

بسم الله الرحمن الرحيم

**Sudan University of Science and Technology**  
**College of Graduate Studies**

**Sero-prevalence of Toxoplasmosis in Sheep and Goats in El-Gadarif State**

الانتشار المصلي لمرض المقوسات للضأن والماعز

بولاية القضارف

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**A thesis submitted to the College of Graduate Studies in the fulfillment of the requirements for the Master degree in Preventive Medicine**

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**Khartoum North, June 2017**

## بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

وَكَذَلِكَ أَنْزَلْنَاهُ قُرْآنًا عَرَبِيًّا وَصَرَّفْنَا فِيهِ مِنَ الْوَعِيدِ لَعَلَّهُمْ يَتَّقُونَ أَوْ  
يُحَدِّثُ لَهُمْ ذِكْرًا ﴿١١٣﴾ فَتَعَالَى اللَّهُ الْمَلِكُ الْحَقُّ وَلَا تَعْجَلْ  
بِالْقُرْآنِ مِنْ قَبْلِ أَنْ يُقْضَى إِلَيْكَ وَحْيُهُ وَقُلْ رَبِّ زِدْنِي عِلْمًا ﴿١١٤﴾

صدق الله العظيم

سورة طه

Dedication

To my parents, my dear wife, my children,  
and my colleagues

Hamadnalla

## **Acknowledgments**

I would like to express my gratitude to my supervisor Prof. Dr. Mohamed Abdelsalam Abdalla for his patience, supervision, guidance and support.

Also I would like to express my sincere thanks and grateful acknowledgement to my co supervisor Prof. salah Hassan Idreis for his assistance and helpful advice.

I also want to express my warm thanks and appreciation to Dr. Yassir Adam Shuaib for the assistance and technical support he offered throughout the work.

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## Abstract

Across-sectional study was conducted from July to November 2015 to estimate the prevalence of anti-toxoplasma antibodies in sheep and goats in El-Gadarif state.

Total of 400 serum samples; 200 sheep and 200 goats, were collected and tested by Toxo-latex agglutination test and iELISA.

The overall sero-prevalence was 52.0% (208/400) using Toxo-latex agglutination test. Using iELISA, 45.7% (42/92) of the sheep and 27.2% (25/92), of the goats were sero-positives. The sero-prevalences among the two investigated species and the two age groups were statistically not different, however, statistically different between localities, different breeds, and between males and females. In the univariate analysis, species ( $p$ -value=0.028) and locality ( $p$ -value=0.001) were associated with Toxo-latex agglutination test positive status. Furthermore, species (sheep) and locality (Al-Fao, Al-Hawatah, and Wasat El-Gadarif) had increased odds of being Toxo-latex agglutination test positive in the multivariate analysis.

It can be concluded that the prevalence of anti-toxoplasma antibodies is relatively high and there was no between-species variation in sero-positivity. These findings warrant further investigations in El-Gadarif and to evaluate burden of the disease and the existed risk to human beings.

## ملخص الدراسة

أُجريت هذه الدراسة خلال الفترة من يوليو الي نوفمبر 2015 لتحديد الإنتشار المصلي للمقوسة القوندية في قطعان الضان والماعز بولاية القضارف.

جمعت 400 عينة (200) عينة من الضان و(200) عينة من الماعز واستخدم إختبار يتراص اللاتكس(LAT)والمقايسة المناعية المرتبطة بالإنزيم غير المباشرة (iELISA) في تحديد الإنتشار المصلي للمقوسة القوندية.

كان الإنتشار المصلي على مستوى النوعين 52% (400/208) باستخدام إختبار يتراص اللاتكس(LAT).معدلا لأجسام المضادة للمقوسة القوندية بواسطة اختبار الاليزا في الضان 45.7% (42/92) وكان المعدل 27.2% (25/92) بالنسبة للماعز. واطهرت النتائج انه ليس هنالك اختلاف معنوي بين النوعين والاعمار المختلفة ولكنها اوضحت ان هناك فروقات معنوية بين المحليات والانواع والذكور والاناث. وكانت الفروقات المعنوية بين الجنسين (p-value=0.028) بينما كانت بين المحليات (p-value=0.001) وكانت النتائج ايجابية بفحص الاتكس. والمحليات هي (الفاو الحوامة ومحلية وسط القضارف)

يستنتج من هذه الدراسة المسحية وجود إصابة واسعة لل *Toxoplasma gondii* عند الضان والماعز علي حد سواء وحيث اوضحت الدراسة انه ليس هنالك اختلافات معنوية بين النوعين في الإصابة. وهذا يتطلب إجراء مسوحات لدا التكسوبلازما بمنطقة القضارف للحيلولة دون اصابة الانسان.

## INTRODUCTION

Toxoplasmosis is an anthrozoonic disease caused by infection with the obligate intracellular parasite *Toxoplasma gondii* and is among the global major health problems (Sarciron and Gherardi, 2000; Kasper, 2005; Petersen et al., 2010; Torgerson and Macpherson, 2011). From medical and veterinary point of view, toxoplasmosis is one of the most important prevalent parasitic diseases. It causes a variety of disease syndromes in humans, ranging from flu-like symptoms in immunocompetent adults, to severe disseminated disease in immunosuppressed individuals, to birth defects in infants when women get exposed during pregnancy (Radostits et al., 2007; Dubey, 2010; Innes, 2010a). The economic burden of toxoplasmosis in animals is because it results in reproductive failure, embryonic death and resorption, fetal death and mummification, abortion, stillbirths, and neonatal death in small ruminants (Marquardt et al., 2000; Dubey, 2009; Dubey, 2010). The severity of infection in sheep is associated with the stage of gestation at which the ewe becomes infected; the earlier in gestation, the more severe the consequences (Dubey, 2009).

In the Sudan, the disease in animals has been reported by a number of researchers in varying prevalence's according to the species of animals investigated and the geographical area (Khalil and Elrayah, 2011; Elfahal et al., 2014). While in humans, the prevalence of toxoplasmosis can go as high as 50.0% (Tamomh et al., 2016). However, only a limited number of these studies took into consideration risk factors that are most important for infection with this parasite (Hammond-Aryee et al., 2014). Besides, there has been an increasing interest in recent years in the prevalence of *T. gondii* infection in small ruminants because of their role on the dissemination of the

protozoan to man through direct contact or by consuming products of animal origin (Cenci-Goga et al., 2013).

**Objective:**

1. Estimating the prevalence of anti-toxoplasma antibodies in sheep and goats in El-Gadarif state.
2. To Investigate individual animal risk factors.

## CHAPTER ONE

### LITERATURE REVIEW

#### 1. Definition and description of disease

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.

## **2. Identification of the agent**

*Toxoplasma gondii* is an obligate intracellular parasite that has a sexual cycle in felidae and a two – stage asexual cycle in all warm –blooded animals. It predominantly comprises three clonal lineages (I, II and III). In the acute phase of infection, tachyzoites multiply in cells to cause varying degrees of tissue destruction and, in fatal cases; tachyzoites may be demonstrated in ascitic fluid or in lung impression smears. With the onset of an immune response, tachyzoites are transformed into bradyzoites that multiply slowly in cells to produce tissue cysts. In aborting sheep, goats and pigs, *T. gondii* is often difficult to find in tissue sections, but is more likely to be seen in sections of brain and placenta. Its identify can be confirmed by immunohistochemistry, while the polymerase chain reaction may be used to identify parasite DNA in tissues. Isolation of *T.gondii* from samples is expensive and slow but, if required, is best achieved by inoculation of mice with tissue homogenate derived from fetal brain or placenta. The sexual life – cycle of the parasite takes place exclusively in epithelial cells of the feline intestine and can result in the excretion of large numbers of oocysts in the faeces. Oocysts may remain viable in the environment for many months.

## **3. Sub-clinical congenital transmission**

The rate of sub-clinical congenital transmission has not been documented because only a few animals are tested once a diagnosis has been established. An example where sub-clinical congenital transmission of *T. gondii* was documented in sheep is provided in the paper of Dubey and Kirkbride (1989b) . In this study, a flock of 80 Hampshire ewes were pastured in 1987 on a farm in South Dakota, USA. The 80 ewes produced 144 lambs of which 30 were stillborn. Toxoplasmosis was confirmed in 11 of 30 aborted

lambs based on fetal serology and immune-histochemical examination. *T. gondii* antibodies (MAT) were found in 68 of 114 (40.3%) surviving lambs 3-4 months after birth; 2 had titers of 1:64, 1 of 1:256, 12 of 1:1024, and 53 had titers of 1:4096. These high titers are indicative of an immune response initiated by an active infection and not due to passive immunity acquired through suckling of colostrum. Eight lambs, with MAT titers of 1:1024 or higher, were slaughtered when they were seven month old. *T. gondii* was isolated from the hearts of three lambs, the tongues of seven lambs, the leg muscles of eight lambs, and from the chops of seven lambs using a bioassay in mice with 100 g samples of each tissue.(Dubey and Kirkbride, 1989b). Serological examination of the ewes 2-3 weeks after lambing revealed that 56 of 80 ewes had MAT titers of 1:64 or more, indicating a high rate of infection in the ewes.

#### **4. Prenatal, post-natal, and repeat transmission of *T. gondii***

Until recently, the prevailing view was that 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 ( Dubey and Beattie, 1988; Buxton et al., 2006, 2007 ) . Evidence for these conclusions is based on three older studies (Hartley, 1961; Watson and Beverley,1971; Munday, 1972) and one recent study (Rodger et al.,2006). Studies reported by Hartley (1961) and Watson and Beverley (1971) studied experimentally infected ewes. Of 38 ewes infected with *T. gondii* during a previous pregnancy, all but one gave birth to uninfected lambs; *T. gondii* was isolated from only one placenta ( Hartley, 1961). Of 26 ewes inoculated with *T. gondii* during a previous pregnancy, 24 had uninfected, live lambs; one aborted twins

and one was barren. *T. gondii* was isolated from the brain of the aborted lamb ( Watson and Beverley, 1971). Of 178 lambs born to 135 persistently naturally infected ewes, none had precolostral *T. gondii* antibodies; the placenta of one was infected with *T. gondii*( Munday, 1972). Recent studies by a group of researchers from Scotland(Buxton et al., 2006; Rodger et al., 2006 ) supported these findings that congenital transmission of *T. gondii* from ewes persistently infected with the parasites is infrequent. Their observations were based on a flock of 46 Scottish black ewes; 31 of these were seropositive and 15 were seronegative for *T. gondii*( Buxton et al., 2006; Rodger et al.,2006 ). Progeny of these ewes and placental tissue were tested using histopathology, PCR, precolostral lamb serology, along with clinical outcome to determine the presence of *T. gondii* . The seropositive ewes delivered 43live and six dead lambs, but none of the lambs were infected with *T. gondii* based on histopathology, DNA analysis, or the presence of *T. gondii* antigen, and/or intact tachyzoites in immune-histochemical analysis of tissues. Antibodies were not found by IFA in fetal fluids from the dead lambs or in precolostral sera from all but two live twin lambs using western blot. Thus at the most, only one of the 31 (3.2%) naturally infected ewes had transmitted the infection transplacentally. The seronegative ewes produced 24 live uninfected lambs. In conclusion, all 4 studies discussed above reached the same conclusion that congenital transmission of *T. gondii* from ewes persistently infected with the parasite, may occur, but is very infrequent. Recently, a series of papers was published from a group of researchers in England ( Duncanson et al., 2001; Morley et al., 2005; Williams et al., 2005; Morley et al., 2008). These authors proposed that repeat transplacental transmission of *T. gondii* in sheep maybe more common than previously believed. However, all the evidence they presented was based on the detection of *T. gondii* DNA by PCR. In the first paper,



placental and fetal tissues from a flock of 88 Suffolk cross sheep in Worcestershire were tested for *T. gondii* DNA. They detected DNA in the placenta of 37 of 70 sheep birthing live lambs, indicating a 42% congenital transmission. *T. gondii* DNA was detected in 17 of 18 placental and fetal tissues; from the brains of 15, and hearts of 14 (Duncanson et al., 2001 ). Similar findings were reported in a study of two pedigree Charollais sheep flocks in Cheshire and a Suffolk flock Worcestershire (presumably the same flock studied by Duncanson et al., 2001). In these three flocks 4.5–18.9% of lambs aborted with a 91% *T. gondii* infectivity rate based on PCR (Williams et al., 2005). More interestingly, 65% of live lambs had evidence of *T. gondii* DNA based on placental or cord samples. These three flocks were geographically separated. The higher abortion rate observed in the Charollais as compared with the Suffolk flock raises the important question of breed susceptibility ( Williams et al., 2005 ), although abortion may be caused by several other agents eg: chlamydomyces abortus , so it is important to do a differential diagnosis on abortion material submitted for examination. In the third paper, abortion and *T. gondii* infection were associated with different families of Charollais sheep within a flock( Morley et al., 2005). In this study, abortion data in 765 ewes from 27 families in one flock were analyzed. The abortion rate varied from 0-100%. In total, tissues of 155 aborted lambs were tested for *T. gondii* DNA. The frequency of *T. gondii* positive lambs also varied from 0 to 100%(Morley et al., 2005), however again the group did not look for the presence of other abortifacient agents in this study. In a fourth paper (Morley et al., 2008), further observations were conducted on the Charollais sheep flock reported on by Morley et al. (2005) . In this study, 29 ewes were selected based on whether they had two or more lambings during the 2000–2003 seasons. Of these 29 ewes, nine (31%) produced *T. gondii* -

positive progeny over two successive lambings. Of the 35 lambs from these nine ewes, 12 were born alive and 22 were aborted or mummified; more importantly 33 of the 35 lambs were PCR-positive (Morley et al., 2008). A major shortcoming of these studies is that their conclusions are based solely on *T. gondii* DNA detection; they have not demonstrated *T. gondii*-associated lesions using conventional histopathology which would have helped to establish a cause-effect relationship nor do they look for the presence of other abortifacient agents.

## **5. Human health risks**

*Toxoplasma gondii* readily infects human beings and while infection is relatively common (approximately 30% of the population depending on age and environment), clinical illness is relatively uncommon. Those particularly at risk of developing clinical illness include pregnant women, as the parasite can pose a serious threat to the unborn child if the mother becomes infected for the first time while pregnant, and individuals who are immune suppressed, such as tissue transplant patients, AIDS patients, patients with certain types of cancer and those undergoing certain forms of cancer therapy. These individuals are at risk of developing acute lethal infection if left untreated. The very young and very old may also be more susceptible. On occasions, people with no apparent immune deficiency may develop an illness characterized by general malaise, fever and lymphadenopathy. The most likely sources of human infection are ingestion of raw or lightly cooked meat containing live *T. gondii* tissue cysts, ingestion of raw or lightly cooked vegetables contaminated with oocysts or exposure to oocysts derived from cat faeces, such as may be encountered in gardens and children's sand pits. Toxoplasmosis is now also recognized to be a water-borne zoonosis (10). This

method of transmission occurs where water treatment is ineffective or non-existent and there is a sizeable local felid population that contaminates surface water with oocysts (1, 10). Linked to this there is now also an appreciation that sea mammals are becoming infected by waters from contaminated land and from untreated urban sewage (15).

## **6. Diagnostic Techniques**

### **6.1. Identification of the agent**

#### **6.1.1. Isolation**

Isolation of *T. gondii* from aborted ovine and caprine fetuses and fetal membranes is best made by inoculation of laboratory mice. The best tissues for inoculation are fetal brain and placental cotyledons, and optimum results are obtained with fresh samples free from contamination. Samples must not be frozen at any stage, as this kills the parasite:

- i) With aseptic precautions, remove 2–5 g of placental cotyledon or brain tissue from the aborted fetus.
- ii) Homogenize the tissue in an equal volume of 0.3 M sterile phosphate buffered saline (PBS), pH 7.4, with added antibiotics (100 International Units [IU]/ml penicillin and 745 IU/ml streptomycin) in a ‘stomacher’ (Seward Laboratory, London) or other suitable macerating equipment. Brain tissue may be effectively homogenized by passing it through a 16-gauge needle ten times by means of a syringe.
- iii) Inoculate each of three Toxoplasma-free mice intra-peritoneally with 0.5 ml of the homogenate.
- iv) Kill the mice 6–8 weeks after inoculation and remove the brains. Blood should also be recovered from the mice at this stage and the serum separated

and stored at  $-20^{\circ}\text{C}$ . Brains from mice that die before 6–8 weeks should also be harvested.

v) Homogenize each mouse brain with an equal volume of sterile PBS by passing through a 16-gauge needle ten times by means of a syringe.

vi) Spread one drop (5  $\mu\text{l}$ ) of a given suspension on each of five slides.

vii) Dry and stain with Giemsa, dehydrate and mount under a cover-slip.

viii) Examine slides under a microscope. Tissue cysts appear as circular structures measuring 5–50  $\mu\text{m}$  filled with blue-staining, crescent-shaped bradyzoites. An alternative method for examining the mouse brain is to take a small portion of forebrain (approximately match-head size) squashed flat with a cover-slip. Tissue cysts should be easily detected under the microscope. If the tissues inoculated are heavily infected with *T. gondii*, mice may die at 1–2 weeks. Failure to demonstrate tissue cysts does not rule out positive diagnosis. Serum from the mice may be analyzed for the presence of antibodies to *Toxoplasma* (e.g. using an indirect fluorescent antibody [IFA] test); if this analysis is also negative, infection with *Toxoplasma* is unlikely.

### ***6.1.2. Tissue sections***

In animals that die with acute toxoplasmosis, focal mononuclear inflammation with or without focal necrosis may be seen in a number of tissues, including the liver, heart and lungs. The latter may be edematous. Lymph nodes may have undergone expansion and there may or may not be focal necrosis with or without hemorrhage. Typically *Toxoplasma* tachyzoites may be demonstrable in association with necrosis and inflammation. In cases of abortion and stillbirth in sheep and goats, affected placental cotyledons typically contain large foci of coagulative necrosis that may have become mineralized with

time. Any associated inflammation is characteristically slight and non-suppurative. Well preserved samples of placental cotyledons may show moderate oedema of the mesenchyme of the fetal villi with a diffuse hypercellularity due to the presence of large mononuclear cells. Sometimes small numbers of intracellular and extracellular toxoplasms are visible, usually on the periphery of a necrotic area or in a villus that is in the early stages of infection. The *Toxoplasma* tachyzoites appear ovoid, 2–6 µm long, with nuclei that are moderately basophilic and located centrally or towards the posterior end. In the fetal brain primary and secondary lesions may develop. Microglial foci, typically with a necrotic and sometimes mineralized centre and often associated with a mild focal lymphoid meningitis, represent a fetal immune response following direct damage by local parasite multiplication. *Toxoplasma* tissue cysts are only rarely found, usually at the periphery of these lesions. Focal leukomalacia is also common and is considered to be due to fetal anoxia in late gestation caused by advanced lesions in the placentome preventing sufficient oxygen transfer from mother to fetus. Such foci occur most commonly in the cerebral white matter cores, but sometimes also in the cerebellar white matter. Focal leukomalacia on its own suggests placental disease or acute insufficiency but the two types of neuro-pathological change seen together are characteristic of *Toxoplasma* infection. Confirmation of the identity of *T. gondii*-like structures in tissue sections from such cases, as well as from instances of acute toxoplasmosis, may be achieved by immunohistochemistry that labels intact *T. gondii* or antigenic debris. The method is both convenient and sensitive and is used with fixed tissues (including archive tissues) that may also exhibit a degree of decomposition, where isolation would not be appropriate or possible. The ABC indirect

immunoperoxidase method and the peroxidase–antiperoxidase (PAP) technique (34) are equally good.

### **6.1.3. Nucleic acid recognition methods**

Several polymerase chain reaction (PCR)-based assays have been developed for the detection of DNA from *T. gondii*. The main target regions are the B1 repetitive sequence (3), the P30 (SAG1) gene (31) and 18S ribosomal RNA (rRNA) (14). The sensitivity of the PCR is dependent on the copy number of the target sequence (P30: 1 copy; B1: 35 copies; rRNA: 110 repeat units). Customised synthetic DNA oligonucleotides are commercially available (e.g. [www.sigma-genosys.co.uk](http://www.sigma-genosys.co.uk)). Recently, the method for amplification of the B1 repetitive sequence has been used to analyse the lens aspirates of congenitally infected human cataract patients (25) and was found to be more sensitive than the conventional method used (enzyme-linked immunosorbent assay [ELISA]). However, although the PCR is extremely sensitive, care should be taken if it is the only test available, as in many situations a more reliable diagnosis will be gained if it is used in combination with other diagnostic data. Recently, a real-time PCR has been developed to allow simultaneous quantification and amplification of DNA. It is very similar to existing PCR methods and can be carried out on 96-well microtitre plates. After each round of amplification, fluorogenic dyes intercalate with the double-stranded DNA and the results, shown on an amplification plot, allow quantification of the parasite DNA in the sample. Real-time PCR has been used to amplify and quantify DNA from the *T. gondii* B1 gene (7, 23). This quantification of parasite DNA can be used to determine the number of parasites in tissues and fluids, such as the amniotic fluid of patients suspected

of being congenitally infected with *T. gondii*. (27). The real-time PCR is a highly sensitive and specific method, however it is expensive and requires specialized detection systems and therefore may only be cost-effective in laboratories where analysis of large numbers of samples is carried out. The following method is a nested form of the PCR, amplifying the B1 repetitive sequence of DNA (36).

Parasite DNA can be extracted and purified from several tissues, including placenta, the central nervous system, heart and skeletal muscle. Contaminating red blood cells in tissues are removed by washing in 10 mM Tris/NH<sub>4</sub>Cl lysis buffer, pH 7.6, followed by centrifugation at 2000 g for 15 minutes. DNA is then extracted from the resultant pellet and resuspended in 10 mM Tris, pH 8.3, 50 mM KCl, 1.5 mM MgCl<sub>2</sub> containing proteinase K 100 µg/ml and 0.5% Tween 20. Samples are incubated at 55°C for at least 1 hour, then the proteinase K is inactivated by boiling. The PCR procedure is performed in 50 µl volumes. Amplification of the B1 gene is performed by modifying the procedure described in ref. 1. The reaction mixture contains 10 mM Tris, pH 8.3, 2.5 mM MgCl<sub>2</sub>, 40 mM KCl, 0.01% gelatin, 0.1 mM dNTPs, 0.2 µM of each primer (oligonucleotide primers are those described in ref. 1), two sense primers P1 and P2 and two antisense primers P3 and P4) and 2.5 units of Taq polymerase. Primary amplification is performed with primers 1 and 4 to give a 193 bp product over 25 cycles of 93°C for 1 minute, 50°C for 1.5 minutes and 72°C for 3 minutes. The amplified product is then diluted 1/20 in distilled water to reduce amplification of non-specific products. Secondary amplification using nested primers 2 and 3 and the same reaction conditions, is carried out over 15 cycles to give a 94 bp product. The final product is then visualised on 1% agarose gels. Southern blotting, using a

labelled probe, can be used to confirm the identity of the B1 PCR products and to distinguish them from non-specific products.

#### **6.1.4. Oocyst detection in drinking water**

*Toxoplasma gondii* oocysts have been detected in drinking water using the method for the detection of *Cryptosporidium* oocysts . The method relies on the collection of a large-volume sample of water and passing it through a cartridge filter. Identification of *Toxoplasma* oocysts was by means of inoculation of rodents.

### **6.2. Serological tests of Toxoplasmosis**

There are several serological tests available for the detection of *T. gondii* antibodies. In one type of test the observer judges the given colour of tachyzoites under a microscope, such as with the dye test (DT) and IFA test. Another depends on the principle of agglutination of *Toxoplasma* tachyzoites, red blood cells or latex particles, as with the direct agglutination test (DAT) and indirect haemagglutination test (IHA) and latex agglutination (LA) test, respectively. With the ELISA, the degree of colour change defines the quantity of specific antibody in a given solution. The DT, IFA test, DAT and ELISA are outlined below and the IFA test is given in more detail. The DT is the so-called ‘gold standard’ serological test for *Toxoplasma* antibody in humans. Live *Toxoplasma* tachyzoites are incubated with a complement-like accessory factor and the test serum at 37°C for 1 hour before methylene blue is added. Specific antibody induces membrane permeability in the parasite so that the cytoplasm is able to leak out and the tachyzoite does not incorporate the dye and so appears colourless. Tachyzoites not exposed to specific antibody (i.e. a negative serum sample) take up the dye and appear blue. The



DT is both specific and sensitive in humans, but may be unreliable in other species. In addition, it is potentially hazardous as live parasite is used. It is expensive and requires a high degree of technical expertise. It should be noted that on animal welfare grounds, tachyzoites should be grown in tissue culture rather than in mouse peritoneum whenever possible. The IFA test (26) is a simple and widely used method. Whole, killed *Toxoplasma* tachyzoites are incubated with diluted test serum, the appropriate fluorescent antispecies serum is added, and the result is then viewed with a fluorescence microscope. Fluorescent-labelled antibodies are available commercially for a variety of animal species, the method is relatively inexpensive and kits are commercially available. However, the method requires a fluorescence microscope and the results are read by eye, so individual variation may occur. It may be difficult to find some species-specific conjugates and there is a risk of possible cross-reactivity with rheumatoid factor and anti-nuclear antibodies. The DAT is both sensitive and specific. Formalinised *Toxoplasma* tachyzoites are added to U-shaped well microtitre plates and dilutions of test sera are then applied. Positive samples will produce agglutination that can be graded, while negative samples will produce a 'button' of precipitated tachyzoites at the bottom of the well. The test is simple and easy to perform although relatively large amounts of antigen are required. Kits are commercially available. The method of growth and harvesting of parasites is given below. A commercially available latex agglutination test (LAT) is also available. The DAT and LAT are not species specific and are suitable for use in all species. The original ELISA uses a soluble antigen preparation made from *Toxoplasma* RH strain tachyzoites (as described below) and layered into wells in a microtitre plate. Test sera (e.g. ovine in origin) are added, followed by an anti-species enzyme-labelled conjugate such as horseradish peroxidase-labelled anti-ovine-IgG.

Any attached conjugate causes a colour change in the substrate that is directly related to the amount of bound antibody, and which can be read with a spectrophotometer at the absorbance specific to the substrate used. The assay is simple, can readily test a large number of samples and is easy to perform with the chosen anti-species conjugate. Defined anti-species conjugates, substrates and whole kits are commercially available. However, the assay does require a spectrophotometer. The ELISA is well suited to laboratories required to analyze large numbers of samples. Recently, a kinetics ELISA (KELA) has been developed. The KELA system measures the rate of reaction between bound enzyme and the substrate solution that leads to development of colour. Three optical densities (OD) are read at 45-second intervals (using the KELA data management program) and the results are reported in terms of slopes. The correlation between the ELISA and the KELA is very high, and therefore, the two tests are very good diagnostic tools, differing only in their convenience of application. To improve the specificity of the conventional ELISA, assays that use recombinant antigens and affinity purified *Toxoplasma*-specific antigens have been developed for use in sheep but these tests are not yet routinely used.

With the conventional ELISA the detection of *Toxoplasma*-specific IgG and IgM antibodies allows a degree of discrimination between acute and chronic toxoplasmosis. More recently avidity assays have been developed. As the immune response matures, after infection is established, so antibodies of increasing avidity (functional affinity) for the antigen develop. This avidity can be measured and used to indicate active or recent *T. gondii* infection. An assay for the detection of avidity of IgG for the P30 antigen of *T. gondii* in sheep has been developed. This test is a good diagnostic tool for discriminating relatively recent from more established infections.

Toxoplasmosis is an important zoonotic disease caused by the intracellular protozoan parasite *Toxoplasma gondii*. Mammals are intermediate hosts while wild and domestic felids are the definitive hosts. The main clinical sign of the disease in sheep, goats and humans is abortion (Dubey, 2009). The life cycle of the parasite consists of asexual reproductive stage in mammalian intermediate hosts and a sexual reproductive stage in the intestinal mucosa of feline definitive hosts (Garcia, 2001). Infection with *T. gondii* is widely prevalent in humans and animals worldwide and can occur pre- or postnatally (Dubey and Beattie, 1988). It has been found that nearly one-third of the human population worldwide is infected (Dubey and Beattie, 1988). Toxoplasmosis causes heavy economic losses to sheep and goat industry and is considered as one of the main causes of infectious ovine and caprine abortion (Buxton et al., 2007). Sero-prevalence of *T. gondii* in sheep varied from 92% in France (Cabannes et al., 1997) to 3 % in Pakistan (Zaki, 1995), while in goats varied from 69% in Austria (Edelhofer and Aspöck (1996), to 4% in Senegal (Deconinck et al., 1996).

## **2.7. Historical background**

*T. gondii* was found for the first time by Nicholls and Bandeaux in 1908 in the liver of the African rodent *Ctenodactylus gondii* (Dubey and Beattie, 1988; Dubey, 2008). Later, it became an important pathogen in livestock species, as reports from New Zealand described the existence of *T. gondii* in placental tissue from aborting sheep and within aborted ovine fetuses (Hartley and Marshall, 1957), but the route of transmission was not clear on that time. After it was found that this organism can infect sheep, as an herbivore animal species, trials were conducted to explore the routes of transmission other than eating grasses. The discovery in the late 1960's that

cats can shed a new environmentally stable form of the parasite in their faeces (Hutchison, 1965), led to the recognition of the cats as the definitive hosts of the parasite (Frenkel *et al.*, 1970) and the oocysts as major source of infection for animals and humans (Dubey 2004). The discovery of *T. gondii* oocysts helped to explain transmission of infection to herbivores and therefore, how the disease spread within and between flocks of grazing animals (Dubey and Beattie, 1988)

## **2.8. Etiology and life cycle**

*T. gondii* belongs to Apicomplexa phylum, Sprozoa class, Eucoccida order, Emmerinae suborder and Sarcocystidae family (James,1992). Toxoplasma has several strains; more than 95% of them are grouped into three genetic types (I, II and III). Type I is highly virulent in mice, type II is the most common type in persistently infected animals (sheep and goats) and type III is defined as no virulent strain. Clinical human infections are more often associated with type II strains (Sibley, 2003). *T. gondii* life cycle includes definitive and intermediate hosts. The sexual and asexual cycle of the parasite can take place in the intestinal epithelial cell of the cat (definitive host), but in the intermediate host only asexual cycle takes place (Dubey 2008; Frenkel *et al.*, 1970; Dubey, 2004). In the cat, following a primary infection, oocysts are produced and shed in the feces. Oocysts require 1-5 days in adequate temperature and moisture to sporulate, before they become infective to birds and mammals (Gajadhar *et al.*, 2004; Dubey *et al.*, 1998a). When an intermediate host (sheep) gets infected by ingestion of contaminated feed or grazes on land with sporulated oocysts, the parasite (zoospores) will release and become able to actively invade and multiply within the gut cells. The tachyzoite stage of the parasite multiplies asexually by a process of

endodyogeny within a parasitophorous vacuole and then the parasites eventually release from the ruptured cell and invade further cells (Lingelbach and Joiner, 1998). By day four following infection, tachyzoites may be found in the mesenteric lymph nodes (Dubey, 1984; Dubey, 2004) and the parasites are also found in the circulation where they can spread throughout the host (Wastling *et al.*, 1993). In pregnant animals, the tachyzoites invade and multiply within the caruncular septa in the placentome and then go on and invade the adjacent foetal trophoblast cells where they can spread to the rest of the fetus (Buxton and Finlayson, 1986). Tissue cysts may develop in visceral organs, including lungs, liver, and kidneys. They are more prevalent in muscular and neural tissues, including the brain, eye, skeletal, and cardiac muscle. Intact tissue cysts are probably harmless and can persist for the whole life of the host (Dubey *et al.*, 1998a). When cats consume infected meat, the wall of the cyst is digested by the proteolytic enzymes in the stomach and small intestine of cats and bradyzoites are released in the gastrointestinal tract. Some of the bradyzoites penetrate the lamina propria of the intestine and multiply. Within a few hours, *T. gondii* may disseminate to extra-intestinal tissues. Other bradyzoites penetrate epithelial cells of the small intestine and initiate development of numerous generations asexually (Dubey and Frenkel, 1972). Oocysts of *T. gondii* are formed only in cats, including both domestic and wild felids. Cats shed oocysts after ingesting tachyzoites, bradyzoites, or sporozoites (Dubey, 2004). About three to ten days after infection, infected cats start to shed oocysts for two to three weeks (Dubey and Beattie, 1988). Each infected cat may shed millions of oocysts to the environment (Dubey and Beattie, 1988), and as few as 200 sporulated oocysts can cause congenital disease in naïve sheep (McColgan *et al.*, 1988). Under laboratory conditions, cats can shed as many as 500 million oocysts after ingesting one *T.gondii*-

infected mouse (Dubey and Frenkel,1972). Millions of oocysts were shed by cats fed even a few bradyzoites (Dubey, 2001). Up to 13 million *T. gondii* oocysts were present per gram of cat feces (Schaes et al., 2008). It has been reported that at any given time, approximately 1% of cats are expected to shed oocysts, based on the observation that most cats shed oocysts for about 1 week in their life (Dubey, 1995; Dubey, 2004). Cats shed millions of oocysts in their faeces that can survive for 12-18 months in the environment, depending on climactic conditions, and are a important sources of infection for grazing animals (Tenter et al., 2000, Innes 2009, Inneset al.,2009). Shedding of oocysts tend to be more extensive amongst younger (neonatal cats) rather than older cats (Jackson and Hutchison, 1989; Buxton and Rodger, 2008).

## **2.9. Toxoplasmosis in cats**

Cats are the definitive hosts of *T. gondii*; in fact they are the only animals that pass oocysts in their feces (Dubey and Beattie, 1988) for only 2 to 3 weeks following primary infection. Mature cats are less likely to shed *Toxoplasma* if they have been previously infected (Dubey and Beattie, 1988). The two main transmission mechanisms of *T. gondii* infection are through ingestion of either oocysts shed into the environment from the faeces of cats or viable tissue cysts found in raw or undercooked meat of intermediate hosts (Dubey and Jones, 2008; Innes et al., 2009). Cats can suffer from clinical toxoplasmosis (Dubey and Jones, 2008). Most infected cats are asymptomatic, but early symptoms include lethargy, persistent fever and anorexia. Dyspnea and other signs of pneumonia are seen in many cats. Infections with severe respiratory signs are often fatal (Neufled, 1974).

## 2.10. Toxoplasmosis in sheep

Sheep are important in many countries economics, as their products are a source of food or other benefits for humans. Sheep are commonly infected with *T. gondii*. Clinical symptoms in sheep include early embryonic death and resorption, mummification, stillbirths, neonatal death or birth of alive but weak lambs (Buxton and Rodger, 2008). Up to 50% of sheep may develop fever, tremor, dyspnea and abortion in the last 4 weeks of pregnancy. Within 3-4 days after birth, death may occur with neonatal nervous sings (Waldeland, 1976). Malformations in the fetus have also been reported (Woods and Anderson, 1992). Severity of infection is associated with the stage of pregnancy at which the ewe becomes infected. Infection during the early stage of gestation can result in fetal death, resorption and abortion, while infection in the latter stage of gestation (fetal immunity is relatively well developed), may have no clinical effect and lambs are usually born normal but infected and immune (Dubey and Beattie, 1988; Buxton et.al., 2007). The main pathological changes in infected animals are small white foci and necrosis on cotyledons and focal necrotic lesions in fetal brain, liver, and lungs (Buxton and Finlayson, 1986). The meat of infected sheep is a source of *T. gondii* infection for humans and carnivore animals (Dubey, 2009). Infection in sheep can occur after consumption of contaminated feedstuffs or grazing land with sporulated oocysts (Innes et al., 2009) and transplacentally (Dubey, 1994; Esteban Redondo et al., 1995). Most infections in sheep occur following birth (Waldeland, 1977;Lunden et al., 1994; Innes et al., 2009). Some recent data suggest that in some circumstances persistently infected sheep may transmit the parasite to the fetus in subsequent pregnancies and abortions may occur (Morley et al., 2005). Transmission of *T. gondii* tachyzoites in un pasteurized sheep or goat milk (Tenter et al.,2001) also may occur via tachyzoites

contained in blood products, tissue transplants, but are not probably important epidemiologically in animals (Tenter et al.,2001). Ovine toxoplasmosis occurs in temperate sheep rearing countries worldwide where the climatic conditions favour oocyst survival (Buxton and Rodger, 2008). Infection occurring at mid-gestation typically results in stillborn or weak lambs accompanied by a small mummified foetus (Buxton and Rodger,2008). Although the basic aspect was that toxoplasmosis can cause abortion only once in infected animals, a series of recently published papers from a group of researchers in England (Buxton et al., 2007; Morley et al., 2005; Morley et al., 2008) have reported that repeated transplacental transmission of *T.gondii* in sheep may be more common than previously believed. All evidences presented were based on the detection of *T. gondii* DNA by Polymerase Chain Reaction (PCR) techniques. However, studies with different results also exist (Rodger et al., 2006).

### **2.11. Toxoplasmosis in goat**

Goats infected by *T. gondii* represent important sources of human infection due to consumption of meat and milk from infected animals (Dubey, 2004). Such fact is extremely important concerning the disease control and mainly for public health; since the consumption of goat milk is increased (Chiari and Neves, 1984; Chiari et al., 1987) *T. gondii* tachyzoites have been also isolated from vaginal mucosa, saliva, nasal secretion and urine of experimentally infected goats (Dubey,1980). The excretion of tachyzoites in the milk of naturally infected goats has also been reported (Chiari and Neves, 1984). A statistically significant correlation between positive serology for *T. gondii* in humans and consumption of goat milk has been found (Chiari et al., 1987). *T. gondii* can cause early embryonic death, resorption , fetal death, mummification, abortion, stillbirth and neonatal death depending on the stage



of gestation (Dubey, 1991; John, 1999). The signs are more severe if the infection occurs in the first half, compared to the second half of gestation (Dubey, 1991).

## **2.12. Toxoplasmosis in cattle**

Natural infection in cattle was first diagnosed in 1953 (Sanger et al., 1953). Further observations showed that toxoplasmosis is uncommon in cattle and does not appear to cause abortion (Dubey, 1986). Calves are more susceptible than adults (Nematollahi and Moghddam, 2008). Clinical signs of orally affected calves include diarrhea, anorexia, poor weight gain, depression, weakness, dyspnea and fever. In some cases just a modest lymphadenopathy may occur. Congenitally infected calves show fever, dyspnea, cough, sneezing and neurological signs, while also stillbirths and neonatal deaths can be observed. If the disease occurs in adults, symptoms may include fever, dyspnea, and nervous signs, followed by lethargy (Dubey, 1986).

## **2.13. 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, 1972; 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 (Ruiz and Frenkel, 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).

#### **2.14. 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/cm<sup>2</sup> (Wainwright et al., 2007b).

## 2.15. 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 viable tissue 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; Dubey, 2004).

## **2.16. 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).

### **2.16.1. 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).

### **2.16.2. Direct smears**

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

### **2.16.3. Serological test**

Serological test is used as common method for diagnosis of toxoplasmosis which includes sabin Feldman dye test, indirect hemagglutination 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 (Markell 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).

#### **2.16.4. 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., 2004; Edvinsson et al., 2006). The PCR assay targeting the B1 gene 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., 2010; 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; 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; 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; Edvinsson et al., 2006).

## **2.17. Risk factors**

### **2.17.1. 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; Kamani et.al., 2009).

### **2.17.2. 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; Cavalcante et al., 2008).

### **2.17.3. 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, 1994; 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).

#### **2.17.4. 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.

#### **2.17.5. 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). 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 dapsona have been used with various results in non-ruminant species and humans (Giadinis et al., 2009).

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 1988; Dubey and Jones, 2008). Ingestion of contaminated food and pasture is the most common source of small animal's infection (Dubey, 2004; 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, 2008; Lopes et al., 2008; 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). 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 significantly improved, compared to unvaccinated sheep in the same flocks (Spence et al., 1992). After subcutaneous inoculation, S48 tachyzoites multiply locally, producing parasitemia 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- $\gamma$  (Wastling et al., 1993; 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 shortcomings of the live vaccine, its short shelf life and safety margins (Stanley et al., 2004).

## CHAPTER TWO

### MATERIALS AND METHODS

#### 3.1. Study area

Eastern Sudan region has national borders with Eritrea and Ethiopia in the East and with Egypt in the North. Within the Sudan, the region borders Khartoum, Gezira, the White Nile and Upper Nile states in the west and the south. The region covers an area of 368,704 km<sup>2</sup> (UN, 2010). The region falls within the Sudano-Sahelian climate zone of Africa. Soils are dark, heavy, deep cracking *vertisol*. Annual rainfall is concentrated in a single relatively short summer season during June to September and amounts to around 680 mm per annum. Temperature ranges from a mean minimum of 17°C in January to a mean maximum of 40°C in April and May (Sulieman and Buchroithner, 2006). The region is rich in fodder, grazing areas, by-products of sorghum and sesame, and in water resources (the Blue Nile and its tributaries Dindir and El-Rahad). Port Sudan, the main port for export of livestock and livestock products is situated in the region. During the rainy season, the region has abundant fodder and water in the northern Butana area. In the summer, when water is scarce, sheep populations are concentrated around the Blue Nile in large sorghum and sesame agricultural schemes and around *hafeers* (artificial water reservoirs) and deep-bore wells. The mixed crop–livestock system, the nomadic, and the semi-nomadic system predominate in the region. Dubassy, Gaash, and Watiesh desert sheep breeds are raised and produced in the region for both domestic and export markets (ILRI, 2009). The region has an estimated livestock population standing at around 13,370,764 animals of which 4,991,763 are sheep (Anonymous, 2008).

### 3.2. Study Population

The study population was all sheep raised in the El-Gadarif state. Different breeds of sheep and goats were sampled from different production systems (nomadic, semi-nomadic, sedentary, and semi-sedentary), husbandry systems and ecological conditions. Normally, after raising, sheep and goats are usually sold in local markets and transported to secondary markets in Um-Durman, Khartoum state, where animals are finally sold and taken for meat or live exports, based on approval to be fit for export by ante-mortem and post-mortem examinations by legal authorities. Animals for meat are slaughtered at Ganawah or Kadarow abattoirs while live animals are transported to Sawakin or Port-Sudan quarantines and then shipped to international markets.

### 3.3. Sample Size

The actual sample size for determining the prevalence rate of toxoplasmosis in sheep and goats in the El-Gadarif state was calculated based on the following parameters: 95% level of confidence,  $\pm 5\%$  desired level of precision, the expected prevalence rate of toxoplasmosis in sheep and goats (Thrusfield, 2007). The prevalence rate of toxoplasmosis in sheep and goats in different regions of the Sudan was not substantially determined in previous studies. Therefore, the sample size in this study was determined by assuming an expected prevalence rate of toxoplasmosis of 50% in sheep and goats in the El-Gadarif state. By using the formula:

$$n = \frac{(1.96)^2 \times P_{exp} \times (1 - P_{exp})}{d^2}$$

where:

n = Required Sample Size

P<sub>exp</sub> = Expected Prevalence Rate, 50%  
d = Desired Absolute Precision, ±5%

The required sample size (n) was determined to be 384 animals from each study area (Thrusfield, 2007).

### **3.4. Samples**

Two serum samples were taken from animals in the selected herds as recommended by OIE (2008). About 5 ml of blood sample was collected from the jugular veins using plain vacutainer tubes. After that, tubes were kept in a slant position and protected from direct sunlight until the blood was clotted and thereafter the serum was separated. The separated serum was transferred to sterile cryovials and kept at -20°C until processed.

### **3.5. Sampling Strategy and Study Design**

A cross-sectional type of epidemiological studies was employed with a multistage sampling strategy with two hierarchical levels of selection. The first level of selection was the state, whereby it was purposively selected based on supplying animal markets. This state is considered important supply hinterland of sheep and goats for both export and domestic markets throughout the year was selected. Within each the state, a number of localities were randomly selected.

### **3.6. Laboratory procedures**

#### **3.6.1. Latex agglutination test**

The Toxo-latex agglutination test kit was obtained from the Spinreact, S.A./S.A.U., Ctra, Santa Coloma, (GI), Spain. The Toxo-latex agglutination test was carried out as described by the manufacturer.

#### **3.6.2. Indirect enzyme linked immunosorbent assay (iELISA)**

The iELISA kit was obtained from the IDvet Innovative Diagnostics, rue Louis Pasteur, Grabeis, France. The kit components were reconstituted as directed by the manufacturer. These included concentrated conjugate (10X), positive and negative control sera, dilution buffer 2 and 3, concentrated wash solution (20X), substrate solution, and stop solution (0.5 M). The test procedure was carried out as per the manufacturer's protocol. A positive/negative cut-off was calculated as S/P% of  $\geq 50\%$ .

### **3.7. Data Analyses**

The Statistical Package for Social Sciences (SPSS) for Windows<sup>®</sup> version 22.0 (SPSS Inc., Chicago, Illinois) was used for all appropriate statistical analyses. Descriptive statistics of the variables were obtained. For each variable (age, sex, breed, and locations), frequencies (number of observations within variable) and prevalence rates by cross-tabbing (number of positive valid samples/number of individuals sampled in the variable) were obtained. Hypotheses of differences of age group, breed, sex, and locations between test-positive and test-negative animals were first tested by univariate analysis by means of the 2-tailed chi-square test. In a second step, a logistic regression model was used to assess the association between the potential risk



factors sex, breed, state, and locality and the outcome variable toxoplasmosis serological status. Associations in the logistic regression model were deemed significant when  $p \leq 0.05$ .

## CHAPTER THREE

### RESULTS

#### 4.1. Frequencies and distributions of the tested samples

A total of 400 serum samples were collected in the period from June to November 2015 from sheep and goats in El-Gadarif state. All samples were tested to estimate the sero-prevalence of anti-toxoplasma antibodies by using latex and iELISA. Frequencies and distributions of the test samples by species, locality, sex, age, and breed, are presented in Table 1.

Table 1: Frequencies and distributions of the tested serum samples by species, locality, sex, age, and breed for anti-toxoplasma antibodies (June to November 2015) in El-Gadarif state.

<b>Risk factors</b>	<b>No. of tested</b>	<b>% of tested</b>
<b>Species</b>		
Sheep	200	50.0
Goats	200	50.0
<b>Localities</b>		
Al-Fao	100	25.0
Al-Hawatah	100	25.0
West El-Gadarif	100	25.0
El-Gadarif Municipality	100	25.0
<b>Breeds</b>		
Wateish sheep	111	27.8
Agbash sheep	89	22.2
Balady goat	128	32.0
Nuoby goat	72	18.0
<b>Age groups (yrs)</b>		
≤ 2	227	56.8
> 2	173	43.2
<b>Sex</b>		
Male	216	54.0
Female	184	46.0
<b>Total</b>	400	100

#### 4.2. The overall sero-prevalence

Generally, anti-toxoplasma antibodies were detected in all the selected localities with variations observed in the sero-prevalences between different

age groups, breeds, and sexes as presented in Table 2. The overall sero-prevalence was 52.0% (208/400) with 95% CI between 47.1 and 56.9, using Toxo-latex agglutination test.

#### **4.3. Sero-prevalence among species**

Sero-prevalences were estimated among sheep and goats with sheep showing a sero-prevalence of 57.5% (115/200), with 95% CI from 50.65 to 64.35, and goats of 46.5% (93/200), with 95% CI between 39.59 and 53.41. The sero-prevalences were statistically not significant using Toxo-latex agglutination test as presented in Table 2. On the other hand, the sero-prevalences using iELISA were 45.7% (42/92) with 95% CI from 35.52 to 55.88 in sheep and 27.2% (25/92), with 95% CI from 18.11 to 36.29 in goats.

#### **4.4. Sero-prevalence in the different surveyed localities**

There were significant statistical differences in the sero-prevalences of the surveyed localities. Al-Fao (73.0%; 73/100, with 95% CI from 64.3 to 81.7) and El-Gadarif municipality (30.0%; 30/100, with 95% CI from 21.02 to 38.98) were showing, respectively, higher and lower sero-prevalences than Al-Hawatah (51.0%; 51/100, with 95% CI from 41.2 to 60.8) and West El-Gadarif (54.0%; 54/100, with 95% CI from 44.23 to 63.77) localities as presented in Table 2.

#### **4.5. Sero-prevalence among breeds**

There were no differences in the sero-prevalences estimated among different breeds. The sero-prevalences ranged from 45.9% to 61.8% with 95% CI from 37.82 to 71.23 as presented in Table 8.

#### 4.6. Sero-prevalence among age groups

There were no statistically significant differences in the sero-prevalences among the two age groups. Animals that were  $\geq 2$  years old were showing a prevalence of 54.6% (124/227, 95% CI 48.12% to 61.08%), and animals that were  $< 2$  years old were showing a prevalence of 48.6% (84/173, 95% CI 41.15% to 56.05%), as presented in Table 2.

#### 4.7. Sero-prevalence among males and females

Sero-prevalences between males and females were significantly different. Females were showing a higher prevalence of 54.9% (95% CI 47.71% to 62.09%) and males were showing a lower prevalence of 49.5% (95% CI 42.83% to 56.17%), as presented in Table 2.

Table 2: Estimated sero-prevalences of anti-toxoplasma antibodies by species, locality, breed, age and sex, from July to November 2015 in El-Gadarif state.

<b>Risk factors</b>	<b>No. of tested</b>	<b>No. of positive</b>	<b>% of positive</b>	<b>95% CI Lower - Upper</b>
<b>Species</b>				
Sheep	200	115	57.5	50.65 - 64.35
Goats	200	93	46.5	39.59 - 53.41
<b>Localities</b>				
Al-Fao	100	73	73.0	64.30 - 81.70
Al-Hawatah	100	51	51.0	41.20 - 60.80
West El-Gadarif	100	54	54.0	44.23 - 63.77
El-Gadarif municipality	100	30	30.0	21.02 - 38.98
<b>Breeds</b>				
Wateish sheep	111	63	56.8	47.58 - 66.02
Agbash sheep	89	52	58.4	48.16 - 68.64
Balady goat	128	58	45.3	36.68 - 53.92
Nuoby goat	72	35	48.6	37.06 - 60.14
<b>Age groups (yrs)</b>				
$\leq 2$	227	124	54.6	48.12 - 61.08
$> 2$	173	84	48.6	41.15 - 56.05
<b>Sex</b>				
Male	216	107	49.5	42.83 - 56.17
Female	184	101	54.9	47.71 - 62.09
<b>Total</b>	400	208	52.0	47.10 - 56.90

#### 4.8. Univariate associations

The proportions of sero-positive differ between some localities, breeds, age groups, and males and females. In the univariate analysis using chi square, species (p-value = 0.028) and locality (p-value = 0.001) were significantly associated with Toxo-latex agglutination test positive status for toxoplasmosis. However, age (p-value = 0.229), breed (p-value = 0.164), and sex (p-value = 0.285) were not significantly associated with a with Toxo-latex agglutination test positive status for toxoplasmosis (Table 3).

Table 3: Univariate association of anti-toxoplasma antibodies positive status with species, locality, breed, age and sex, from July to November 2015 in El-Gadarif state.

<b>Risk factors</b>	<b>No. of tested</b>	<b>No. of positive</b>	<b>% of positive</b>	<b>X<sup>2</sup></b>	<b>df</b>	<b>p-value</b>
<b>Species</b>				4.484	1	0.028
Sheep	200	115	57.5			
Goats	200	93	46.5			
<b>Localities</b>				37.260	3	0.001
Al-Fao	100	73	73.0			
Al-Hawatah	100	51	51.0			
West El-Gadarif	100	54	54.0			
El-Gadarif municipality	100	30	30.0			
<b>Breeds</b>				5.104	3	0.164
Wateish sheep	111	63	56.8			
Agbash sheep	89	52	58.4			
Balady goat	128	58	45.3			
Nuoby goat	72	35	48.6			
<b>Age groups (yrs)</b>				1.450	1	0.229
≤ 2	227	124	54.6			
> 2	173	84	48.6			
<b>Sex</b>				1.141	1	0.285
Male	216	107	49.5			
Female	184	101	54.9			

#### 4.11. Multivariate analysis

Results of the logistic regression analysis assessing the combined relationship between species, localities, and breeds with the positive reaction for toxoplasmosis in the Toxo-latex agglutination test positive status are

presented in Table 4. The regression coefficients (Exp(B)) express ‘odds ratios’ (OR) (= the increased or decreased probability (OR ≠1) of seropositivity occurrence in comparison to the reference (OR = 1). The factors found significantly associated with increased odds of being Toxo-latex agglutination test positive were species (sheep) and locality (Al-Fao, Al-Hawatah, and West El-Gadarif).

Table 4: Multivariate association of anti-toxoplasma antibodies positive status, from July to November 2015 in El-Gadarif state.

<b>Risk factors</b>	<b>No. of tested</b>	<b>No. of positive</b>	<b>% of positive</b>	<b>Exp(B)</b>	<b>p-value</b>	<b>95% CI Lower - Upper</b>
<b>Species</b>						
Goats	200	93	46.5	ref		
Sheep	200	115	57.5	2.00	0.022	1.105 – 3.528
<b>Localities</b>						
El-Gadarif municipality	100	30	30.0	ref		
Al-Fao	100	73	73.0	6.71	0.001	3.589 – 12.52
Al-Hawatah	100	51	51.0	2.44	0.003	1.353 – 4.381
West El-Gadarif	100	54	54.0	2.87	0.001	2.868 – 5.170
<b>Breeds</b>						
Balady goat	128	58	45.3	ref		
Wateish sheep	111	63	56.8	1.58	0.178	0.949 – 2.643
Agbash sheep	89	52	58.4	1.20	0.258	0.982 – 2.930
Nuoby goat	72	35	48.6	1.14	0.640	0.640 – 2.036

## CHAPTER FOUR

### DISCUSSION

Toxoplasmosis is one of the most not uncommon parasitic diseases world-wide (Tenter et al., 2000; Hammond-Aryee et al., 2014). It has been reported in many species of animals including cats, dogs, small and large ruminants and equines. Besides, circa 2 billion people are chronically infected with this disease around the globe (Tenter et al., 2000; Prandota, 2009; Cenci-Goga et al., 2013; Hammond-Aryee et al., 2014).

The overall sero-prevalence of anti-toxoplasma antibodies reported in this study was comparable to the sero-prevalence reported by Khalil and Elrayah (2011) in sheep was higher than the overall sero-prevalences reported by Elfahal (2014). It was also higher than the seroprevalence estimated in the southern part of Africa which was found to be 4.5% (n= 600) in sheep. On the other hand, it was lower than the one reported in Zimbabwe (67.9%; n= 335) in small ruminants. In general, varying seroprevalences were reported from different countries and different animal species; for examples Hammond-Aryee et al. (2014) found out that 38.2% and 26.8% of the sheep and goats investigated in Ghana were seropositive and as high as 98.4% of the tested sheep in Egypt. Moreover, toxoplasmosis has been detected in horses, donkeys, camels and humans and in wild animals in the Sudan (Tenter et al., 2000; Elnahas et al 2003; Seri et al., 2003; Khalil et al., 2012; Khalil et al., 2013; Shadia et al., 2013; Abdel-Raouff and Elbasheir, 2014; Gebremedhin et al., 2014; Hammond-Aryee et al., 2014). Density of domestic cat population and frequency and amount of contact between cats and other animals could probably lead to a variation in the prevalence of toxoplasmosis. Many sheep and goats owners keep sheep and goats in their houses in the investigated area. These animals graze in the backyard or in the close vicinity, hence, the contact

between them and domesticated cat is considerable, consequently, higher risk of contracting toxoplasmosis.

The results of the present study indicated that there is no between-species differences in sero-positivity, which was disagreeing with the findings of Khalil and Elrayah (2011) and Elfahal et al. (2013) who found out that 57.5% of the surveyed sheep samples were toxoplasmosis-positive, 32.0% of the cattle samples, and 20.0% of the camel samples. As the already shed infective stage of this protozoan parasite and disseminated in the environment is being swallowed with the ingesta, the dissimilarity of feed intake and the feeding behaviors of different animal species might likely result in diverse sero-positivity among these animal species. Grazers, such as cattle, browsers like goats and camels, and sheep which are classified as intermediate have different grazing behaviors such as searching for and selecting the type of grasses or feed and grasping and taking the selected grasses or feed. Cattle are less selective to their forage in comparison to small ruminants and so they eat more dead material than sheep and goats that have narrower mouths and more flexible lips. These mouthparts also make it more difficult for cattle to select leaves of woody plants (browse) as camels do (Iqbal and Khan, 2001; Mosavat and Chamani, 2013).

Concerning the prevalence in different age groups, the findings of the present study disagreed with the findings of Nematollahi and Moghddam (2008) and Elfahal et al. (2013) who found that the prevalence of *T. gondii*-infection was significantly higher in young animals less than in old animals. Yin et al. (2015) found positive samples in all age groups with variations but the highest prevalence was detected in young animals. This may reflect the dominance of maternally acquired antibodies in this age group or that the cattle unless re-infected, deplete antibodies as their age increases. Age was an



important factor for being seropositive as a measure of the cumulated life-time risk.

There were significant statistical differences between the sero-prevalences of the surveyed localities. This was in disagreement with the results of Elfahal et al. (2013) who found that there was no significant relationship between antibodies prevalence and location and the prevalence was 12.7% in Khartoum state and 14.9% in Al-Gazira state whereas the by locality prevalences were 12.9%, 14%, and 10.3% in Khartoum, Khartoum North and Omdurman localities, respectively, and 25% in Alkamleen and 12.8% in Wad Madani localities. However, Yin et al. (2015) reported that the seroprevalence of toxoplasmosis in Tibetan sheep in Tianzhu and Maqu areas were statistical different. The data reported by Hammond-Aryee et al. (2014) suggested a geographical trend of toxoplasmosis sero-prevalence in humans and animals. Elfahal et al. (2013) also indicated that geographical variations might occur not only among different countries but also within countries.

There was significant statistical difference between the sero-prevalences of the different breeds. This was in disagreement with the results of Elfahal et al. (2013) who found that there was no significant relationship between antibodies prevalence and breed. Ahmad et al. (2015) found that no statistically significant breed wise difference of seroprevalence was observed in goats. However, seroprevalence was significantly high in salt range sheep as compared to other breeds of sheep.

There was significant statistical difference between the sero-prevalences of males and females. This was in agreement with the results of Elfahal et al. (2013) who found out that significantly higher seroprevalence of *T. gondii* was observed in males (30.8%) than in females (11.9%). Moreover, Yin et al. (2015) noted that the prevalence in females (19.2%, 95% CI = 17.01-21.46)

were lower than in males (22.8%, 95% CI = 19.22-26.36). However, several studies indicated that the prevalence in females were higher than males which was probably due to the lower levels in immune response or antibody persistence of females in some periods of their lives (van der Puije et al., 2000; Lopes et al., 2010; Kamani et al., 2010). Ahmad et al. (2015) indicated that the likelihood of infection increased with age in sheep and goats. Female animals were more likely to be infected as compared to males. Females have longer production life span in comparison to males and subjected to more stressors (pregnancy and lactation) make less resistant to toxoplasmosis.

Among the risk factors investigated by Rêgo et al. (2016), extensive rearing system, number of domestic cats on the farm, and domestic and wild dogs access to the water trough used by the sheep. In goats, significant risk factors were: cats feeding on placenta remains, the number of domestic dogs on the farm, sex, and breeding from the two investigated areas in Brazil.

Ahmad et al. (2015) found that statistically significant risk factors in sheep were poor hygienic conditions, presence of cats, extensive farming practice, flock size larger than 50 individuals and pregnancy. Similarly statistically significant risk factors in goats were poor hygienic condition, usage of outdoor water source, presence of cats, extensive farming practice and flock sized larger than 30 and 50 individuals. Yin et al. (2015) in Tibetan sheep, and the results indicated that age, gender, and numbers of past pregnancies of Tibetan sheep were not a significant risk factors ( $P > 0.05$ ) and left out of the final model, however, season and geographical origin were considered as risk factors for the infection. Cenci-Goga et al. (2013) five variables (flock size, production system, stagnant water, resident cats, access of stray cats to water) were selected for inclusion in the multivariable Poisson

model. The final Poisson model contained only two predictors (flock size and access of cats to water)

### **Conclusions and recommendations**

- 1- It can be concluded that the prevalence of anti-toxoplasma 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.
- 2- These findings warrant further in-depth investigations to be carried out to allow better understanding of the epidemiology of the disease not just in sheep and goats but in other animal species also in El-Gadarif and to evaluate burden of the disease and the existed risk to human beings.
- 3- Molecular characterization of the parasite is of high importance in the Sudan.

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