et al.,* 1996; Vesco *et al.,* 2007). In addition to the presence of cats, Vesco *et al.,* (2007) stated that using of surface water for drinking and farm size were factors associated with *T. gondii* seropositivity in Italy. *T. gondii* was demonstrated in water surface and underground water by DNA amplification in France (Villena *et al.,* 2004). Gebremedhin *et al.,* (2013) indicated that the probability of acquiring *T. gondii* was higher in females than in males, in adults than in young animals, in small than in large flocks and in sheep that were given tap water and river water than in those that drink water from mixed sources. In Brazil, no significant differences were observed for risk factors associated with the occurrence of toxoplasmosis in humans due to contaminated sources such as fresh milk, cheese/sausage, and contact with felines or other animals (Santos *et al.,* 2009). The presence of felines can indicate the likelihood of a contaminated environment, posing a risk to the human

population and other animals. It has been recently established that infectious environmentally resistant oocysts shed in the faeces of felids can be transported via fresh water runoff into the marine ecosystem, where represent a major source of infection and mortality in some marine mammals (Conrad *et al.,* 2005). Gilot-Fromont *et al.,* (2009) and Clun *et al.,* (2006) stated that, the seroprevalence decreased when the herd size increased. The fore authors also stated that, the presence of cat in farms had no effect on seroprevalence. Both extensive and intensive management system were identified as risk factors associated with goat toxoplasmosis (Neto *et al.,* 2008). Toxoplasmosis is an opportunistic infection. Antibodies to *T. gondii* are detected more frequently in sick (Immunosuppressed) animals and any complications of primary disease (Svobodova *et al.,* 1988; Svobodova *et al.,* 1998). Infections by Cytomegalovirus and *T. gondii* are endemic in Chile and only low proportion of infected individuals has clinical manifestations (Abarca *et al.,* 1997). *Toxoplasma* seropositive was associated with a positive *Toxocara* titer (Taylor *et al.,* 1997). Antibody against *T. gondii* was detected in *Brucella abortus, Listeria monocytogenes* and *Neospora caninum* seropositive animals (Huong *et al.,* 1998; Figliuolo *et al.,* 2004; Yildiz *et al.,* 2009). Reactivation of latent infection following immunosuppression was most likely cause of disseminated toxoplasmosis (Burnsteen *et al.,*

1999; Nath *et al.,* 1987; Nath *et al.,* 1993). The latter author stated that toxoplasmosis can be fatal complication in renal transplant recipient. Seroposistive donors not to be used for seronegative recipient and seropositive recipients should be monitored closely after surgery for clinical signs of toxoplasmosis. Public health concerns associated with *T. gondii* clearly indicate the need for sero-epidemiological investigation of toxoplasmosis.

2.1.3.6. Diagnosis of Toxoplasmosis:

Because of the lack of specific clinical manifestations during acute infection, *T gondii* is mainly a laboratory diagnosis. Diagnosis of *T. gondii* in animal and human is very difficult and recourse must be made demonstrate either the organism or the antibodies against it (Taylor *et al.,* 2007). The most convincing diagnostic tools include: serological tests, Bioassay, Histopathology, Immunohistochemistry and molecular techniques as well as tissue impression smear. The diagnosis of congenital toxoplasmosis can be performed by identifying the agent using histological slides and the polymerase chain reaction (PCR) with aborted fetuses and placentas (Pereira-Bueno *et al.,* 2004). Pregnant women at risk of toxoplasmosis infection in France have been required to undergo monthly serological testing (Baril *et al.,* 1999).

It is Papini *et al.,* (2006) who stated that, a direct measure of the environmental contamination by oocysts counting is unfeasible for technical reasons. An interesting alternative

for measuring *T. gondii* environmental spreading is the seroprevalence in animal reservoirs (Papini *et al.,* 2006) that can be used as a good indicator in urban areas.

2.1.3.6.1. Serological Techniques:

Since direct observation of tissue cysts is not a suitable diagnostic method to be carried out on live animals, the serological techniques appear to be the method of choice (Hossein *et al.,* 2008).

There are variable serological tests in different laboratories for diagnosis of toxoplasmosis in animal and human including Sabin Feldman Dye Test (SFDT) (Sabin and Feldman 1948), Indirect immuno-fluorescent Antibody Technique (IFAT) (Remington *et al.,* 1968; Remington *et al.,* 2004), direct and indirect Haemo-agglutination Test (HAT&IHAT) (Desmonts and Remington, 1980; Remington, 2004; Weiss and Kim, 2007), Complement Fixation Test (CFT) (Marquardt and Demaree, 1985), Enzyme Linked Immunosorbent assay (ELISA) (Jacobs *et al.,* 1960; Piergili, 2004), Modified Agglutination Test (MAT), and Latex Agglutination Test (LAT) (Dubey *et al.,* 1985; Zhang and Wei, 2001). Using MAT, sera with antibodies in a 1:25 dilution were considered to have *T. gondii* infection (Hove and Dubey 1999). The SFDT is still considered as the “gall standard” for serological diagnosis of

Toxoplasmosis in animals and humans (Tenter *et al.,* 2000). However, the ELISA test is often used because of its high sensitivity and specificity when compared with other methods (Salant and Spira 2004). There was no correlation between the Sabin-Feldman Dye Test (DT) and the Modified Direct Agglutination Test (MAT) when North American horses were tested for *T. gondii* (Dubey *et al.,* 1999). LAT is considered as one of the most specific and sensitive serologic test for the detection of *T. gondii* antibodies in animals (Hashimi-Fesharki 1996). The later author stated that, LAT is more sensitive compared with IHAT. Svobodova *et al.,* (1998) believe that, the more suitable diagnostic purpose is the demonstration of specific immunoglobulin classes. They also stated that, generally, the prevalence of IgM antibodies is very low as well as the demonstration of oocyst in cat's faeces.

Almost perfect agreement was found between MAT and ELISA tests (Negash *et al.,* 2004). Using the MAT as a reference, the sensitivity and specificity of the ELISA were 98.4% and 90.9%.

Cross-reactivity between antibodies against *T. gondii* and *Sarcocystis neurona* in horses was not detected (Dubey *et al.,* 1999). The convenience of the Dot-ELISA test is obvious when compared with other serological tests for both laboratory and field surveys, mainly due to its features of practicability and reagent stability (Ferreira *et al.,* 1997). It is

important to mention that, even in cats, many scientists stated that serological techniques are more useful for measurement of environmental contamination (Papini *et al.,* 2006) and epidemiological data on *T. gondii* infection (Dubey and Frenkel 1972).

2.1.3.6.2. Bioassay:

Inoculation of suspected material into Toxoplasma free mice intra-peritoneal or intra-cerebral rout is quite promising (Soulsby, 1986; Urquhart *et al.,* 1996; Taylor *et al.,* 2007; Ragozo *et al.,* 2009). Its disadvantage is that unless the strain of *T. gondii* is highly virulent, it requires three weeks before the inoculated mice can yield recognizable *T. gondii* cysts (Taylor *et al.,* 2007). Bioassay was found to be less effective for detection of parasitaemia than PCR (Burney *et al.,* 1999). Arias *et al.,* (1994) isolated *T. gondii* from several organs of cattle from Costa Rica using mice bioassay and carbon immunoassay (CIA) testing. Using bioassay in mice, Pop *et al.,* (1989) isolated about (9.8%) *T. gondii* from pools samples of diaphragmatic muscles from cattle. *T. gondii* have been found in mutton from sheep, goat and beef from Iran (Hoghooghi and Afraa1993). Although it is very important issue, evidence of *T. gondii* tissue cyst in cattle was not fully documented (Dubey 1995; Hashimi-Fesharki 1996; Canada *et al.,* 2002).

2.1.3.6.3. Histopathology:

In abortion cases, the multifocal necrosis and calcification in the placenta could be demonstrated microscopically using histopathological sections. Additionally, parasites can be detected in the placenta, and in the foetal heart, brain, liver or lung (Dubey, 2008). Necrosis might be seen microscopically in foetal cerebellum. Moreover, Lymphocyte proliferation and micro necrosis may be presented in foetal kidneys, adrenals, lymph nodes or brain (Dubey and Jones, 2008). Myocarditis, non superlative encephalitis, hepatitis and diffuse interstitial pneumonia were the most predominant histopathological changes observed (Ahmed *et al.,* 2008) in the aborted foetuses of sheep and goats in Egypt. *T. gondii* was not found in tissue of 300 aborted foetuses from Iranian cows by direct microscopy and bioassay in mice (Hashimi-Fesharki, 1996).

2.1.3.6.4. Impression Smears:

Direct smears from affected tissues to detect tachyzoites proved to be rapid and easy diagnostic method (Terpsidis *et al.,* 2009).

2.1.3.6.5. Immunohistochemistry:

Immunohistochemical tests allow visualization of intact *T. gondii* and antigenic debris in tissue sections of aborted materials. The method is convenient and sensitive compared to attempts of isolation and detection of *Toxoplasma* antigen even in decomposed tissues (Buxton, 1998; Dubey and Jones, 2008).

2.1.3.6.6. Molecular Technique:

Several molecular techniques (PCR based Techniques) have been developed and used -worldwide including the Sudan- for *T. gondii* molecules detection using different gene targets (Owen *et al.,* 1999; Ahmed *et al.,* 2008; Abdel-Hafez 2013). Isolation of cysts from naturally infected cattle is rare (Canada *et al.,* 2002). Using polymerase chain reaction, Aspinall *et al.,* (2002) detected 27 (38%) positive sample among 71 pieces of bovine meat. On the contrary, Dubey *et al.,* (2005) found no positive sample among 2094 pieces of cattle meat in USA. This discrepancy merits further investigation in the future. Whole blood PCR was found to be not helpful as a diagnostic test for clinical feline toxoplasmosis (Burney *et al.,* 1999).

2.1.3.6.7. Diagnosis of Cat Toxoplasmosis:

Diagnosis of cat's toxoplasmosis is challenging (Suzan 2008). In addition to faecal examination and the above mentioned diagnostic methods it may involve X-Ray. It is important to mention that, a vaccinated cat can have false positive anti-*T. gondii* antibodies. According to Suzan (2008), this effect can persist for up to 10 months after administration of any routine vaccination.

2.1.4. Sero-prevalence of *T. gondii*:

Serological techniques appear to be the diagnostic method of choice (Weiss and Kim, 2007; Taylor 2007; Hossein *et al.,* 2008) for *T. gondii* infection. Different antibody detection

methods were used for different animal species and humans using different cut-off points. Thus the seroprevalence of *T. gondii* showed wide variation among different animal species in different continents (Weiss and Kim, 2007).

2.1.4.1 Sero-prevalence of *T. gondii* Antibodies in Cattle:

Through the world, the current literature presents values ranges from 0 to 99% for the presence of anti-*T. gondii* antibodies in cattle (Tenter *et al.,* 2000; Hall *et al.,* 2001; Santos *et al.,* 2009). The worldwide average value of seroprevalence of *T. gondii* infection in cattle was 9% (Dubey, 2004). The level of infection in cattle herd has been less studied. In France, the crude seroprevalence of 1329 beef cattle (24 herds) at threshold 1:24 using MAT was 7.8% (Gilot-Fromont *et al.,* 2009). Positive individuals were found in 21 herds (87.5%) out of 24 herds. Small size and isolate herds showed the highest seropositivity and the presence of cats modified the age-prevalence relationship (maximal seroprevalence was observed in oldest cows in farms without cats, and in youngest individuals in farms with cats). Cows in France are often exposed to toxoplasmosis, however, landscape characteristics (water point, isolation) and herd management (Herd size, cats) may affect seroprevalence (Gilot-Fromont *et al.,* 2009). In Serbia, Clun *et al.,* (2006) reported a seroprevalence of 76.3% in yearling and adult of

both sexes. They also considered small herd size and farm location as a risk factor for cattle toxoplasmosis. Reports on seroprevalence of cattle toxoplasmosis In Turkey varies between 7.6% and 41.6% (Yildiz *et al.,* 2009). There are many reports on cattle toxoplasmosis from Iran. In 1996 Hashemi-Fesharki (1996) fail to detect antibodies against *T. gondii* in cow sera diluted 1:8 and 1:64 using LAT and IHAT respectively. However, Ciamak-Ghazaoi, (2005) and Nematollahi and Moghaddam (2008) reported 9% and 15.9% seroprevalence of cattle toxoplasmosis in Iran using ELISA and IFAT respectively. Nematollahi and Moghaddam (2008) reported significant differences between age and sex groups. The rate of infection was higher in cattle less than one year old. *T. gondii* have been found in mutton from sheep, goat and beef from Iran (Hoghooghi and Afraa1993). Santos *et al.,* (2009) reported 71% (1420/2000) anti-*T. gondii* antibodies seroprevalence in Southwest region of the Mato Grosso State, Brazil using IFAT. There was no positive association between the prevalence of *T. gondii* in cattle and the presence of cats (Santos *et al.,* 2009). The role of cat probably, not by direct contact, but by contaminating the environment by millions of oocysts. Although cattle may be seropositive, viable *T. gondii* is rarely found in beef (Dubey, 1996) and rare to find tissue cyst in bovine tissues (Dubey, 1986). Adult cows that were experimentally infected with *T. gondii* oocysts became seropositive, but the number of

tissue cysts was below the detection of bioassay using mice (Dubey, 1996).

Data concerning cattle sero-prevalence of *T. gondii* in Africa is very few. The available reports are that of Sudan (Elfahal *et al.,* 2013; Khalil and Elrayah, 2011; Zein Eldin *et al.,* 1985) and Gabon Mbiye *et al.,* (2013) who reported a sero-prevalence of 26% in cattle from Gabon.

2.1.4.1.1. Seroprevalence of *T. gondii* Antibodies in Cattle from the Sudan:

The first available data on cattle toxoplasmosis in the Sudan was that (40%) reported by Zein Eldin *et al.,* (1985) using IHAT. Thereafter, Khalil and Elrayah (2011) reported 32% using LAT. The antibody intensity in the investigated cattle was 20% (10), 8% (4) and only 4% (2 animals) for 1:8, 1:16 and 1:32 serum dilution respectively, (Khalil and Elrayah 2011). Recently, Elfahal *et al.,* (2013) assayed 181 sera of dairy cattle from Khartoum and Gazira States with reproductive problems for antibodies to *T. gondii* by ELISA. The prevalence rate of *T. gondii* antibodies in cattle at herd level was 44.8% (13/29). Herd level infection rates were 50% and 33.3% in Khartoum and Gazira States, respectively. The overall prevalence of *T. gondii* at individual level in both states was 13.3% (24/181). The prevalence was 12.7% (17/134) and 14.9% (7/47) in Khartoum and Gazira State, respectively. The authors observed significant effect of age and sex on the seropositivity while no significant relationship

was discerned regarding breed, location, season, or signs of reproductive diseases.

2.1.4.2. Sero-prevalence of *T. gondii* Antibodies in Sheep:

Toxoplasmosis occurs in all breeds (Ragozo *et al.,* 2009), sex (Bonyadian *et al.,* 2007) and age (Bahrieni *et al.,* 2008; Anamaria *et al.,* 2008) groups of ewes and their foetuses. Ovine toxoplasmosis occurs in temperate sheep rearing countries worldwide where the climatic conditions favour oocyst survival (Buxton and Rodger 2008). Infection in sheep is associated with contamination of feed or grazing range land with Sporulated oocysts (Innes *et al.,* 2009) and transplacentally (Dubey, 1994). Field investigations have shown an association between cats on farms and exposure of sheep to *T. gondii* (Skjerve *et al.,* 1998). Serological based tests can be useful aids to diagnosis in sheep flocks and to provide epidemiological information (Innes, 2011). According to Fayer *et al.,* (1981), the world average of seroprevalence of sheep and goats was 31%. There are numerous reports on seroprevalence of toxoplasmosis in sheep worldwide, ranging from 0% to 100% (Fayer *et al.,* 1981; Tenter *et al.,* 2000; Ortega-Mora *et al.,* 2007). About 34-40% of the world's adult sheep have been infected with *T. gondii* (Smith, 1991). The average value (31%) that reported by Fayer (1981) is higher when compared to other animals such as goats (15%) and (9%) for cattle (Dubey, 2004). Bahrieni *et al.,* (2008) found

that seropositive animals (sheep and goats) more than one year 1.6 times more likely to be seropositive than younger ones. The same author observed that sheep were 1.5 times more likely to be infected than goats. Seroprevalence reported in intensively managed sheep was lower than that in semi-intensively managed sheep (Nada *et al.,* 2007). In USA, the seroprevalence of ovine toxoplasmosis using MAT was ranging from 64% to 80% in adult sheep (Dubey and Jones, 2008). In United Kingdom Helmick *et al.,* (2002) obtained 167 positive (39.5%) for *T. gondii* by Latex agglutination test (LAT ≥1:64) and recent prevalence of 50% was reported by Mason *et al.,* (2010). In Irish (Mulvihill 2009) reported a seroprevalence of 43% and 36% in sheep using ELISA and LAT respectively. Using ELISA, Anamaria *et al.,* (2008) reported 45.7% seroprevalence in sheep slaughtered for human consumption in Romania. No statistically significant differences between the age groups (Anamaria *et al.,* 2008). Clun *et al.,* (2006) reported a seroprevalence of 84.5% by the MAT in Serbia.

Seroprevalence of 13.9% and 28.5% was reported in sheep under intensive and extensive management systems respectively in Uruguay using MAT (Savio and Nieto 1995). Antibodies to *T. gondii* were observed in 34.7% of the tested sheep sera in Brazil (Figliuolo *et al.,* 2004) using the indirect fluorescent antibody test (IFAT). Ragozo *et al.,* (2008) reported a seroprevalence of 24.2% with a significant higher

prevalence in ewes kept under semi-intensive management system in the same country. Indirect Immunoflourecent Test (IIFT) was found to perform significantly (28%) better than IHAT which reported 12% positive cases in sheep (408 head) examined in Chile (Gorman *et al.,* 1999). No differences were observed between geographical locations or sex of the sampled sheep regarding serological detection of *T. gondii* antibodies in these animals.

In Asian countries, such as Iran many studies have been done in sheep toxoplasmosis (Hashemi-Fesharki 1996; Ciamak-Ghazaoi, 2005; Bonyadian *et al.,* 2007; Sharif *et al.,* 2007; Bahrieni *et al.,* 2008; Hossein *et al.,* 2008). They reported seroprevalence of 24.5%, 30%, 29.1%, 35%, 24.7 and 72.6% respectively using different serological tools. Hossein *et al.,* (2008) detected 109/150 (72%) by ELISA and 104/150 (69.3%) by MAT. Their results suggested that ELISA and MAT were good tools for epidemiological studies of *T. gondii* infection in sheep. Bonyadian *et al.,* (2007) indicated that there was no significant difference between the two sexes of Iranian sheep. However, there was a significant difference between the townships located in east and west of the province. Bahrieni *et al.,* (2008) reported over all (19.6%) seroprevalence in Iranian sheep and goat. In Turkey, the prevalence rates were varies from 7.1% to 88% on regional basis (Murat *et al* 2005). In Pakistan, the seroprevalence was 19.9% using LAT and ELISA (Lashari and Tasawar 2010) with

significant higher prevalence in male, lambs and animals with low weights. It was Sharma *et al.,* (2008) who reported a seroprevalence of 3.8% in Indian sheep.

In Africa, there is very little work on toxoplasmosis. Prevalence rate ranging from 4.3% to 63% have been reported (Abu Samra *et al.,* 2007; Deconinck *et al.,* 1996; Bekele and Kasali 1989; Okoh *et al.,* 1981) in sheep. In Egypt, serological surveys indicated that the incidence of infection average was 9.4 and 6.9% using DAT in sheep and goats respectively (Hassan *et al.,* 2000) and 31.1% in sheep using ELISA (Ghazi *et al.,* 2006). Abu Samra *et al.,* (2007) reported overall seroprevalence of 5.6% and 4.3% in sheep from South Africa using IFA and ELISA respectively. In Gabon, the overall prevalence of antibody against *T. gondii* in the domestic animals in Gabon was 26.4% (Mbiye *et al.,* 2013) who reported 75% prevalence of *T. gondii* antibodies in sheep. The overall flock and animal level seroprevalence in Ethiopia were 70.48% (160/227) and 31.59% (357/1130) respectively (Gebremedhin *et al.,* 2013). They identified sex, age, flock size and source of water as important risk factors to acquire the infection. Seroprevalence of 52.6% and 56% in sheep were reported by Modefied Direct Agglutination Test (MDAT) and ELISA test (Negash *et al.,* 2004). Sawadogo *et al.,* (2005) reported a seroprevalence of 45.5% in Morocco using IHAT.

2.1.4.2.1. Seroprevalence of *T. gondii* Antibodies in Sheep from the Sudan:

The earliest studies on sheep toxoplasmosis were carried out by Elbedawi *et al.,* (1984). Then Zein Eldin *et al.,* (1985) reported seroprevalence of 34% using IHAT and CFT in slaughtered male sheep from Kordofan and Central Sudan. Khalil and Elrayah (2011) reported seroprevalence of 57.5% in ElKadaro slaughter house using LAT. They recorded 1:8 (38.7%), 1:16 (17.5%), 1:32 (1.3%) as antibody titres of the seropositive sera of male sheep slaughtered in ElKadaro abattoir. The most recent serological studies in sheep from the Red Sea State indicated that 45.4% (318/700) of Beja sheep were positive for anti-*T. gondii* antibodies, using LAT (Abdel Hafez 2013). The author reported 1:8 (4.4%), 1:16 (21.1%), 1:32 (16.9%), 1:64 (44.60%), 1:128 (11%), and 1:256 (2.5%) as antibody titres of the seropositive sera. According to Abdel Hafez (2013), sex had no significant effect on the prevalence rate of *T. gondii* infection, which was 46% in female and 41% in male. Management systems had no effect on the prevalence rate of *T. gondii* infection, where it was 47.3% in semi-intensive and 43% in extensive system. Histopathological examination of the tissue samples from the seroposistive Beja sheep revealed no *T. gondii* tissue cyst. However, 16% of the examined tissues were molecularly positive using real-time PCR (Abdel Hafez 2013).

2.1.4.3. Sero-prevalence of *T. gondii* Antibodies in Goats:

Goats are economically important in many countries (Ragozo *et al.,* 2009) including Sudan (Ismail *et al.,* 2015). Goat meat and milk can be a source of infection for humans (Riemann *et al.,* 1975; Sacks *et al.,* 1982; Skinner *et al.,* 1990). In Bulgaria, the prevalence was 59.8% using IHAT (Prelezov *et al.,* 2008). A pilot study was carried out on ten Dutch goat farms to see whether there is relationship between farm management factors and the occurrence of toxoplasmosis (Antonis *et al.,* 1998). The mean prevalence was 47% (range 5-90%). The presence of kittens on a farm was a risk factor for higher prevalence of toxoplasmosis (Antonis *et al.,* 1998). Antibodies to *T. gondii* were found in 26 of 386 old goats (≥ 4years) from 8 of 10 farms in Venezuela (Nieto and Melendez 1998); antibody titers were 1:64 (10 does), 1:256 (3 does), 1:512 (5 does), 1:1024 (1 does), and 1:4096 (7 does). The overall seroprevalence of goat toxoplasmosis in Brazil was 32.2% (Ragozo *et al.,* 2009). They reported higher prevalence in adult nanny goats (66.7%) and in goat raised in intensive system (48.4%). Using ELISA, Ciamak (2005) and Bahrieni *et al.,* (2008) reported 15% and 15.8% *Toxoplasma* seroconversion in goats from Iran. Moreover, 19.3% and 30% were found by Hashemi-Fesharki (1996) and Sharif *et al.,* (2007). It is important to mention that the fore author used 1:8≤positive titer≤1:64 for LAT as cut-off point. A

seroprevalence of 72.7% (200/275) was reported in indigenous goats (Ataseven *et al.,* 2006) in Eastern and Southeastern of Turkey using Sabine-Feldman Dye Test. In Egyptian goats, the prevalence rate was ranging from 6.9% to 35.4% (Hassan *et al.,* 2000; Ramadan *et al.,* 2007) with about 50% in adults. Negash *et al.,* (2004) reported 24% and 25.9% seroprevalence in Ethiopia, by MDAT and ELISA tests respectively. Significant titers of *Toxoplasma* antibodies were present in various populations of sheep, goats and pigs in Nigeria (Okoh *et al.,* 1981). In other African countries such as Botswana, Uganda, Zimbabwe, the prevalence rates were 10% (Binta *et al.,* 1998), 31% (Bisson *et al.,* 2000) and 67.7% (Hove *et al.,* 2005) respectively.

2.1.4.3.1. Seroprevalence of *T. gondii* Antibodies in Goat from the Sudan:

Since (Zein Eldin *et al.,* 1985) who reported seroprevalence of 63% in slaughtered goats in the Sudan using IHAT and CFT, there is no data on goat toxoplasmosis available in the Sudan.

2.1.4.4. Seroprevalence of *T. gondii* antibodies in Equine:

Antibodies to *T. gondii* were found by the MAT in 124 (6.9%) out of 1788 sera from horses slaughtered for food in North America (Dubey *et al.,* 1999); the titers were 1:20 up to 1:160. Mayer *et al.,* (1987) reported 97.1% seroprevalence of equine toxoplasmosis in Argentina using IHAT. Many studies

on equine toxoplasmosis were carried out in Egypt. The seroprevalence in horses ranged from 31.7% to 51.7% and 52.6% using ELISA and MAT respectively (Eman *et al.,* 2005; Ghazy *et al.,* 2007; Shaapan and Ghazy 2007). A prevalence of 65% was reported in Egyptian donkeys (El-Ghaysh 1998). In Nigeria, a seroprevalence of 37.1% was reported using IHAT in horses (Aganga *et al.,* 1983).

2.1.4.4.1. Seroprevalence of *T. gondii* Antibodies in Equine in the Sudan:

Before our recent publications on Horses (Ibrahim *et al.,* 2014) and donkeys (Shadia *et al.,* 2013), there was no data available on *T. gondii* infection in equine in the Sudan. The overall sero-prevalence of the investigated equines was 32.7%. Antibodies to *T. gondii* were found in 27.6% of 105 donkeys while prevalence in horses ranged from 23.3% to 38% in different seasons (Ibrahim *et al.,* 2014).

2.1.4.5. Seroprevalence of *T. gondii* Antibodies in Camels:

Seroprevalence of *T. gondii* infection in the worldwide camel's zone is extremely variable. Using ELISA and MAT in the United Arab Emirates (UAE), Abu-Zaid (2002) and Abu-Zaid *et al.,* (2006) reported prevalence rate of 31.4% and 22.4% respectively. Hussein *et al.,* (1988) recorded 16% in Kingdom Saudi Arabia (KSA) using IHAT. Studies in Egypt revealed 17.4% (Hilali *et al.,* 1998), 25.6% (Shaapan and fathia 2005) and 30.7% (Shaapan and fathia 2008) using

direct agglutination test (DAT) on sera of slaughtered camels in Cairo. Higher seroprevalence (46% and 54.2%) of Camel toxoplasmosis was reported previously in Egypt by Rifaat *et al.,* (1979) and Derbala *et al.,* (1993) respectively. Although significant titers of *Toxoplasma* antibodies were present in various populations of sheep, goats and pigs in Nigeria (Okoh *et al.,* 1981), but *Toxoplasma* seroconversion was not detected in camel sera collected from abattoir in Kano, Nigeria. These camels were originated from area bordering Niger.

2.1.4.5.1. Seroprevalence of *T. gondii* Antibodies in Camels from the Sudan:

Most of the work on animal toxoplasmosis in the Sudan was conducted in camels (Seri *et al.,* 2003). Since Zein Eldin (1985), there were many reports in camel toxoplasmosis in the Sudan (Abass *et al.,* 1987; Bornstein and Musa 1987; Elamin *et al.,* 1992; Manal 2003). The last study was that of Husna *et al.,* (2012) who reported seroprevalence of 44% in camels from Tumbool Slaughterhouse using LAT. Khalil and Elrayah (2011) reported seroprevalence of 20% in camel from El-Kadaro area using the same technique. The later authors reported antibody titres ranging from 1:8 (17.1%), 1:16 (2.9%) and 1:32 (0.0%). Zein Eldin (1985) reported 54% seroprevalence in slaughtered camels from Kordofan and central region of the Sudan. Their results showed widespread of *Toxoplasma* among meat producing animals in the Sudan. More widespread seroprevalence (61.7%) and (67%) of *T.*

gondii in camels were reported by Manal (2003) and Elamin *et al.,* (1992) respectively. Elamin *et al.,* (1992) reported seroprevalence of 67% prevalence rate in pastoral camels from Butana plains using LAT (Two fold dilutions ranging from 1:8 to 1:256). The prevalence rate increased significantly with age (74.2% in camels aged over 7 years). The prevalence rate of seropositivity decreased proportionally with the level of serum dilution. At dilution of 1:32 and above, the prevalence rate was 25.9%. There were no sex linked differences (p>0.05) in seropositivity. The overall prevalence among female was 22.7% and male 29.1% camels. Using LAT, Manal and Majid, (2008), reported over all prevalence of 51.3% of anti-*T. gondii* antibodies from sera of calf-camels with diarrhoea from different parts of the Sudan.

2.1.4.6. Seroprevalence of *T. gondii* Antibodies in Cats:

Although cat is the reservoir animal of *T. gondii* infection, only a few reports are available on the prevalence of *T. gondii* oocysts in faeces (Papini *et al.,* 2006). For example Pampiglone *et al.,* (1973) reported a prevalence of 0.4% (1/250) for *T. gondii* infection revealed by microscopic examination of faeces. Therefore, serological surveys in cat were more useful than faecal examination for measurement of environmental contamination (Papini *et al.,* 2006) and epidemiological data (Dubey and Frenkel 1972). Based on

serological surveys, about 74% of adult cats are infected with *T. gondii* (Tenter *et al.,* 2000). The seroprevalence increased with age and higher in free roaming cats than the domestic cats which are often fed with preserved food (Dubey 1973). Thirty nine percent of cats were found positive for *T. gondii* IgG in Melbourne. Older cats tend to have higher titers. There was no significant difference in the *T. gondii* antibody titers between males and females or between cats living in urban areas and cats from rural areas (Sumner and Ackland 1999). A significant proportion of cats from Melbourne have been exposed to *Toxoplasma*. This may have implications for health of wildlife and humans. A serological survey among stray cats collected in Hyogo Prefecture in 1990-1991 indicated that 44 (19.0%) of 231 cats were positive for *Toxoplasma* antibody as determined by the LAT (Khin-Sane-Win. *et al.,* 1997). A seroprevalence of 40.7% (233/573) was reported in stray cats from Italy using DAT (Papini *et al.,* 2006). *T. gondii* is more prevalent in female and younger cats. A serological survey with LAT was conducted in 800 serum samples collected from domiciled cats in Japan. The overall prevalence was 6.0% (48/800). Among 48 positive there was no specific strength of antibody titers; the titer was 1:64 in 8 cats, 1:128 in 12, 1:256 in 8, 1:512 in 10, 1:1024 in 8 and 1:2048 in 2. The prevalence in the outdoor cats (11.1%) was significantly higher than that in the free group (4.8%). Epidemiological aspects observed in

the domiciled cats were different from those reported in the stray cats (Nogami *et al.,* 1998). Clinically unapparent toxoplasmosis, manifested by the presence of IgG antibodies is frequent in cats; the opportunistic character of the infection is important (Svobodova *et al.,* 1998). Using IFAT, they reported a seroprevalence of 61.3% in cats free of Feline Lukaemia Virus (FLV) and Feline Immunodeficiency Virus (FIV), with only one cat (0.28%) showing specific IgM antibodies. Oocyst shedding was demonstrated in only one cat (0.28%) showing IgG. In cats infected with the above mentioned viruses the seropositivity was 63.6%. Neither IgM nor oocysts were detectable in this group. The titer of antibodies found to be lower in the group of cats infected with the viruses. *C. parvum* specific IgG antibodies was detected most frequently in *T. gondii* specific IgG seropositive cats, outdoor cats and cats with gastro-intestinal signs (McReynolds *et al.,* 1999). In South Africa, Cheadle *et al.,* (1999) reported 74% (50/68) cat's serum samples tested positive for antibodies to *T. gondii*.

On the other hand, several authors (Dubey and Bettie, 1988; Dubey *et al.,* 1995; Monica *et al.,* 2002) have determined the seroprevalence of antibodies to *T. gondii* in wild feline (39-68%) and rodents (0.8-6.3%). The LAT and IHAT were found to be less sensitive than MAT for diagnosis of *T. gondii* in foxes. Antibodies were not detected by LAT (titer 1:64) in the two examined foxes (Dubey et al., 1999).

2.1.4.6.1. Seroprevalence of *T. gondii* Antibodies in Cats from the Sudan:

With the exception of our recent report (Ismail *et al.,* 2013) in Khartoum State and Siddig (2010) in Red Sea State, there is no consistent data on *T. gondii* infection in cats from the Sudan.

2.1.4.7. Seroprevalence of *T. gondii* Antibodies in Dogs:

According to Santos *et al.,* (2009), about 88.5% (54/61) of Brazilian dogs were found to be seropositive for *T. gondii* using IFAT. The authors considered dog as a common source of infection. Lin (1998) stated that, pet dogs had seroprevalence of 7.9% and had IgG and IgM geometric mean titers of 1:50 and 1:31, respectively. Older or heavier pet dogs had higher odds of seropositivity than younger or lighter dogs. Also mixed-breed dogs had higher odds of seropositivity than pure-bred dogs (Lin 1998). Fan *et al.,* (1998) reported prevalence of (19.6%) in dogs from Atayal, Taiwan using LAT. It is important to mention that, the results of Lindsay *et al.,* (1997) support the hypothesis that dogs may involved in the mechanical transmission of *T. gondii* to humans. On the other hand, there is no any data available on *T. gondii* infection in dogs from the Sudan.

2.1.4.8. Seroprevalence of *T. gondii* Antibodies in Wild Animals:

T. gondii infections were detected in (22%) of the deer in Sao Paulo, Brazil (Ferreira *et al.,* 1997) using Dot-ELISA. Hill

et al., (1998) considered 1:32 as indicative of infection in free-ranging mammals in Iowa using MAT. Dubey *et al.,* (1999), and Hove and Dubey (1999) studied *T. gondii* infection in foxes, where he found that LAT and IHAT were less sensitive than MAT for the diagnosis of *T. gondii* infection in foxes, pigs and wild games of Africa.

2.1.4.9. Seroprevalence of *T. gondii* antibodies in Pigs:

A serological survey indicated that, 23% were seropositive out of 11229 slaughtered pigs in USA (Dubey 1996). The seroprevalence was directly related to the hygienic conditions under which these animals were kept (Hove and Dubey 1999). These authors reported lowest (19.8%) prevalence in fattening pigs in large commercial farms compare to (42%) in backyard scavenging pigs. In Serbia, Clun *et al.,* (2006) reported a seroprevalence of 28.9%. A titer of 1:25 dilution was considered indicative to *T. gondii* infection using MAT in pigs of New England States (Gamble *et al.,* 1999), where the prevalence rate was 47.4% (900/1897). Out of 85 herds tested, 77 had at least one positive pig for a herd prevalence rate of 90.6%. Within herd prevalence ranged from 4% to 100% (mean=48.4%). Seroprevalence ranged from 3.5 to 13.2% in the different regions of Canada (Gajadhar *et al.,* 1998). Anti-*Toxoplasma gondii* IgG at titers of ≥ 1:32 were found in 240 Canadian market-age pigs examined by commercial LAT (Gajadhar *et*

al., 1998). In Gabon, 16.6%, prevalence of *T. gondii* antibodies in pigs was reported (Mbiye *et al.,* 2013). It worth mentioning that, pig's farms as a source of food are now available around the Khartoum State and elsewhere in the Sudan (MLFR, Personal communication 2014). However, data concerning *T. gondii* infection in pigs from the Sudan was not available yet.

2.1.4.10. Seroprevalence of *T. gondii* Antibodies in Fowl:

A seroprevalence of 16.9 and 44.4% were reported In USA (Dubey *et al.,* 2005). However, in Columbia, South America it was 77% in free ranging chickens (Dubey *et al.,* 2005). They considered the seroprevalence of toxoplasmosis in free-ranging chickens as a good indicator of general prevalence of *T. gondii* oocysts in the soil. Seroprevalence of 24.2% was reported in free-range chickens from commercial farms in Israel (Dubey, 2004). Antibodies ≥ 1:25 to *T. gondii* were found in 39.5% of chickens sera in Madras, India (Devada *et al.,* 1998) using MAT incorporating mercaptoethanol. In Iran (Ciamak 2005) reported 0% seroprevalence in slaughtered chickens kept under intensive management system. The overall seroprevalence of fowl toxoplasmosis in Egypt was 18.7% (Dubey and Hassanein 2005). These authors indicated that, anti-*Toxoplasma gondii* antibody in backyard Egyptian chickens was 30%, and in chickens kept in intensive system was 11.1%. Mbiye *et al.,* (2013) reported 17.7%, prevalence

of *T. gondii* antibodies in chicken from Gabon. Although chickens are one of the most important sources of food in the Sudan, we have no doubt that; there is no any data on *T. gondii* infection in fowl in the country.

2.1.4.11. Seroprevalence of *T. gondii* Antibodies in Humans:

Serological surveys have been done in several parts of the world showed that more than one third of the human populations have antibodies against *T. gondii* (Dubey, 2004; Weiss and Kim, 2007). This high seroprevalence demonstrate it's important as zoonotic disease. This has even more relevance now than when most seroprevalence studies were originally carried out, because of the current high risk of toxoplasmosis for HIV/AIDS positive people (Dubey, 2004). Latent toxoplasmosis is the most widespread parasitic infection in developed and developing countries (Tenter *et al.,* 2000). The prevalence varies from 20-80% in different countries. This form of toxoplasmosis is generally considered to be asymptomatic. Recently, the published results suggested that, personality of infected subjects differ from those of uninfected controls (Flegr and Havlicek 1999). Meat consumption is an important risk factor for infection (Roghmann *et al.,* 1999). Professional groups, such as abattoir workers, butchers and hunters may be infected during evisceration and handling of meat (Buzby and Roberts 1997). In order to reduce the danger of a primary infection in

pregnant women a screening before pregnancy is recommendable (Fiedler *et al.,* 1999). Repeated screening during pregnancy would be expensive, but would detect and possibly prevent infection in approximately 10 neonates per 10000 women (Allian *et al.,* 1998). The New England Newborn Screening Programme has included congenital toxoplasmosis since 1986 (Petersen and Eaton 1999). Since 1992 all pregnant women at risk of Toxoplasma infections in France have been required to undergo monthly serological testing (Baril *et al.,* 1999). Prevention campaigns among pregnant women could be improved and should focus on eating habits, hand hygiene and cats. Infection by *T. gondii* had a global prevalence of 24.6%. The rates of susceptible individuals were 80% and 50% in high and medium-low socioeconomic levels respectively (Abarca *et al.,* 1997). One hundred and thirty-two out of 280 (47.1%) pregnant women showed seropositivity to anti-*T. gondii* IgG in Jordan (Qublan *et al.,* 2002) using IFAT and ELISA. Ashrafunnessa *et al.,* (1998) concluded that toxoplasmosis is endemic in Bangladesh. They tested Sera of 286 pregnant women for toxoplasma IgG antibody by applying ELISA technique. Among the 286 sera, 88 samples were randomly selected and tested for toxoplasma IgM using ELISA. 110 (38.5%) were positive for toxoplasma IgG antibody and only 1.1% was positive for toxoplasma IgM (Ashrafunnessa *et al.,* 1998). The seroprevalence of antibody was higher among

the women in poor (53.0%) than the upper socio-economic class (22.0%) and among women with jobs (55.0%) than housewife group (35.0%). The seropositivity was higher among women living in rural areas, which are using rainwater to drink, ingesting undercooked meat and who have contact with soil. Santos *et al.,* (2009) reported seroprevalence of 97.4% (113/116) in Brazil using IFAT. They considered contact with other animals as a risk factor. Using LAT, no significant different in the positive rates between males (22.1%) and females (21.4%) or between human (21.8%) and dogs (19.6%) in Atayal, Taiwan (Fan *et al.,* 1998). Significant differences were observed between the positive rates in adults (28.3%) and children (18.7%). The consumption of raw liver of wild animals or insufficiently cooked meat was suggested to be the major mode of transmission of toxoplasmosis in Taiwan (Fan *et al.,* 1998). Using indirect latex agglutination test (ILAT), 7.7% Korean Children under 10 years old (Kook *et al.,* 1999) showed positive titers higher than 1:32 without significant differences between males (7.3%) and females (8.5%). The sero-positive rate increased with age (Kook *et al.,* 1999). Some correlations were suggested between toxoplasmosis and congenital anomalies in Korea (Kook *et al.,* 1999). Seroprevalence was higher in country children (16.6%) than (10.2%) in town (Taylor *et al.,* 1997). The proportion testing positive increased with age in both town and country

children (Taylor *et al.,* 1997). No association with cat ownership was found. Lacks of energy or tiredness in the last 12 months were the only clinical features associated with positive titer (Taylor *et al.,* 1997). The latter author stated that, *Toxoplasma* seropositive was associated with a positive *Toxocara* titer. Between HIV-seropositive and HIV-seronegative pregnant women in Thailand, antibody rates to *Toxoplasma gondii* were 21.2% and 13.2% respectively (Chintana *et al.,* 1998). However, prevalence of antibodies was higher in controls (69%) than in patients (47%) in Spanish (Gongora-Biachi *et al.,* 1998). This justifies the routine determination of antibodies and the use of therapeutic protocols for preventing encephalitis by *Toxoplasma* in HIV patients as a high percentage of them would be at risk of developing it (Gongora-Biachi *et al.,* 1998). Boto de los Bueis *et al.,* (1998) reported 36.7% positive IgG anti-toxoplasma in the group of HIV-1 infected Spanish patients, non-drugs addicts, against 30.9% in the group of HIV-1 drug addicts patients. The control group showed a *Toxoplasma* Seroprevalence of 26.7%, without statistically significant against the drug addicts HIV group. Chintana *et al.,* (1998) reported a seroprevalence of 21.1% and 13.1% in sera of HIV seropositive and HIV seronegative respectively. Antibody titers were higher in HIV infected persons than in those who were uninfected (Woldemichael *et al.,* 1998). Toxoplasmic encephalitis will be a common

opportunistic infection among HIV-infected Ethiopians. On the other hand, the Seroprevalence of IgM and IgG was 2.4% and 32.1% respectively in blood donors in Czech Republic (Svobodova and Literak 1998).

2.1.4.11.1. Seroprevalence of *T. gondii* Antibodies in Humans in Africa:

Human toxoplasmosis has been well studied elsewhere in Africa. An estimated seroprevalence of 45.7% was reported in Tanzania (Swai and Schoonman 2009). Seroprevalences of 42.6% and 52.4% were reported from slaughter house workers in Djibouti and Egypt, respectively (Chantal *et al.,* 1996; Ibrahim *et al.,* 1997). Occupational contacts including butchers, slaughter house workers, milkers, and cow attendants in Nigeria revealed 22.6% were infected (Osiyemi *et al.,* 1985). Cattle breeders and abattoir personnel from Benin gave 87% positives (Fayomi *et al.,* 1987) and high-risk groups from city of Pointe-Noire in Congo and Addis Ababa in Ethiopia showed a seroprevalence between 41.9% and 80%, respectively (Candolfi *et al.,* 1993; Woldemichael *et al.,* 1998). In Egypt, Hamadto *et al.,* (1997) reported 24%, 15%, 17.5%, 28.6% and 40% in obstetric cases, hepato-splenomegalic, prolong fever, ophthalmic and localized lymphadenopathy cases attending clinics of Benha University hospital respectively. Recently, IgG antibody against *T. gondii* was detected in 81.4% of women from Ethiopia (Gebremedhin *et al.,* 2013).

Based on IgG anti-toxoplasma antibodies status the seroprevalence of toxoplasmosis in HIV patients was 94% in a hospital in Ethiopia (Yizengaw 2013) but with no IgM antibody detection. The author found that, consumption of raw vegetables and not having primary information about toxoplasmosis were the significant risk factors having association with the presence of anti-toxoplasma antibody.

2.1.4.11.2. Seroprevalence of *T. gondii* Antibodies in Humans in the Sudan:

Although human toxoplasmosis in the Sudan was recorded since 1966 (Carter and fleck, 1966), there are very few reports available (Khalil *et al.,* 2013). Though few, most of these reports were on pregnant women from hospitals of Khartoum and Gezira States (Abd Elhameed, 1991; Adnan, 1994; Elnahas *et al.,* 2003; Satti, 2003; Khalil *et al.,* 2012; Maha *et al.,* 2012; Abdel-Raouf and Elbasheir 2014). The only serological data available on human toxoplasmosis in the eastern part of the Sudan was from Read Sea State. Musa, (2008) reported 68% seropositive pregnant women in Port-Sudan city. After that, Siddig, (2010) reported 44.4% prevalence rate of Toxoplasmosis in the same area. Another recent data (Anon, 2010) collected from private Medical diagnostic laboratories in the same city, indicated over all seroprevalence of 78% of *T. gondii* infection in pregnant women during 2006 to 2010.

2.1.5. Control of Toxoplasmosis:

2.1.5.1. Treatment of Toxoplasmosis:

Although it is very difficult, treatment of infected animals can reduce the economic losses due to toxoplasmosis in unvaccinated flocks. There are several drugs with good results such as decoquinate, monesin, clindamycine and sulphadimidine (Buxton *et al.,* 1996; Weiss and Kim, 2007; Giadinis *et al.,* 2009). The use of Combinations of pyrimethrine and sulphadimidine, vacuiloprium and sulphadimidine or trimethoprim and sulphadimidine (Buxton *et al.,* 1993b) were also recommended.

2.1.5.2. Prevention Measures:

Food and water should be kept away from cat's faeces and any contaminative environment (Dubey, 1991). Other control measures include minimizing number of cats shedding oocysts (Dubey and Jones, 2008) by limiting the breeding of cats. In addition to adequate and continuous control programs of stray and feral cats can reduce the risk of *T. gondii* transmission, beside controlling the rodent's population (Buxton and Rodger, 2008; Lopez *et al.,* 2008). Education of farmers on the principles of the rout of infection and measures that reduce the prevalence of clinical cases as

well as vaccination will reduce animal and human toxoplasmosis (Buxton *et al.,* 2007; Ogendi *et al.,* 2013).

Prevention measures for *T. gondii* infection in humans have not been reached (Bout *et al.,* 2002; Camossi *et al.,* 2013). Heating of meat to 67ºC or higher is considered sufficient to immediately kill tissue cysts (Dubey 2000).

2.1.5.2.1. Vaccination:

Following primary infection, most animals develop protective immunity. Therefore, controlling the disease using vaccines is a feasible goal (Innes and Vermeulen, 2006). The later author stated that, different vaccines are required including, vaccines to prevent congenital toxoplasmosis, vaccine to reduce or eliminate tissue cysts and vaccine to prevent oocysts shedding.

Alive vaccine from congenital toxoplasmosis (Buxton and Innes, 1995) is now commercially available in Europe. This vaccine (*"Toxovax",* Intervet) used for the control of toxoplasmosis in sheep is based on live attenuated *T. gondii* strain S48 tachyzoites obtained from mice (Buxton 1993). Other vaccine trials (Mateus-Pinilla *et al.,* 1999) with a positive titer set at the 1:25 dilution. Moreover, *T. gondii* tachyzoites irradiated vaccine was evaluated in Wistar female rats (Camossi *et al.,* 2013). They verified that the vaccine reduced the parasitic load in most analyzed organs and showed favorable effect on birth rate, but not prevent the establishment of infection with the parasite.

2.1.5.3. Control of Toxoplasmosis in the Sudan:

In order to build control strategy, data on seroprevalence of *T. gondii* infection is considered as indicator of environment contamination with the parasite. However, there are very few works in animal and human toxoplasmosis in the Sudan. Though few, most of the available data was on camel toxoplasmosis (Seri *et al.,* 2003). The other available data on animal toxoplasmosis in the Sudan are collected from few slaughtered animals (Zein Eldin *et al.,* 1985; Khalil and Elrayah, 2011; Abdel-Hafez, 2013). Consistent data on human toxoplasmosis in the Sudan have not been reached (Musa 2008; Anon 2010; Siddig, 2010). Seroprevalence of *T. gondii* infection in food animals in the Sudan is recommended, in order to build control strategies for reducing *T. gondii* infection in Sudanese people and their livestock. Therefore, the present work is unique in that, it is the first area wide and large scale study on seroprevalence of *T. gondii* in live dairy animals in the Sudan. Moreover, some economic impact and risk factors of toxoplasmosis affecting these dairy farms were discussed in this study. This cross-sectional survey was planned to assess the prevalence of *Toxoplasma* infection in farm animal's populations in the Khartoum State as the richest area of dairy farm industry in the Sudan. The shedding of oocysts in cat's faeces is generally considered to be a primary factor in the dissemination of the disease (Dubey, 2004). In view of this

attempts were made to establish the possible degree of contact with cats in the surveyed populations.

2.2. *Neospora caninum* (*N. caninum*):

N. caninum is a recent recognized protozoan parasite of animals which until 1988 was misidentified as *T. gondii* (Dubey and Schares, 2011; Dubey and Lindsay, 1996). *N. caninum* is a heteroxenous cyst forming coccidian of animals closely related to *T. gondii* (Dubey *et al.,* 2002). It was first recognized in 1984 in dogs (Dubey *et al.,* 2002). Neosporosis is the major cause of abortion in cattle in many countries (Dubey 2003; Ortega-Mora *et al.,* 2007; Dubey and Schares, 2011). It is also important cause of neuromuscular paralysis in dogs (Dubey and Lindsay, 1996). *N. caninum* can cause abortion and neonatal mortality in cattle, sheep, goats, deer and horses in many countries (Dubey and Lindsay, 1996; Youssefi *et al.,* 2009).

2.2.1. History of *N. caninum*:

N. caninum is structurally and biologically similar to *T. gondii* and until 1988 it was misdiagnosed as *T. gondii* (Dubey *et al.,* 1988). Neosporosis was first described in dogs in Norway in 1984 causing neuromuscular degeneration leading to hind limb paralysis (Bjerkas *et al.,* 1984). Later in 1988, it was described as a cause of abortion in cattle (Dubey and Schares, 2011; Dubey *et al.,* 1988). Discovery and naming of two new organisms, *Neospora caninum* (Dubey *et al.,* 1988) and *Sarcocystis neurona* (Dubey *et al.,* 1999), which were

previously thought to be *T. gondii,* resulted in new information on the host distribution of *T. gondii*.

2.2.2. General biology of *N. caninum*:

N. caninum has a wide host range, but its zoonotic potential is unknown (Dubey and Lindsay 1996; Taylor *et al.,* 2007). Several surveys indicated that wide range of domestic and wild animals have been exposed to *N. caninum*. However, viable parasite has been only isolated from few hosts including cattle, sheep, water buffalo, dogs, horses and camels (Youssefi *et al.,* 2009; Manal *et al.,* 2013) from different countries. Isolates of *N. caninum* from various hosts are genetically similar (Dubey and Schares, 2011). Previously, according to Dubey and Lindsay (1996), its life cycle is unknown because experimental studies have failed to identify the definitive host (Urquhart *et al.,* 1996). However, transplacental transmission is the only recognized mode of transmission. Recently it was stated that, the definitive host for *N. caninum* is the dog and frank clinical disease manifestations were mainly seen in dogs and cattle (Dubey, 2004; Taylor *et al.,* 2007; Manal *et al.,* 2013).

Oocysts, the key of epidemiology of neosporosis, are environmentally resistant like other oocysts of other coccidian protozoa (Neto *et al.,* 2011; Dubey and Schares, 2011). The number of oocysts shed by dogs is usually low (Dubey and Schares, 2011). Previous reports showed that oocysts stage of the parasite are resistant to environmental

conditions and convert to sporulation within 24 hours after excretion from the definitive host (Lindsay *et al.,* 1999). This stage is often not available (Dubey and Lindsay 1996; Dubey and Schares, 2006). Therefore, often tachyzoites are used for experimental infection. *N. caninum* often dies together with host tissue (Dubey and Schares, 2011). Few experimental infection of mice showed tissue cyst of *N. caninum* infective even after 13 months of subcutaneous inoculation and were resistant to 4 ºC for 7-14 days, and were killed after 1 day exposure to -20 ºC (Lindsay *et al.,* 1992). *N. caninum* is considered as one of the major causes of abortion worldwide. There are reports of infection in other animals including sheep, goats, deer and horses (Youssefi *et al.,* 2009; Dubey and Schares, 2011). Congenital transmission of *N. caninum* can frequently occur in the same animal (Taylor *et al.,* 2007; Youssefi *et al.,* 2009; Dubey and Schares, 2011).

2.2.2.1. Pathogenesis of *N. caninum* Infection:

Bovine neosporosis is mainly a disease of placenta and foetus. Following parasitaemia, *N. caninum* is able to establish itself in the maternal caruncular septum before crossing to the foetal placental villus causing damage to the foetus or its placenta as well as hormonal deregulation. Thus beside many factors the direct injury of the foetus by tachyzoites multiplication may determine the survival of the

foetus (Innes, 2007). *N. caninum* is an important cause of neuromuscular disease in dogs (Dubey and Lindsay, 1996).

2.2.3. Epidemiology of *N. caninum* Infection:

2.2.3.1. Transmission:

Vertical transmission from dam to the foetus during pregnancy (Anderson *et al.,* 1997; Innes, 2007), and postnatal ingestion of tissues harbouring cysts and oral uptake of oocysts containing sporozoites sheds in dog faeces (Lindsay *et al.,* 1999; McAllister *et al.,* 1998) are the only demonstrated modes of transmission in cattle. Calves born carrying the infection are capable of infecting their offspring (Williams *et al.,* 2009). *N. caninum* is one of the most efficiently transplacentally transmitted parasites among known microbes in cattle. Transplacental transmission plays a major role in the maintenance and spread of the disease (Davison *et al.,* 1999). According to Williams *et al.,* (2009), transplacental transmission can occur in postnatally acquired infections by ingestion of oocysts (exogenous) or reactivation of infection in chronically infected cow (endogenous). Serological examination of precolostral serum is a convenient way of measuring congenital infection. Passively acquired *N. caninum* antibodies persisted for five weeks (Cardoso *et al.,* 2008).

The ingestion of *N. caninum* oocysts from the environment is the only demonstrated natural mode of infection in cattle after birth (Neto *et al.,* 2011; Dubey and Schares, 2011). The

seroprevalence increase with age indicated significant postnatal transmission in the area under examination (Eiras *et al.,* 2011).

Other modes of transmission suggested are via pooled colostrums or milk (Uggla *et al.,* 1998) and semen (Ortega-Mora *et al.,* 2003) could also be possible. Venereal transmission is possible, but unlikely since large numbers of tachyzoites were necessary for experimental infection (Ferre *et al.,* 2008). Moreover, dams naturally bred with experimentally infected bulls failed to seroconvert (Osoro *et al.,* 2009).

2.2.3.1.1. Transmission of *N. caninum* in Dogs:

Advances concerning *Neospora* life cycle have proved dogs to be both intermediate and definitive hosts (McAllister *et al.,* 1998), and cattle and other animals to be its natural intermediate hosts (Dubey, 1999).

Vertical transmission of *N. caninum* was first recognized in dogs. The parasite is considered to be transmitted from dam to neonates during terminal stages of gestation or postnatally via milk (Dubey and Schares, 2011).

Carnivorism (the ingestion of infected tissue) is the major rout of infection to dogs (Bandini *et al.,* 2011). Infectious materials, like foetal membrane (placenta), aborted foetus, dead calves or/and cows could be major sources of infection to dogs.

2.2.3.2. Risk factors Associated with *N. caninum* Infection:

The knowledge on risk factors for herds to acquire *N. caninum* infection and *N. caninum* associated abortion is important for the development and implementation of measures to control bovine neosporosis (Dubey and Schares, 2011). It is now generally accepted that the presence of farm dogs increases the chance of *N. caninum* infection in cattle (Lindsay *et al.,* 1999; Dubey *et al.,* 2007a). Dogs as definitive hosts increase the chance of potential infection via oocyst contamination of the cattle food or environment. Specific feeding habits of farm dogs, like feeding on aborted foetuses and placenta may increase the chance of seropositivity in cattle (VanLeeuewn *et al.,* 2010a). Infectious materials, like foetal membrane (placenta), aborted foetus, dead calves or/and cows could be major sources of infection to dogs.

Differences in the farm management (feeding, pasture management, cattle density and housing) may have an influence on infection risk. Moore *et al.,* (2009) in Argentina suggested a higher risk of seropositivity in dairy than in beef herds. *N. caninum* is considered a primary pathogen. However, concurrent infection was found to be a potential risk factor for bovine neosporosis in Italy (Rinaldi *et al.,* 2007) and Vietnam (Duong *et al.,* 2008).

2.2.4. Neosporosis:

N. caninum is one of the most important causes of abortions in ruminants (Dubey and Lindsay, 1996).

2.2.4.1. Cattle Neosporosis:

Bovine Neosporosis is an important cause of reproductive failure in cattle worldwide. The definitive host for *N. caninum* is the dog and frank clinical disease manifestations were mainly seen in dogs and cattle. Neosporosis may be transmitted to cattle through consumption of infective oocysts in contaminated feeds and/or water or via vertical transmission *in utero* (Dubey *et al.,* 2007). The common signs (abortion, mummification and re-absorption following foetal death) are seen in developing foetus (Buxton *et al.,* 2002).Timing and duration of parasitaemia in the pregnant dam are the factors affecting the disease severity and outcome (Innes, 2011). Several reports emphasize the high rate of vertical transmission associated with *N. caninum* which can occur over several generations and in successive pregnancies (Dubey *et al.,* 2006, Thurmond and Hietala, 1997, Pare *et al.,* 1997). *N. caninum* seropositivity had a negative effect on reproduction parameters like first–service conception and calving intervals (Dubey and Schares, 2011). There are also more reports that neosporosis can cause repeated abortions in cattle (Anderson *et al.,* 1995, Pabon *et al.,* 2007).

2.2.4.1.1. Clinical signs of Neosporosis in Cattle:

N. caninum is a major cause of abortion in cattle (Dubey *et al.,* 2006), and worldwide seroprevalence was reviewed by

Dubey and Schares, (2011). Cows of any age may abort from three months gestation to term with abortion occurring at five to six month gestation. Fetuses may die in utero, be resorbed, mummified, autolized, stillborn, born alive with clinical signs or born clinically normal but persistently infected (Ortega-Mora *et al.,* 2007). In some epidemic herd outbreaks as many as 57% of dairy cows have been reported to abort over just few weeks up to months. Abortion outbreaks have been defined as epidemics when more than 10% or 12.5% of cows at risk abort within six to eight weeks. Small proportion (less than 5%) of cows may have repeated abortion due to neosporosis (Dubey *et al.,* 2007).

Clinical signs, other than abortion, which have only been reported in calves more than two months of age, include neurologic signs, an inability to rise and below average birth weight. The hind limbs or the forelimbs may be flexed or hyper-extended.

2.2.4.2. Neosporosis in dogs:

N. caninum was first detected in 1984 in dogs with myositis, lameness and encephalitis (Bjerkas *et al.,* 1984; Dubey 1999b) Most canine isolates of *N. caninum* were obtained from sick dogs (Ghalmi *et al.,* 2008).

N. caninum is a primary pathogen in dogs and can cause clinical disease in dogs of all ages. Most clinical canine neosporosis have been in congenitally infected dogs. In most instances, dogs are born asymptomatic and being to develop

clinical signs three or more weeks after birth (Taylor *et al.,* 2007). Paralysis of limbs, often with contracture is the most consistent sign of neonatal neosporosis.

In study in Mashhad, Iran, on 174 dog's faeces, only 3 samples were positive where oocyst was found in faeces (Ramzi 2008). Serological surveys in dogs indicate widespread exposure to the parasite worldwide (Malmasi *et al.,* 2007; Dubey and Schares, 2011).

2.2.4.3. Camels Neosporosis:

Serological studies suggest that camelids could potentially act as intermediate hosts for *N. caninum* (Wolf *et al.,* 2005). Investigation for determination of *N. caninum* as causes for abortion in Peruvian llamas (Serrano-Martinez *et al.,* 2007) showed that *N. caninum* was detected by either immunohistochemistry or specific PCR in 14 out of 50 fetuses (28%). A few studies have been performed for serodiagnosis of *N. caninum* in camels; 6 of 161 (3.72%) camels from Egypt (Hilali *et al.,* 1998), 4.6% of 308 camels from Argentina (More *et al.,* 2008) and 7 of 120 (5.83%) camels in Mashhad Iran were reported infected (Sadrebazzaz *et al.,* 2006). Relatively high seroprevalence of *N. caninum* infection in dogs in Iran (Malmasi *et al.,* 2007) support the possible role of the dogs as definitive hosts of the parasite and a possible infection source for camels.

2.2.4.4. Neosporosis in Sheep and Goats:

N. caninum occurs less frequently in ovine and is associated with abortion and weak preterm offspring (Lindsay *et al.,* 1995). Ovine neosporosis and foetal injuries are similar to those caused by *T. gondii* (Dubey and Lindsay 1990; Dubey *et al.,* 1990).

N. caninum can cause abortion, neonatal mortality and perhaps clinical signs in adult sheep (West *et al.,* 2006; Masala *et al.,* 2007; Howe *et al.,* 2008; Abo-Shehada and Abu-Halaweh 2010; Bishop *et al.,* 2010; Hassan *et al.,* 2000). Sheep are an excellent ruminant model for testing efficacies of vaccine against neosporosis abortion (Weston *et al.,* 2009). Antibodies against *N. caninum* were detected from sera of goats (Dubey and Schares, 2011; Masala *et al.,* 2007).

2.2.4.5. Equine Neosporosis:

Another species of *Neospora*, *N. hughesi* is considered to parasitize equids. These species may cross-react serologically (Gondim *et al.,* 2009, Kilbas *et al.,* 2008). Clinical neosporosis in adult horse has been reported only from USA (Finno *et al.,* 2007; Finno *et al.,* 2010). One of these cases was 23 years old mule that had myeloencephalitis (Finno *et al.,* 2010). Dubey *et al.,* (1999) concluded that *S. neurona* is a common cause of fatal encephalomyelitis in horses in the Americas**.**

Antibodies against *N. caninum* were not detected in any examined horses (101 heads) in Brazil (Dubey *et al.,* 1999)

and Argentina (Dubey *et al.,* 1999) using *N. caninum* agglutination test.

2.2.4.6. The Economic Losses due to Neosporosis:

N. caninum is a worldwide distributed pathogen which causes abortion in cows leading to economic and reproductive losses in cattle industry (Dubey 1999a; Trees *et al.,* 1999). The major economic loss due to neosporosis is reproductive failure in cattle in many countries. In addition to the direct costs involved in foetal loss, indirect costs include professional help and expenses associated with establishment of diagnosis, breeding, possible loss of milk yield (Tiwari *et al.,* 2007) and replacement costs if aborted cows are culled. The diagnosis of neosporosis-associated abortion is difficult and expensive. Postnatal losses due to neosporosis are difficult to document because there are no obvious ill effects in adult cattle other than foetal loss (Ortega-Mora *et al.,* 2007). Culling perhaps accounts for the major loss associated with neosporosis. There are difficulties in monitoring when small foetuses are expelled in the first trimester, and so there are no accurate assessments of *N. caninum*-induced losses in cattle (Taylor *et al.,* 2007). The economic production losses, other than reproduction are difficult to assess because there are no clinical signs in adult cattle, although neosporosis has been reported to decrease milk production and weight gain (Tiwari *et al.,* 2007).

The direct economic losses associated with neosporosis include reproductive failure and replacement costs associated with culling of infected stock (Dubey *et al.,* 2007). The indirect costs are associated with diagnostic investigations and re-breeding. Loss of milk yields associated with neosporosis was also reported (Thurmond & Hietla, 1997; Hernandez *et al.,* 2002).The magnitude of the economic losses incurred by primary producers around the world due to Neospora-associated abortion has been estimated to exceed hundreds of millions of dollars per year (Dubey *et al.,* 2007). In 108 randomly selected Dutch dairy herds, 76% of seropositive reference herds had no economic losses, whereas in the remaining 24% of herds, the economic losses went up to maximally €2000 per year, economic losses continued after the actual event of the abortion epidemic for at least two more years with average costs of €50 per animal per 2 years. (Bartels *et al*, 2006). Yearly economic loss due to neosporosis in Australia in beef and dairy cattle is considered 85 and 25 million $ respectively (Dubey *et al.,* 2007). The estimation in dairy cattle for New Zealand and California is 17.8 and 35 million $ respectively (Dubey *et al.,* 2007). They suggested that, the real economic loss should be more than this estimation.

2.2.4.7. Diagnosis of *N. caninum* Infection:

Diagnosis of neosporosis abortion is difficult, and often expensive. Serologic examination of dams and foetus, and the detection of lesions and *N. caninum* in a foetus by immune-histology and PCR can all aid diagnosis. As clinical diagnosis is difficult, serological tests are necessary for an exact diagnosis (Nourollahi *et al.,* 2008). Many serological tests are available for diagnosis of bovine *N. caninum* infection (Bjorkman and Uggla, 1999; Dubey and Schares 2006) including, Enzyme Linked Immunosorbent Assay (ELISA), the Indirect Fluorescent Antibody Technique (IFAT) (Schares *et al.,* 1998), the Direct Agglutination Test (DAT), and Immuno-Blotting (IB). All serological assays are based on tachyzoites antigens (Dubey and Schares, 2006).

Molecular tools are being developed to distinguish isolates which will be very valuable for epidemiological studies (Regidor-Cerrillo *et al.,* 2006; Regidor-Cerrillo *et al.,* 2008).

N. caninum was isolated from water buffaloes from Brazil by feeding tissues of naturally infected animals to dogs (Neto *et al.,* 2011). But, there is no report of clinical neosporosis in buffalo.

The first bioassay and molecular study in dogs and mice of *N. caninum* in camels in the Sudan was reported by Manal *et al.,* (2013).

2.2.4.7.1. Serologic Prevalence of *N. caninum*:

At present, the two main types of serological tests most commonly used for diagnosis of *N. caninum* infection are IFAT (Schares *et al.,* 1998) and ELISA (Nourollahi *et al.,* 2008). These tests are valuable for identifying sera with moderate to high levels anti-*Neospora* antibodies.

There are considerable differences in serologic prevalence of *N. caninum* among countries, within countries, between regions, among different animals and between beef and dairy cattle (Dubey and Schares, 2011; Bartels, 2006). However, differences in serological techniques, study design and sample size used should be considered in the evaluation of these results (Dubey *et al.,* 2007a).

Bulk milk serology is an economical way of estimating *N. caninum* prevalence on herd basis (Schares *et al.,* 2009), but this method is not as accurate as the detection of antibodies in the serum. There are indications that the *N. caninum* seroprevalence differs according to cattle breed and production system used (Munhoz *et al.,* 2009). However, in study with large sample size, seroprevalence in beef cattle and dairy cattle were similar (Eiras *et al.,* 2011).

2.2.4.7.2. Seroprevalence of *N. caninum* Infection in Cattle:

Antibodies to *N. caninum* were found in 36 of the 285 (12.6%) sera of cattle using ELISA (Nourollahi *et al.,* 2008) in Iran. With regard to seropositivity, no significant differences were observed regarding origin, sex and age. Youssefi *et al.,*

(2009) reported seroprevalence of (32%, 76/237) antibodies to *N. caninum* in cattle in Northern Iran. They also observed a significant difference regarding *N. caninum* infection in rural (43.9%, 36/82) and industrial (25.8%, 40/155) cattle. Seroprevalence of 7%, 45.2% and 57.5% were observed for cattle from three different climatic regions of Iran (Youssefi *et al.,* 2010). Gestation number, abortion number and age of cattle had specific effects on *N. caninum* seroprevalence. Cattle with history of one abortion and gestation had highest seroprevalence. The first case of confirmed neosporosis in aborted foetuses in dairy cattle from Mashhad, Iran was reported in 2007, when 25.8% (123/810) were positive with IFAT (Ramzi *et al.,* 2007). In turkey, seroprevalence of cattle neosporosis varies between 2% and 32.7% (Yildiz *et al.,* 2009). There is no significant difference in seropositivity among cattle breeds (Yildiz *et al.,* 2009) who reported *N. caninum* seroprevalence of 10.8% (60/557). Significant levels of *N. caninum* antibodies were detected in 5.5% of the cattle sera by ELISA in Southern Vietnam (Huong *et al.,* 1998).

2.2.4.7.2.1. Seroprevalence of *N. caninum* infection in Cattle from the Sudan:

There is meager information about the frequency and etiological factors of abortion in dairy cattle other than *Brucella* and none is known about *T. gondii* and *N. caninum*. The first preliminary study on *N. caninum* in the Sudan reported seroprevalence rate of 43.7% (7/16) at herd level and 9% (18/200) at individual level (Amira *et al.,* 2012) in

the Khartoum State. In this report, Prevalence rates of 6.6%, 14.9%, and 4.7% were recorded in Khartoum, Khartoum North and Omdurman districts, respectively. No significant difference due to locality, animal breed, sex and age were observed. At the same time Hussein *et al.,* (2012) reported 15.9% seroprevalence of *N. caninum* in sera of cattle from different States.

2.2.4.7.3. Seroprevalence of *N. caninum* Infection in Other animals:

There are very few data available on other animals neosporosis worldwide (Hosseininejad *et al.,* 2009; Dubey and Schares, 2011) compare to that on cattle. For example, the only available reference of *N. caninum* infection in cats was that of South Africa, (Cheadle *et al.,* 1999) who reported 5.9% (4/68) cat's serum samples positive for antibodies to *N. caninum*.

2.2.4.7.3.1. Seroprevalence of *N. caninum* Infection in Sheep and Goats:

In United Kingdom, Helmick *et al.,* (2002) obtained only three positive (0.45%) out of 660 sera of aborting sheep for *N. caninum* using IFAT. *Neospora* prevalence values of 9.5 were found in reactive ovine in Brazil (Figliuolo *et al.,* 2004). Recent serological survey in sheep indicate very low (0.6% in New Zealand) to high (30.8% in Brazil) prevalence in asymptomatic sheep (Dubey and Schares, 2011). IgG antibodies directed against *N. caninum* were observed in 9.2% of the tested sheep sera from Brazil using the IFAT

(Figliuolo *et al.,* 2004). Only 12 sheep (32.14% n=38) and 2 goats (n=4) were positive for *N. caninum* in the tested domestic animals in Gabon (Mbiye *et al.,* 2013). In their work, none of the cattle (n=46) or pigs were found positive for antibodies against *N. caninum*. Serologic surveys indicate 2-23% prevalence in goats (Dubey and Schares, 2011; Masala *et al.,* 2007).

2.2.4.7.3.2. Seroprevalence of *N. caninum* Infection in Camels:

To our knowledge, very few serologic references were available on camel neosporosis (Hilali *et al.,* 1998; Sadrebazzaz *et al.,* 2006; Hosseininejad *et al.,* 2009). Antibodies to *N. caninum* were found in 6 of 161 (3.7%) camels in titers of 1:40 (2 camels) and 1:80, 1:160, 1:640 and 1: 1280 in 1 camel, using *N. caninum* formalin preserved whole tachyzoites as antigen (Hilali *et al.,* 1998) using *Neospora* Agglutination Test (NAT) as described by Romand *et al.,* (1998). A prevalence of 3.22% and 5.83% of *N. caninum* antibodies were reported from Iranian camels using IFAT (Hosseininejad *et al.,* 2009; Sadrebazzaz *et al.,* 2006).

2.2.4.7.3.3. Seroprevalence of *N. caninum* Infection in Water buffaloes:

The first data on *N. caninum* seroprevalence in water buffaloes was reported by Dubey *et al.,* (1998) in Egypt. Antibodies to *N. caninum* were found in 51 (68%) of 75 buffaloes in titers of 1:20 (6 buffaloes), 1:40 (15 buffaloes), 1:160 (1 buffalo), 1:320 (1 buffalo) and ≥ 1:640 (28

buffaloes), using *N. caninum* formalin-preserved whole tachyzoites as antigen (Dubey *et al.,* 1998). Significant levels of *N. caninum* antibodies were detected in 1.5% of the water buffalo sera by enzyme-linked immunosorbent assay in Southern Vietnam (Huong *et al.,* 1998). This is the first report of serological evidence of *N. caninum* infection in the water buffalo from Vietnam.

2.2.4.8. Control of *N. caninum* Infection:

2.2.4.8.1. Treatment of Neosporosis:

Treatment of cattle appears to be uneconomical due to the fact that it can only be used as a preventive measure and hence it must be long-term and likely produce unacceptable milk or meat residues (Taylor *et al.,* 2007; Dubey and Schares, 2011). Currently, there is no chemotherapy for bovine neosporosis that has been shown to be safe and effective (Dubey and Schares, 2011). Treatment of clinical neosporosis with currently available drugs including clindamycine is only partially effective. None of the drugs kill *N. caninum* tissue cyst (Ramzi, 2008; Tenter, 2000).

2.2.4.8.2. Prevention Measures:

Many control measures have been discussed to reduce *N. caninum* infection in cattle (Dubey *et al.,* 2007a), including embryo transfer (de Oliveira *et al.,* 2010), artificial insemination (Yaniz *et al.,* 2010), culling, replacement heifers, chemotherapy, and vaccination (Dubey and Schares, 2011). Annual serological screening could be useful in a

control strategy because long term study showed that *N. caninum* seropositivity was very stable during the observation period and *N. caninum* seroposistive cows showed a high rate of repeat abortion (Pabon *et al.,* 2007).

2.2.4.8.3. Vaccination:

At present there is no commercial vaccine for neosporosis. However, studies indicated that cattle develop cellular immunity after inoculation with killed vaccine formulations (Innes *et al.,* 2007; Moore, 2011). There is much interest in the prospect of developing an effective vaccine against *N. caninum* in cattle to contribute to the efforts aimed at preventing abortions in cattle (Innes *et al.,* 2002; Innes and Vermeulen, 2006).

2.3. Mix-infection of *N. caninum* with *T. gondii* and Other Abortifacient Pathogens:

N. caninum may cause increased susceptibility to other infectious agents (Bjorkman *et al.,* 2000; Yildiz *et al.,* 2009). Co-existence of *N. caninum* seropositivity was frequently observed with specific antibodies against the other infectious agent tested such as *T. gondii*, *Brucella abortus* and *Listeria monocytogenes* (Yildiz *et al.,* 2009). Co-infection of *T. gondii* could occur with *N. caninum* infection, and should be considered in differential diagnoses (Dubey and Schares, 2011). Additionally, toxoplasmosis is an opportunistic infection where antibodies to *T. gondii* are detected more frequently in sick (Immunosuppressed) animals and any

complications of primary disease (Svoboda *et al.,* 1988; Svoboda *et al.,* 1998) such as neosporosis and brucellosis (Huong *et al.,* 1998; Hilali *et al.,* 1998; Figliuolo *et al.,* 2004; Yildiz *et al.,* 2009).

2.3.1. Mix-infection in Cattle and Water buffaloes:

Mixed infection of *N. caninum* with *T. gondii* in cattle was reported in Vietnam (Huong *et al.,* 1998). They stated that, about 10.5% of the cattle sera seropositive to anti-*N. caninum* antibodies were found to contain *T. gondii* antibodies (Huong *et al.,* 1998). The co-existence rate of *N. caninum* seropositivity with *T. gondii*, *Brucella abortus* and *Listeria monocytogenes* was 24.77% (138/557), 13.82% (77/557) and 42.85% (162/378) respectively in Turkey (Yildiz *et al.,* 2009). Only one animal had significant antibody titers for all above mentioned analyzed infectious agents. Co-existence of *N. caninum* seropositivity was frequently observed with specific antibodies against the other infectious agent tested: *N. caninum-T. gondii* (8/60, 13.33%), *N. caninum*-B. abortus (6/60, 10%), and *N. caninum-L. monocytogenes* (4/60, 6.66%), *N. caninum-T. gondii-B. abortus* (6/60, 10%), *N. caninum-T. gondii-L. monocytogenes* (1/60, 1.66%) and *N. caninum-T. gondii-B. abortus -L. monocytogenes* (1/60, 1.66%).

Antibodies to *T. gondii* were not found in a 1:100 dilution of serum of any of the 75 *N. caninum* seropositive buffaloes,

using *T. gondii* as antigen, indication specificity in the detection of antibodies to *N. caninum* (Dubey *et al.,* 1998). However, about 3% of the water buffalo sera seropositive to anti-*N. caninum* antibodies were found to contain *T. gondii* antibodies in Vietnam (Huong *et al.,* 1998).

2.3.2. Mix-infection in Sheep and Goats:

In United Kingdom (Helmick *et al.,* 2002) obtained only three positive (0.45%) out of 660 sera of aborting sheep for *N. caninum* using IFAT and 167 (39.5%) for *T. gondii* by Latex agglutination test (LAT ≥1:64). IgG antibodies directed against *N. caninum* were observed in 9.2% of the tested sheep sera using IFAT from Brazil, (Figliuolo *et al.,* 2004). About 3.5% of these tested sheep found to be infected with both *T. gondii* and *N. caninum*. All farms had at least one positive animal for *T. gondii*, and 26 of the 30 farms had at least one positive animal for *N. caninum*. Mbiye *et al.,* (2013) reported co-existence of *N. caninum* with *T. gondii* in 32.14% of the investigated sheep from Gabon. However, none of the examined cattle and pigs showed the co-infection.

2.3.3. Mix-infection in Dogs and Cats:

A bitch that had high *N. caninum* IFAT titer during pregnancy delivered pups coinfected with *T. gondii* and *N. caninum* (Al-Qassab *et al.,* 2009b).

In South Africa, Cheadle *et al.,* (1999) reported 5.9% (4/68) cat's serum samples positive for antibodies to *N. caninum* and 74% (50/68) samples tested positive for antibodies to *T.*

gondii. The four animals tested positive to both *N. caninum* and *T. gondii.*

2.3.4. Mix-infection in Camels:

Participation of *N. caninum* and *T. gondii* in abortion cases was investigated in South American camelids. Results of this investigation showed that these two closely related protozoan parasites were important causes of abortions in camel population (Serrano-Martinez *et al.,* 2007). Base on serologic tests, South American camelids (Coppens 2006; Dubey *et al.,* 1992) and *Camelus dromedaries* (Hilali *et al.,* 1998; Sadrebazzaz *et al.,* 2006; Hosseininejad *et al.,* 2009) are suggested to be potential intermediate hosts for *N. caninum* and *T. gondii*. Mix-infection in camels was examined in Egypt by Hilali *et al.,* (1998) during their first report of *N. caninum* seroprevalence in camel. Antibodies to *N. caninum* were detected in 6 camels (3.7%) and *T. gondii* were found in 17.4% of 166 camels in titers of 1:25 (3 camels), 1:50 (18 camels) and ˃ 1:500 (8 camels) using *T. gondii* tachyzoites. All 6 camels with *N. caninum* antibodies had no *T. gondii* antibodies in 1:4 dilution of serum, indicating specificity of the reaction. Similarly, none of the 10 (3.22%) positive serum samples for *N. caninum* showed positive results for *T. gondii* in Iranian camels (Hosseininejad *et al.,* 2009). *N. caninum* was detected in 14 out of 50 fetuses (28%) of Peruvian llamas while *T. gondii* DNA was not detected in any of the analyzed fetuses (Serrano-Martinez *et al.,* 2007).

2.4. *N. caninum* Infection in the Sudan:

The first bioassay and molecular study in dogs and mice for *N. caninum* in camels in the Sudan was reported recently by Manal *et al.,* (2013).

Only two reports are available for the occurrence *N. caninum* in cattle in the Sudan (Amira *et al.,* 2012; Hussein *et al.,* 2012). The cattle tested in their studies are very few. Therefore, the both authors recommended a large scale seroepidemiological investigation of *N. caninum* in cattle and other animals. Therefore, the present study was planned to assess a large scale sero-prevalence of *N. caninum* infection in farm animals in the Khartoum State as the richest area of dairy animals in the country. The study also compared the prevalence of *N. caninum, T. gondii* and the most recent available data on *B. abortus* as well their co-existence in sera of the examined dairy cows and the co-herded camel, sheep and goats. This may help in the evaluation of the economical value of these protozoal abortifacients (*N. caninum* and *T. gondii*) compared to the well known bacterial abortifacient (*B. abortus*). Risk factors such as farm management and presence of dogs, associated with the seropositive farms were also discussed in this study.

3. MATERIALS AND METHODS

The investigation was carried out in compliance with animal welfare code in the Sudan.

3.1. The Study Design:

Observational descriptive method was used in this study.

3.2. The Study Area Description:

Khartoum State, the capital State of the Sudan, is one of the eighteen States of the country (fig. 1). Although it is the smallest State area wise (22,142 km^2), yet it is the most populous with approximately 5,274,321 inhabitant (2008 census), and growth rate of 8.9 % (The Economic Review, 2002). The State is located in the central region of the country. The state lies between longitudes 31.5 to 34 °E and latitudes 15 to 16 °N (table 3.2). It is surrounded by River Nile State in the North-east, in the North-west by the

Northern State, in the East and Southeast by the States of Kassala, Gedarif and Gezira, and in the west by North Kordofan. The Northern part of the State is mostly deserting because it receives barely any rainfall. The other parts have semi-desert climates. The weather is rainy in the summer, and cold and dry in the winter. The average rainfall reaches 100–200 mm in the Northeastern areas and 200–300 mm in the Northwestern areas of the State. The temperature in summer ranges from 25 to 40 °C from April to June, and from 20 to 35 °C in the months of July to October. In winter, the temperature declines gradually from 25 to 15 °C between March and November. The River Nile, Blue Nile and White Nile divide the state into three districts (towns) namely, Khartoum, Khartoum north and Omdurman (Table 3.2). The State has seven localities, two in Khartoum, two in Khartoum North and three in Omdurman (table 3.3). In the countryside most people are engaged in agriculture and animal grazing and thus they supply the capital, Khartoum, with vegetables, fruits, and dairy products. Sudan dairy farms industries of different production systems are mainly concentrated in the Khartoum State (MLFR 2014).

According to Anonymous (2012), the State has an estimated livestock population standing at around 1,348,676 heads (table 3.1).

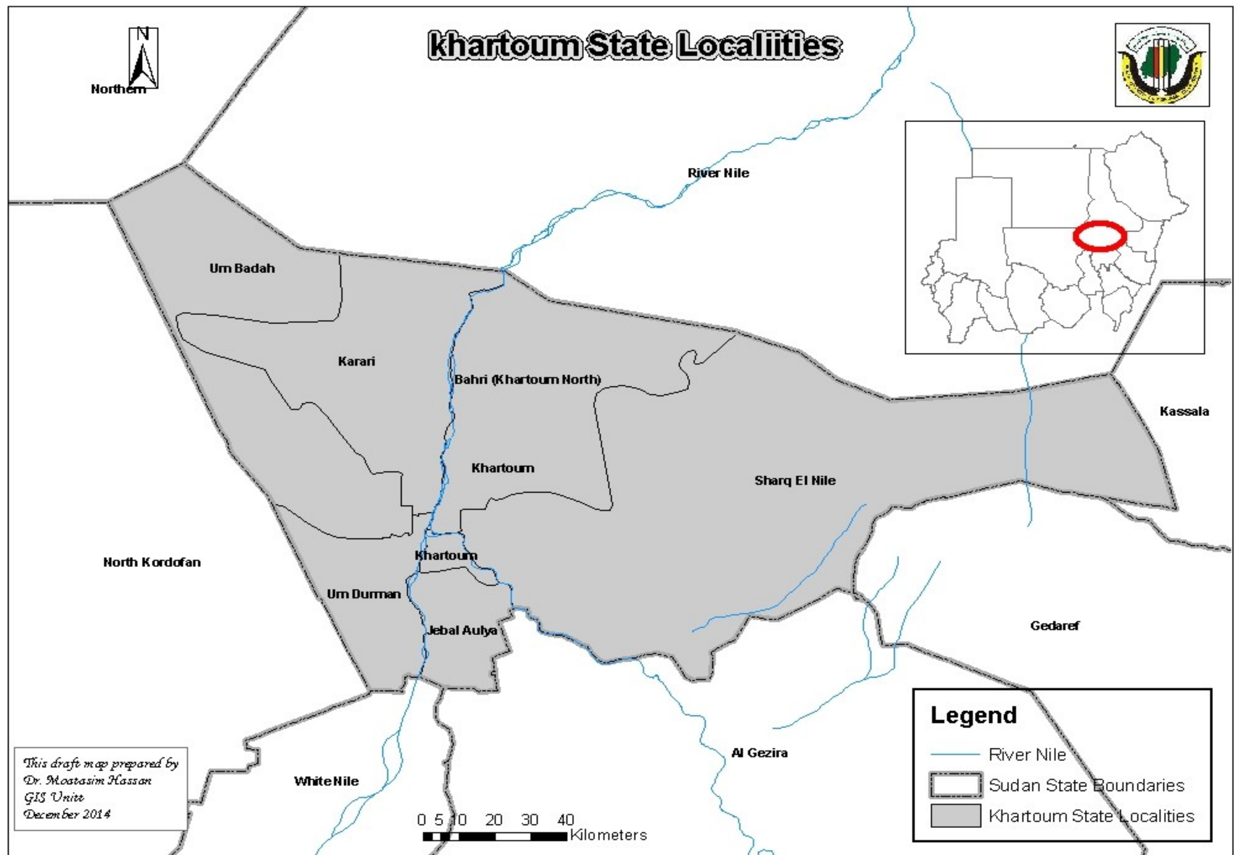


Fig 1: Map of Sudan showing the area of the Study (The Khartoum State).

3.2.1. Criteria for Selection of the Study Area:-

a) Large numbers of dairy farms of different production systems (intensive and semi-intensive) are available in the Khartoum State.

b) Dairy cattle of different breeds (crossbred and foreign) are found in the State.

c) Sheep and goats originating from different parts of the country as well as foreign breeds are also available.

d) Camels were recently introduced to be used for milk production in the State (Plate 3.2).

3.3. Study Population:

There are two study populations including human (Farm personnel) and their animals (Dairy cows, camels, sheep and goats).

i. Human Population: Dairy farm personnel including owners, workers or farm managers were interviewed using guide questionnaire for their awareness on these abortifacients.

ii. Animal Population: Farm animals including, cattle, camels, sheep and goats. The presence of domestic or stray dogs and cats in the investigated dairy farms were also considered in the guided questionnaire.

3.3.1. Animals:-

3.3.1.1. Cattle: Dairy cattle were the main target farm animals in this study (plate 3.1). Because:

(i) They constitute the majority of dairy farms in the study area.

(ii) Dairy cattle herders continuous complain of reproductive failure.

(iii) *Neospora caninum* is believed to be the major cause of abortion in cattle rather than other domestic animals.

(iv) Cattle are the major source of diet (meat and milk) in the State.

3.3.1.2. Sheep, Goats and Camels:

Representative sheep, goats and camels were sampled when found co-herded with dairy cattle in the same farm (Plate 3.1) or near the sampled dairy farm for comparison in seroconversion against *N. caninum* and *T. gondii*.

The few newly introduced specialized camel dairy farms were also sampled for the same purpose (Table 3.4).

3.3.1.3. Dogs and Cats: Those dogs and cats (the final hosts) observed within the vicinities of the investigated farms were documented and if possible sampled for presence of Oocyst in their faeces.

3.3.1.4. Inclusion and Exclusion Criteria of the investigated Animals:

i. Dairy farms in the seven localities of the three Districts of the State were visited for samples collection.

ii. Adult (≥2 years old) female dairy animals (Cows, She-camel) and (≥1 years old) ewes and does were tested to avoid measuring antibodies passively transferred in colostrum.

iii. Animals had no any overt symptoms of disease at sampling.

iv. The tested animals were not vaccinated against any of the agent investigated in this study.

v. Blood samples from dairy cows were collected through a systemic random sampling procedure (Thrusfield 1995). However, the low population density of the other investigated dairy animals or co-herded (camel, sheep and goats) in the visited farms enacted the sampling of available ones (Plate 3.1).

3.4. Sampling (Samples Design and Size):

A cross section survey was conducted to collect the required epidemiological data. The need to use the herd as the basic statistical unit for the economic study, beside the lack of an appropriate sampling frame, cluster sample (Otte and Gumm 1997) was used in this study.

The State was divided in to three Districts (strata), then into seven localities (substrata) according to the administrative division, these are: Khartoum North (Bahri), Eastern Nile, Khartoum, Jebel Aolia, Omdurman, Ombadda and Karary.

The number of clusters (herds) in each stratum was calculated according to Bennett *et al.,* (1991) following formula: $C= P(1-P)D/SE^2\, n$

Where C is the number of clusters to be sampled, P is the expected prevalence, D (=4) the design effect of using cluster sample instead of simple random sample,

SE (=0.05) is the standard error of the estimate and n (=20) is the average cluster size.

It worth mentioning that, because of the lack of any previous large scale prevalence of Toxoplasmosis or/and neosporosis in any of the selected (sampled) localities, therefore, we used the previous prevalence of Brucellosis reported by Anon (2011). Number of clusters for each locality was presented in table (3.3). Details of sample sites and their number in different localities were presented in table (3.5).

Individual animals were sampled from the randomly selected herds in the selected cluster either randomly or conveniently. Extra animals were sampled to compensate for problems encountered while transporting or handling the sera. More than 2 years old female crossbreed of Zebu (Local) and Friesian dairy cattle were sampled. All animals were adult unvaccinated cows from private farms keeping 5 up to 600 dairy cows. These herds were divided in to two categories including (i) small scale herds, containing from 5-100 dairy cows and (ii) large scale herds, containing more than 100 dairy cows.

3.5. Collection of Epizootiological Data:

A questionnaire survey for herders was filed in at the time of blood collection. The questionnaire (Appendix 4) included the following data:

(i) Dairy farm personnel (owners, farm manager or worker) of the sampled farm were interviewed about farm and animal management, reproductive background, dealing with the aborted or infertile cows, the age of the aborted cow and the age of the aborted foetus, and the information obtained recorded.

(ii) Data concerning production parameters and socio-economic characteristics of animal's keepers, community familiarity, awareness and perception about causes of abortion were collected.

(iii) Occurrence and frequency of reproductive failure problems (repeat breeding, still-birth, repeated abortion); data needed to estimate the economic losses due to these problems were also gathered and reported.

(iv) Information on the possible potential risk factors such as herd size/farm type, type of housing, feeding and watering practice was reported. On the other hand, data concerning presence of domestic or/and stray dogs and cats were thoroughly documented.

3.6. Samples Collection:

The survey was carried out during 20 months period between October 2012 and June 2014. Blood samples of a total 1477 animals (1216 cattle, 100 sheep, 100 goats and

61 camels) were collected from 177 dairy farms (132 cattle, 16 sheep, 16 goats and13 camel's herds), taking the advantage of blood drawing for our national ongoing research project on brucellosis testing programme carried by Angara *et al.,* during 2012 - 2014.

3.6.1. Serum Samples:

Approximately, 5-8 ml of blood from jugular vein was aseptically collected in plain vacutainers for serum collection. All samples were immediately transported on ice to the Laboratory of Parasitology, College of Veterinary Medicine (CVM), Sudan University of Science and Technology (SUST). After clotting, serum was removed after centrifugation at 5000xg for 10 min. Each serum sample was divided into three cryotubes (aliquots), labeled for Toxo-latex, Toxo-EIA and Neospora cELISA, and stored at -20 ºC until needed for laboratory testing. At the end of the study a total of 1477 serum samples were collected and investigated.

3.6.2. Faecal Samples:

Faecal samples were collected from dogs and cats when available for oocysts identification.

3.7. Serological Tests:

T. gondii specific IgG antibodies were detected by both Latex Agglutination Test (LAT) and Indirect Enzyme Linked Immuno Sorbent Assay (ELISA). However, *N. caninum* specific IgG

antibodies were detected using competitive ELISA (cELISA) as follows:

3.7.1. Detection of Antibodies Against *Toxoplasma gondii*:

Specific anti- *T. gondii* IgG antibodies in the sera of all surveyed animals were determined using latex agglutination test (LAT) as screening (reference) test, and indirect enzyme linked immunosorbent assay (ELISA) for confirmation and validation of the screening test in cows sera. The level of agreement between the two tests as well as the sensitivity and specificity of the two tests in the detection of antibodies to *T. gondii* in dairy cows sera were calculated using Kappa test and Receiver Operating Characteristic (ROC) Curve (Landis and Kock 1977; Dohoo *et al.,* 2003; Thrusfield 2005; Viera and Garrett 2005; Fan *et al.,* 2006),

3.7.1.1. Latex Agglutination Test (LAT):

Toxo-Latex reagent is a suspension of Polystyrene latex particles coated with soluble *T. gondii* antigen. The Toxo-Latex diagnostic kits *(Spinreact, S.A./S.A.U, Spain)* were purchased from *Shifak Company*, Khartoum, Sudan (Appendix 1). *T. gondii* RH strain tachyzoites salt soluble antigen was used in these kits.

3.7.1.1.a. Screening Test:

Initially, all sera were examined against *T. gondii* RH antigen for detection of specific anti- *T. gondii* IgG antibodies qualitatively based on the LAT manufacturer's instructions.

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 respective circle on the slide and a drop of the antigen suspension (25µl) was placed on the corresponding circle. After mixing the contents of each circle uniformly over the entire cycle by separate sticks, the slide was then rocked gently to and forth for ≤4 minutes and then examined for agglutination visually. Visible clumps indicate positive agglutination (Plate 3.3). Positive and negative controls must be observed. If the screening test was positive, the serum sample was subjected to semi-quantitative test to obtain the level of antibody titration to the specific antigen.

3.7.1.1.b. The Level of Antibody Titration:

After screening of the serum samples and the positive group was assorted, for titration of these positive sera, serial double dilutions of 1:2 up to 1:256 were prepared from serum using normal saline and examined by the same method of screening test mentioned above. Diluted serum-antigen mixture was mixed well as in the qualitative method using separate stick for each dilution (Plate 3.3). The highest dilution with positive agglutination was read as the titer of the respective serum sample. Sera having titer of ≥1:2 were considered positive for antibodies to *T. gondii*.

The herd considered positive when at least one animal was found positive. There is no clear assessment of which positivity threshold should be considered using LAT in cattle. Therefore, we analyzed data using threshold 1:2 against

ELISA in order to examine consistency between results obtained at different threshold.

3.7.1.2. Indirect ELISA:

This serological test was performed in dairy cows sera only. According to the results of LAT, animals (dairy cows) were divided in to two groups; LAT seropositive group and LAT seronegative one.

Commercial Enzyme Immuno-assay (EIA) test kits (DRG instruments GmbH, Germany) were purchased from Shifak Company, Khartoum, Sudan (Appendix 2). They were used according to the manufacturer's recommendation with the exception of that; bound IgG was detected with species specific anti-IgG Peroxidase conjugate as follow:

a. **Conjugate:** Anti-bovine IgG Peroxidase (Sigma) was donated from the Department of Parasitology, Veterinary Research Institute (VRI), Khartoum, Sudan, and it was diluted 1:30000 in 0.1M (pH 7.4) Phosphate Buffered Saline (PBS).

b. **Serum Dilution:** Test serum was diluted 1:400 in the Diluents' buffer of the commercial Diagnostic (DRG) kit.

c. **Cut-off point:** from the LAT negative serum samples group, 32 sera (eight groups 4 each) were tested by ELISA to calculate the cut-off point (OD=1.86). It was calculated from the mean OD of these negative controls plus two standard deviations. Optical density

was measured in a microplate automatic reader (*Biotek, USA,* and the programme used in the reader is *KCjunior*) at a wave-length of 620 nm (Plate 3.4).

Serum considered positive if the OD of the well was equal to or greater than 1.86. A strong positive and negative control obtained from LAT seropositive and confirmed by ELISA was included in each plate assay for calibration.

3.7.2. Detection of Antibodies Against *Neospora caninum*:

The level of antibodies directed to *N. caninum* in the sera of investigated animals was determined using a commercially available cELISA kits for detection of specific anti-*N. caninum* IgG. The kits were imported from VMRD, USA, (VMRD, Inc., WA, USA) through *Autma Development Company*, Khartoum, Sudan (Appendix 3). The test is based on the principles of competitive ELISA in which, sample serum with antibodies to *N. caninum* inhibits binding of Horse Radish Peroxidase (HRP)-labeled *N. caninum* specific monoclonal antibody to *N. caninum* antigen coated to the microplates. Binding the HRP-labeled monoclonal antibody conjugate is detected by the addition of enzyme substrate and quantified by subsequent colour produced (Plate 3.4). Weak colour, development due to inhibition of the conjugate indicates the presence of the parasite antibodies in the examined sera. The cut-off value

of antibody percentage inhibition (pi) is ≥30 i.e. all samples recorded antibody pi ≥30 were considered positive.

The kit was used according to manufacturer’s instructions. Briefly, serum samples were thawed and after vortexing, 50µl of undiluted sera was loaded into the *N. caninum* antigen coated plate, and incubated at room temperature for one hour. Then, the wells were washed 3 times with the wash buffer and 50µl of HRB conjugated with anti *N. caninum* monoclonal antibody were added to each well and incubated for 20 minutes at room temperature. The plate was washed again and 50µl of substrate solution was added and incubated at room temperature for 20 min. then 50µl of stop solution were added and the plates were read in an ELISA micro-plate automatic reader (*Biotek, USA*) at a wave-length of 620 nm. Two of each negative and positive control sera were included in each run of analysis, and the mean of the OD of the two negative controls (NC) was calculated. The optical density (OD) of the ELISA was recorded and the pi values of the tested samples were calculated by the following formula: Pi= 100[1-(sample OD/NC OD)]. A herd was considered positive when at least one animal with positive antibody reaction was detected.

3.8. Detection of Antibodies Against *Brucella abortus*:

Two serological tests were used in this study for detection of *B. abortus*. The Rose Bengal Plate Test (RBPT), was used as

screening test (Barroso *et al.,* 2002). The positive results were confirmed by c-ELISA and only animals positive on both RBT and c-ELISA were classified *Brucella* seropositive.

3.8.1. Rose Bengal Plate Test (RBPT):

All serum samples were screened by the RBPT for the presence of antibodies against *B. abortus*. The antigen and the serum samples were removed from the refrigerator to room temperature before use. After shaking properly, equal quantity of serum sample and (RBPT) antigen (30µl) were taken on an enamel plate, mixed thoroughly with metal stick. The plate was then shaken on a rocker for 4 min. Clear agglutination was considered as positive reaction (Alton *et al*., (1988).

3.8.2. Competitive enzyme linked Immuno-sorbent Assay (c-ELISA):

This test was used as a confirmatory test to eliminate any positive reaction in the (RBPT) due to vaccination or cross reaction. The c-ELISA kit was obtained from (Veterinary Laboratory Agency, New Haw, Addlestone, Surrey KT 15 3NB United Kingdom). Result was used for comparison, association between these abortifacient agents and serological co-existence of *B. abortus* with *T. gondii* and *N. caninum* in the investigated dairy farms.

3.9. Data Management and Statistical Analysis:

All collected data were entered, coded and stored in a Microsoft® Excel spread sheet for Windows® 2007 data base. The Statistical Package for Social Sciences (SPSS) for Windows® version 17 was used for all appropriate statistical analysis. Descriptive statistics for the variables were obtained. For each variable frequencies and seroprevalence were obtained by cross-tabbing. Seroprevalence was defined as the percentage [number of positive results (p)/total animal population (N)]. Hypothesis of differences of variables (factors) were first tested statistically by univariate analysis via the 2-tailed chi-square test (x^2) of independence to determine significant association or differences between infection by *N. caninum* or/and *T. gondii* and other factors studied. The differences were considered statistically significant when $p \leq 0.05$. In the next step, all potential risk factors with $p \leq 0.25$ were entered into a logistic regression model to assess the association between the potential risk factors and the outcome variable *T. gondii* or *N. caninum* serological status. Association in the logistic regression model were deemed significant when $p \leq 0.05$.

The magnitude of risk association was determined by the occurrence probability ratio (odds ratio, OR), and significance was determined for a 95% confidence

interval. In addition to the risk factors collected from the questionnaire, opinions, perception and comments collected from dairy farm personnel were also reported and calculated.

The level of agreement between the LAT and ELISA tests was calculated using Kappa test (Dohoo *et al.,* 2003; Thrusfield 2005), and evaluated based on Landis and Kock (1977) and Viera and Garrett (2005). Moreover, the sensitivity and the specificity of the two tests were calculated using Receiver Operating Characteristic (ROC) Curve (Fan *et al.,* 2006).

Maps were produced using *Arc GIS version 10.2.2 (ESRI, Redlands, California)* to show the study area and distribution of the seroprevalence of *T. gondii* and *N. caninum* in the different localities of the State.

Plate 3.1: Co-herded Dairy Farms (Cattle, Camels, Sheep and Goats as well as horses).

Plate 3.2: Profesional Camels dairy Farms in Eastern Nile and Bahri

Table 3.1: Live stock estimate in the Khartoum State (MLFR 2012).

Total	Camels	Goats	Sheep	Cattle	State
1,348,676	6,651	651,052	446,284	244,688	**Khartoum**

Table 3.2: Some Geographical information about the study area.

District	Latitude	Longitude	E. Sea Level
Khartoum	15º 35’17”N	32º32’3”E	1250ft (381m)
Khartoum North	15°32′47″ N	32°32′00″ E	1243 ft (379 m)
Omdurman	15º38’09”N	32º26’13”E	1276ft (389m)

Table 3.3: Distribution of dairy herds/animals sampled for detection of antibodies directed to *T. gondii* and or/and *N. caninum* in the seven localities of the Khartoum State.

Sampled Area			No of samples	
District	**Locality**	**N of areas**	**N of Herds**	**N of Animals**
Kh. North	E. Nile	6	28	301
	Bahri	6	29	328
Sub total		**12**	**57**	**629**
Khartoum	Jabal Aolia	8	58	378
	Khartoum	2	12	96
Sub total		**10**	**70**	**474**
Omdurman	Omdurman	2	32	186
	Ombadda	4	11	105
	Karary	1	7	83
Sub total		**7**	**50**	**374**
Total	7	**29**	**177**	**1477**

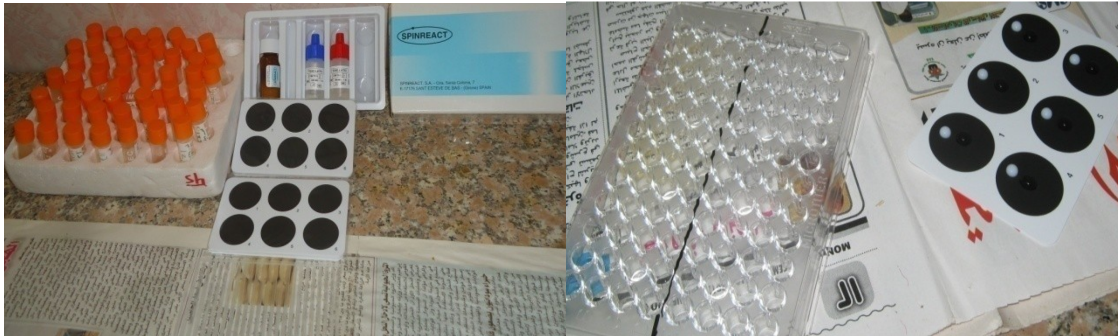
Table 3.4: Number of animals sampled for detection of antibody against *T. gondii* or/and *N. caninum* in the three district of the Khartoum State.

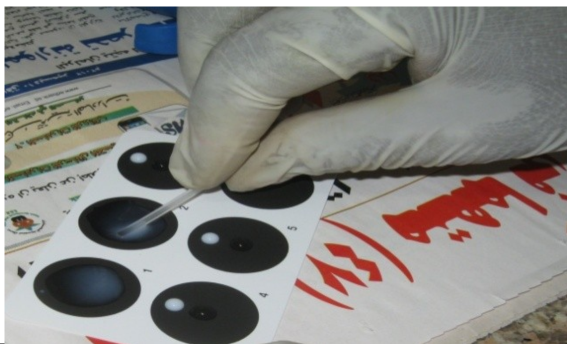
Animals District	**Total**	**Number of Animals sampled (N of herds)**			
		Cattle	**Sheep**	**Goats**	**Camels**
Kh. North	629 (57)	569 (47)	0 (0)	17 (3)	43 (7)
Khartoum	474 (70)	292 (39)	89 (14)	75 (11)	18 (6)
Omdurman	374 (50)	355 (46)	11 (2)	8 (2)	0 (0)
Total	**1477(177)**	**1216 (132)**	**100 (16)**	**100 (16)**	**61 (13)**

Table 3.5: Details of the sampled sites of dairy farms tested for detection of antibodies directed to *N. caninum* or/and *T. gondii* in the State.

District	Locality	Site	N of herds	No. of Animals
Khartoum North	E. Nile	Ed Babekir	4	40
		Haj Yosif	7	74
		Mahlab3	9	67
		Soba East	5	75
		Elafoon	1	22
		Um-Dawanban	2	23
Sub Total		**6**	**28**	**301**
	Bahri	Bahri	14	163
		Halfaya	4	47
		Faki-Hashem	4	33
		Tibna	3	58
		Kadarou	3	25
		Shambat	1	2
Sub Total		**6**	**29**	**328**
Khartoum	Jabal Aolia	Saig project	11	66
		Zarayeb Shock	3	21
		Taiba Elhasanab	10	84
		Shigailab	8	63
		Soba project	9	60
		Sundus Project	6	42
		Um-Arda	3	25
		Jabal Aolia	8	17
Sub Total		**8**	**58**	**378**
	Khartoum	Jeraif West	6	43
		Azozab	6	53
Sub Total		**2**	**12**	**96**
Omdurman	Omdurman	Omdurman	21	115
		Fetaihab	11	71
Sub Total		**2**	**32**	**186**
	Karary	Karary	**7**	**83**
	Ombadd	Elssalam	5	48

	a			
		Elahamda	2	13
		Elsarha	1	6
		Rudwan project	3	38
Sub Total		**5**	**11**	**105**
		29	**177**	**1477**





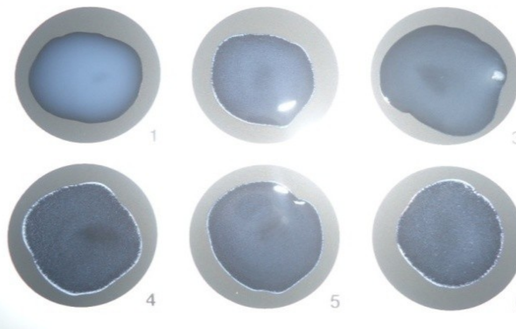


Plate 3.3: Latex Agglutination Test for detection of Antibody against *T. gondii* (Qualitative and quantitative).

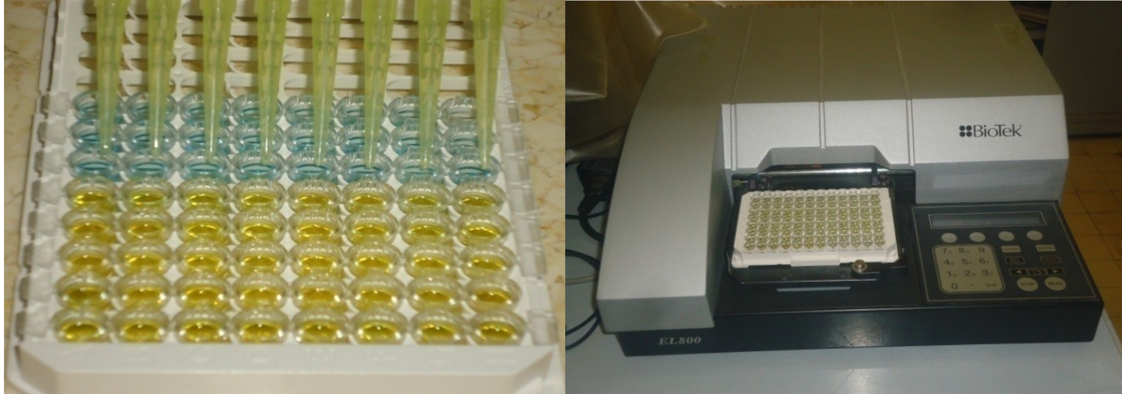


Plate 3.4: Indirect ELISA (DRG) and cELISA (VMRD) for detection of Antibody against *T. gondii* and *N. caninum.*

4. RESULTS

4.1. *Toxoplasma gondii* infection:

4.1.1. Sero-prevalence of *T. gondii* Infection Using LAT:

4.1.1.1. Sero-prevalence of *T. gondii* Infection at herd level:

The overall seroprevalence of *T. gondii* infection in dairy herds from the Khartoum state was 92.7%. The within herd seroprevalence was ranging from 8% up to 100% with mean of 51.3±24.3% in different herds of different dairy animals species in the State (Table 4.1.1). The majority of examined herds (86, 52.4%) recorded more than 50% within herd seroprevalence rate with highly statistically significant differences ($p=0.000$) at $p<0.01$ (Table 4.1.2).

Table 4.1.1: Sero-prevalence of *T. gondii* infection in Dairy herds from the Khartoum State.

LAT	N of herds	Percent	Prevalence Range%	Mean±SD
Positive	164	92.7	8 - 100	51.3±24.3

Negative	13	7.3
Total	**177**	**100**

Table 4.1.2: Frequency distribution of Sero-prevalence rate of *T. gondii* infection in the State.

Herd Prevalence Rate	N. of positive Herds	Percent	*P-value*
1-24%	21	12.8	
25-49%	57	34.8	
50-74%	54	32.9	0.000
75-100%	32	19.5	
Total	**164**	**100**	

The highest seroprevalence rate was reported in herds from Omdurman District (100%) followed by Khartoum North and Khartoum which recorded 98.2% and 82.9% seroprevalence respectively (Table 4.1.3). The differences between the three district was statistically highly significant (p<0.01). Similar statistically significant differences (p= 0.005) were also reported within localities (Table 4.1. 4).

Table 4.1.3. Sero-prevalence of *T. gondii* infection in Dairy herds from the three districts of the State.

District	N of Herds examined	Positive (%)	Negative (%)	*P-value*
Khartoum	70	58(82.9)	12 (17.1)	
Khartoum North	57	56(98.2)	1 (1.8)	0.000
Omdurman	50	50 (100)	0 (0)	
Total	**177**	**164 (92.7)**	**13 (7.3)**	

Table 4.1.4. Sero-prevalence of *T. gondii* infection in dairy herds from the seven localities of the State.

Locality	Herds examined	Positive (%)	Negative (%)	*P-value*
Khartoum	12	11(91.7)	1(8.3)	
Jabal Aolia	58	47 (81)	11(19)	
Eastern Nile	28	27(96.4)	1(3.6)	0.005
Bahri	29	29(100)	0(0.0)	
Omdurman	32	32(100)	0(0.0)	
Karary	7	7(100)	0(0.0)	
Ombadda	11	11(100)	0(0.0)	
Total	177	164(92.7)	13(7.3)	

The differences in the frequency distribution of within herd seroprevalence rate of the examined herds revealed insignificant differences (p>0.05) at both district (Table 4.1.5) and locality level (table 4.1.6). There was significant differences (p<0.05) among herds of the 29 sample sites investigated from the State in the seroprevalence and in the range of prevalence rate of *T. gondii* infection.

Table 4.1.5: Frequency distribution of within herd prevalence rate of *T. gondii* infection in the three districts of the State.

District	Total Positive	Herd Prevalence rate				*P value*
		1-24%	**25-49%**	**50-74%**	**75-100%**	
Khartoum	**58**	8(13.8)	19(32.8)	14(24.1)	17(29.3)	0.34
Kh. North	**56**	7(12.5)	20(35.7)	21(37.5)	8(14.3)	
Omdurm	**50**	6(12)	18(36)	19(38)	7(14)	

an					
Total	**164**	**21(12.8)**	**57(34.8)**	**54(32.9)**	**32(19.5)**

Table 4.1.6: Frequency distribution of within herd prevalence rate of *T. gondii* infection in the seven localities of the State.

Locality	Total Positive	Herd Prevalence Rate Range (%)				*P value*
		1-24%	**25-49%**	**50-74%**	**75-100%**	
Khartoum	**11**	2(18.2)	6(54.5)	3(27.3)	0(0.0)	
Jabal Aolia	**47**	6(12.8)	13(27.7)	11(23.4)	17(36.2)	0.06
Eastern Nile	**27**	5(18.5)	8(29.6)	11(40.7)	3(11.1)	
Bahri	**29**	2(6.9)	12(41.4)	10(34.5)	5(17.2)	
Omdurman	**32**	4(12.5)	7(21.9)	15(46.90	6(18.8)	
Karary	**7**	0(0.0)	5(71.4)	1(14.3)	1(14.3)	
Ombadda	**11**	2(18.2)	6(54.5)	3(27.3)	0(0.0)	
Total	**164**	**21(12.8)**	**54(34.8)**	**57(32.9)**	**32(19.5)**	

With statistically significant ($p<0.05$) differences among different animal species herds in the State, camels and cattle herds reported the highest seroprevalence rate followed by goats and Sheep respectively (Table 4.1.7).

Table 4.1.7: Seroprevalence of *T. gondii* infection in dairy herds of different animals in the State.

Animal Species	Herds examined	Seropositive (%)	Seronegative (%)	*P value*

Cattle	132	126 (95.5)	6 (4.5)	
Camel	13	10 (96.9)	3 (3.1)	
Sheep	16	13 (81.3)	3 (18.8)	0.025
Goat	16	15 (93.8)	1 (6.3)	
Total	**177**	**164 (92.7)**	**13 (7.3)**	

The differences in the frequency distribution of seroprevalence rate of *T. gondii* infection in the different dairy animals herds was statistically highly significant (p<0.01). Most of sheep herds (76.9%) reported the highest range (75-100%) of within herd prevalence rate followed by goats herds (46.9%) and camels herds (40.0%). The lowest seroprevalence rate (8.7%) for the same range (75-100%) was reported in cattle herds (Table 4.1.8).

Table 4.1.8: Frequency distribution of within herd prevalence rate of *T. gondii* infection in different dairy animals in the State.

Herd	**Total Positive**	**Herd Prevalence rate**				**P value**
		8-24%	**25-49%**	**50-74%**	**75-100%**	
Cattle	**126**	21(16.7)	51(40.5)	43(34.1)	11(8.7)	0.00
Camel	**10**	0(0.0)	1(10.0)	5(50.0)	4(40.0)	
Sheep	**13**	0(0.0)	1 (7.7)	2(15.4)	10(76.9)	
Goat	**15**	0(0.0)	4(26.7)	4(26.7)	7(46.7)	
Total	**164**	**21(12.8)**	**57(34.8)**	**54(32.9)**	**32(19.5)**	

4.1.1.2. Ser-prevalence of *T. gondii* infection at individual level:

The overall sero-prevalence of *T. gondii* in dairy animals in the State was 45.3% (table 4.1 9).

Table 4.1.9. Sero-prevalence of *T. gondii* infection in dairy animals from the Khartoum State.

Tested samples for antibody against *T. gondii* using LAT		Percent
Positive	669	45.3
Negative	808	54.7
Total	1477	100.0

There was no significant differences (p>0.05) in the seroprevalence among the three districts of the State. However, there were statistically significant variations in the distribution of antibody titrations of their positive sera (table 4.1.10).

The examined localities of the State showed highly (p<0.01) significant differences in both, the seroprevalence and the distribution of antibody titration (table 4.1.11).

Table 4.1.10. Sero-prevalence of *T. gondii* infection in dairy animals from the three districts of the State.

District	Animals Tested	*P+ve (%)	**Distribution of specific antibody titers to *T. gondii* positive reaction (%)							Negative (%)
			1:2	1:4	1:8	1:16	1:32	1:64	1:128	
Khartoum	474	**226 (47.7)**	28(12.4)	53(23.5)	60(26.5)	36(15.9)	42(18.6)	4(1.8)	3(1.3)	248 (52.3))
Khartoum North	629	**282 (44.8)**	30(10.6)	54(19.1)	79(28.0)	43(15.2)	74(26.2)	1(0.4)	1(0.4)	347 (55.2)
Omdurman	374	**161 (43.0)**	20(12.4)	33(20.5)	58(36.0)	6(3.7)	44(27.3)	0(0.0)	0(0.0)	213 (57.0)
Total	**1477**	**669 (45.3)**	**78(11.7)**	**140(20.9)**	**197(29.4)**	**85(12.7)**	**160(23.9)**	**5(0.7)**	**4(0.6)**	**808 (54.7)**

*Insignificant (p=0.3) at P≤0.05. **Significant (p=0.002) at P≤0.05.

Table 4.1.11. Sero-prevalence of *T. gondii* infection in dairy animals from the seven localities of the State.

Locality	Anima	**Distribution of specific antibody titers to *T. gondii* positive

	ls Tested	*P+ve (%)	reaction (%)							Negative (%)
			1:2	1:4	1:8	1:16	1:32	1:64	1:128	
Khartoum	96	**34(35.4)**	1(2.9)	1(2.9)	14(41.2)	3(8.8)	15(44.1)	0(0.0)	0(0.0)	**62(64.6)**
Jabal Aolia	378	**192 (50.8)**	27(14.1)	52(27.1)	46(24.0)	33(17.2)	27(14.1)	4(2.1)	3(1.6)	**186(49.2)**
Eastern Nile	301	**123(40.9)**	12(9.8)	25(20.3)	33(26.8)	19(15.4)	34(27.6)	0(0.0)	0(0.0)	**178(59.1)**
Bahri	328	**159(48.5)**	18(11.3)	29(18.2)	46(28.9)	24(15.1)	40(25.2)	1(0.6)	1(0.6)	**169(51.5)**
Omdurman	186	**93(50.0)**	13(14.0)	19(20.4)	33(35.5)	5(5.4)	23(24.7)	0(0.0)	0(0.0)	**93(50.0)**
Karary	83	**32(38.6)**	1(3.1)	7(21.9)	11(34.4)	1(3.1)	12(37.5)	0(0.0)	0(0.0)	**51(61.4)**
Ombadda	105	**36(34.3)**	6(16.7)	7(19.4)	14(38.9)	0(0.0)	9(25.0)	0(0.0)	0(0.0)	**69(65.7)**
Total	1477	**669(45.3)**	**78(11.7)**	**140(20.9)**	**197(29.4)**	**85(12.7)**	**160(23.9)**	**5(0.7)**	**4(0.6)**	**808(54.7)**

*Significant (p=0.002) at P≤0.05. **Significant (p=0.001) at P≤0.05.

As shown in table (4.1.12), sheep were the superior in the seroprevalence of *T. gondii* infection (75.0%). Cattle revealed the lowest seroprevalence (40.9%). The positive sera of sheep and goats recorded the highest level of antibody titration (1:128) with highly statistically significant differences (p<0.01), compared to cattle and camels ones which did not reach 1:64 dilution (table 12). Most of the seropositive dairy cows by LAT revealed high antibody titration (positive at >1:8 serum dilution). The titration of 1:8 and 1:32 were the usual threshold in the seroconvert dairy cows. Antibody against *T. gondii* was not detected in dairy cows sera diluted to 1:64 (table 3).

Table 4.1.12. Sero-prevalence of *T. gondii* infection in different dairy animal species from the State.

Animals	Tested	*P+ve (%)	**Distribution of specific antibody titers to *T. gondii* positive reaction (%)							Negative (%)
			1:2	1:4	1:8	1:16	1:32	1:64	1:128	
Cattle	1216	**497(40.9)**	56(11.3)	101(20.3)	152(30.6)	46(9.3)	142(28.6)	0(0.0)	0(0.0)	**719(59.1)**

Camels	61	**33(54.1)**	7(21.2)	10(30.6)	5(15.2)	7(21.2)	4(12.1)	0(0.0)	0(0.0)	**28(45.9)**
Sheep	100	**75(75.0)**	10(13.3)	16(21.3)	19(25.3)	21(28.0)	6(8.0)	2(2.7)	1(1.3)	**25(25.0)**
Goats	100	**64(64.0)**	5(7.8)	13(20.3)	21(32.8)	11(17.2)	8(12.5)	3(4.7)	3(4.7)	**36(36.0)**
Total	**1477**	**669(45.3)**	**78(11.7)**	**140(20.9)**	**197(29.4)**	**85(12.7)**	**160(23.9)**	**5(0.7)**	**4(0.6)**	**808(54.7)**

*Significant (p=0.00) at P≤0.05. **Significant (p=0.00) at P≤0.05.

4.1.2. Detection of *T. gondii* infection Using ELISA:

4.1.2.1. Seroprevalence of *T. gondii* at herd level Using ELISA:

The overall seroprevalence of *T. gondii* infection in the dairy herds of cattle from the State using ELISA was 89.3% when only 14 herds (10.7%) were found to be clean from *T. gondii* infection. The minimum within herd prevalence rate was 12%. The range increased to reach 100% in several herds with mean prevalence rate of 56.3±22.5% in different herds of dairy cattle in the State (Table 4.1.2.1).

Table 4.1.2.1: Sero-prevalence of *T. gondii* infection in Dairy herds using ELISA.

ELISA	N of herds	Percent	Range%	Mean±SD
Positive	117	89.3	**12 - 100**	**56.3±22.5**
Negative	14	10.7		
Total	131	100		

The highest seroprevalence rate was reported in herds from Omdurman District (97.8%) followed by Khartoum North which recorded 87.2% seroprevalence (Table 2). The

differences among the three district was statistically significant ($p<0.05$) when the lowest prevalence rate at herd level (81.6%) was reported in Khartoum District (Table 4.1.2.2).

Most of the examined dairy herds (83/117, 70.9%) in the State recorded more than 50% seroprevalence rate of *T. gondii* infection. eleven of these dairy herds recorded 100% seroprevalence rate. Although there was no any statistically significant differences ($p>0.05$), most of dairy herds (78.05%, 32/41) from Khartoum North District reported the highest range (50% up to 100%) compered to the other two districts (Table 4.1.2.2).

Table 4.1.2.2: Seroprevalence and Frequency Distribution of Prevalence rate of *T. gondii* infection within herds of Dairy Cattle in the three Districts of the State using ELISA.

District	Herds examined	*P+ve (%)	**Within Herd Prevalence rate				N-ve (%)
			1-24%	25-49%	50-74%	75-100%	
Khartoum	38	31 (81.6)	5(16.1)	8(25.8)	11(35.5)	7(22.6)	7 (18.4)
Kh. North	47	41(87.2)	2 (4.9)	7(17.1)	22(53.7)	10(24.4)	6 (12.8)
Omdurman	46	45 (97.8)	3(6.7)	9(20)	21(46.7)	12(26.7)	1 (2.2)
Total	**131**	**117 (89.3)**	**10(8.5)**	**24(20.5)**	**54(46.2)**	**29(24.8)**	**14 (10.7)**

*Significant (p=0.04) at P≤0.05. **Insignificant (p=0.5) at P≤0.05.

As showen in table (4.1.2.3), seroprevalence rate of *T. gondii* infection was significantly ($p<0.05$) varies among the seven localities of the State. The lowest seroprevalence rate was reported in dairy herds from Jabal Aolia locality of Khartoum

District. However, most herds of Khartoum locality reported the higher range of seroprevalence rate (75-100%) withou any statistically significant differences (p>0.05) compare to other localities (Table 4.1.2.3). There was significant differences (p<0.05) among herds of the 24 sample sites investigated from the State in the seroprevalence and in the range of within herd prevalence rate of *T. gondii* infection.

Table 4.1.2.3: Seroprevalence and Frequency Distribution of Prevalence rate of *T. gondii* infection within herds of Dairy Cattle in the seven localities of the State using ELISA.

Locality	Herds examined	*P+ve (%)	**Herd Prevalence rate				N-ve (%)
			1-24%	25-49%	50-74%	75-100%	
Khartoum	12	11(91.7)	0(0.0)	4(36.4)	3(27.3)	4(36.4)	1(8.3)
Jabal Aolia	26	20(76.9)	5(25.0)	4(20.0)	8(40.0)	3(15.0)	6(23.1)
Eastern Nile	22	19(86.4)	2(10.5)	3(15.8)	9(47.4)	5(26.3)	3(13.6)
Bahri	25	22(88.0)	0(0.0)	4(18.2)	13(59.1)	5(22.7)	3(12.0)
Omdurman	28	27(96.4)	2(7.4)	4(14.8)	14(51.9)	7(25.9)	1(3.6)
Karary	7	7(100)	1(14.3)	1(14.3)	3(42.9)	2(28.6)	0(0.0)
Ombadda	11	11(100)	0(0.0)	4(36.4)	4(36.4)	3(27.3)	0(0.0)
Total	**131**	**117(89.3)**	**10(8.5)**	**24(20.5)**	**54(46.2)**	**29(24.8)**	**14(10.7)**

*Significant (p=0.03) at P≤0.05. **Insignificant (p=0.4) at P≤0.05.

4.1.2.2. Seroprevalence of *T. gondii* at individual level Using ELISA:

The overall Sero-prevalence of *T. gondii* in dairy cows in the State using ELISA was 49.9% (table 4.1.2.4). There were statistically significant differences (p<0.05) among the three districts (Table 4.1.2.5). Omdurman district revealed the highest prevalence rate (53.7%), followed by Khartoum North (51.9%). However, the Khartoum district revealed the lowest (42.0%) prevalence rate.

Table 4.1.2.4. Sero-prevalence of *T. gondii* in dairy cattle from the Khartoum State, using ELISA.

Samples examined for antibody against *T. gondii* using ELISA		Percent
Positive	371	49.9
Negative	373	50.1
Total	744	100.0

Table 4.1.2.5. Sero-prevalence of *T. gondii* infection in Dairy Cattle from the three districts of the Khartoum State using ELISA.

District	Tested Samples	Positive (%)	Negative (%)
Khartoum	193	81(42.0)	112 (58.0)
Khartoum North	335	174(51.9)	161 (48.1)
Omdurman	216	116 (53.7)	100 (46.3)
Total	**744**	**371 (49.9)**	**373(51.1)**

Significant (p=0.03) at P<0.05.

As presented in table (4.1.2.6), Khartoum Locality reported the highest seroprevalence rate (56.9%) with statistically significant variations among the seven localities of the State.

Table 4.1.2.6. Sero-prevalence of *T. gondii* infection in dairy cattle from the seven localities of the Khartoum State using ELISA.

Locality	Tested Samples	Seropositive (%)	Negative (%)
Khartoum	51	29(56.9)	22(43.1)
Jabal Aolia	142	52 (36.6)	90 (63.4)
Eastern Nile	177	86 (48.6)	91(51.4)
Bahri	158	88(55.7)	70(44.3)
Omdurman	133	74(55.6)	59(44.4)
Karary	33	17(51.5)	16(48.5)
Ombadda	50	25(50.0)	25(50.0)
Total	**744**	**371 (49.9)**	**373(51.1)**

Significant ($p=0.02$) at $P<0.05$.

4.1.3. Detection of Antibody against *T. gondii* in Dairy Cows Using LAT and ELISA:

Out of the 744 dairy cows examined by both LAT and ELISA, antibody against *T. gondii* was detected in 575 dairy cows (77.3%) by either LAT or/and ELISA (table 4.1.3.1). However, 169 dairy cows (22.7%) were seronegative (clean) by both tests.

Table 4.1.3.1. The overall prevalence rate of *T. gondii* in dairy cows in the Khartoum State using LAT and ELISA tests.

Samples examined for antibody against *T. gondii* using LAT and ELISA		Percent
Positive Animals by at least one of the two tests	575	77.3
Clean animals by the both tests	169	22.7
Total	744	100.0

4.1.3.1. The Level of agreement between LAT and ELISA:

As shown in table (4.1.3.2), most of the positive reacted sera 296 (79.8%) with LAT were confirmed positive by ELISA. ELISA

test also detected extra 75 samples (20.2%) that reacted negative by LAT. The level of agreement between ELISA and LAT with chance was 64%. The level of agreement beyond the chance was found to be fair using Kappa test (K value=0.286). The sensitivity and specificity of the two tests was found to be fair with area under the curve (0.7) and (0.02) St. Error using Receiver Operating Characteristic (ROC) Curve (Fig. 4.1.3.1).

Table 4.1.3.2: The level of agreement between ELISA and LAT in the detection of *T. gondii* infection in dairy cattle from the Khartoum State.

*		**Toxo-LAT Result**		**Total**
		P+ve (%)	**N-ve (%)**	
Toxo-EIA result	P+ve	296 (79.8)	75 (20.2)	**371(49.9)**
	N-ve	191(51.2)	182(48.8)	**373(50.1)**
Total		487(65.5)	257(34.5)	**744(100)**

*Measure of agreement was fair using Kappa test (K=0.286) according to Landis and Koch (1977).

Fig.4.1.3.1: ROC Curves of LAT and ELISA for detection of *T. gondii* antibodies in Dairy cows (Area under the curve = 0.7).

4.1.3.2. The effect of the level of antibody titration on ELISA:

Table (4.1.3.3) showed that, the confirmation of LAT positive samples by ELISA increased significantly (p=0.000) with the increasing of antibody titration. About eighty percent of positive sera that revealed LAT titration of ≥1:32 were also positive by ELISA. Additionally, most of the ELISA negative samples were the samples that scored lower LAT titration (<1:8 dilution). Similar level of agreement (fair) was revealed when we analyze ELISA against LAT considering ≥1:8 as cut-off point (table 4.1.3.3), but the degree of agreement between the two tests was increased (K value = 0.33).

Table 4.1.3.3: The degree of agreement between ELISA and the level of antibody titration using LAT in the detection of *T. gondii* infection in dairy cattle from the State.

		****LAT titration**					**Total**
		1:2	**1:4**	**1:8**	**1:16**	**1:32**	
***Toxo-ELISA**	P+ve	22(39.0)	47(48.9)	92(60.3)	25(56.8)	110(79.7)	296(60.8)
	N-ve	34(60.0)	51(52.1)	59(39.7)	19(43.2)	28(20.2)	191(39.2)
Total		**56**	**98**	**151**	**44**	**138**	**487**

* Measure of agreement was slightly increased (K=0.33) when 1:8 used as cut-off point.
**Significant (p=0.000) at P<0.05.

4.1.4. Analysis of Risk Factors Associated with Seroprevalence of *T. gondii*:

4.1.4.1. Univariate analysis of risk factors:

Zoographic and farm management characteristics of the investigated population are presented in table (4.1.4.1&2). Univariate analysis of risk factors showed that region, animal

species, herd type, source of fodder, source of water, Neosporosis, keeping cats, keeping both dogs and cats, and presence of both stray dogs and cats were significantly ($p<0.05$) associated with the seropositivity of *T. gondii*. Districts, Production system, herd size, source of concentrate, brucellosis and presence of stray cats were not significant risk factors (Table 4.1.4.1&2).

4.1.4.2. Multivariate Analysis of Risk Factors:

Results of multivariate analysis revealed more statistically significant likelihood of infection in Bahri ($p=0.000$) and Omdurman ($p=0.044$) than in Ombadda locality and in sheep ($p=0.006$) than in cattle. The effect of source of water (common canals) and source of fodder (odds=2.652) were significantly ($p=0.027$ and $p=0.004$) associated with *T. gondii* infection (Table 3). Localities (Eastern Nile), keeping cats, keeping both dogs and cats, and presence of both stray dogs and cats were the factors found not to be significantly associated ($p>0.05$) but with increasing odds of being LAT positive (Table 4.1.4.3). Localities (Eastern Nile), keeping cats, keeping both dogs and cats, presence of both stray dogs and cats and source of fodder were the factors found not to be significantly associated ($p>0.05$) but with increasing odds of being LAT positive (Table 4.1.4.3).

Mapping of the distribution of seroprevalence of *T. gondii* in the different localities of the State was presented in fig. (4.1.4).

Table 4.1.4.1: Estimated Seroprevalence of *T. gondii* infection in Dairy animals from Khartoum State and Univariate analysis for the associated Risk factors using Chi square.

Risk Factors		**N of Animals examined**	**N of P+ve**	**%**	***p-value***
Districts	Khartoum	474	226	47.7	
	Kh. North	629	282	44.8	0.386
	Omdurman	374	161	43.0	
Localities	Khartoum	96	34	35.4	
	Jabal Aolia	378	192	50.8	
	Eastern Nile	301	123	40.9	
	Bahri	328	159	48.5	0.002
	Omdurman	186	93	50.0	
	Karary	83	32	38.6	
	Ombadda	105	36	34.3	
Animal sp.	Cattle	1216	497	40.9	
	Camels	61	33	54.1	0.000
	Sheep	100	75	75.0	
	Goats	100	64	64.0	
Prod. system	Intensive	161	78	48.4	0.395
	Semi-intensive	1316	591	44.9	
Total		**1477**	**669**	**45.3**	

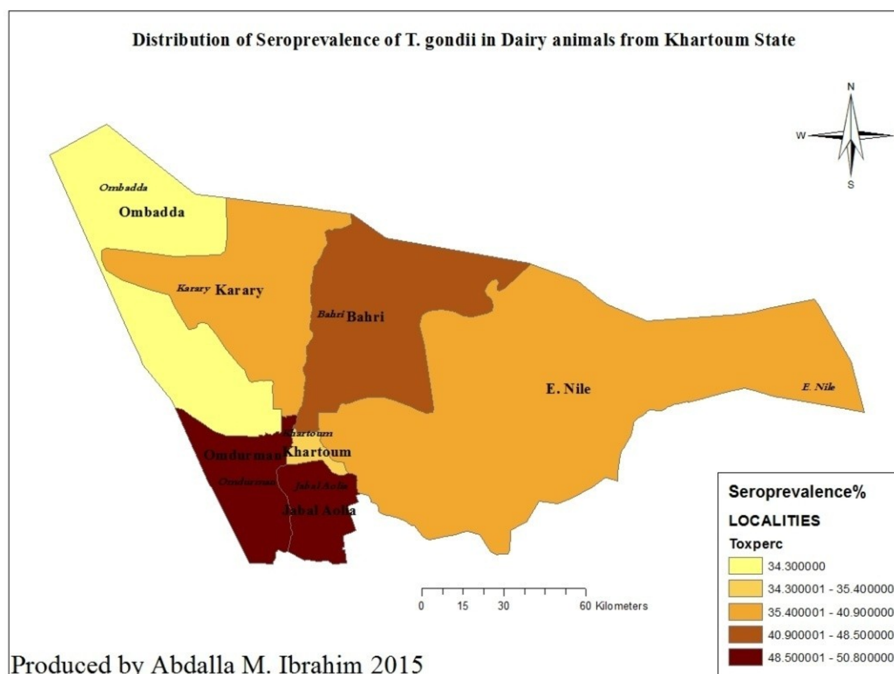


Fig. 4.1.4: Distribution of seroprevalence of *T. gondii* in Dairy Animals in different Localities of the Khartoum State.

Table 4.1.4.2: Results of univariate association of Risk factor with LAT toxoplasma seropositivity in dairy animals from the state using Chi square.

Risk factors		**Animals examined**	**N of P+ve**	**P+ve %**	**p-value**
Herd size	Large	939	425	45.3	0.973
	Small	538	244	45.4	
Herd type	One species	1176	512	43.5	0.007
	Multi-species	301	157	52.2	
Source of concentrate	Ready made	547	236	43.1	0.203
	Prepared in farm	930	433	46.6	
Source of fodder	By from market	848	349	41.2	0.000
	Cut from farm	629	320	50.9	
Source of water	Tap water/well	1225	511	41.7	0.000
	Common canals	252	158	62.7	
*Other Diseases	Brucella P+ve	311	129	41.5	0.260
	Brucella N-ve	1087	490	45.1	
	Neospora P+ve	80	36	45.0	0.041
	Neospora N-ve	826	470	56.9	
Keeping cats	Yes	285	151	53.0	0.004
	No	1192	518	43.5	
Stray cats	Yes	712	340	47.8	0.067
	No	765	329	43.0	
Keeping dogs and cats	Yes	188	121	64.4	0.000
	No	1289	584	42.5	
Stray dogs and cats	Yes	607	294	48.4	0.043
	No	870	375	43.1	
Total		**1477**	**669**	**45.3**	

*Total animals examined for Brucella and Neospora were 1398 and 906 respectively.

Table 4.1.4.3: Results of Multivariate association of Risk factor with LAT toxoplasma seropositivity in dairy animals from the state using Chi square.

Risk factors		N of Animals examined	N of P+ve (%)	Wald (L.R)	p-value	Exp(B)	95% CI for Exp(B)	
							Lower	Upper
Localities	Ombadda	105	36(34.3)			Ref.		
	Khartoum	96	34(35.4)	3.772	0.052	0.318	0.100	1.010
	Jabal Aolia	378	192(50.8)	0.700	0.403	0.669	0.261	1.714
	E. Nile	301	123(40.9)	2.090	0.148	1.883	0.798	4.440
	Bahri	328	159(48.5)	15.707	0.000	0.076	0.021	0.272
	Omdurman	186	93(50.0)	4.067	0.044	0.347	0.124	0.971
	Karary	83	32(38.6)	2.478	0.115	0.383	0.116	1.265
Animal sp.	Cattle	1216	497(40.9)			Ref.		
	Camels	61	33(54.1)	1.167	0.280	0.359	0.056	2.303
	Goats	100	64(64.0)	3.336	0.068	0.255	0.059	1.105
	Sheep	100	75(75.0)	7.499	0.006	0.111	0.023	0.536
Herd type	One species	1176	512(43.5)			Ref.		
	Multi-species	301	157(52.2)	0.008	0.927	1.024	0.615	1.705
Other diseases	Brucella P+ve	311	129(41.5)			Ref.		
	Brucella N-ve	1087	490(45.1)	3.713	0.054	1.480	0.993	2.204
	Neospora P+ve	80	36(45.0)			Ref.		
	Neospora N-ve	826	470(56.9)	0.750	0.387	0.791	0.466	1.344
Keep cats	No	1192	518(43.5)			Ref.		
	Yes	285	151(53.0)	0.047	0.829	1.075	0.558	2.070
Keep dogs and cats	No	1289	584(42.5)			Ref		
	Yes	188	121(64.4)	1.5	0.20	2.82	0.56	14.25

				88	8	9	1	8
Stray cats	No	765	329(43.0)			Ref.		
	Yes	712	340(47.8)	1.577	0.209	0.638	0.317	1.286
Stray dogs and cats	No	870	375(43.1)			Ref.		
	Yes	607	294(48.4)	0.464	0.496	1.272	0.637	2.541
Source of concentrate	Ready made	547	236(43.1)			Ref.		
	Prepared in farm	930	433(46.6)	3.087	0.079	0.552	0.285	1.071
Source of water	Tap water/well	1225	511(41.7)			Ref.		
	Common canals	252	158(62.7)	4.879	0.027	0.421	0.195	0.907
Source of fodder	By from market	848	349(41.2)			Ref.		
	Cut from farm	629	320(50.9)	8.121	0.004	2.652	1.356	5.186

4.2. Results of *N. caninum* infection Using cELISA:

4.2.1. Ser-prevalence of *N. caninum* in dairy farms at herd level:

The overall seroprevalence of *N. caninum* in dairy herds from the Khartoum State was 32.2% based on cELISA. The within herd seroprevalence was ranging from 7% up to 75% with mean of 27.2±15.4% in the different herds of different dairy animals species (Table 4.2.1). The highest pi in the investigated herd's sera was 93% with mean of 38.7±12.3.

Table 4.2.1: Sero-prevalence of *N. caninum* and percent inhibition in dairy herds in the Khartoum State.

Herds Examined for *N. caninum* infection		**percent**	**Prevalence rate%**		**Percent Inhibition (pi)**	
			Range	Mean±SD	Range	Mean±SD
Positiv	56	32.2	7 - 75	27.2±1	30 - 93	38.7±12

e			5.4	.3
Negative	118	67.8		
Total	174	100		

About 12.5% of the seropositive herds recorded more than 50% seroprevalence rate without statistically significant differences at P≤0.05 among the frequency distribution of seroprevalence rate of *N. caninum* infection (Table 4.2.2) in the investigated herds.

Table 4.2.2: Frequency distribution of sero-prevalence rate of *N. caninum* in dairy herds from the State.

Herd Prevalence Rate	No. of Herds	Percent	*P value*
*1-24%	27	48.2	
25-49%	22	39.3	
50-74%	6	10.7	0.08
75-100	1	1.8	
Total	**56**	**100**	

*7% was the lower within herd seroprevalence rate reported.

As presented in table (4.2.3), although Khartoum North District reported the highest prevalence rate, the seroprevalence of *N. caninum* among the herds of the three districts of the State revealed no statistically significant (p>0.05) differences. Similarly, the highest seroprevalence among the seven localities was found in Eastern Nile Locality, but also without statistically (p>0.05) significant differences (Table 4.2.4). The examined herds of a single locality (Karary) found to be clean when no antibody against *N. caninum* was detected in dairy animals of this locality. The

differences in the frequency distribution of seroprevalence rate of the examined herds revealed insignificant differences (p>0.05) at both district (Table 4.2.3) and locality level (table 4.2.4).

Table 4.2.3: Sero-prevalence and frequency distribution of prevalence rate of *N. caninum* within herds of the three districts of the State.

District	Herds Examined	*Positive Herds (%)	**Herd Prevalence rate				Negative Herds (%)
			1-24%	25-49%	50-74%	75-100%	
Khartoum	70	22(31.4)	13(59.1)	7(31.8)	2(9.1)	0(0.0)	48 (38.6)
Khartoum North	56	19(33.9)	9 (47.4)	8(42.1)	2(10.5)	0(0.0)	37 (66.1)
Omdurman	48	15 (31.3)	5(33.3)	7(46.7)	2 (13.3)	1 (6.7)	33 (68.8)
Total	**174**	**56(32.2)**	**27(48.2)**	**22(39.3)**	**6(10.7)**	**1(1.8)**	**118 (67.8)**

*Insignificant (p=0.9) at P≤0.05. ** Insignificant (p=0.08) at P≤0.05.

Table 4.2.4: Seroprevalence and Frequency distribution of prevalence rate of *N. caninum* in herds of the seven localities of the State.

Locality	Herds Examined	*Positive Herds (%)	**Herd Prevalence rate				Negative Herds (%)
			1-24%	25-49%	50-74%	75-100%	
Khartoum	12	1(8.3)	0(0.0)	1(100)	0(0.0)	0(0.0)	11(91.7)
Jabal Aolia	58	21(36.2)	13(61.9)	6(28.6)	2(9.5)	0(0.0)	37(63.8)
Eastern Nile	28	13(46.4)	6(46.2)	6(46.2)	1(7.7)	0(0.0)	15(53.6)
Bahri	28	6(21.4)	3(50.0)	2(33.3)	1(16.7)	0(0.0)	22(78.6)
Omdurman	32	12(37.5)	5(41.7)	4(33.3)	2(16.7)	1(8.3)	20(62.5)

Karary	6	0(0.0)		0(0.0)			6(100)
Ombadda	10	3(30.0)	0(0.0)	3(100)	0(0.0)	0(0.0)	7(70.0)
Total	**174**	**56(32.2)**	**27(48.2)**	**22(39.3)**	**6(10.7)**	**1(1.8)**	**118(67.8)**

*Insignificant (p=0.09) at P≤0.05. **Insignificant (p=0.1) at P≤0.05.

Without statistically significant differences (p>0.05) among different dairy animals species herds in the State, camels and cattle herds reported the highest seroprevalence rate of *N. caninum* followed by goats and sheep respectively (Table 4.2.5). As presented in table (4.2.5), the differences in the frequency distribution of seroprevalence rate of *N. caninum* infection in the different dairy animals herds were statistically insignificant (p>0.05). The prevalence rate in sheep herds did not exceed 24%, however, cattle herds reported the highest range 75% of within herd prevalence rate followed by camel and goat (25-49%).

Table 4.2.5: Sero-prevalence and frequency distribution of prevalence rate of *N. caninum* within herds of different dairy animals species from the State.

Animal Herd	Herds Examined	*Positive Herds (%)	**Herd Prevalence rate				Negative Herds (%)
			1-24%	25-49%	50-74%	75-100%	
Cattle	129	43 (33.3)	20(46.5)	16(37.2)	6(14.0)	1(2.3)	86 (66.7)
Camel	13	5 (38.5)	2(40.0)	3(60.0)	0(0.0)	0(0.0)	8 (61.5)
Sheep	16	3 (18.8)	3(100)	0(0.0)	0(0.0)	0(0.0)	13 (81.3)
Goat	16	5 (31.3)	2(40.0)	3(60.0)	0(0.0)	0(0.0)	11 (68.8)
Total	**174**	**56**	**27(48.**	**22(39.**	**6(10.**	**1(1.8)**	**118(67**

(32.2) 2) 3) 7) .8)

*Insignificant (p=0.6) at P<0.05. ** Insignificant (p=0.7) at P<0.05.

The minimum *N. caninum* seroconversion in the within different herds of dairy farms was 7% and the maximum was 75%. Although there was no significant differences between the tested animal herds (p>0.05), cattle and camels herds are more susceptible to *N. caninum* infection than sheep and goats ones. Cattle and camel showed higher within herd's prevalence rate reaching 75% and 34% respectively, and higher percent inhibition reaching 93% and 56% pi respectively. Goat's herds were more susceptible to *N. caninum* infection than sheep ones. The highest prevalence rate reported in goat herds was 25% against only 14% within herd seroprevalence rate in sheep.

4.2.2. Ser-prevalence of *N. caninum* in the dairy farms at individual level:

The overall Sero-prevalence of *N. caninum* in dairy animals from the State was 8.8% (table 4.2.6). The pi of the seroconvert animals to *N. caninum* was 30 up to 93% pi, with Mean (39.3±13.9) pi. About 15% of seroconvert animals reported more than 50% up to 93% pi (table 4.2.7).

Table 4.2.6: Sero-prevalence of *N. caninum* and percent inhibition in dairy animals from the Khartoum State.

Results of *N. caninum* cELISA		**Percent (%)**	**PI Range %**	**Mean±SD**
Positive	80	8.8	30 - 93	39.3±13.9
Negative	826	91.2		

Total	**906**	**100.0**

Table 4.2.7: Frequency distribution of Percent Inhibition (pi) among seropositive dairy animals to *N. caninum*.

Percent Inhibition	**No. of Animals**	**Percent**
30-49%	68	85
50-74%	8	10
75-100%	4	5
Total	80	100

Although Khartoum North was the superior, there was no significant differences (p>0.05) in the seroprevalence of *N. caninum* in dairy animals among the three districts of the State, but the frequency distribution of the pi in Omdurman district was found to be lower compared to that of the other two districts when all seropositive animals from Omdurman did not reach 50% pi (table 4.2.8).

Table 4.2.8: Seroprevalence of *N. caninum* and the percent inhibition in dairy animals from the three districts of the State.

District	**Animals Examined**	***P+ve (%)**	****Percent Inhibition (pi)**			**N-ve (%)**
			30-49	**50-74**	**75-100**	
Khartoum	369	30(8.1)	26(86.7)	3(10.0)	1(3.3)	339 (91.9)
Khartoum North	335	32 (9.6)	24(75.0)	5(15.6)	3(9.4)	303(90.4)
Omdurman	202	18 (8.9)	18(100)	0(0.0)	0(0.0)	184 (91.1)
Total	**906**	**80 (8.8)**	**68(85.0)**	**8(10.0)**	**4(5.0)**	**826 (91.2)**

*Insignificant (p=0.8) at P<0.05. **Insignificant (p=0.2) at P<0.05.

The mapping of the distribution of seroprevalence of *N. caninum* in the different localities of the State was presented in fig. (4.2). The examined localities also showed insignificant differences (p>0.05) in both, the seroprevalence and the frequency distribution of pi (table 4.2.9). The highest prevalence rate was observed in Ombadda locality followed by Omdurman and eastern Nile localities (Fig. 4.2). The 22 animals examined from Karary locality were free from antibody against *N. caninum*.

Table 4.2.9: Seroprevalence of *N. caninum* and the percent inhibition in dairy animals from the seven localities of the State.

Locality*	**Animals Examined**	***P+ve (%)**	**Percent Inhibition (pi) 30-49**	**50-74**	**75-100**	**N-ve (%)**
Khartoum	46	1(2.2)	1(100)	0(0.0)	0(0.0)	45(97.8)
Jabal Aolia	323	29(9.0)	25(86.2)	3(10.3)	1(3.4)	249(91.0)
Eastern Nile	207	20(9.7)	15(75.0)	3(15.0)	2(10.0)	187(90.3)
Bahri	128	12(9.4)	9(75.0)	2(16.7)	1(8.3)	116(90.6)
Omdurman	151	15(9.9)	15(100)	0(0.0)	0(0.0)	136(90.1)
Karary	22	0(0.0)				22(100)
Ombadda	29	3(10.3)	3(100)	0(0.0)	0(0.0)	26(89.7)
Total	906	80(8.8)	**68(85.0)**	**8(10.0)**	**4(5.0)**	826(91.2)

*Insignificant (p=0.5) at P<0.05. **Insignificant (p=0.8) at P<0.05.

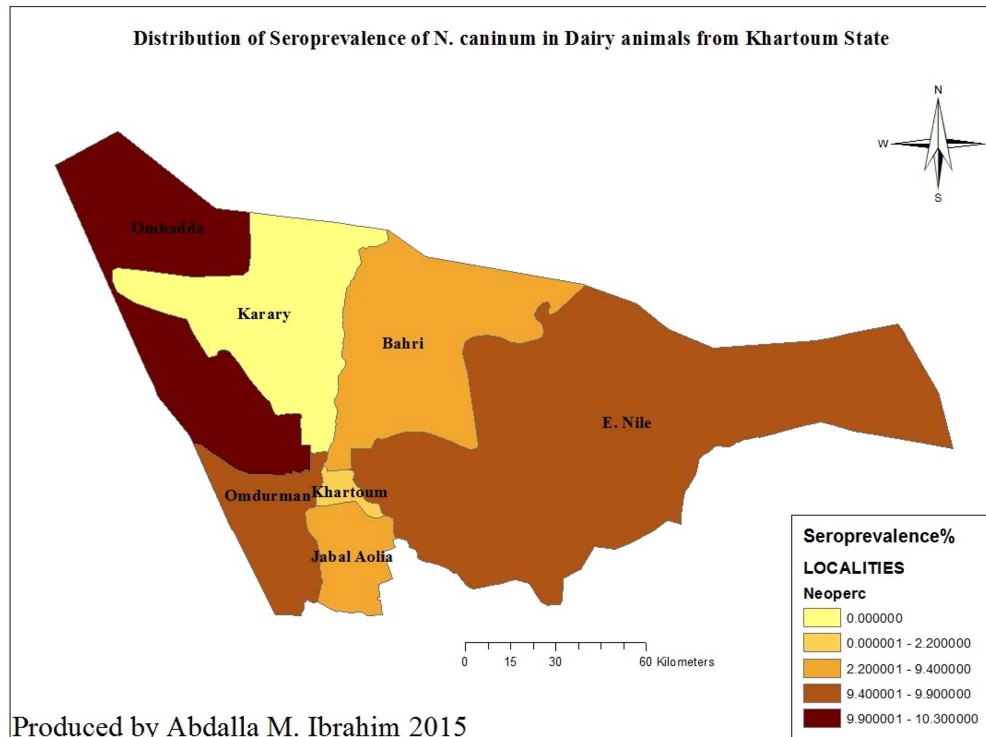


Fig. 4.2: Distribution of seroprevalence of *N. caninum* in Dairy Animals in different Localities of the Khartoum State.

As shown in table (4.2.10), sheep revealed the lowest seroprevalence of *N. caninum* (4.0%) followed by goats (6.0%). However, cattle revealed the highest seroprevalence (9.9%) followed by camels (9.8%). Without any statistically significant differences (p>0.05), the sera of cattle and camels recorded the highest frequency distribution of pi rate (50% up to 93%). While the sera of sheep and goats did not reached 50 pi (table 4.2.10).

Table 4.2.10: Seroprevalence of *N. caninum* and percent inhibition in dairy animals from the three districts of the State.

Animal species	Animals Examined	*P+ve (%)	**Percent Inhibition (pi) 30-49	50-74	75-100	N-ve (%)

Cattle	645	64(9.9)	54(84.4)	6(9.4)	4(6.3)	581(90.1)
Camel	61	6(9.8)	4(66.7)	2(33.3)	0(0.0)	55(90.2)
Sheep	100	4(4.0)	4(100)	0(0.0)	0(0.0)	96(96.0)
Goat	100	6(6.0)	6(100)	0(0.0)	0(0.0)	94(94.00
Total	**906**	**80(8.8)**	**68(85.0)**	**8(10.0)**	**4(5.0)**	**826(91.2)**

*Insignificant (p=0.1) at P<0.05. **Insignificant (p=0.4) at P<0.05.

4.2.3. Analysis of Risk Factors Associated with Seroprevalence of *Neospora caninum*:

4.2.3.1. Univariate analysis of Risk factors Associated with *N. caninum* infection:

Zoographic and farm management characteristics of the investigated population are presented in table (4.2.3.1&2). The univariate analysis of risk factors showed that production system, source of concentrate, other diseases (Toxoplasma) and keeping dogs in farms were significantly (p<0.05) associated with the seropositivity of *N. caninum*. Regions (Districts and Localities), animal species, herd size, herd type, source of fodder, Source of water, brucellosis and presence of stray dogs were not significant (p>0.05) risk factors associated with *N. caninum* cELISA seropositivity (Table 4.2.3.1&2).

Table 4.2.3.1: Estimated Seroprevalence of *N. caninum* infection in Dairy animals from the Khartoum State and Univariate analysis for the associated Risk factors.

Risk Factors	Animals	N of P+ve	Seroprevalence (%)	*p-valu*

		examined			***e***
Districts	Khartoum	369	30	8.1	
	Kh. North	335	32	9.6	0.801
	Omdurman	202	18	8.9	
Localities	Khartoum	46	1	2.2	
	Jabal Aolia	323	29	9.0	
	Eastern Nile	207	20	9.7	
	Bahri	128	12	9.4	0.517
	Omdurman	151	15	9.9	
	Karary	22	0	0.0	
	Ombadda	29	3	10.3	
Animal sp.	Cattle	645	64	9.9	0.177
	Camels	61	6	9.8	
	Sheep	100	4	4.0	
	Goats	100	6	6.0	
Prod. system	Intensive	98	19	19.4	0.000
	Semi-intensive	808	61	7.5	
Total		**906**	**80**	**8.8**	

4.2.3.2. Multivariate analysis of Risk factors Associated with *N. caninum* infection:

The results of multivariate analysis revealed only production system (Intensive p=0.019) and source of concentrate (readymade, p=0.007) as statistically significant risk factors associated with Neospora cELISA seropositivity at ($p<0.05$). Animal species (sheep), keeping dogs, stray dogs, keeping both dogs and cats and presence of both stray dogs and cats were the factors showed increasing odds of being cELISA positive (Table 4.2.3.3) and found not to be significantly associated ($p>0.05$) with *N. caninum* infection.

Table 4.2.3.2: Results of Univariate association of Risk factor with cELISA Neospora seropositivity in dairy animals from the Khartoum state.

Risk factors		**Animals examined**	**N of P+ve**	**P+ve %**	**p-value**
Herd size	Large	658	55	8.4	0.415
	Small	248	25	10.1	
Herd type	One species	668	57	8.5	0.597
	Multi-species	238	23	9.7	
Source of concentrate	Ready made	333	45	13.5	0.000
	Prepared in farm	573	35	6.1	
Source of fodder	By from market	521	41	7.9	0.236
	Cut from farm	385	39	10.1	
Source of water	Tap water/well	704	65	9.2	0.425
	Common canals	202	15	7.4	
*Other Diseases	Brucella P+ve	155	17	11.0	0.328
	Brucella N-ve	672	57	8.5	
	Toxoplasma P+ve	506	36	7.1	0.041
	Toxoplasma N-ve	400	44	11.0	
Keeping dogs	Yes	326	20	6.1	0.032
	No	580	60	10.3	
Stray dogs	Yes	644	52	8.1	0.209
	No	262	28	10.7	
Keeping dogs and cats	Yes	177	10	5.6	0.096
	No	729	70	9.6	
Stray dogs and cats	Yes	387	27	7.0	0.090
	No	519	53	10.2	
Total		**906**	**80**	**8.8**	

*Total animals examined for Brucella were 827.

Table 4.2.3.3: Results of Multivariate association of Risk factor with cELISA Neospora seropositivity in dairy animals from Khartoum state.

Risk factors		N of Animals examined	N of P+ve (%)	Wald (L.R)	p-value	Exp(B)	95% CI for Exp(B)	
							Lower	Upper
Animal sp.	Sheep	100	4(4.0)			Ref.		
	Cattle	645	64(9.9)	2.721	0.099	0.270	0.057	1.279
	Camels	61	6(9.8)	0.780	0.377	0.493	0.102	2.369
	Goats	100	6(6.0)	0.149	0.699	0.766	0.199	2.957
Prod. System	Semi-intensive	808	61(7.5)			Ref.		
	Intensive	98	19(19.4)	5.500	0.019	0.385	0.174	0.855
Other diseases	Toxoplasma P+ve	506	36(7.1)			Ref.		
	Toxoplasma N-ve	400	44(11.0)	1.284	0.257	0.753	0.460	1.230
Keep dogs	Yes	326	20(6.1)			Ref.		
	No	580	60(10.3)	0.033	0.85	1.083	0.458	2.560

					6			
Keep dogs and cats	Yes	177	10(5.6)			Ref		
	No	729	70(9.6)	0.530	0.467	1.684	0.414	6.852
Stray dogs	Yes	644	52(8.1)			Ref.		
	No	262	28(10.7)	1.865	0.172	1.566	0.823	1.980
Stray dogs and cats	Yes	387	27(7.0)			Ref.		
	No	519	53(10.2)	0.161	0.689	1.158	0.564	2.378
Source of concentrate	Prepared in farm	573	35(6.1)			Ref.		
	Ready made	333	45(13.5)	7.182	0.007	0.374	0.182	0.768
Source of fodder	By from market	521	41(7.9)			Ref.		
	Cut from farm	385	39(10.1)	0.494	0.482	0.789	0.408	1.527

4.3. Co-existence of *T. gondii* and *N. caninum*:

Out of 906 animals examined for both *T. gondii* and *N. caninum*, 601 (66.3%) were found to be seropositive for either of the two parasite or both. Antibody against *T. gondii* was detected in 86.7% (521/601) of the seropositive animals and 13.3% (80/601) were seropositive to *N. caninum* (table 4.3.1). The differences between the three district of the State was not statistically significant ($p>0.05$). Approximately, 50% of the *Neospora* seropositive animals have also anti-*T. gondii* antibodies in their sera (table 4.3.2). Differences between the three districts in this mix-infection was statistically significant ($p<0.05$).

Table 4.3.1. Occurrence of *T. gondii* and *N. caninum* infection in Dairy Animals from the three Districts of the Khartoum State.

District	Animals Examine	Infected animals (%)			Clean (%)
		Over all	Toxoplasm	Neospora	

	d	(%)	a (%)	(%)	
Khartoum	369	247(66.9)	217(87.9)	30(12.1)	122(33.1)
Khartoum North	335	218(65.1)	186(85.3)	32(14.7)	117(34.9)
Omdurman	202	136(67.3)	118(86.8)	18(13.2)	66(32.7)
Total	**906**	**601 (66.3)**	**521(86.7)**	**80(13.3)**	**305 (33.7)**
p-value		0.8		0.7	

Table 4.3.2. Co-infection among *N. caninum* seropositive Dairy animals from the three districts of the Khartoum State.

District	Positive animals	Co-existence	Single infection	p-value
Khartoum	30	22(73.3)	8(26.7)	
Khartoum North	32	7(21.9)	25(78.1)	0.00
Omdurman	18	10(55.6)	8(44.4)	
Total	**80**	**39(48.8)**	**41(51.3)**	

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No anti-*N. caninum* antibody detected in the animals from Karary locality (Table 4.3.3.). The differences between the localities in the seropositivity of either *T. gondii* or/and *N. caninum* was statistically highly significant ($p<0.01$). However, there were no significant differences in the occurrence of the two parasites in different localities. The highest seroprevalence was recorded in Bahri locality while the higher anti-*N. caninum* antibodies were detected in animals from Eastern Nile locality (table 4.3.3).

Highly statistically significant differences were observed between the localities in the occurrence of mix-infection of the two parasites ($p<0.01$). The highest mix-infection of *T.*

gondii with *N. caninum* infection was reported in Jabal Aolia locality (table 4.3.4).

Table 4.3.3. Occurrence of *T. gondii* and *N. caninum* in Dairy Animals from the seven localities of the Khartoum State.

Locality	Animals examined	Infected animals (%)			Clean (%)
		Over all (%)	Toxoplasma (%)	Neospora (%)	
Khartoum	46	34(73.9)	33(97.1)	1(2.9)	12(26.1)
Jabal Aolia	223	213(65.9)	184(86.4)	29(13.6)	110(34.1)
Eastern Nile	207	116(46.0)	96(82.8)	20(17.2)	91(44.0)
Bahri	128	102(79.7)	90(88.2)	12(11.8)	26(20.3)
Omdurman	151	101(66.9)	86(85.1)	15(14.9)	50(33.1)
Karary	22	17(77.3)	17(100)	0(0.0)	5(22.7)
Ombadda	29	18(62.1)	15(83.3)	3(16.7)	11(37.9)
Total	**906**	**601 (66.3)**	**521(86.7)**	**80(13.3)**	**305 (33.7)**
p-value		0.001	0.2		

Table 4.3.4. Co-infection among *N. caninum* seropositive dairy Animals in the different localities of the Khartoum State.

*Locality	Positive Animals	Co-existence (%)	Single infection (%)	p-value
Khartoum	1	1(100)	0(0.0)	
Jabal Aolia	29	21(72.4)	8(27.6)	
Eastern Nile	20	3(15.0)	17(85.0)	0.002
Bahri	12	4(33.3)	8(66.7)	
Omdurman	15	9(60.0)	6(40.0)	
Ombadda	3	1(33.3)	2(66.7)	
Total	**80**	**39(48.8)**	**41(51.3)**	

*6 localities because *N. caninum* was not detected in the investigated dairy herds from Karary locality.

As presented in table (4.3.5) Sheep was the superior in the overall seroprevalence (78.0%) and in the *T. gondii* seropositivity (94.0%) without statistically significant differences (p>0.05). However, sheep was the lowest in *N. caninum* seropositivity (5.1%) and in the mix-infection (25%) of the two parasites (table 4.3.6). Higher seroprevalence of *N. caninum* was reported in dairy camels (16.2%) followed by dairy cows (15.2%) and goats (9.2%) without any statistically significant differences (p>0.05). The co-existence was higher in goats followed by camel and cattle (table 4.3.6).

Table 4.3.5. Occurrence of *T. gondii* and *N. caninum* in different animal species from the Khartoum State.

Locality	Animals examined	Infected animals (%)			Clean (%)
		Over all (%)	Toxoplasma (%)	Neospora (%)	
Cattle	645	421 (65.3)	375(84.8)	64(15.2)	224 (34.7)
Camels	61	37(60.7)	31(83.8)	6(16.2)	24(39.3)
Sheep	100	78(78.0)	74.4(94.9)	4(5.1)	22(22.0)
Goats	100	65(65.0)	59(90.8)	6(9.2)	35(35.0)
Total	**906**	**601(66.3)**	**521(86.7)**	**80(13.3)**	**305(33.7)**
p-value	**0.06**		**0.7**		

Table 4.3.6. Co-infection among *N. caninum* seropositive Dairy animals from the Khartoum State.

Animal sp.	Neospora P+ve	Co-existence (%)	Single infection (%)	p-value
Cattle	64	30(46.9)	34(53.1)	
Camel	6	3(50.0)	3(50.0)	
Sheep	4	1(25.0)	3(75.0)	0.2
Goat	6	5(83.3)	1(16.7)	
Total	**80**	**39(48.8)**	**41(51.3)**	

T. gondii infection showed statistically significant (p=0.041) association with *N. caninum* seropositivity in the univariate analysis (table 4.4.7), but multivariate analysis (table 4.3.8) revealed insignificant association (p=0.346) with increasing odds ratio (odds=1.410, 95% CI=0.690 – 2.882).

Table 4.3.7: Univariate association of *T. gondii* infection as Risk factors with *N. caninum* seropositivity in dairy animals using Chi square.

Risk factors		N of Animals examined	N of P+ve	P+ve %	p-value
Toxoplasma gondii	P+ve	506	36	7.1	**0.041**
	N-ve	400	44	11.0	

Table 4.3.8: Multivariate association of *T. gondii* infection as Risk factors with *N. caninum* seropositivity in dairy animals from Khartoum State using Chi square.

Risk factors		N of Animals examined	N of P+ve (%)	Wald (L.R)	p-value	Exp(B)	95% CI for Exp(B)	
							Lower	Upper
T. gondii	P+ve	506	36 (7.1)	Reference				
	N-ve	400	44 (11.0)	0.889	0.346	1.410	0.690	2.882

4.4. Co-existence of *T.gondii, N.caninum* and *B.abortus*:

The overall seroprevalence of the three abortifacient agents at herd level was 94.9% (Table 4.4.1). Antibody against *T. gondii, B. abortus and N. caninum* was detected in 92.7%, 65.6% and 32.2% of the tested herds respectively. The minimum and the maximum within herd prevalence rate were presented in table (4.4.2). The majority of the herds that infected with *T. gondii* recorded more than 50% prevalence rate, while the herds having *B. abortus* or *N. caninum* recorded less than 50% prevalence rate (Table 4.4.3).

Table 4.4.1: Over all Seroprevalence of three abortifacients in dairy farms from the Khartoum State.

Tested Samples for anti body against *Toxoplasma gondii*, *Brucella abortus* and *Neospora caninum*

	At Herd Level		At Individual Level	
Results	**N**	**%**	**N**	**%**
Seropositive	168	94.9	895	64
Clean	9	5.1	503	36
Total	**177**		**1398**	

Table 4.4.2: Seroprevalence of the three abortifacients in dairy herds from the Khartoum State.

Antibody Against	N Tested	N P+ve	Prevalence%	Range %	Mean±SD
Toxoplasma	177	164	92.7	8 - 100	51.27±24.34
Brucella	163	107	65.6	4 - 100	30.64±18.06
Neospora	174	56	32.2	**7 - 75**	27.21±15.43

Table 4.4.3: Frequency distributions of prevalence rates of the three abortifacients in dairy herds from the Khartoum State.

Abortifacient agent	1-24%	25-49%	50-74%	75-100%	P+ve Herds (%)
	N of herds (%)				
Toxoplasma	21(12.8)	57(34.8)	54(32.9)	32(19.5)	164(92.7)
Brucella	47 (43.9)	42 (39.3)	15 (14.0)	3 (2.8)	107(65.6)
Neospora	27(48.2)	22(39.3)	6(10.7)	1(1.8)	56(32.2)

The overall seroprevalence of the three agents at individual level was 64.02%. *T. gondii*, *B. abortus* and *N. caninum* seroprevalence were 45.3%, 22.2, and 8.8 respectively. Highly statistically significant ($p<0.01$) differences were reported among animals species in cases of *T. gondii* and *B. abortus*, while no significant ($p>0.05$) difference in case of *N. caninum* infection among the different dairy animals (Table 4.4.4). Mix-infection with either *T. gondii* or *N. caninum* was reported in 47.9% of *B. abortus* seropositive animals.

Table 4.4.4: Seroprevalence and Co-existence of three abortifacients in different dairy animals from the Khartoum State.

Animal	Cattle		Sheep		Goats		Camels		p-value	Total	
Antibody	N	P+ve (%)	N	P+ve (%)	N	P+ve (%)	N	P+ve (%)		N	P+ve (%)
Toxoplasma	1216	497(40.9)	100	75(75.0)	100	64(64.0)	61	33(54.1)	0.000	**1477**	**669(45.3)**
Brucella	1216	301(24.8)	89	4(4.5)	75	5(6.7)	18	1(5.6)	0.000	**1398**	**311(22.2)**
Neospora	645	64(9.9)	10	4(4.0)	10	6(6.0)	6	6(9.8)	0.177	**90**	**80(8.8)**

***Mix-infection**	301	140(46.5)	04	3(75)	05	5(100)	11	1(100)	0.047	**6311**	**149(47.9)**

*Mix-infection among Brucella Seropositive animals.

The mix-infection was detected in 176 heads (19.7%) out of 895 seropositive animals, where 9 (5.11%) of them recorded the antibodies to the three abortifacient agents (Table 4.4.5). Out of the 311 Brucella positive animals 149 (47.9%) showed mix-infection, within which 9 (6.04%) have antibody of *B. abortus* with *T. gondii* and *N. caninum* (Table 4.4.6).

Table 4.4.5: Distribution of Mix infection among over all seropositive dairy animals from the Khartoum state.

Mix Infection	N	Percent	Valid %
Single Toxoplasma infection	527	58.9	
Single Brucella infection	162	18.1	
Single Neospora infection	30	3.4	
Toxoplasma with Neospora	27	3.0	15.34
Brucella with toxoplasma	132	14.7	75.00
Brucella with Neospora	8	0.9	4.55
Brucella with Toxoplasma and Neospora	9	1.0	5.11
Total Co-existence		**176**	
Total Seropositive animals		**895**	

Table 4.4.6: Mix-infection among Brucella positive dairy animals from the Khartoum State.

Mix Infection	N	Percent	Valid percent
Brucella with toxoplasma	132	42.4	88.59
Brucella with Neospora	8	2.6	5.37
Brucella with Toxoplasma and Neospora	9	2.9	6.04
Single Brucella infection	162	52.1	
Total Brucella Mix infection	**149**	**47.9**	**100.00**
Total of Brucella Seropositive		**311**	

animals

As presented in table (4.4.7), the univariate analysis showed no significant (p>0.05) association between *B. abortus* seropositivity and the two protozoal infections (Toxoplasma (p=0.260) and Neospora (p=0.328). In the multivariate analysis, seropositive Toxoplasma (p=0.269) and Neospora (p=0.805) factors showed increasing odds of being Brucella seropositive and found not significantly associated (p>0.05) with *B. abortus* infection (table 4.4.8).

Table 4.4.7: Univariate association of protozoal abortifacients as Risk factors with *B. abortus* seropositivity in dairy animals from Khartoum State.

Risk factors		**Animals examined**	**N of P+ve**	**P+ve %**	**p-value**
T. gondii	P+ve	619	129	20.8	0.260
	N-ve	779	182	23.4	
N. caninum	P+ve	74	17	23.0	0.328
	N-ve	753	138	18.3	

Table 4.4.8: Multivariate association of protozoal abortifacients as Risk factors with *B. abortus* seropositivity in dairy animals using Chi square.

Risk factors	N of Animal	N of P+ve (%)	Wald (L.R)	p-value	Exp(B)	95% CI for Exp(B)

		s examined					Lower	Upper
T. gondii	N-ve	779	182(23.4)	Reference				
	P+ve	619	129(20.8)	1.223	0.269	1.264	0.834	1.914
N. caninum	N-ve	753	138(18.3)	Reference				
	P+ve	74	17(23.0)	0.061	0.805	1.102	0.510	2.383

4.5. Summary of the Questionnaire Results:

As presented in table (4.5.1), the occurrence of abortion in the investigated herds was very high (72.4%), and there were no significant differences among the three district and the localities of the State in the problem of abortion complains. Even there was no different whether the respondent is the owner, worker or the farm manager. Most of these abortions (65.5%) occurred during the first or the second trimester of the pregnancy (2-5 months), and 45.9% of the aborted cows age were less than 6 years old while the rest (54.1%) were older (more than 6 years old) when they aborted. About 18.2% of these herds recorded repeated abortion. The majority of the dairy farms (76.3%) were complaining from repeat breeding. Cases of stillbirth were reported in 18.4% of the interviewed dairy farms. Although some responders denied keeping dogs and cats in their farms, but these animals were observed in around their farms (plate 4.1).

Almost, all the investigated farms were suffering from neonatal deaths after sever diarrhoea (plate 4.1).

Table 4.5.1: Results of owner's interview on occurrence of abortion, repeat breeding and still-birth among the dairy herds in the Khartoum State.

Reproductive problem	N of Herds	N of Yes	Percent
Occurrence of abortion	76	55	72.4
Occurrence of repeated abortion	55	10	18.2
Occurrence of repeat breeding	76	58	76.3
Occurrence of still-birth	76	14	18.4

Plate 5.1: Dogs were seen inside the farm, in the feed storage and around dairy farms eating dead and/or aborted calves.

4.5.1. Herders Awareness and Perception:

The summary of response of the farm owners on awareness and concept of reproduction failure was presented in table (4.5.2). The majority of the dairy farm personnel (68.4%) did not know causes of abortion. Ten persons (41.7%) out of the 24 (31.6%) herders who knew the causes had mentioned *Brucella* as a cause of abortion. The others (58.3%) mentioned different causes including FMD, CBPP, Oxytetracycline injection and starvation, as well as young age of pregnant cow or uses of young bull. Concentrates of specific companies and out grazing were also incriminated as causes of abortion.

Only 4 (5.3%) respondents know *Toxoplasma gondii* and only 3 (3.9%) of them know *Neospora caninum*. Dairy animals with reproductive problem usually (65.8%) left without any intervention. However, the majority of farm owners (61.8%) send dairy animals with reproductive problems to slaughter houses.

Table 4.5.2: Results of owner's awareness on causes of abortion.

Awareness	N of Respondent	Yes (%)	No (%)
Know cause of abortion	76	24(31.6)	52(68.4)
Know Toxoplasmosis	76	4(5.3)	72(94.7)
Know Neospora	76	3(3.9)	73(96.1)
Intervention	65	15(23.1)	50(76.9)
Culling (Slaughtering)	76	47(61.8)	29(38.2)

5. DISCUSSION

In the current study, two important reproductive protozoal diseases (Toxoplasmosis and neosporosis) of farm animals which cause reproduction and economic loss were investigated. Additionally, one of these two parasites, *T. gondii* infections have significant threatens the public health impact. Monitoring and surveillance programmes for *T. gondii* infections in animals used for human consumption are essential to elucidate the relative importance of the various sources of human infections, to control disease and to prevent reduction in quality of human life caused by *T. gondii* (EFSA 2007. However, only a few countries regularly monitor toxoplasmosis in humans, and thus far, no country monitors *T. gondii* infection in animals (Tenter, 2009).

The present study pointed out that, the seroprevalence rate of *T. gondii* infection in sheep (75.0%), goats (64.0%) and cattle (40.9%) were higher than the world average (30%, 15%, and 9%) respectively (Fayer 1981; Dubey, 2004). The present result in sheep was within the range (64%-88%) that reported in adult sheep from Gabon (Mbiye *et al.,* 2013),

Turkey (Murat *et al* 2005), USA (Dubey and Jones, 2008) and Iran (Hossein *et al.,* 2008). Our results were higher than the results obtained from slaughtered animals in different abattoirs in the Sudan (Zein Eldin *et al.,* 1985; Khalil and Elrayah 2011; Abdel Hafez 2013), Egypt (Hassan *et al.,* 2000; Ghazi *et al.,* 2006; Ramadan *et al.,* 2007), and South Africa (Abusamra *et al.,* 2007). The higher infection rate in sheep than the goats is a uniform finding in many seroprevalence studies worldwide (Bahrieni *et al.,* 2008) and in the Sudan (Zein Eldin *et al.,* 1985). Lower infection rates in goats, camel and cattle compared to those in sheep may be attributed to the differences in susceptibility to *T. gondii* and to the feeding habits of these animals (Bahrieni *et al.,* 2008). The high prevalence of ovine toxoplasmosis could also be linked to the low resistance of sheep to the parasite and to the fact that sheep grazing system exposed them to contact with oocysts (Dubey and Hamir 2002).

The seroprevalence of *T. gondii* infection in dairy camels (54.1%) was found to be higher than that reported in camels from UAE (Abu-Zaid 2002; Abu-Zaid *et al.,* 2006), KSA (Hussein *et al.,* 1988) and Egypt (Hilali *et al.,* 1998; Shaapan and fathia 2005; Shaapan and fathia 2008) although similar seroprevalence (54.1%) was obtained from camels in Egypt by Derbala *et al.,* (1993). No anti-*T. gondii* antibodies were detected in camels from Nigeria (Okoh *et al.,* 1981). The current result in Sudanese camels was closely similar to that

reported in slaughtered camels (54%) by Zein Eldin (1985), higher than that of Manal and Majid, (2008) and lower to that (67% and 61.7%) reported by Elamin *et al.,* (1992) and Manal (2003). This is may be because of the differences in the management system of the examined camels. Camels may play a serious role in the epidemiology of human toxoplasmosis in the Sudan if we consider the culture of drinking raw milk, and eating raw or undercooked liver and meat of this animal. Generally, the present study was the first area wide and large scale study on seroprevalence of *T. gondii* infection in different farm animal species co-herded in dairy farms in the Sudan while most of the previous data was in camels or in abattoirs (table 1.1).

The overall flock and individual seroprevalence of *T. gondii* infection (92.7% and 45.3%) were higher than that (70.5% and 31.6%) reported in Ethiopia (Gebremedhin *et al.,* 2013). In this study, the seroprevalence of *T. gondii* infection in dairy cattle at herd and individual level (95.5% and 40.9) were found to be higher than that (87.5% and 7.8%) of French beef cattle (Gilot-Fromont *et al.,* 2009). Our result on dairy cattle toxoplasmosis was approximately similar to that reported from slaughtered cattle in the Sudan (40%) and Turkey (41.6%) by Zein Eldin (1985) and Yildiz *et al.,* (2009) respectively. These results were also higher compared to that (50% and 12.7%) reported by Elfahal *et al.,* (2013) in dairy cattle from the Sudan, although the number of herds

and animals tested in our study was extremely higher (132 herds and 1216 cow) than theirs (29 herds and 134 cattle). That difference is also may be because we examined adult female animals only, but their samples included different ages and sexes. Some researchers (Samad *et al.,* 1993; Nematollahi and Moghaddam 2008) considered bulls as more susceptible than cows to *T. gondii* infection. However, many authors (Alexander and Stimson 1988; Pita *et al.,* 1999; Van der Puije *et al.,* 2000; Gebremedhin *et al.,* 2013) believe that cows were more seropositive than bulls. We examined only female cattle (Dairy cows) to trigger the economic importance of these protozoal causes of reproductive failure in dairy industry. Although *N. caninum* has recently been recognized as the primary cause of abortion in dairy cattle (Ortega-Mora 2007), *T. gondii* has not been excluded as a potential causal factor (Oliveira *et al.,* 2001; Gilt-Fromont *et al.,* 2009).

In agreement with many authors (Wiss and Kim 2007; Taylor *et al.,* 2007; Ortega-Mora *et al.,* 2007; Innes, 2011), the present work also cofirmed that, sheep and goats were more susceptible to *T. gondii* infection than any other farm animals when they recorded the highest seroprevalence and antibody titration (1:128). The highest antibody titration reported in Iranian sheep and goats using LAT was ≤1:64 (Hashimi-Fesharki, 1996). The later author found no positive reaction against *T. gondii* in sera of 2000 cows. Therefore, he

and Dubey (1986) supported the hypothesis that cattle are not favored hosts for *T. gondii*. The antibody titers in animals can often be linked to chronic infection. Since the work of Dubey and Schmitz (1981) there is evidence that seropositive animals may harbour cysts of *T. gondii* in their internal organs and muscles. The level of specific antibody determined in our examined cattle and camel were relatively low (≥1:32<1:64) compared to the co-herded sheep and goats. The antibody titration of 1:32 was the highest level reported by Khalil and Elrayah (2011) in only 3 (1 sheep and 2 cattle) out of 76 positive sera of their examined slaughtered animals. At least one outbreak of human toxoplasmosis whose source was raw beef has been documented (Smith 1993). In addition 25% of beef samples randomly chosen from UK tested positive for *T. gondii* by PCR (Aspinall *et al.,* 2002). These facts along with the circumstantial evidence provided by other authors (Klun *et al.,* 2006) on the high prevalence of cattle infection, all suggest that the role of cattle as reservoir for human toxoplasmosis should be reconsidered. The importance of beef as source of food and discrepancies on cattle toxoplasmosis make it an important issue (Dubey 1995). We thus examined our dairy cows using two different serological techniques (LAT and ELISA). In agreement with Mulvihill (2009) these tests could be considered as good tools for serological diagnosis of *T. gondii* infection. The study

revealed that, the level of agreement between the two tests, and the sensitivity and specificity were found to be fair. Higher agreement was reported between ELISA and MAT (Hossein *et al.,* 2008; Negash *et al.,* 2004) in sheep. In addition to cattle resistant to *T. gondii* infection than sheep (Munday 1978; Dubey *et al.,* 2003), the lower agreement and area under the ROC curve reported in this study may be just because of the high dilution (1:400) and the high cut-off point (OD≥1.86) used for ELISA in our work. The presence of antibodies against *T. gondii* in animals only indicates exposure to the parasite. However, since direct observation of cysts in tissues is not a suitable diagnostic method to be carried out on live animals, the serological techniques appear to be the method of choice (Hossein *et al.,* 2008). Oocysts stages excreted in the faeces of feline are unsporulated and, thus, are not immediately infective. Therefore, direct contact with cats usually does not result in *T. gondii* infection (Tenter *et al.,* 2000; Cook *et al.,* 2000). However, consumption of undercooked meat was identified as the principle risk factor in several case control studies on Toxoplasma infection in human (Cook *et al.,* 2000). Although small ruminants play more important role as a source of human toxoplasmosis than cattle do (Weiss and Kim, 2007; Cook *et al.,* 2000; Dubey 1986), bovine protein cannot be ruled out as a significant source of infection for human (Ciamak 2005).

In the Sudan people prefer sheep mutton, however, the majority of them consume beef (Naema and Angara 2014), thus increasing the importance of cattle as a source of local toxoplasmosis infection, particularly when we know that -during this study- most of the dairy animals with infertility problems or reproduction failure were sent to slaughter houses. Records on human toxoplasmosis in the Sudan are not available, yet from the present results among animals (Reservoirs), it can be assumed that human exposure to the infection may be very high.

Undercooked meat of cattle, camels, sheep and goats are widely consumed in the Sudan. Almost all of the products (milk and meat) obtained from cattle were consumed in the local markets. Cows more than 5 years were most likely culled due to increased infertility problems (Yildiz *et al.,* 2009). Similarly our survey results revealed that most cows with reproductive problems (failure) were sent to the slaughter houses in their third or fourth production season (5-6 years old).

In the present study, the statistically significant ($p<0.05$) risk factors for dairy animals toxoplasmosis included source of water (common canals), source of fodder, farm location and animal species. Similar observations were reported by Villena *et al.,* (2004); Clun *et al.,* (2006) and Vesco *et al.,* (2007). The latter author stated that using of surface water for drinking and farm size were factors associated with *T.*

gondii seropositivity in Italy and the first one demonstrated *T. gondii* in water surface and underground water by DNA amplification in France.

The results of the current study reflected an increasing odds of infection without statistically significant association ($p>0.05$) in risk factors like Localities (Eastern Nile), keeping cats, keeping both dogs and cats, and presence of both stray dogs and cats. Interestingly and in agreement with (Figliuolo *et al.,* 2004; Santos *et al.,* 2009) and in contrast with several authors (Dubey 1980; Dubey and Bettie, 1988; Mainar *et al.,* 1996; Vesco *et al.,* 2007), presence of cats was not significantly associated with Toxoplasma seropositivity in dairy farms. That is may be because stray cats were observed in or near to all farms premises, even when farm personnel/owners denied their presence.

Based on the results of this study, the contaminative factors such as source of water and feeds played an effective role on *T. gondii* infection in dairy animals. Similar observation was reported by (Villena *et al.,* 2004; Vesco *et al.,* 2007). In comparison with the previous studies (table 1.1), this study provides more comprehensive information on the seroprevalence and risk factors of *T. gondii* infection in dairy animals in the Sudan. Our results indicate the persistence of the necessary epidemiological conditions for the parasite life cycle and for the source of this agent to humans and other domestic and wild animals. This was clearly explained when

we found that the environmental factors (region, animal species, source of water and source of food) were the main risk factors associated with toxoplasmosis seropositivity. We tried to support these findings by our recent reports on working horses and donkeys (Shadia *et al.,* 2013) as well as the stable horses (Ibrahim *et al.,* 2014) in a cooperative work during this study. It is also believed that our results may contribute to an updated informational bank of data on food animal toxoplasmosis throughout the world. As cattle, sheep, goats and camels are the main source of meat and milk in the Sudan, the results of the present study suggest that consumption of raw or undercooked meat and milk of these animals may be probable source in the transmission of human toxoplasmosis. The high flock and individual animal level seroprevalence of toxoplasmosis in farm (food) animals is a good marker of the potential risk of human infections.

Before Dubey (1988) *N. caninum* was misdiagnosed as *T. gondii*. Now it has been recognized worldwide as one of the most important abortifacient agents in cattle (Dubey and Lindsay 1996; Ortega-Mora 2007; Dubey and Schares, 2011). Although there is no cross reactivity between *N. caninum* and *T. gondii* (Romand *et al.,* 1998), we use the very specific VMRD cELISA (Dubey and Schares, 2011) in our study. The study revealed overall flock 32.2% and individual 8.8% seroprevalence of *N. caninum* infection in dairy animals using cELISA. There are considerable differences in serologic

prevalence of *N. caninum* among countries, within countries, between regions and between beef and dairy cattle (Dubey and Schares, 2011). Our result in cattle (33.3%) was lower than that obtained by Amira *et al.,* (2012) at herd level (43.7%). That is may be just because of the drastic differences in the number of herds examined. Also may be because, we have only examined adult female animals, but they have tested different sexes and different ages. Interestingly, at individual level our work revealed higher seroprevalence (9.9%). This may reflect that the infection is increasing in the area. Higher prevalence rate (15.9%) was reported by Hussein *et al.,* (2012) outside the Khartoum State. The difference is may be just because most of their animals examined were from extensive production system, because animal breed has no significant effect on *N. caninum* infection (Yildiz *et al.,* 2009) while the production system do (Anderson *et al.,* 1991, 1995). Moreover, the only two Sudanese authors examined very few number of cattle compared to the examined population (dairy cows) in the current study.

There are very few data on seroprevalence of *N. caninum* in other animals worldwide (Dubey and Schares, 2011). For camels, sheep and goats, our results 9.8%, 4.0% and 6.0% were the first serological evidence of occurrence of *N. caninum* in these animals in the Sudan, but the results were within the world range 0.6 up to 30.8% (Huong *et al.,* 1998;

Hilali *et al.,* 1998; Figliuolo *et al.,* 2004; Hosseininejad *et al.,* 2009; Yildiz *et al.,* 2009; Dubey and Schares, 2011). The serological evidence of *N. caninum* infection in camel in this study may support the report of Manal *et al.,* (2013) who succeeded to isolate the parasite from camel tissues using bio assay and molecular technique. Our results revealed higher seroprevalence of *N. caninum* in camel than that reported in Egypt (3.7%) by Hilali *et al.,* (1998) using *Neospora* Agglutination test (NAT) and that (3.22%) of Iranian Camels using IFAT (Hosseininejad *et al.,* 2009).

In this study, the minimum *N. caninum* seroconversion in the different dairy farms was 7% and the maximum was 75%. Although there was no significant differences between the tested animal herds (p>0.05), cattle and camel are more susceptible to *N. caninum* infection than sheep and goats. Cattle and camel showed higher within herd's prevalence rate reaching 75% and 34% respectively, and higher percent inhibition reaching 93% and 56% pi respectively. Goat's were more susceptible to *N. caninum* infection than Sheep. The highest within herd prevalence rate reported in goat's herds was 25% against only 14% in sheep herds. Similar results were reported by Dubey and Schares (2011).

The absence of significant differences in the seroprevalence among the studied variables such as area and animals species reported in the present study was consistent with various studies worldwide (Nourollahi *et al.,* 2008; Youssefi

et al., 2009; Dubey and Schares, 2011). This may support that, the usual method of *N. caninum* transmission is transplacental (Dubey and Lindsay 1996; Taylor *et al.,* 2007; Ortega-Mora *et al.,* 2007; Dubey and Schares, 2011). The present study revealed that seroprevalence of *N. caninum* (9.9%) was much lower than that of *T. gondii* (40.9%) in adult dairy cows. It was suggested that, infected cows can infect foetus, and if these calves have not been re-infected, antibody titers decline over time, resulting in an apparent decrease in seroprevalence with cow age (Sanderson *et al.,* 2000).

In the present study, with the exception of the production system (p=0.019) and the source of concentrate (p=0.007), the majority of the possible risk factors investigated were not significantly (p>0.05) found to be associated with Neospora cELISA seropositivity. Anderson *et al.,* (1991, 1995) stated that, *N. caninum* is cited as the cause of abortion in many countries and regions with intensive dairy industry. Even the source of concentrate (readymade concentrates) showed significant association, may be just because it is usually adopted in the intensive system. An increasing odds of infection without statistically significant association (p>0.05) was found in risk factors like presence of dogs and animal species. That is may be because stray dogs were observed in or near to all farm premises, even when farm personnel/owners denied their presence (Plate 4.1). Similar

observation was reported by (Figliuolo *et al.,* 2004). Based on these results, unlike *T. gondii* infection (Villena *et al.,* 2004; Vesco *et al.,* 2007), the environmental factors does not play an effective role on *N. caninum* infection in dairy animals. We thus found to be in agreement with many authors (Dubey and Lindsay 1996; Figliuolo *et al.,* 2004; Ortega-Mora *et al.,* 2007; Dubey and Schares, 2011) who believed that the main rout of transmission of *N. caninum* is vertical.

The majority of abortion cases in dairy farms originate from infectious agents (Ortega-Mora *et al.,* 2007). The present study revealed that dairy animals were widely exposed to protozoal abortifacients (*T. gondii* and *N. caninum*) infection together with the well known agent *Brucella abortus*. Similar results of co-existence were reported by Huong *et al.,* (1998) Yildiz *et al.,* (2009). In agreement with the later authors, *T. gondii* was found to be more prevalent than *B. abortus* and *N. caninum*. Toxoplasmosis is an opportunistic infection. Antibodies to *T. gondii* are detected more frequently in sick (immunosuppressed) animals and any complications of primary disease (Svoboda *et al.,* 1988; Svoboda *et al.,* 1998; Bjorkman *et al.,* 2000). The results of co-existence in the present study were consistent with authors who claimed that the ratio of *N. caninum* seropositivity is higher in *T. gondii* seropositive cattle (Gonzalez-Warleta *et al.,* 2008). Among *N. caninum* positive animals 48.8% were co-infected with *T.*

gondii and 23.0% with *B. abortus*. Lower results were reported (13.33% and 10% respectively) in Turkey (Yildiz *et al.,* 2009). Our results (seroprevalence and mix-infection) were also higher compared to that reported in Vietnam (Huong *et al.,* 1998). The results of the present study were consistent with authors who claimed that there is no cross reaction between antibodies against *T. gondii* and antibodies to *N. caninum* (Dubey and Lindsay 1996; Ortega-Mora *et al.,* 2007; Dubey and Schares, 2011).

Univariate analysis revealed significant association (p=0.041) between *T. gondii* and *N. caninum* infection. However, the outcome of the multivariate analysis was somehow found to be contradictory with the reports (Yildiz *et al.,* 2009) claiming that the ratio of *N. caninum* seropositivity is higher in *T. gondii* seropositive animals. Because, in our study seronegative *T. gondii* showed insignificant association with an increasing odd ratio of being *N. caninum* seropositive. Interestingly, although the association was insignificant (p>0.05), seropositive *T. gondii* and *N. caninum* showed an increasing odds ratios of being *B. abortus* seroposistive. The mix-infection of *T. gondii* with *N. caninum* reported in sheep in our work (25%) was higher compared to that reported (3.5%) in Brazil (Figliuolo *et al.,* 2004). In agreement with several authors (Seri *et al.,* 2003; Wiss and Kim 2007; Taylor *et al.,* 2007; Ortega-Mora *et al.,* 2007; Innes, 2011), the present study revealed that, *T. gondii*

seropositivity was significantly higher in sheep, goats and camels while *Brucella abortus* and *N. caninum* seropositivities were significantly higher in cattle.

Summary of the questionnaire results of this study found that, causes of abortion other than *Brucella* in the Sudan are completely neglected, although most of the abortions occur in the first or the second trimester of the pregnancy. Moreover, in agreement with many reports (Anderson *et al.,* 1995; Ortega-Mora *et al.,* 2007; Pabon *et al.,* 2007), a number of repeated abortions and stillbirth were reported during this study. *N. caninum* seroprevalence is significantly higher in aborted cattle than in healthy ones, and there is strong evidence for a possible link between *N. caninum* seropositivity and abortion (Yildiz *et al.,* 2009; Youssefi *et al.,* 2010). Gamble *et al.,* (1999) suggested including of small producers in the education on farm management.

Infectious materials, like foetal membrane (placenta), aborted foetus, dead calves or/and cows which could be major sources of infection to dogs were fully observed in and around the investigated dairy farms during this work (plate 4.1). Thus the high seroprevalence of *N. caninum* might be related to the presence of both domestic and stray dogs reported and noticed in the investigated dairy farm premises at the time of the study visits (Plate 4.1). Stray dogs are very present; when the farm personnel mentioned that dogs eat the retained placenta directly from the vagina causing

serious damage (pyometra/infertility) to the urogenetal tract of the cow. The interview (questionnaire) revealed high prevalence of reproductive failure problems including repeated abortions, where *N. caninum* infection suggested (Ortega-Mora *et al.,* 2007). The basic aspect was that *T. gondii* can cause abortion only once in infected animals. However, recent references (Morely *et al.,* 2005; Buxton *et al.,* 2007; Morely *et al.,* 2008) indicated that repeated transplacental transmission may be more commonly occur than previously believed. Co-existence of *T. gondii* and/or *N. caninum* and *B. abortus* may play an effective role in the incidence of abortion and reproductive failure complains in this study. In particular, almost, all the visited dairy farms in addition to abortion suffer from frequent repeat breeding. Many of herders believe on cutting part and cauterization of the vagina of the cow as well as air pumping as a treatment of repeat breeder cows. The neonatal mortality (one day old calve with severe diarrhoea) was one of the most important complains of farmers (plate 5.1). Manal and Majid, (2008) reported an association between fatal diarrhoea in camel-calve and *T. gondii* seropositivity.

6. CONCLUSION AND RECOMMENDATIONS

6.1. Conclusions:

1. In addition to providing large scale and comprehensive serological data on *T. gondii* and *N. caninum* infection in dairy animals as well as their possible socio-economical

impact, this study provide the first serologic evidence of *N. caninum* infection in camels, sheep and goats in the Sudan. Furthermore, it is the first report on analysis of potential risk factors associated with *N. caninum* infection in the country.

2. Dairy animals including cattle, camels, sheep and goats are widely exposed to *T. gondii* and *N. caninum* infection and their role in the economic losses affecting dairy industry (abortion and reproduction failure) could be more than the expected in the Sudan. Moreover, since the seroprevalence of *T. gondii* in animals (reservoirs) is an indicator of the status in humans, the role of dairy animals in the epidemiology of human toxoplasmosis is strongly suspected, particularly when we know that -during this study- most of the dairy animals with infertility problems or reproduction failure were sent to slaughter houses.
3. Based on the results of this study, the contaminative factors such as source of water and source feeds played an effective role on *T. gondii* infection in dairy animals. However, they showed no role on *N. caninum* infection. Our current understanding of the epidemiology of toxoplasmosis leads us to believe that farm animals acquire infection by ingestion of grass and water contaminated with *T. gondii* oocysts shed by cats or

transported by dogs. The differences in rate of infection could be attributed to susceptibility to *T. gondii*.

4. The LAT and IgG-ELISA tests detected relatively similar proportion of Toxoplasma positive serum samples of dairy cows. Therefore both are reliable for population screening tests. However, the need of species specific conjugates and spectrophotometer (automatic ELISA reader) for ELISA test may limit its use in the Sudan. From the results of the present study, LAT positive sera diluted to ≥1:32 could be strongly considered seropositive without confirmation by any other serological test like ELISA. Other LAT seroposistive titrations should be confirmed by ELISA particularly, dilution of 1:2 and 1:4. These findings may add to the available clear and consistent assessment of which positivity threshold should be considered using LAT in cattle or any other intermediate hosts including humans, and it may also help in the economy of *T. gondii* diagnosis particularly in poor areas or laboratories.
5. Co-existence of *N. caninum, T. gondii* and *B. abortus* is frequently occur in dairy animals and should be considered in differential diagnosis. Generally, we believe that, the comprehensive data generated through this study can significantly contribute to understanding of serologic association of *N. caninum*

with *T. gondii* or/and *B. abortus* in dairy industry. These findings indicate that *T. gondii* and *N. caninum* are highly prevalent together with *B. abortus* in the major area of dairy industry in the Sudan, and this calls for control strategies to be implemented in both veterinary and medical/public health as well as extension fields.

6. From the results of interview (questionnaire), incidence of neonatal deaths, abortion and reproductive failure are very high in the Sudan with inadequate awareness, diagnostic data and prophylaxis. The economic losses due to neonatal mortalities and missed lactation are probably higher than the expected.

6.2. Recommendations:

- Further studies on clinical toxoplasmosis and neosporosis in dairy animals as well as the role of dairy animals in the epidemiology of human toxoplasmosis are recommended using other diagnostic tools.
- The results obtained here suggest that education on dairy farm management practices to reduce exposure to *T. gondii* and *N. caninum* should be targeted to include small producers and all dairy farm personnel.
- Consistent data on both immunocompetent and immunocompromized people in the Sudan is needed. Nevertheless, Sudanese should be educated to avoid the behaviour of eating and drinking raw and undercooked meat and milk.

- Further in-depth interdisciplinary research project on protozoal, bacterial, viral and genetically abortifacients is suggested to evaluate their role and economic consequences. Moreover, etiological laboratory diagnosis of animal’s abortions should not be restricted to common bacterial abortifacients.

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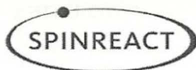
APPENDICES

Appendix 1: Latex Agglutination Test Kits used in this study:



TOXO-LATEX

Toxo-Latex

Slide agglutination

Qualitative determination of anti-toxoplasma antibodies
IVD

Store at 2 - 8ºC.

PRINCIPLE OF THE METHOD

The Toxo-latex is a slide agglutination test for the qualitative and semi-quantitative detection of anti-toxoplasma antibodies.
Latex particles coated with soluble *Toxoplasma gondii* antigen are agglutinated when mixed with samples containing antibodies anti-Toxoplasma.

CLINICAL SIGNIFICANCE

Toxoplasmosis is an infectious disease affecting both animals and humans, which is caused by the protozoan parasite *Toxoplasma gondii.* Acquired toxoplasmosis is usually asymptomatic and benign. Adults, depending on the geographical area and age, would contain antibodies in more that 50% of cases, being protected to a new infection. In its congenital form may be devastating, causing mental retardation, ocular disease, and death in newborn. In adults, the parasite may be responsible for some forms of eye disease; individuals with impaired immunologic competence are also at serious risk.
Infection in pregnant women acquires a special significance as the parasite may enter the fetal circulation through the placenta and causes congenital toxoplasmosis especially during the first trimester of pregnancy. The consequences range from spontaneous abortion, early delivery or fetal death.

REAGENTS

Latex	Latex particles coated with soluble *T. gondii* antigen, pH, 7.5. Preservative
Control +	Animal serum with an antibody anti-Toxoplsma concentration > 4 IU/mL. Preservative
Control -	Animal serum. Preservative

PRECAUTIONS

Sodium azide may react with lead or copper plumbing to form explosive compounds. When disposing of this product through plumbing fixtures, flush with plenty of water. Require Safety Data Sheet for more information.
Personal protection: Wear suitable protective gloves.

CALIBRATION

The Toxo-latex sensitivity is calibrated against the 3rd International Standard for anti-Toxoplasma (WHO).

STORAGE AND STABILITY

All the kit components are ready to use, and will remain stable until the expiration date printed on the label, when stored tightly closed at 2-8ºC and contaminations are prevented during their use. Do not freeze: frozen reagents could change the functionality of the test.
Reagents deterioration: Presence of particles and turbidity.

ADDITIONAL EQUIPMENT

- Mechanical rotator with adjustable speed at 80-100 r.p.m.

SAMPLES

Fresh serum. Stable 7 days at 2-8ºC or 3 months at –20ºC.
Samples with presence of fibrin should be centrifuged.
Do not use highly hemolized or lipemic samples.

PROCEDURE

Qualitative method

1. Allow the reagents and samples to reach room temperature. The sensitivity of the test may be reduced at low temperatures.
2. Place 50 µL of the sample and one drop of each Positive and Negative controls into separate circles on the slide test.
3. Swirl the Toxo-latex reagent gently before using and add **25 µL** of this reagent next to the samples to be tested.
4. Mix the drops with a stirrer, spreading them over the entire surface of the circle. Use different stirrers for each sample.
5. Place the slide on a mechanical rotator at 80-100 r.p.m. for **4 minutes**. False positive results could appear if the test is read later than four minutes.

Semi-quantitative method

1. Make serial two fold dilutions of the sample in 9 g/L saline solution.
2. Proceed for each dilution as in the qualitative method.

READING AND INTERPRETATION

Examine macroscopically the presence or absence of visible agglutination immediately after removing the slide from the rotator.
The presence of agglutination indicates an antibody concentration equal or greater than 4 IU/mL.
The titer, in the semi-quantitative method, is defined as the highest dilution showing a positive result.

CALCULATIONS

The approximate anti-Toxoplasma concentration in the patient sample is calculated as follows:

4 x anti-Toxo Titer = IU/mL

QUALITY CONTROL

Positive and Negative controls are recommended to monitor the performance of the procedure, as well as a comparative pattern for a better result interpretation.

REFERENCE VALUES

Up to 4 IU/mL.
Each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

1. **Analytical sensitivity:** 4 (3-7) IU/mL, under the described assay conditions
2. **Prozone effect:** Up to 200 IU/mL. Occasionally a prozone effect may be observed with strong positive sera. Therefore in these cases where a suspected case of toxoplasmosis gives a negative result, the test should be repeated using 1/5 serum dilution in NaCl 9 g/L.
3. **Diagnostic sensitivity:** 96.1%
4. **Diagnostic specificity:** 89.6%

INTERFERENCES

Hemoglobin (10 g/L), bilirrubin (20 mg/dL), lipemia (10 g/L), and rheumatoid factors (300 IU/mL) do not interfere. Other susbstances may interfere[6].

LIMITATIONS OF THE PROCEDURE

- False positive results may be obtained with hepatocellular diseases. A 25% of serum containing heterofile antibodies may give false positive results.
- All positive sera should be tested with a confirmatory test.
- Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

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PACKAGING

Cod.: 1201002 100 test		: 2.5 mL Toxo-Latex
	Cont.	: 1 mL Control +
		: 1 mL Control -
		: 18 x 6 disposable slides

SGIS10-I 30/06/11

SPINREACT,S.A./S.A.U Ctra.Santa Coloma, 7 E-17176 SANT ESTEVE DE BAS (GI) SPAIN
Tel. +34 972 69 08 00 Fax +34 972 69 00 99 e-mail: spinreact@spinreact.com

Appendix 2: Enzyme Linked Immuno-sorbent Assay (ELISA) Kits used in this study:

User´s Manual

Toxoplasma gondii IgG ELISA

IVD

EIA-3519

96 Wells

Legal Manufacturer:

DRG Instruments GmbH, Germany
Division of DRG International, Inc
Frauenbergstr. 18, D-35039 Marburg
Telefon: +49 (0)6421-17000 Fax: +49-(0)6421-1700 50
Internet: www.drg-diagnostics.de
E-mail: drg@drg-diagnostics.de

Distributed by:

Appendix 3: Competitive Enzyme Linked Immuno-sorbent Assay (cELISA) Kits used in this study:

vmrd Veterinary Medical Research & Development

FOR VETERINARY USE ONLY

USDA Product Code 5N05.20

NEOSPORA CANINUM ANTIBODY TEST KIT, cELISA

Assay instructions for catalog numbers: 280-2 and 280-5

General Description

This competitive enzyme-linked immunosorbent assay (cELISA) detects antibody to *Neospora caninum* in bovine sera. Sample serum antibody to *N. caninum* inhibits binding of horseradish peroxidase (HRP)-labeled *N. caninum*-specific monoclonal antibody to *N. caninum* antigen coated on the plastic wells. Binding of the HRP-labeled monoclonal antibody conjugate is detected by the addition of enzyme substrate and quantified by subsequent color product development. Strong color development indicates little or no blockage of HRP-labeled monoclonal antibody binding and therefore the absence of *N. caninum* antibody in sample sera. Weak color development due to inhibition of the monoclonal antibody binding to the antigen on the solid phase indicates the presence of *N. caninum* antibodies in sample sera.

Kit Contents

Component	280-2	280-5
A Antigen-Coated Plates	2 plates	5 plates
B Positive Control	3.6 ml	3.6 ml
C Negative Control	3.6 ml	3.6 ml
D 100X Antibody-Peroxidase Conjugate	0.3 ml	0.5 ml
E Conjugate Diluting Buffer	30 ml	60 ml
F 10X Wash Solution Concentrate	120 ml	2 × 120 ml
G Substrate Solution	30 ml	60 ml
H Stop Solution	30 ml	60 ml
This insert		

Materials Required But Not Included in the Test Kit

Single and multichannel adjustable-volume pipettors and disposable plastic tips, test tubes or non-antigen-coated transfer plate(s), ELISA microplate reader or spectrophotometer with 620, 630 or 650 nm filter, deionized or distilled water, paper towels, graduated cylinder, timer, multichannel pipettor reservoirs, wash bottle, manual multichannel washing device or automatic plate washer

Appendix 4: Questionnaire sheet for Neosporosis and Toxoplasmosis as causes of abortion in farm animals in Khartoum State, Sudan, 2012-2013

Date: ………………………………………….. Serial No………………

Name (farm owner): ………………………………………Address (Phone): ……………

1. District: ………………..2. Locality: ……………..3. Administrative Unit……..
4. **Respondent**: Name…………………………………. (i) Owner (ii) Worker (iii) Farm Manager
5. **Animal Health Data**:

Do you have abortion cases in your farm? (i) Yes (ii) No

	Goats	sheep	Cattle	Camel
Age of aborted foetus				

6. **Frequency of abortion: (i) Repeatedly (iii) Once**
7. **Do you know the causes of it (abortion)? (i) Yes (ii) No**
8. **If yes, what are they? (i) (ii) (iii)**
9. **Do you know Toxoplasmosis? (i) Yes (ii) No**
10. **Do you know Neosporosis? (i) Yes (ii) No**
11. **Do you have Repeat Breeder cases in your farm? (i) Yes (ii) No**
12. **Do you have still birth cases in your farm? (i) Yes (ii) No**
13. What do you do for an animal that aborted or repeat breeder?

i. Nothing ii. Treatment iii. Call/consult a veterinarian cost……

14. How do you deal with animals that proved to have abortion?
(i) Sell them for slaughtering (ii). Sell them to other farm (iii) Treat them (iv) Leave them within the herd without treatment (v) Keep them in a separate place

15. **Herd management data**:
A. Do you keep (i) dogs (ii) cats (iii) Both in your farm? (i) Yes (ii) No.
B. Do stray (i) dogs (ii) cats (iii) both enter your farm? (i) Yes (ii) No.

Case Recording Form (infected animals)

1	Farm umber/Name											**Rem.**
2	Sample Number											
3	Animal ID											
4	Age of the animal											
5	Animal breed											
6	Number of birth											
7	Number of abortions											
8	Age of aborted foetus											
9	repeat breeding											
11	LAT result											
12	Elisa result											

Total/Remarks											

16. **Any additional notice ……………………………………………………………………………………… ……… ……… ……… ………**
17. **Faecal Sample of: Dog……………………………………………………………………………………………………… … Cat…… .**
18. **Interviewer: Name & Sign. ……………………………………..Phone……………………………………..**

Appendix 5: Declaration of Publications:

The following papers were extracted from the Thesis and published during this study as one of the thesis examination requirements in the Sudan University of science and Technology:

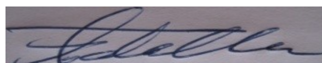
1. **Ibrahim A.M.** Ismail A. A. Angara T. E.E. Osman M. O. **(2015).** Detection of Antibodies against *Toxoplasma gondii* and *Neospora caninum* in Dairy Camels from the Khartoum State, Sudan. Proceedings of The Regional Scientific Conference of Camel management and Production Under open range system (RCCMPR), Sudan University of Science and technology, 2-4th March 2015. Khartoum, Sudan.

2. **Ibrahim A.M.** Ismail A. A. Angara T. E.E. Osman M. O. **(2014).** Area-wide Detection of Anti-*Toxoplasma gondii* Antibodies in Dairy Animals from the Khartoum State, Sudan. Journal of life sciences, Vol. 8: (9), Pp.723-730. DOI: 10.17265/1934-7391/2014.09.001.

3. **Ibrahim A.M.** Ismail A. A. Angara T. E.E. Osman M. O. **(2014).** Serological survey on Toxoplasma gondii infection in Dairy Cows from the Sudan using ELISA.

Global Journal of Animal Sciences, Livestock Production and Animal breeding, Vol. 2: (3), pp. 114-118.

4. **Ibrahim A.M.** Ismail A. A. Angara T. E.E. Osman M. O**. (2014).** Seroprevalence of *Neospora caninum* in Dairy Cattle and the Co-herded camels, sheep and goats in Dairy farms in the Khartoum State, Sudan. *Journal of Applied and Industrial S*ciences, 2(5): 206-212*.*



Abdalla Mohamed Ibrahim Abdalla
Date of Thesis Submission: May *2015*