Introduction

Toxoplasmosis is a widely prevalent zoonosis**,** caused by facultative two – host protozoan toxoplasma *gondii* **(**Prelezov, *et al*., 2008 and Limon *et al*., 2016 ***)***. The definitive hosts of the parasite are domestic and wild cats. intermediate host all mammals including man) are infected by ingestion of sporulated oocyst, cyst-contaminated meat , milk contaminated by tachyzoites or transplacentarily ***(****Prelezov, et al., 2008****)***. Infection more common in warm climates and in low- laying areas than in cold climates and mountainous regions, where condition for sporulation and survival of oocyst are less favorable. The presence of parasite in tissues among live stock is most common in pigs, sheep and goats, followed by rabbits and poultry. Although most infections in small ruminant are asymptomatic there can be abortion, foetal mummification, stillbirth and birth of weak lamb –kids ***(****Dubey , JP 2009 ****)***. In cattle natural toxoplasma *gondii* infection does not appear to cause clinical disease or abortion. There for interest in toxoplasma *gondii* in catlle stems mainly from a public health perspective; if cattle carry infectious tissue cyst they may be an important source of human infections since beef is often consume undercooked ***(***Gasior *et al* ., 2013 ***)***.

In the Sudan, the disease in animal has been reported by number of researcher in varying prevalence according to the species of animal investigated and the geographical area while in human, the prevalence of toxoplasmosis can go as 50 %. However, only a limited number of these studies took into consideration risk factor that are most important for infection with this parasite ***(***Atail, H. 2017***)***. A more recent study recorded prevalence rates of toxoplasma antibodies of 20%, 32%, and 57.5% in camels, cattle, and sheep, respectively Prevalence rates of up to 73% were recorded in childbearing age women and up to 100%in camel herders in Sudan The limited studies of toxoplasmosis in cattle, the increased risk of infection from consumption milk and meat because of its little price, entails the necessity of increase the inter site for research.

Objective of this study :

1-To estimate the prevalence of anti toxoplasma in cattle using LAT and ELISA test at different localities in Gedarif state.

2- To investigate risk factor associated with toxoplasma in animal .

CHAPTER ONE

Literature review

1.1. Definition and description of the disease Toxoplasmosis:

Toxoplasmosis is a zoonotic disease of animals and humans which caused by the protozoan parasite toxoplasma *gondii* **(**Sarciron and Gherardi, 2000; Kasper, 2005; Petersen *et al*., 2010; Torgerson and Macpherson, 2011**).**This parasite has the capacity to infect all warm-blooded animals. While infection does not cause clinical illness in the majority of animal species, in some, it causes acute life-threatening disease and in others, particularly in sheep and goats, it may manifest itself as a disease of pregnancy by multiplying in the placenta and fetus. In these latter animals it can result in the abortion or the birth of weak lambs/kids, which may be accompanied by a mummified fetus. Characteristically, in these cases, the placental inter-cotyledonary membranes are normal, but white foci of necrosis, approximately 2-3mm in diameter, may be visible in the cotyledons. Microscopically, these foci appear as areas of coagulative necrosis that are relatively free of inflammation. Inflammation, when present, is non-suppurative. Toxoplasma tachyzoites are seen only rarely in association with these foci, usually at the periphery of the lesion. Examination of the brain may reveal focal microgliosis. The lesion often have a small central focus of necrosis that might be mineralized. Focal leukomalacia in cerebral white matter , due to anoxia arising from placental damage but may occur in other pathological condition where the placenta is compromised , including , though rarely , ovine chlamydiosis. Infection in pigs may cause severe fetal losses in pregnant sows, but more usually is mild and unnoticed. Acute fatal infections affect.New World monkeys, marsupials and certain others animals.

1-2 Etiology and life cycle :

Toxoplasma.*gondii* belongs to Apicomplexa phylum , sprozoa class , Eucoccida order , Emmerinae suborder and sarcocystidae famly **(**James , 1992**)** . Toxoplasma has several strains ; more than 95 % of them are grouped into three gentic types (1 . 11 . 111) . Type 1 is highly virulent in mice, type 11 is most common type in persistently infected animals (sheep and goat) and type 111 is define as no virulent

strain . clinical human infection are more often associated with type 11 strain ***(***Sibley , 2003***)***.

Toxoplasma *gondii* life cycle include definitive and intermediate host . The sexual and a sexual cycle of the parasite can take place in the intestinal epithieal cell of the cat (defentive host) , but in the intermediate host only asexual cycle take place ***(***Dubey , 2008 ; Frenkel , et al 1970 ; Dubey . 2004***)***.

In the cat, fallowing a primary infection, oocyst are produced and shed in the feces. oocyst require 1-5 days in adequate temperature and moisture to speculate, before they become infective to birds and mammals ***(***Gajadhar et al . , 2004 ; Dubey et al . ,1998***)***. When an intermediate host (sheep) gets infected by ingestion of contaminated feed or grazes on land with spourlated oocyst, the parasite (porosities) will be released and becomes able to actively invade and multiply with in the gut cell. The tachyzoite stage of the parasite multiplies asexually by a process of endodyogeny with in parasitophorous vacuoles and then the parasite eventually release from the rupture cell and invade further cell ***(***Lingelbach and Joiner , 1998***)***. By day four fallowing infection, tachyzoites may be found in the mesenteric lymph nodes ***(***Dubey, 2004***)*** and the parasite are also found in the circulation where they can spread throughout the host **(** Wastling *et al* 1993 ***)***.

In pregnant animals , the tackyzote invade and multiply within the caruncular speta in the placentome and then go on and invade the adjusting fetal trophoblast cells where they can spread to the rest of the fetus ***(****Buxton and Finlayson , 1986****)***.

Tissue site may develop in visceral organ, including lungs , liver and kidneys . They are more prevalent in muscular and neural tissues including the brain , eye , skeletal and cardiac muscle . intact tissue cyst are probably harmless and can persist for hall life the host ***(***Dubey *et al . ,1898****)***. When cat consume infected meat the wall of the cyst is digested by the proteolytic enzymes in the stomach and small intestine of cats and bradizoyte are release in the gastrointestinal tract . some of the bradyzoites penetrate the lamina propria of the intestine and multiply. within a few hours, T. *gondii* may desminated to extra intestinal tissue. Other bradizoyte penetrate the epithelial cell of the small intestine and initiate development of numerous generation asexually ***(***Dubey and Frankel , 1972***)***. oocyst of toxoplasma gondii are form only in cats , including both domestic and wild felids . cat shed oocyst after

ingestion tchyzote, bradizoyte, or sprosities (Dubey 2004). about three to ten days after infection , infected cat star to shed oocyst for two to three weeks (Dubey and beattie , 1988). Each infected cat may shed million of oocyst in environment (Dubey and beattie , 1988) and as few as 200 spourlated oocyst can cause congenital disease in naïve sheep (M cColgan et al ., 1988). Under labotary condition , cats can shed as many as 500 million oocyst after ingestion one toxplasma gondii infected mouth (Dubey and Frankle 1972). Million of oocyst were shed by cats fed even a few bradyzoite (Dubey, 2001) up to 13 million T.gondii oocyst were present per gram of cat feces (Schares et al .,2008). it has been reported than at any given time approximately 1% of cats are expected to shade oocyst , base on the observation that most cats shed oocyst for about 1 week in their life (Dubey , 1995 ; Dubey , 2004). Cat shed million of oocyst in their feces that can survival for 12- 18 month in the environment depending on climatic conditions , and are an important scours of infection for grazing animal (Tenter et al., 2000 , Innes 2009 , Innes et al., 2009). shedding of oocyst tend to be more extensive amongst younger cats rather than older cats (Jackson and Hutchison , 1989 ; Buxton and Rodger , 2008).

1-3 Toxoplasmosis in cattle :

Natural infection In cattle was first diagnosed in 1953 (Sander et al., 1953) . Further observation showed that toxoplasmosis is uncommon in cattle and dose not appear to cause abortion (Dubey , 1986). Calves are more susceptible than adult (Nematollahy and Moghddam , 200). Clinical sings of orally affected calves include diarrheoa ,anorexia , poor weight gain , depression , weakness , dyspnea ,and fever . in some cases just a modest lymphadenopathay may occur . Congenatally nfected calves show fever , dyspnea ,cough , sneezing and neurological sings , while also stillbirth and neonatal death can be observed . If the disease occuar in adult, sysptom may include fever, dyspnea and nervous sings fallowed by lethargy (Dubey , 1986) .

The variations in the infection rate as observed in the different geographical regions might be associated with the serological test employed and other factors such as management, hygienic standards, cat population and environmental conditions. The latter factors may also perhaps be applicable to negative serodetection of T. *gondii* antibody in farm borne cattle of organized farm compared to positive report observed in open grazed cattle (Kalita and Sarmah, 2015).

In Sudan Seroprevalence of *T. gondii* reported in the Khartoum State attains 13.3% and 26.1% using ELISA and LAT respectively*(*Alfahal *et al* ., 2014 *)*. More recently, Khalil and Intisar *(2011)* reported 32% (16/50) seroprevalence of T. gondii in cattle in Khartoum State using LAT test. Ibrahim *et al (* 2014*)* reported that dairy cattle are widely exposed to T. *gondii* infection and the role of toxoplasmosis in the economic losses affecting dairy farm industry due to abortion and reproduction failure could not be neglected or excluded. Moreover, the role of dairy cattle in the epidemiology of human toxoplasmosis is strongly suspected.

1.4. Epidemiology of toxoplasmosis :

Role of cats: T. *gondii* oocysts are shed by domestic cats and other felids resulting in widespread contamination of the environment *(*Dubey and Beattie, 1988*)*. Domestic cats are the major source of contamination as they are common reservoir of infection and excrete large numbers of oocysts *(*Dubey and Frenkel,1972and Dubey, 2001*)*, while only a few cats may shed T. *gondii* oocysts at any given time .Latently infected cats can shed oocysts after being challenged by infection *(*Dubey, 1995*)*, while congenitally infected kittens can also excrete oocysts *(*Dubey and Carpenter, 1993b*)*. Infection rates in cats are largely determined by the rate of infection in the local avian and rodent populations, which serve as a food source *(*Ruiz and Frenkel, 1980a*)*. For example, T. *gondii* oocysts were found in 23.2% of cats in Costa Rica where infection in local rodents and birds was much higher *(, 1980a)*. For epidemiologic surveys sero-prevalence data for cats are more useful than results of fecal examination because cats with antibodies have probably already shed oocysts and are indicators of environmental contamination *(*Dubey and Frenkel, 1972*)*. Under laboratory conditions, cats can shed as many as 500 million oocysts after ingesting one T. *gondii* infected mouse **(**Dubey and Frenkel, 1972*)*. Cats fed even a few bradyzoites can shed millions of oocysts *(*Dubey, 2001*)*.

1.5. Environmental resistance of oocysts:

Sporulated oocysts can survive for long periods under moderate environmental conditions. For example, they can survive in shaded and moist soil for months to years **(**Dubey and Beattie, 1988; Frenkel et al.,1975*)*. T. *gondii* oocysts are highly resistant to disinfectants, but are killed at temperatures above 60 °C *(*Dubey, 2004; Wainwright et al., 2007a*)*. Under laboratory conditions, oocysts

remained infective from 30 days (in uncovered dishes at 37 °C) to 410 days or more, in covered and uncovered dishes at 4°C.Outdoors, infectivity varies from 46 days (uncovered, exposed to direct sunlight, mean air temperature is 20 °C) to 410 days or more (covered in shade and air temperature is 19.5°C). T. *gondii* oocysts may remain infective for a year in warm climates and even longer in cool climates or in air-conditioned buildings ***(***Yilmaz and Hopkins, 1972***)***. Inactivation of T. *gondii* oocysts occurred with exposure to pulsed and continuous UV radiation at doses of > 500 mJ/cm2 **(**Wainwright et al., 2007b***)***.

1.6. Mode of transmission :

Ingestion of contaminated water, food or unpasteurized milk with fecal oocysts shed by cat or oocysts from contaminated hands, utensils or surface (indirect transmission) is the most common mode of transmission ***(***Dubey and Beattie, 1988; Dubey, 2008***)***. Most sheep acquire T. gondii infection after birth. Although exact data are not available, it is thought that < 2% of sheep become congenitally-infected with T. *gondii*, and less than 4% of persistently infected sheep transmit it to the next generation ***(***Buxton et al., 2007; Dubey, 2009;Higa et al., 2010***)***. However, transplacental transmission from mother to fetus through infected placenta has been reported ***(***Dubey and Sharma, 1980; Moura *et al.,* 2007; Dubey, 2008; Dubey and Jones, 2008; Lopes et al., 2009; Scarpelli *et al*., 2009***)***. Also, T. gondii has been isolated from the semen of experimentally infected rams ***(***Lopes et al., 2009***)***, bucks ***(***Dubey and Sharma, 1980***)***, swine ***(***Moura et al., 2007***)***, bulls and male dogs ***(***Scarpelli et al., 2009; Arantes et al., 2009***)***.The main source for human infection is ingestion of uncooked meat containing viabletissue cysts or by ingesting food or water contaminated with oocysts from the feces of infected cats ***(***Dubey, 2004***)***, as well as unpasteurized milk ***(***Higa et al., 2010***)***. Water-borne transmission of T. gondii was considered uncommon but a large human outbreak linked to contamination of a municipal water reservoir in Canada by wild felids and the widespread infection by marine mammals has been detected ***(***Dubey, 2004;Dubey, 2008***)***. Furthermore, oocysts can be spread mechanically in the environment by flies, cockroaches, dung beetles and earthworms ***(***Kniel et al., 2002 and Dubey, 2004***)***.

1.7. Diagnosis :

Histopathology: In abortion cases, multifocal necrosis and calcification might be seen in the placenta. The placental cotyledons can be bright to dark red ***(***Dubey and Beattie, 1988; Buxton, 1998***)***. Parasites can be detected in the placenta and in

the fetal heart, brain, lung or liver **(**Dubey, 2008***)***. Microscopically, necrosis might be found in the white matter of the fetal cerebellum and cerebrum. Focal lymphoid-cell proliferations and micro necrosis might be presented in fetal kidneys, adrenals, lymph nodes or brain ***(***Buxton, 1998; Dubey, 2008; Dubey and Jones, 2008***)***.

1.7.1. Immunohistochemistry :

Immuno-histochemical techniques allow visualization of both intact T. *gondii* and antigenic debris in tissue sections of aborted materials; they are convenient, sensitive methods and have the advantage, when compared with attempts at isolation, of detecting toxoplasma antigen even in decomposed tissues ***(***Buxton, 1998; Dubey and Jones, 2008***)***.

1.7.2. Direct smears :

Direct smear from affected tissue proved rapid and easy diagnostic method ***(***Terpsidis et al., 2009***)***.

1.7.3. Serological test :

Serological test is used as common method for diagnosis of toxoplasmosis which includes sabin Feldman dye test, indirect heamagglutination test (IHT), indirect fluorescent antibody test *(IFAT)*, complement fixation test (CFT) and intra dermal test (IDT) ***(***Jacobs *et al*., 1960; Dubey,2008***)***. Sabin–Feldman dye test was developed in 1948 by Albert Sabin and Harry Feldman ***(***Dubey, 2008***)***. The dye test is highly sensitive and specific with no evidence for false results in humans. The ability to identify T. *gondii* infections based on a simple serological test opened the field for extensive epidemiological studies on the incidence of infection ***(***Dubey, 2008; Dubey, 2009***)***, however it is very expensive, time consuming and not without hazard as it requires alive tachyzoites as antigen ***(***Buxton, 1998***)***. The IHT is a simple, fast and inexpensive test using nonliving antigen; it's very practical and useful in veterinary and small diagnostic laboratories. This test measures antibodies that appear after two weeks or more after primary infection which mean no value for the test immediate infection but have less sensitivity than sabin Feldman dye test or IFAT ***(***Jacobs *et al.*, 1960***)***. IFAT requires intact tachyzoites and is more sensitive and specific compared to IHA and Enzyme-Linked Immuno-sorbent Assay (ELISA) that is used in the diagnosis of ovine toxoplasmosis *(*Jacobs *et al*., 1960; Piergili, 2004***)***. The ELISA for T. *gondii* antibodies has been adapted for use in most domestic animals including sheep and goat ***(***Dubey, 2008; Dubey, 2009***)***. There is

specific ELISA assays for both IgM and IgG subtypes. These ELISA assays are ideally suited to screen large numbers of samples and looking at the IgM/IgG ratio. The IgM/IgG ratio can be used to distinguish between the acute and chronic infections ***(****Denmark and Chessum, 1978****)***. Prenatal diagnosis of congenital toxoplasmosis may be made by detecting specific antitoxoplasma IgM antibodies in fetal blood ***(***Markel*l et al., 1992****)***, but congenital infections may be difficult to diagnose serologically, as maternal IgG crosses the placental barrier and will appear and persist for several months in the circulation of the newborn. Since IgM antibodies do not cross the placenta, demonstration of anti-toxoplasma IgM at birth or up to several months of age is presumptive evidence of congenital toxoplasmosis ***(****Brown and Neva, 1987****)***. The presence of specific antibodies in serum or tissue fluid from stillborn lambs or kids or in precolostral serum from live offspring indicates uterine infection ***(****Buxton, 1998****)***. Serological analysis using IFAT and ELISA has been widely employed in order to detect herds contaminated by toxoplasma, including swine and sheep flocks ***(***Van der Puije *et al.,* 2000***)***.

1.7.4. Molecular diagnosis :

Burg et al. (1989) detected T. *gondii* DNA from a single tachyzoites using the B1 gene by PCR method for the first time. Several subsequent PCR tests have been developed using different gene targets. In general, this technique has been proven as a useful method in diagnosis of clinical toxoplasmosis ***(***Dubey, 2008**)**. The B1 gene referred to as B1 repeat, is a 2214 base pair (bp) sequence with unknown function that is repeated 35 time in the genome of T. gondii **(**Jalal *et al.,* 2004an*d* Edvinsson *et al.,* 2006**)**. The PCR assay targeting the B1gene has been used extensively ***(***Jalal *et al*., 2004***)***. Recently, B1-PCR has been shown as the most sensitive protocol to detect T. *gondii* ***(***Mason *et al., 2010****)***. Although some previous studies have reported the higher sensitivity of PCR targeting AF146527 over that of B1 gene which is usually used for diagnosis of toxoplasmosis, some recent studies suggests that the AF146527 element was absent in 4.8% of human T. gondii-positive samples, which may prove the B1 PCR technique as the choice one (Wahab *et al., 2010and Menotti et al., 2010****)***. More recently, a 200-300-fold repeated (that exists in 200-300 copies/genome) 529 bp element of unknown function has been described in the genome of T. *gondii* **(**Edvinsson *et al., 2006 and Kasper et al., 2009****)***. The higher sensitivity and accuracy of the 529- bp PCR assay even in a faster protocol compared to B1 gene was reported **(**Edvinsson *et al.,* 2006 and Kasper *et al., 2009****)***.

It has been postulated that an increased analytical sensitivity is achieved when a repeated DNA element is amplified, although some studies suggested no difference in analytical performance depending on the number of repeats *(*Wastling *et al., 1993 and* Edvinsson *et al., 2006)*.

1.8. Risk factors:

1.8.1. Age

It has been reported that age can be associated with the sero-prevalence of toxoplasmosis, as older sheep and goats had a higher prevalence of toxoplasmosis infection compared to younger sheep *(*Cavalcante *et al.,* 2008, Ramzan *et al.,* 2009 and Kamani *et.al.*, 2009*).*

1.8.2. Gender:

It has been shown that female sheep and goats are more susceptible than males to toxoplasma infections ***(****Ramzan et al., 2009)*. Although there are other reports did not show significant correlation between toxoplasma infection and the gender of the animals ***(****Caballero-Ortega et al., 2008 and Cavalcante et al., 2008)*.

1.8.3. Animal presence:

The high sero-prevalence of T. gondii antibodies in sheep may be associated with the presence of cats in almost every farm sampled. Newborn kittens are more dangerous than old cats ***(***Dubey, 1994and Buxton and Rodger, 2008***)***. Infected cats excrete toxoplasma oocysts which, after sporulation, become infectious to man and animals and remain infectious for a long period of time ***(***Dubey and Jones, 2008***)***. Also, multivariate analysis showed that the probability of infection was higher in herds where more than 10 cats were present. This might be related to greater environmental contamination by oocysts defecated in cat feces **(**Cavalcante *et al.,* 2008**)**.

1.8.4. Climate:

Higher prevalence rates of toxoplasmosis in warm and moist areas compared to those which are cold and dry is attributed to the longer viability of T. *gondii* oocysts in moist or humid environments ***(***Van der Puije *et al*., 2000***)***. A new study conducted in Mexico ***(****Caballero-Ortega et al., 2008)* revealed that altitude and farm size, affects infection rate, as prevalence was higher at low altitudes and on large farms.

1.8.5. Management system:

In extensive management systems, cats can be attracted to pen where animals are herded. It will also happen in free roaming pastures during the day. This may increase the chance of environment, food and water contamination ***(***Cavalcante *et al*., 2008*).* Sero-prevalence in intensively managed sheep was lower than in semi-intensively managed (Ragozo *et al.*, 2008***)***. A recent study *(*Neto *et al.*, 2008***)*** showed that both extensive/semi-intensive management systems were identified as risk factors associated with toxoplasmosis in goats. Use of wooden feeding troughs was also associated with goat toxoplasmosis. This might be due to fact that oocysts survive longer in moisture. The lack of feeding troughs also increased the probability of infection from pasture or water contaminated with sporulated oocysts ***(***Cavalcante *et al.,* 2008***)***.

1.8.6.Pharmaceuticals :

To reduce economic losses due to toxoplasmosis, chemotherapeutic treatment of infected animals is essential in unvaccinated sheep flocks. Several drugs were used with good results such as decoquinate ***(****Buxton et al., 1996****)***, combination of pyrimethamine and sulfadimidine, vaquilopruim and sulfadimidine or trimethoprim and sulfadimidine ***(****Buxton et al., 1993b****)***. Injecting sulfadimidine in dose 33 mg/Kg/48 h, 4 injections in total, seems to be very effective in controlling toxoplasmic abortions in sheep flocks ***(***Giadinis *et al.*,2009***)***. Moreover, monensin, given in the food during pregnancy, significantly reduced toxoplasma infection in sheep **(***Buxton et al., 1988****)***. Furthermore, clindamycin, spiramycin, atavaquone, arithromycin, clarithromycin and dapsone have been used with various results in non-ruminant species and humans ***(***Giadinis *et al*., 2009***)***.

1.8.7. Prevention and control :

Cats are born free of toxoplasma infection and start to excrete oocysts following a primary infection ***(***Dubey and Jones, 2008***)***. Cat faeces can create a large, potent, long lasting source of infection for sheep. Oocyst contamination of farm foods and bedding, as well as pasture, is a threat to susceptible, pregnant sheep and goats, related to the number and distribution of cats ***(***Dubey, 2008; Dubey *and Jones, 2008****)***

in the environment. It is estimated that at any time given, about 1% of cats shed oocysts (Dubey and Beattie,1988). Persistently infected mice, voles, shrews, rats, rabbits and small birds are the most important sources of cat infections(Jackson and Hutchison, 1989). Cats are considered as the main source of infection for sheep and goats (Dubey and Beattie 1988and Dubey and Jones, 2008). Ingestion of contaminated food and pasture is the most common source of small animal's infection (Dubey, 2004and Dubey and Jones, 2008). Water can be a real threat not only to animals but also to humans (Bowie *et al., 1997)*. Fields treated with manure and bedding from farm buildings where cats live can transmit oocysts and cause infection (Faull *et al.,* 1986). Cats defecating in farm feeds, such as hay and stored grain, will pose a risk for animals (Plant *et al*., 1974). A single defecation may contain millions of oocysts (Lopes *et al.,* 2008). Further processing of the food disperse these oocysts evenly throughout the grain which can infect many sheep in flocks (McColgan *et al.*, 1988). During pregnancy in which the majority of herds are seronegative to *T. gondii*, all food and water should be kept away from cat's faeces and contaminated environment (Dubey, 1991; Hye-Youn Kim *et al*., 2009). Other measures to reduce environmental contamination by oocysts should be aimed to minimize the number of cats capable of shedding oocysts (Dubey and Jones, 2008). These would include limiting the breeding of cats, maintaining healthy adults and attempts to control future breeding, adequate and continuous control programs of stray cats to reduce the risk of transmission of *T. gondii* and not allowing animals to live or stay outdoors, which will prevent them from hunting. Feeding cats with commercial diets or with food processed either by cooking or freezing can reduce the risk of disease transmission. Maintenance of a small healthy population of mature cats will reduce oocysts excretion, besides controlling the rodents population (Buxton and Rodge,r 2008, Lopes *et al.,* 2008and Hye-Youn Kim et al., 2009). In the case of ovine toxoplasmosis, educating farmers to the principle infection root which is contamination of the environment with Toxoplasma oocysts via cat faeces and also measures that reduce the incidence of clinical disease, including good management of food and water, as well as vaccination with the live vaccine *(Toxovax; Intervet B.V.)* will reduce the disease occurrence (Buxton *et al., 2007)*.But further studies are needed to explore whether some sheep breeds have a particular genetic susceptibility to *T. gondii* (Buxton *et al*., 2007).

1.8.8.Vaccination :

Natural infection with *T. gondii* stimulates protective immunity in both sheep and goats ***(***McColgan *et al*., 1988***)*** but inactivated toxoplasma tachyzoites, either alone ***(***Beverley *et al*., 1971**)** or in Freund's incomplete adjuvant **(**Wilkins *et al*., 1987***)*** do not protect pregnant sheep against experimental challenge with the parasite. The failure of these killed preparations in sheep may be partly because, in natural infections, persistence of the parasite in tissues continually stimulates immunity, as suggested in human toxoplasmosis ***(***McHugh *et al*., 1997***)***. However experiments in which mice and hamsters were infected with a live temperature-sensitive mutant of T. *gondii,* which does not persist in the host, showed that it cannot form bradyzoites and cannot therefore form tissue cysts **(**Buxton, 1998**)**. A live vaccine (*Toxovax*) is commercially marketed in the UK, France and New Zealand for reducing losses to the sheep industry from congenital toxoplasmosis ***(***Buxton and Innes,1995***)***. This vaccine was initially developed in New Zealand ***(***Wilkins *et al*., 1988***)***. The vaccine consists of a modified strain (S48) of *T. gondii,* which were originally isolated by mouse injection from a case of ovine abortion in New Zealand. After around 3000 passes twice weekly in laboratory mice, it was shown to lose its ability to develop bradyzoites in tissue cysts. The commercial vaccine consists of live cell culture-grown tachyzoites that have a shelf life of 10 days. It is recommended to be given 3 weeks before mating. One subcutaneous injection of this 2 ml suspension induces protective immunity for at least 18 months ***(***Buxton and Innes, 1995***)***. Abortions were reduced and lambing percentages significantlyimproved, compared to unvaccinated sheep in the same flocks (Spence *et al*., 1992). After subcutaneous inoculation, S48 tachyzoites multiply locally, producing, parastemia and fever .

Tachyzoites are controlled by the host immune response as soon as 10 days post infection and are not detectable by bioassays at 6 month post infection (Buxton et al., 1993b). Vaccinated sheep develop humeral and cellular immunity involving CD4, CD8 T cells, and IFN-*y* ***(***Wastling *et al*., 1993and Wastling et al., 1994***)***. The mechanism of this persistent immunity in the absence of detectable live T. gondii is most intriguing and needs further research. It must be handled with care strictly according to the manufacturer's recommendations. As with sheep, the majority of goats previously exposed to infection with *T. gondii* develop a protective immunity to the parasite so that they are protected against subsequent challenge during pregnancy ***(***Obendorf *et al., 1990)***.The search for a non-infectious vaccine should

continue because of the existing short comings of the live vaccine, its short shelf life and safety margins ***(***Stanley *et al.*, 2004***)***.

Chapter Two

Materials and Methods

2.1 Study area:

The study was carried out in Gedarif state which is lies between 16.4º-14.4º latitude and 33.35º -35º longitude. It is boarded by Sinaar state, Kassala, Khartoum and Elgaziara state and by Ethiopia .

Map of Gedarif State

The dominant climate is semi arid to poor savannah climate and the rainfall ranges between 300 to 800 mm annually. In the autumn season, all animals are oriented through different animal path from Gedarif different localities and neighbouring state to Sahal Elbuttana.

In the 1940th the space of pasture was 86% of the total land but now due to extention of the mechanized agriculture, it was decreased to 6%. The pastures covered by Elseha, Elhantot, Eltabar, elghobash and Elsafari grasses. Gedarif state depends on seasonal rivers, pools and shallow wells as the water sources. Livestock kept under the pastoralist system, include Camels, Cattle, sheep and goats. The famous tribes which are breeding camel are El- lahwyeen (nomads), Bawadra, Dhabina and El shookria.

The total Animal population in Gedarif state is seven million and two hundred sixty thousand and 070 heads **(**Ministry of Animal Resource, 2013***)*** .

Table number different species in Gedarif state

Cattle	Camel	Goat	Sheep	Total
1.587.134	594.756	906.686	4.171.494	7.260.070

2-2 Samples collection:

Three hundred blood samples (represent 122 males &178 females) were collected by jugular vein puncturein sterile tubes without anti-coagulant and labeled samples were kept at−20∘C and stored for further analysis. Serum samples collected from different localaties in gedarif state (100samples from baladeyat El-Gedrif, 90from Elfashaga, 60from El-Glabat EL-shergia and 50 samples from AL-Butana). serum samples were collected from different localities during the period from July to December /2015.

2-3: Laboratory kits:-

2-3-1: Latex agglutination test (LAT):

The serum samples and Toxoplasma antigen **(***Spinreact, S.A./S.A.U., Ctra. Santa Coloma, Spain)* were kept one hour in room temperature before beginning of the test. A total of 50 µl of each serum to be tested was placed on a LAT plate. Then the vial of antigen was shacked gently and 25 µl of antigen was put beside each of the sera. The antigens and the serum were mixed on the plate with a stirrer and spread over the entire circle. Then the plate was rotated manually for 4 minutes and the reading was taken immediately. Any agglutination was considered as positive,

whereas no reaction (negative) was indicated as the absence of Toxoplasma antibody in the sera.

2-3-2: Enzyme-linked immunosorbentassay ELISA:

ELISA uses crude soluble antigens adsorbed onto the walls of microtiter plate wells and the antigen-antibody reaction is enhanced by the addition of a secondary enzyme-linked antibody, and the reaction can be assessed objectively by quantization of the colour that developed by an ELISA reader. *ELISA Technique.* Commercial iELISA kits (Ruminant SerumToxoplasmosis) for detection of anti-*T. gondii* antibodies were purchased from Lsivet (Nouzilly, France). Positive serum samples will present yellow colour; the colour visualized in each well is proportional to the titer of antibody specific to *T. gondii* present in the diluted sample (1/400). All samples which have antibody titer ≥30 are considered positive.

2-4: Statistical Analysis:

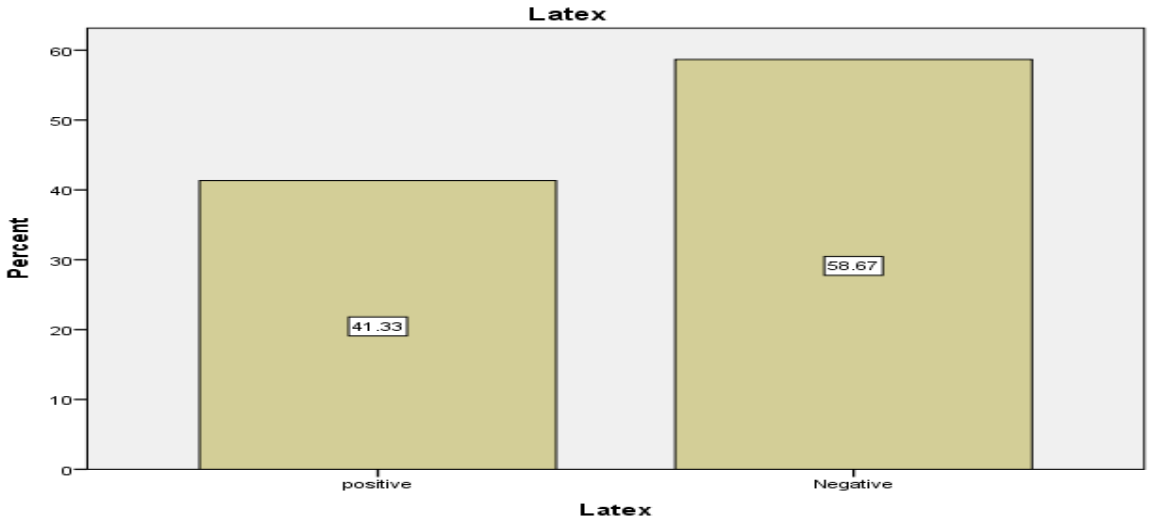
The serological results and other information gathered during this investigation such as location, sex, and age of the sampled animals were edited and analyzed statistically using statistical package(SPSS version 21). To identify the association of the risk factors with the chi-square(χ2 test) and one-way ANOVA were used. The statistical significance level used was $p \leq 0.05$.

CHAPTER THREE

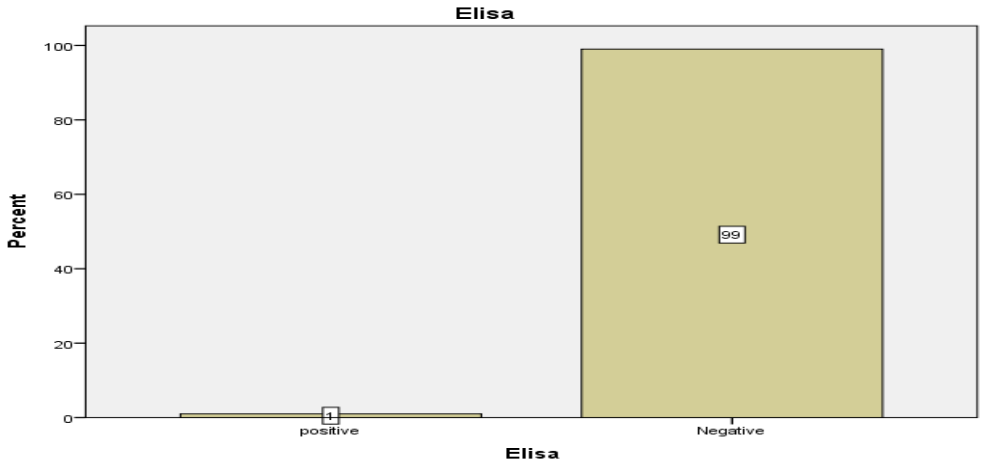
Results

3-1 Seroprevelence of toxoplasmosis using Latex and Elisa tests :

In total 300 cattle sera were tested from different localities in Gedarif state (Baladyat EL-Gedarif, EL-Shwak, EL-Glabat and EL-Butana) using the latex agglutination test and Elisa the positive results was 41.3%, whereas the negative result was 5.7% and in latex and Elisa respectively **Fig (1)**



Fig(1-A) the positive and negative result using latex test



Fig(1-B) the positive and negative result using Elisa test

3.2 Seroprevelance of toxoplasmosis in association with risk factor:

The seroprevelance of toxoplasmosis in association with risk factor was estimated and the result revealed that, high prevelance of disease in El-Gedarif locality(33,3%) fallowed by Fashag(30%) , Glabat EL-shargiea (20%) and finally Butana (16,7%) **Table 1.** The disease show high prevalence in Arshy breed (37%), (23.7%) in Butana breed and (19.7%) in umbrarow and Ethiopain breed **Table2** and **Appendex1**. There was high prevalence of disease in female (59.35%), whereas low in male (40.7%)**Fig2 and Appendex2**. From the view point of age the disease was

had high prevalence in small aged animals 1month- 2 years, 2-4 years, 4-6year and more than6 year 44%, 31%, 23% and 1,7% respectively. **Fig3 and Appendex3**

Table 1: Seroprevelance of toxoplasmosis in association with localities as risk factor:

Locality		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Algadaref	100	33.3	33.3	33.3
	Galabat	60	20.0	20.0	53.3
	Fashaga	90	30.0	30.0	83.3
	Botana	50	16.7	16.7	100.0
	Total	300	100.0	100.0	

3.3 sero-positive of toxoplasma *gondii* in cattle using latex and Elisa test in different localities :

In total 300 sample there was 124 positive and 176 negative using latex test. Elisa test show 3 positive and 297 negative. In El- Gedarif locality there was 34samples positive and 66 negative in 100 samples . El-Glabat El-shargiea was revealed 30 positive and 30 negative in total 60 samples. In 90samples from Fashaga there was 40 positive and 50 negative, in butana locality was 20 positive and 30 negative in total 50 samples. Elisa test show three positive result in each of El-

Gedarif (1), El-Glabat El-shargiea (1) and Fashaga (1) localities **(Fig 4and appendix 5)**

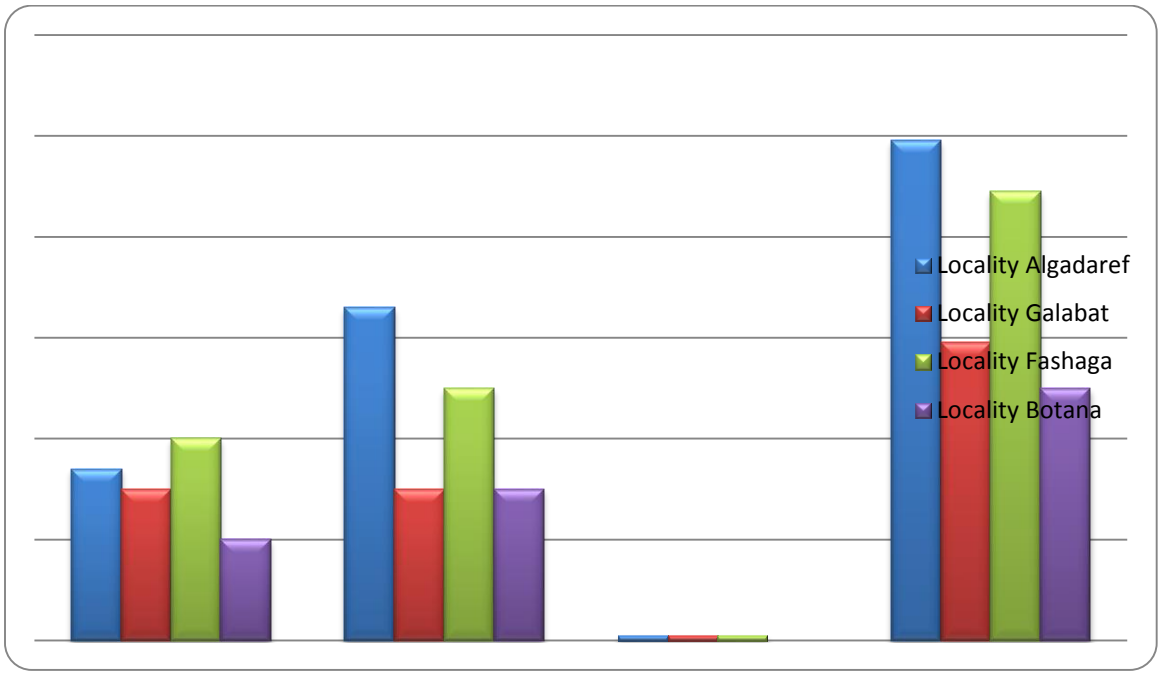


Fig 4: Sero-positive of Toxoplasma *gondii* in cattle using latex and Elisa test in different localities

3.4 Sero-positive of Toxoplasma gondii in cattle using latex and Elisa test in different ages:

The serum sample from cattle aged 1month-2years (132), the positive result was 55 and 1 using latex and Elisa respectively. 2-4years (93) the positive result was 37 4-using latex and 2 using Elisa test.6 year (69) and more than 6 year (5). The positive result was **30 and** 5 respectively using latex test, while Elisa test did not gave a positive result in those age **Fig 5.**

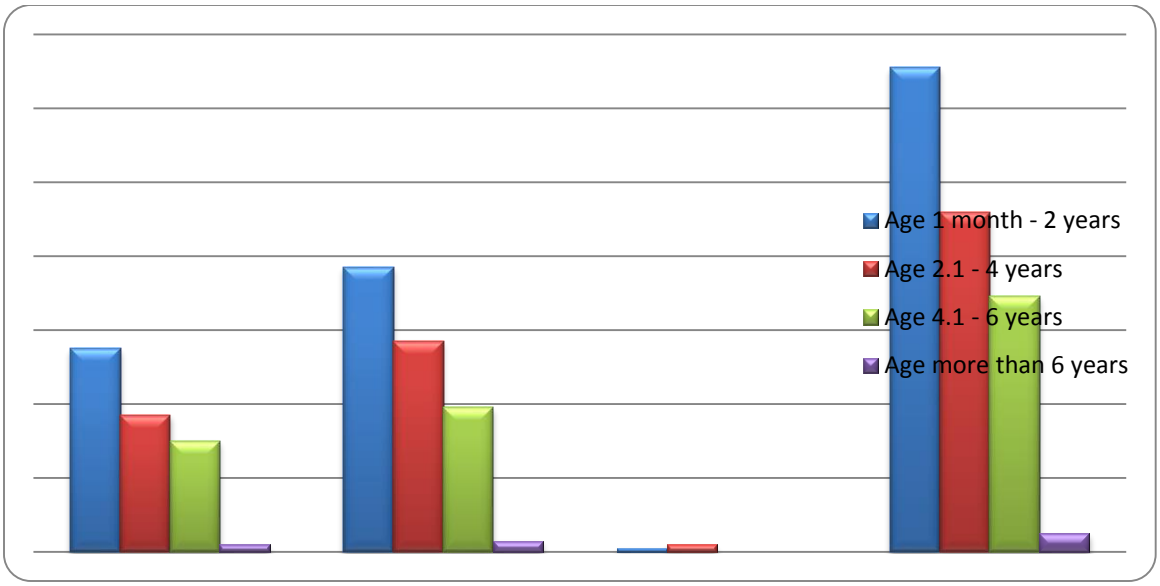


Fig 5: Sero-positive of Toxoplasma *gondii* in cattle using latex and Elisa test in different ages

3.5 Sero-positive of toxoplasma *gondii* in cattle using latex and Elisa test in different breeds :

The positive result of T.*gondii* was follows 31 from Butana breed out of 71 sample. (50/111) in Arshay, (20/59) in umbararow and (23/59) in Ethiopian using latex test . Elisa show 3 positive result 2 in Ethiopian breed and 1 in Butana **(Fig 6and Appendix4).**

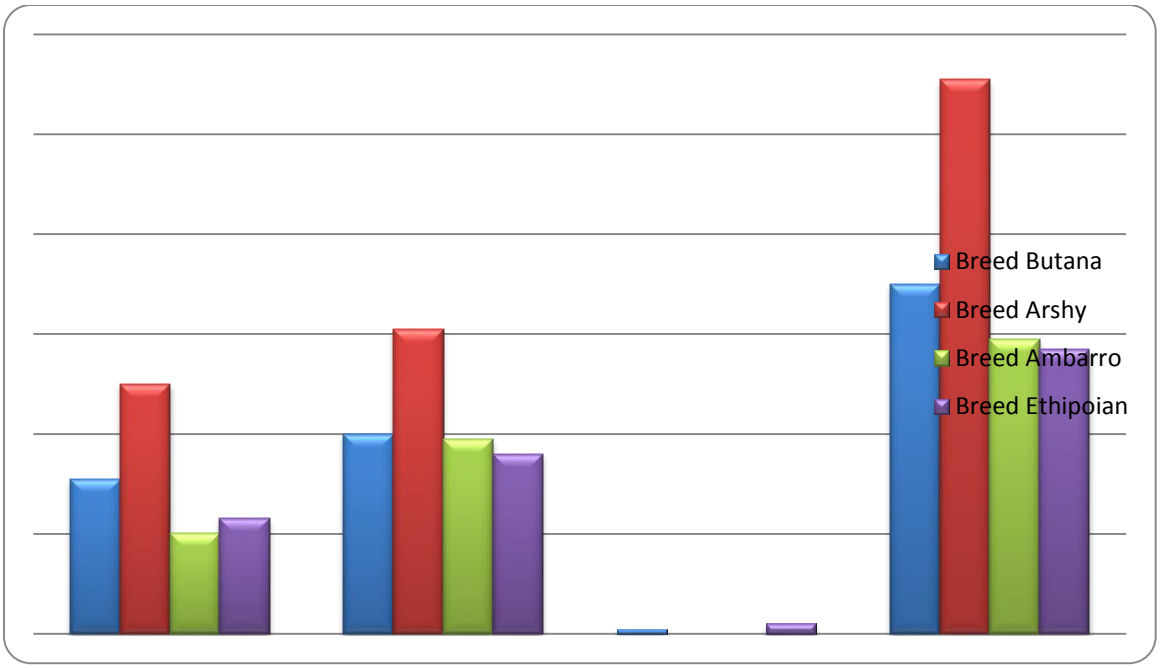


Fig 6: Sero-positive of Toxoplasma *gondii* in cattle using latex and Elisa test in different breeds

3.6 Sero-positive of Toxoplasma *gondii* in cattle using latex and Elisa test in two sexes according to gender :

All serum sample 300, number of females178 and males 122, positive sample was85 in females and in males 39 using latex test , but in Elisa test the positive result was 2 in females and 1 in males. This result revealed there is significant differences ($P<0.05$) between males and females infection (**Fig7 and Appendix 6.)**

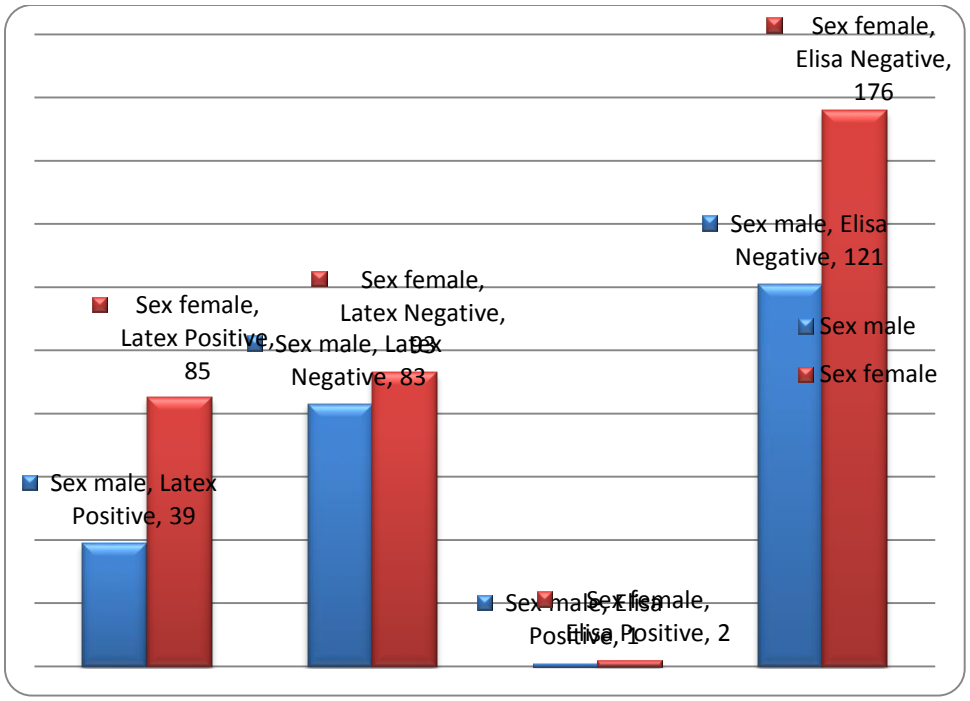


Fig 7 Sero-positive of Toxoplasma *gondii* in cattle using latex and Elisa test in two sexes

CHAPTER FOUR

Discussion

In This study at Gedarif state the estimated seroprevalence of antitoxoplasma antibody using latex test was 41.3% . whereas Afahal *et al* (2014) found infection rate was 50% in Khartoum state and 33% In Gazira State .

Abdalla *et al* (2014) In his study of serological survey of toxoplasma *gondii* in dairy Cattle found the seroprevelance of antitoxoplasma antibody using Elisa test was 89.3% (117/131) but the within herds seroprevelance was ranging from 12% up to 100% and found the overall seroprevalence at individual level is 49.9% (371/774) our result is similar to the finding in Khartoum and Gazera.

From the findings of the present study, cattle could be considered one of the important reservoir, moreover role of cattle in epidemiology of human toxoplasmosis should be considered . it concluded that cattle are more exposed to toxoplasmosis which may result in abortion ,reproductive failure and infertility . in the current study using Elisa estimated seroprevolance was 1 % (3/300) this finding .

Disagree with study that reported 32% (16/50) using LAT (Khalil and Intisar (2011)). In this study frequency of tested samples showed presence of the antibodies on both sexes . with 40.7% (39/122) . in males and 59.3% (85/178) in females ,this findings indicated that the disease could be found in both sexes ,on the other hand in this current study the lowest percentage was in Butana locality 16.7% (20/50) This could be due to climatic difference because Butana is likely desert , on the other hand, in Gedarif locality percentage was 33.3 % (34 /100) because it Gedarif is poor savanna climate .

Abdalghafar *et al., (2013)* in his study at serological seroprevalance of cows with history of reproductive problem found the percentage is 12.7% and 14.9 % and this is higher than reported in china 2.3% but in Iran 15.9%

In this study age was the main risk factor In cow toxoplasmosis, the highest antitoxoplasm antibodies in age range from 1-2 years with 44 % (55 /132) and the lowest is in age 6 years and above 1.7 % (5/30) this could be due to number of

samples which were tested but also in the range (4-6)years was low and it unknown why factors may be involved .

Findings of this study regarding breed risk factor which is first report in gedarif is low in (*Karur*) Ethiopian breed as well as Ambararo breed with 19.7 % and this could be due to nomadic system of these breeds. On the other hand Arshy seroprevalance was the highest with 37 % (50/111) and Butana breed was 23.7% (31/71) ,choose breeds are perhaps settled and they are not exposed to toxoplasma infection.

Conclusion :

It can be concluded that the prevalence of anti-toxopalsma antibodies is relatively high, species and locality were significant risk factors in toxoplasmosis enhancing sero-positivity and there was no between species variation in the sero-prevalence.

Recommendations :

1- Further investigation should be carried out about the epidemiology of the disease in cattle , other species and human .

2- Molecular characterization should be using because the parasite is high importance in the Sudan.

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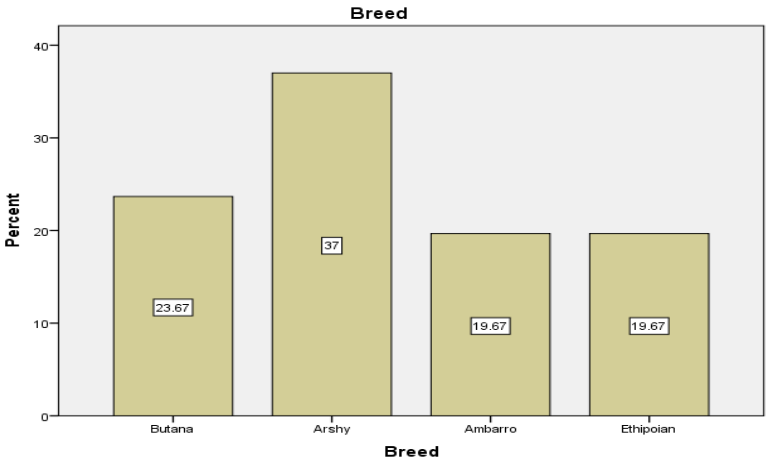
Appendix

Appendix 1
Sreoprevelance of toxoplasmosis in association of risk factor breed



Appendix 2
Sreoprevelance of toxoplasmosis in association of risk factor sex

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	male	122	40.7	40.7	40.7
	female	178	59.3	59.3	100.0
	Total	300	100.0	100.0	

Appendix 3

Sreoprevelance of toxoplasmosis in association of risk factor age

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1 month - 2 years	132	44.0	44.0	44.0
	2.1 - 4 years	94	31.3	31.3	75.3
	4.1 - 6 years	69	23.0	23.0	98.3
	more than 6 years	5	1.7	1.7	100.0
	Total	300	100.0	100.0	

Appendix 4

sero-positive of toxoplasma gondii in cattle using latex and Elisa test in different breed

		Latex		Elisa	
		Positive	Negative	Positive	Negative
Breed	Butana	31	40	1	70
	Arshy	50	61	0	111
	Ambarro	20	39	0	59
	Ethipoian	23	36	2	57
Total		124	176	3	297
Chi-Square (P value)		.519		.155	

Appendix 5

sero-positive of toxoplasma gondii in cattle using latex and Elisa test in different localities

		Latex		Elisa	
		Positive	Negative	Positive	Negative
Locality	Algadaref	34	66	1	99
	Galabat	30	30	1	59
	Fashaga	40	50	1	89
	Botana	20	30	0	50
Total		124	176	3	297
Chi-Square (P value)		.215		.853	

Appendix 6

sero-positive of toxoplasma gondii in cattle using latex and Elisa test in different sex

		Latex		Elisa	
		Positive	Negative	Positive	Negative
Sex	Male	39	83	1	121
	female	85	93	2	176
Total		124	176	3	297
Chi-Square (P value)		.004<0.05 significante		.639	

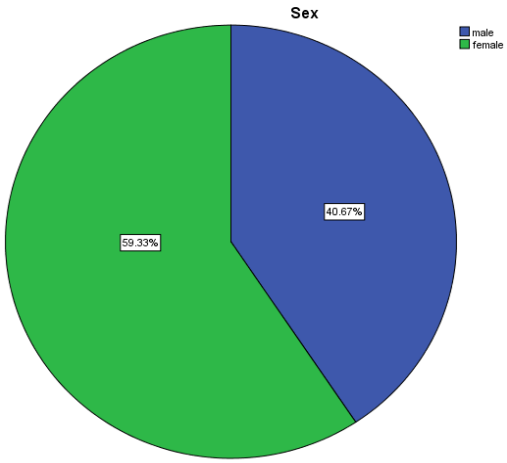
Appendix 7

Sex * Latex Crosstabulation

			Latex		Total
			positive	Negative	
Sex	male	Count	39	83	122
		% within Sex	32.0%	68.0%	100.0%
		% within Latex	31.5%	47.2%	40.7%
	female	Count	85	93	178
		% within Sex	47.8%	52.2%	100.0%
		% within Latex	68.5%	52.8%	59.3%
Total		Count	124	176	300
		% within Sex	41.3%	58.7%	100.0%
		% within Latex	100.0%	100.0%	100.0%

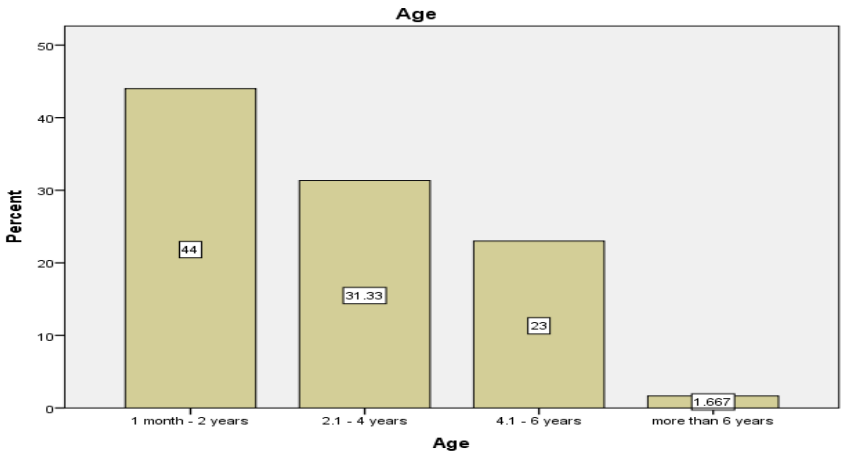
Appendix 8

Seroprevelence of toxoplasmosis using Latex and elisa tests



Appendix 9

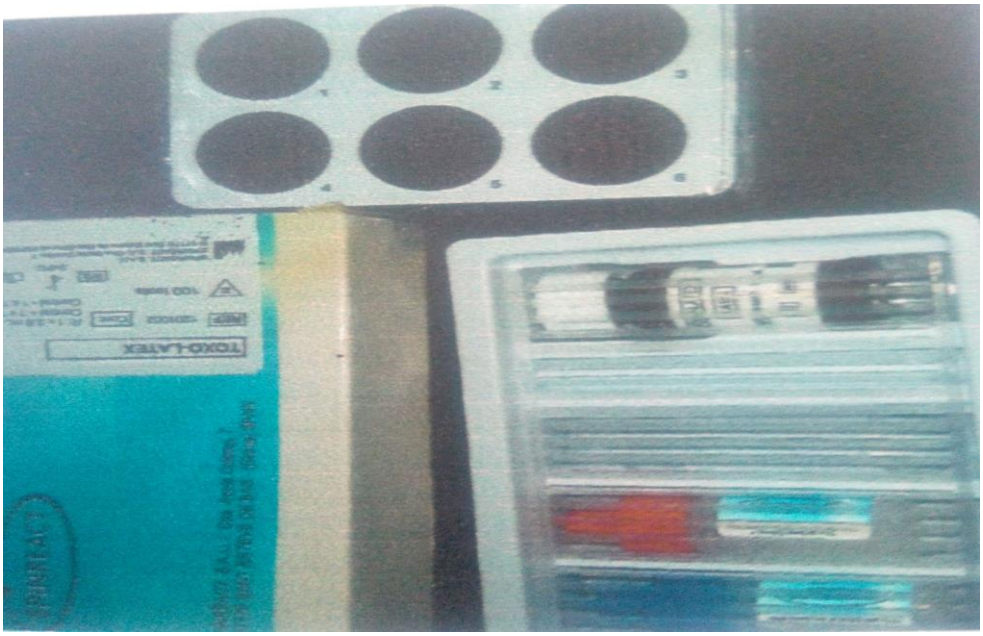
Seroprevelance of toxoplasmosis in association with risk factor



Appendix 10

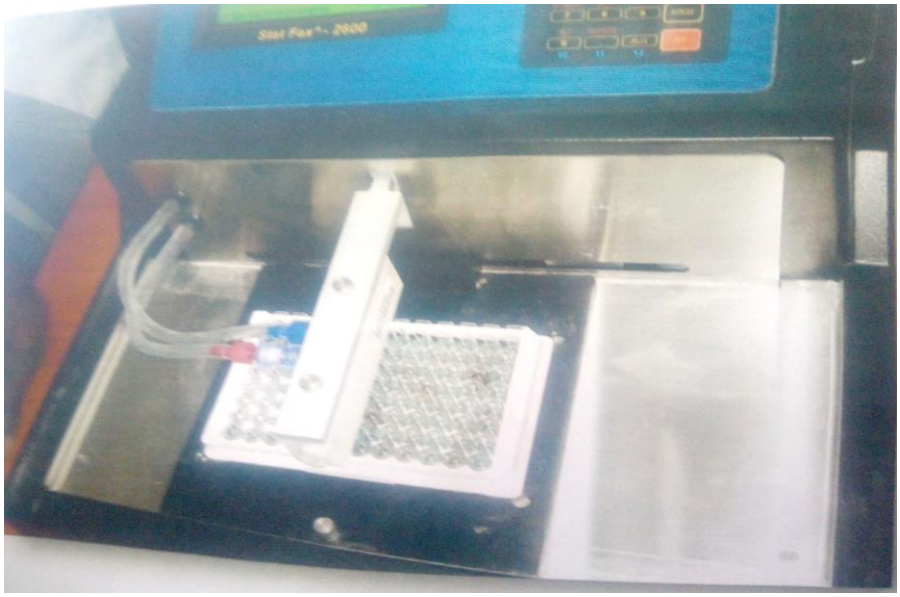
Samples collection

Appendix 12



Latex agglutination test

Appendix 10



Enzyme – linked immunosrbent assay(ELISA)