Toxoplasma gondii infection in slaughtered domestic ruminants in Northwest Ethiopia: occurrence, bioassay and virulence assessment

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Abstract

Purpose

This study investigated the seropositivity, isolation and virulence of *Toxoplasma gondii* in slaughtered domestic ruminants in Gondar city, Northwest Ethiopia.

Methods

Three hundred thirty-five blood samples (135 sheep, 50 goats and 150 cattle) were collected from slaughterhouses. Antibodies against *T. gondii* were assayed using a commercial Toxo-Latex agglutination test. Tissue digestion was also conducted on 39 heart muscles of seropositive animals using the pepsin enzyme, and isolation of viable *T. gondii*, from seropositive ruminants, was also performed in white *albino* mice.

Results

The overall occurrence of *T. gondii* infection was 55.8%. The occurrence of *T. gondii* antibodies in cattle, goats and sheep was 59.3%, 58%, and 51.1%, respectively. *Toxoplasma gondii* antibodies prevalence was significantly higher in females ($\chi 2 = 4.55$, $p < 0.033$) and adults of sheep ($\chi 2 = 7.57$, $p < 0.006$). Similarly, in cattle, old groups ($\chi 2 = 7.81$, $p < 0.005$) and cross-breeds ($\chi 2 = 6.30$, $p < 0.012$) have presented association with presence of *T. gondii* antibodies. However, in goats, no association was observed either with sex or age groups. In bioassayed mice, the overall viable *T. gondii* isolates were 38.5% and the parasites were isolated from samples of sheep (8/16), cattle (3/14) and goats (4/9), and most of these isolates (87.2%) were avirulent.

Conclusion

The high occurrence of *T. gondii* antibodies and a high proportion of viable *T. gondii* observed in this study indicated the prevalent nature of the parasite and its zoonotic importance in the study areas, where slaughtered domestic ruminants serve as an important human protein source. Education of the public about routes of *T. gondii* transmission and control methods is imperative to prevent *T. gondii* transmission.

1. Introduction

Toxoplasma gondii is an obligate intracellular parasite, which can infect all warm-blooded vertebrates including humans, mammals, and birds (Schlüter et al., 2014). It infects up to 30% of the human population in the globe (Dubey, 2010). Humans can acquire the infections by ingesting sporulated oocyst-contaminated food, vegetables, and water; by consuming raw or undercooked meat containing viable

tissue cysts of this parasite from infected food animals, and congenitally from an infected mother to the foetus (Dubey, 2010; Schlüter et al., 2014). Felids play a key role in the epidemiology of *T. gondii* infection in animals and humans as final hosts by shedding millions of environmentally resistant viable oocysts through their faeces (Dubey, 2010; Schlüter et al., 2014). Feline become infected through the consumption of tissues containing viable cysts from intermediate hosts (Dubey, 2010; Robert-Gangneux and Darde, 2012) or, less effectively, through the ingestion of sporulated oocysts (Dubey 2006; Saadatnia and Golkar, 2012; Cornelissen et al., 2014; Shapirro et al., 2019; Attias et al., 2020).

Ruminants are considered important in the epidemiology *of T. gondii* infection worldwide (Tenter, 2009; Dubey, 2010). The ingestion of infected meat from food animals serves as a direct source of infection for humans and felines. In most areas of the world, *Toxoplasma* infection is prevalent in meat-producing animals (Tenter, 2009; Tonouhewa et al., 2017; Stelzer et al., 2019) and pose a risk to public health (Garcia-Bocanegra et al., 2013; Gharbi et al., 2013; Gazzonis et al., 2020) because humans can acquire the pathogen from infected food animals through raw or undercooked meat and milk consumption (Yang et al., 2012; Al-Kappany et al., 2018). Infection of pregnant women results either in abortion or congenital infection of the foetus. The congenital infection of foetuses can results in hydrocephalus, intracranial calcification and retinochoroiditis (Schlüter et al., 2014; Tonouhewa et al., 2017; Hosseini et al., 2019).

Several serological surveys have indicated *T. gondii* infection is prevalent in Ethiopian sheep, goats, and cattle (Negash et al., 2004; Teshale et al., 2007; Gebremedhin et al., 2013; Zewdu et al., 2013; Tegegne et al., 2016; Tilahun et al., 2018; Esubalew et al., 2020). However, little is known about the viability and virulence of *T. gondii* isolates detected in the meat of such seropositive animals. Therefore, this study was conducted: 1) to estimate the prevalence of *T. gondii* antibodies in sheep, goats and cattle destined for slaughter in Gondar city, Northwest Ethiopia; 2) to assess the viability and virulence of *T. gondii* isolate using mice bioassay.

2. Materials And Methods

2.1. Study Design and Sampling Technique

A cross-sectional study design was employed to collect blood and tissue samples from slaughtered domestic ruminants at Gondar ELFORA abattoir and four local slaughterhouses in Gondar city. A laboratory-based experimental follow up in bioassayed mice was also performed from November 2018 to June 2019. A total of 335 animals (135 sheep, 50 goats and 150 cattle) were sampled purposively for the study. The age of the animals was determined by observing the erupted permanent incisors (Taylor, 1984; Awgichew & Abegaz, 2008). The approximate age of the animals was categorised and recorded as young, adult and old. Sheep and goats ≤ 1 year were considered as young, while those over one year were considered adult. In the case of cattle, those with ≥ 7 years were considered as old, while those having four to seven years old were considered adults.

2.2. Sample collection and transportation

A total of 335 blood samples, each about 5–10 ml whole blood, were collected using plain sterile tubes during exsanguination or intracardially at the slaughter line. Out of the total 335 blood samples, 200 had matched heart tissue samples (75 from cattle, 75 from sheep and 50 from goats) for microscopic examination and bioassay in mice. Each collected tissue sample weighed about 50–60 grams. The samples were labelled and transported with a cold box to the Veterinary Parasitology laboratory, College of Veterinary Medicine and Animal Sciences, University of Gondar.

2.3. Serological assay

The blood samples were allowed to clot in a slant position for a few minutes and centrifuged at 4000 rpm for 5 min to separate the sera. Subsequently, sera were decanted into 1.5 ml Eppendorf tubes. *Toxoplasma gondii* antibodies were assayed in the collected serum samples using the Toxo-latex slide agglutination test (SPINREACT, S.A/S.A.U, GIRONA, SPAIN) following the previous reports (Ibrahim et al., 2014; Tegegne et al., 2016) and manufacturer's recommendations. Agglutinates were detected using the naked eye and stereomicroscope. The presence of agglutination was considered as positive at a titre of ≥ 1:2 and indicates an antibody concentration equal to or greater than 4 IU/ml. The laboratory procedures were performed at the University of Gondar, College of Veterinary Medicine and Animal Sciences, Veterinary Parasitology Laboratory.

2.4. Tissue digestion and Bioassay in mice

Tissue samples from seropositive animals were digested as described by Dubey (1998). Briefly, tissue samples weighing 50 grams were minced and digested in a pepsin acid solution (pH 1.1–1.2) at 37°C for 1hr. After filtration, neutralisation was executed one time with 1.2% sodium bicarbonate solution (pH = 8.3) and then centrifugation was performed. Then, the tissue digest was examined microscopically at 10x and 40x magnification power to confirm the presence of liberated bradyzoites and/or tissue cysts of *T. gondii* from the tissue digests. Off microscopically positive samples, only the 39 positive digested tissue samples were inoculated subcutaneously into 5 mice/sample (1 ml suspension per mouse) to assess the viability and virulence of the detected cysts. Before mice inoculation performing, the samples were diluted in 5–10 ml of antibiotic saline solution (1000 IU/ml penicillin and 100µg streptomycin/ml in saline solution). Five to six weeks of aged female white *albino* mice weighing 20–25-gram were used for *T. gondii* isolation. Approximately 5ml aliquots from the suspension were leftover and stored at + 4°C until it was inoculated in the same mice next day (Beltrame et al., 2012). After inoculation, mice were followed for 49 days for the occurrence of clinical signs and/or death. At 49 days, all survivors were euthanised through cervical dislocation after anaesthetising with diethyl ether and blood was then collected through cardiac puncture. The blood was allowed to clot for about 3 to 4 hours and then the top part was pipetted and centrifuged at 4000 RPM for 4 minutes. The serum was tested by the presence of *T. gondii* antibodies on the same day by LAT.

Further, the brain of the euthanized mouse was homogenised in 1ml phosphate-buffered saline (pH = 7.2) using a mortar and pestle to detect the cysts microscopically. The number of cysts in the brain of each mouse was determined by converting the sum of cysts in 30 µl to the whole of the brain homogenates

(Goodwin et al., 2008; Fritz et al., 2012). The bioassay was considered as positive if at least one *T. gondii* cyst is detected in any inoculated mouse or if at least one of the mice sera reacts positively by the LAT (Dubey et al., 1995). The virulence of the parasite was classified according to Pena et al. (2008) based on mice mortality rate within four weeks of infection (without prior information on infecting dose). Then, we categorized isolates into three groups: virulent (if there were 100% death of mice within four weeks), intermediate virulent (30% to less than 100 % death within four weeks), and non-virulent (< 30 % death within four weeks).

2.5. Data Analysis

The data obtained were stored in a Microsoft Excel spreadsheet (2010) and analysed using SPSS version 20 (SPSS Inc., Chicago, IL, USA). The data were summarized using descriptive statistics. The association of seropositivity for *T. gondii* and, age (young, adult and old), sex (male and female), breed (local and cross) and species (cattle, sheep and goat) was tested using the chi-square test. The 95% confidence level was used and statistical analyses were considered significant at $P < 0.05$.

3. Results

3.1. Occurrence of *Toxoplasma gondii* antibodies

The overall occurrence of *T. gondii* antibodies in slaughtered domestic ruminants was 55.8% (95% CI: 50.1–61.2). At species level, it was relatively higher in cattle 59.3% (95% CI: 51.4–67.3) followed by goats 58% (95% CI: 44.0–72.0) and sheep 51.1% (95% CI: 43–60). But, this difference was not significant.

Toxoplasma gondii antibodies occurrence was significantly higher in females ($\chi2 = 4.55$, $p < 0.033$) and adults of sheep ($\chi2 = 7.57$, $p < 0.006$). Similarly, in cattle, old groups ($\chi2 = 7.81$, $p < 0.005$) and cross-breeds ($\chi2 = 6.30$, $p < 0.012$) have presented association with presence of *T. gondii* antibodies (Table 1). However, in goats, no association was observed either with sex or age groups.

Table 1: Association between hosts and risk factors analysed and occurrence of T. gondii **antibodies**

3.2. Bioassay in mice

Thirty-nine positive tissue samples (14 from cattle, 16 from sheep and 9 from goats) containing *T. gondii* tissue cysts and/or liberated bradyzoites (Fig. 1) were bioassayed in mice. The total viable *T. gondii* isolated from the three species of animals was 38.5% (15/39) (Table 2). At the species level, viable *T. gondii* was isolated from 8/16 of sheep, 4/9 of goats and 3/14 of cattle samples. The summary of viability data as indicated by serological and cyst positivity in inoculated mice is presented in Table 2 and detailed data for each tested isolate is provided in Table 3.

Figure 1: T. gondii **tissue cysts (I) and liberated bradyzoites (II).**

Table 2: Summary serological and cyst positivity of inoculated mice by T. gondii **isolate from slaughtered domestic ruminants**

Table 3: Detailed viability data for each bioassayed isolate as indicated by serological and cyst positivity in the inoculated mice.

For seropositivity and virulence analysis, we have used a total of 195 mice. However, at the time of inoculum injection and during follow up period (post-infection) we lost one and twenty-nine mice, respectively. All these lost mice were not included in the seropositivity test and cyst detection (due to autolysis). Therefore, the overall seropositivity of *T. gondii* antibodies in experimentally infected surviving mice from all animal species was found to be 16.4% (27/165). The seropositivity of *T. gondii* antibodies in experimentally infected mice using inoculum from sheep, goat and cattle was 18.2% (12/66), 16.7% (6/36) and 14.3% (9/63), respectively. A total of 21 experimentally infected mice were found to harbour *T. gondii* tissue cysts. Some of the tissue cysts detected in inoculated mice are presented in Fig. 2.

Figure 2: Tissue cysts of T. gondii **isolated from the brain of mice: from sheep sample inoculum [A = unstained 40x], goat sample inoculum [B = unstained; C = impression smear 100x], and cattle [D impression 100x smear]. Note the thin cyst wall (arrow) enclosing bradyzoites.**

The overall mean cyst detected and enumerated from experimentally infected mice was 162.57 ± 34.840 (mean ± SE=) cysts per brain of mice. Overall, higher mean cyst counts in the brains of mice inoculated with heart homogenates from goats (n = 278.4) were observed when compared to those enumerated in the bioassay performed on the sheep (n = 129.57) and cattle (n = 104.0) samples (Table 4).

Table 4: Mean cyst counts in the brains of mice inoculated with heart homogenates of slaughtered animals.

3.3. Virulence of isolates and clinical signs in mice

During the follow-up, most inoculated mice were asymptomatic. However, 29 mice (7 from cattle, 13 from sheep and 9 from goats) died before the 49th day. More specifically, 9 mice (5 from sheep and 4 from goat's inoculum) died on 3rd and 4th days after inoculation while 20 mice (8 from sheep, 7 from cattle and 5 from goats' inoculum) died after the 4th day's post-inoculation (pi). Most of the recovered isolates (87.2%) in this study were avirulent to the mice. One (1/8) highly virulent isolate was suggested from sheep inoculum (**sp**67) by which all inoculated mice (5/5) were killed on 3rd and 4th day PI. Four intermediate virulent isolates (1/16 sheep, 1/14 cattle and 2/9 goats) were suggested (Table 3). The clinical symptoms observed in symptomatic mice were death, ruffled, stiff, arched back, leg paralysis, tachypnoea, inappetence, rough hair coat, dullness and neurological signs (paralysis).

4. Discussion

4.1. Prevalence of *T. gondii* antibodies in slaughtered domestic ruminants

The overall occurrence of *T. gondii* antibodies among slaughtered domestic ruminants was found to be 55.8%. This finding is higher than the reported prevalence of 22.2% from Eastern Ethiopia (Tilahun et al., 2018) and 37% from Tunisia (Lahmar et al., 2015. This discrepancy may be related to the sample size and the age of population studies and methods used. Indeed, previous studies were carried out using an ELISA and DAT technique which are less sensitive than the latex agglutination test used in the present study.

In this study, *Toxoplasma gondii* antibodies occurrence was significantly higher in females and adults of sheep. Previous reports across the globe (Yibeltal, 2008; Ramzan et al., 2009; Gebremedhin et al., 2013; 2014) also reported a higher occurrence of *T. gondii* antibodies in females and adults. It might be due to that females are kept for breeding purposes and are lived for a long time and hence, they have a higher chance to harbour the parasite for their life. It is also attributed to the high chance of exposure to the source of infection as age increases and suggests that most sheep acquire the infection postnatal (Andrade et al., 2013; Opsteegh et al., 2016). In cattle, older and cross-breed animals also showed significantly higher *T. gondii* seropositivity than adult and local breed cattle. Older animals as they lived longer might be more likely to be exposed to the infectious agent from different sources (Jittapalapong et al., 2005; Teshale et al., 2007; Ramzan et al., 2009; Andrade et al., 2013; Opsteegh et al., 2016; Amdouni et al., 2017). According to Furtado et al., (2013), cross-breed cattle also had a higher chance to acquire *T. gondii* infection than local breeds; this may be ascribed to the genetic and immune status of the host.

4.2. Bioassay in mice

The overall viable *T. gondii* isolation rate of 38.5% in the current study shows a lower percentage compared to the report of Berhanu (2015) who reported 67.6%. It is also higher than the report by Elfadaly et al. (2017) from Egypt (8.57%) in domestic ruminants (sheep, goats and cow). The isolation rate of viable *T. gondii* in this study in sheep (50%) is comparable with the report of 57.45% from central Ethiopia (Gebremedhin et al., 2014) and higher than the findings from France with the isolation rates of 26.7% (Dumètre et al., 2006) and from Egypt with isolation rate of 32% (Younis et al., 2015). In contrast, the current result is lower than the report from the USA with the isolation rates of 77.9% (Dubey et al., 2008) and higher than the report from Brazil 19.5% (Ragozo et al., 2008) from MAT seropositive sheep. In goat, isolation rates of viable *T. gondii* (44.4%) in the current study are in parallel with the reported isolation rate of 45.45% in central Ethiopia (Gebremedhin et al., 2014) and 46.15% in Sao Paolo, Brazil (Ragozo et al., 2009). However, this disagrees with records of Berhanu (2015) 75% from Eastern Ethiopia, (Dubey et al., 2011) 26% from Brazil and (Opsteegh et al., 2011) 62.8% from the USA. The differences between these reports may be due to the density of *T. gondii* in tissues of sheep and goats, the type of tissue sampled and the strain or genotype of *T. gondii*.

Attempts to isolate viable *T. gondii* from cattle tissues have been extremely rare. Only a few successful tissue cyst recoveries have been reported (Scarpelli et al., 2009). This study in cattle on the isolation rate of viable *T. gondii* (21.43%) via bioassay in mice is comparable with the report of Opsteegh et al. (2016) who demonstrated 3.3% of viable *T. gondii* from selected European countries. However, the finding of the current study is higher than the reported isolation rate of 0% from Ethiopia in cattle (Berhanu, 2015) 0%

from Egypt in cows (Elfadaly et al., 2017) through bioassay in mice and lower than the reported isolation rate of 100% (18/18) in experimentally infected cattle confirmed via bioassay in mice (Scarpelli et al., 2009). The differences between this work and aforesaid reports may be due to the density of *T. gondii* in tissues of cattle, the type of tissue sampled, the digestion method used and the strain or genotype of *T. gondii* (Dubey, 2010).

The overall prevalence of *T. gondii* antibodies in experimentally infected and surviving mice, from all animal species, was 16.4% (27/165). This finding is in agreement with the report of Gebremedhin et al. (2015) who reported 16.8% seroprevalence in experimentally infected mice with heart homogenates of the pig. However, it is lower than the report of Endrias *et al.* (2015) who reported 30.58% seroprevalence. The overall prevalence of *T. gondii* tissue cysts were detected in the brains of experimentally infected mice was 12.7%. This finding is lower than the reported cyst percentage of 17.6% by Gebremedhin et al., (2015) and the reported cyst percentage of 28.82% by Endrias et al., (2015). The mean cyst count per brain of mice in this study was 162.57 (mean ± SE = 162.57 ± 34.84). This finding is comparable with the mean tissue cyst count of 157.2 from central Ethiopia (Gebremedhin et al., 2015). However, it is lower than 277.97 mean cyst count per brain from central Ethiopia (Gebremedhin et al., 2014), tissue cyst counts ranging from 297 to 1380 by Brown et al. (1995) and the mean number of tissue cysts of 600 by Goodwin et al. (2008), but higher than the previous report by Tesfamariam (2013) in free-range chicken from Ada'a Liben, Central Ethiopia with the mean tissue cyst count of 57.4 per brain of mice. *Toxoplasma gondii* cyst burden in mice is only unassociated with inoculum dose and route, but also by the inoculated parasite strain (Waree et al., 2007) or by the genotype of mice (Brown et al., 1995; Waree et al., 2007). Besides, the number of live bradyzoites in the digested heart tissue of study animals inoculated into mice could contribute to the variation of the counted tissue cysts that are if inoculated bradyzoites are few; few of them reach the brain of mice, there will be fewer numbers of tissue cyst formed (Brown et al., 1995). Thus, microscopic counting of brain cysts in mice may indicate the infectious burden of *T. gondii* in ruminants (Bourguin et al., 1993).

4.3. Clinical signs and virulence of T. gondii isolates in mice

During the follow-up, most inoculated mice were asymptomatic. However, 29 mice (7 from cattle, 13 from sheep and 9 from goats) died before the 49th day. The observed clinical picture in mice in this study was similar to that observed by Gebremedhin et al. (2014b; 2015), and (Kyan et al., 2012), who observed that clinical conditions varied across seropositive mice and include arched back, ruffled, stiff, depression and forced breathing and most of these symptomatic surviving mice recovered to a normal condition.

The majority (87.2%) of the isolates recovered, based on the observation of mouse bioassay, were avirulent to white *Swiss Albino* mice. This virulence assessment is in agreement with previous reports in Ethiopia (Gebremedhin et al., 2014; 2015). However, one isolate (sp67) from sheep inoculum was highly virulent killing all inoculated mice (5/5) on the 3rd and 4th day of post-infection. According to Pena et al., (2008), 100% of death within four weeks of post-infection implies high virulence of *T. gondii* in mice. Besides, 4/5 and 2/5 of mice were killed due to isolates from goat tissue sample inoculum code (Gt6 and Gt 28, respectively), within 4 weeks pi, and 2/5 of mice from each sheep (sp26) and cattle (B65) sample

inoculum died within four weeks postinfection. This may be an indication of intermediate virulence *of T. gondii* strains (Cook et al., 2000; Pena et al., 2008). Alternatively, *T. gondii* was also isolated from a homogenate of the intestine of the cow (Dubey 1992). According to Dubey et al., (2002; 2007b), *T. gondii* isolates differ markedly in their virulence to outbred mice. The avirulent strains were defined as having no mortality at any dose, whereas a "low-dose survivability" phenotype was defined by survival time after injection of 100 parasites (Kyan et al., 2012). It has been suggested the *T. gondii* virulence in mice depends on several factors, including the stage of the parasite, route, dose, types of mice used, host and strain of the parasite (Dardé, 2004; Endrias et al., 2015).

In conclusion, this finding indicates a high overall seroprevalence of *T. gondii* infection in domestic ruminants slaughtered for human consumption. More importantly, the current result showed a high isolation rate of viable *T. gondii* from seropositive domestic ruminants slaughtered for human consumption. However, most of the recovered viable isolates were avirulent. Only one isolate from sheep inoculum was found virulent and four intermediate virulent isolates; one from sheep, one from cattle inoculum and two from goat inoculum. Therefore, the findings of high seropositivity, detection of viable *T. gondii* tissue cysts with some of them a variable degree of virulence coupled with currently increasing trends of beef, mutton and goat meat consumption in the study area signifies the public importance of the disease, particularly in vulnerable groups. Butchers and slaughterhouse workers that handle carcases and organs infected with *T. gondii* are also at risk of getting an infection with toxoplasmosis. Based on the findings of the study, the education of the public about routes of *T. gondii* transmission and control methods is imperative to prevent *T. gondii* transmission to humans. Further advanced diagnostic techniques should be used to identify the genotype and population structure of *T. gondii* strains.

Declarations

Ethics approval and consent to participate

The animal welfare committee of the College of Veterinary Medicine and Animal Sciences, University of Gondar approved the project (Ref. No. O/V/P/RCS/05/1237/2018).

Consent for publication

Not applicable.

Competing interests

The authors have declared that no competing interests exist.

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Authors' contribution

ZST and SD designed the study and read the manuscript. MM collected field samples and compiled the data. DD, AK, and BA conducted data analysis and interpreted the result. MM and AM drafted the manuscript.

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Availability of data and materials

The data supporting the conclusions of this article are included within the article. The data are available from the first author and corresponding authors on reasonable request to be used only within the context of this study.

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Tables

Table 1

Risk factors		Category	No. of samples	No. infected (%)	χ2	*P*-value
Sheep	Sex	Female	49	31 (63.3)	4.55	0.033
		Male	86	38 (44.2)		
	Age	Adult	102	59 (57.8)	7.57	0.006
		Young	33	10 (30.3)		
Cattle	Age	Old	22	19 (86.36)	7.81	0.005
		Adult	128	70 (50.5)		
	Breed	Cross	20	17 (85.0)	6.30	0.012
		Local	130	72 (55.38)		
Goats	Sex	Female	21	12 (57.1)	0.01	0.920
		Male	29	17(58.6)		
	Age	Young	11	5(45.5)	1.60	0.210
		Adult	39	24(61.5)		

Table 2

Sample Source	No. of isolates inoculated	Number of samples induce seropositivity in mice (%)	Number of samples induce cyst positivity in mice (%)	Overall samples being viable in mice during PI (%)
Sheep	16	7(43.8)	7(43.8)	8 (50.0)
Cattle	14	3(21.4)	2(14.3)	3 (21.4)
Goat	9	4(44.4)	3(33.3)	4(44.4)
Total	39	14(35.9)	12 (30.8)	15(38.5)

Table 3

Species	ID	Seropositive/examined mice	Cyst positive/examined mice	Mice dead/No. inoculated mice	Days of mice death PI (No. of mice)
Sheep	Sp13	0/3	0/3	1/4	13(1)
	Sp15	2/4	2/4	1/5	8(1)
	Sp17	2/5	2/5	0/5	All survived
	Sp19	2/4	2/4	1/5	9(1)
	Sp25	2/4	1/4	1/5	21(1)
	Sp26	2/3	2/3	2/5	16(1), 23(1)
	Sp28	1/4	0/4	1/5	30(1)
	Sp35	0/5	0/5	0/5	All survived
	Sp43	0/5	0/5	0/5	All survived
	Sp49	0/4	2/4	1/5	30(1)
	Sp51	0/5	0/5	0/5	All survived
	Sp55	0/5	0/5	0/5	All survived
	Sp62	1/5	3/5	0/5	All survived
	Sp63	0/5	0/5	0/5	All survived
	Sp66	0/5	0/5	0/5	All survived
	Sp67	0/5	0/5	5/5	3(3), 4(2)
Cattle	B10	0/5	0/5	0/5	All survived
	B14	3/3	1/3	2/5	34(1), 36(1)
	B17	0/4	0/4	1/5	26(1)
	B21	0/4	0/4	1/5	35(1)
	B28	0/5	0/5	0/5	All survived
	B35	0/5	0/5	0/5	All survived
	B36	0/5	0/5	0/5	All survived
	B40	0/5	0/5	0/5	All survived
	B48	3/4	0/4	1/5	40(1)
	B52	0/5	0/5	0/5	All survived
	B54	0/5	0/5	0/5	All survived

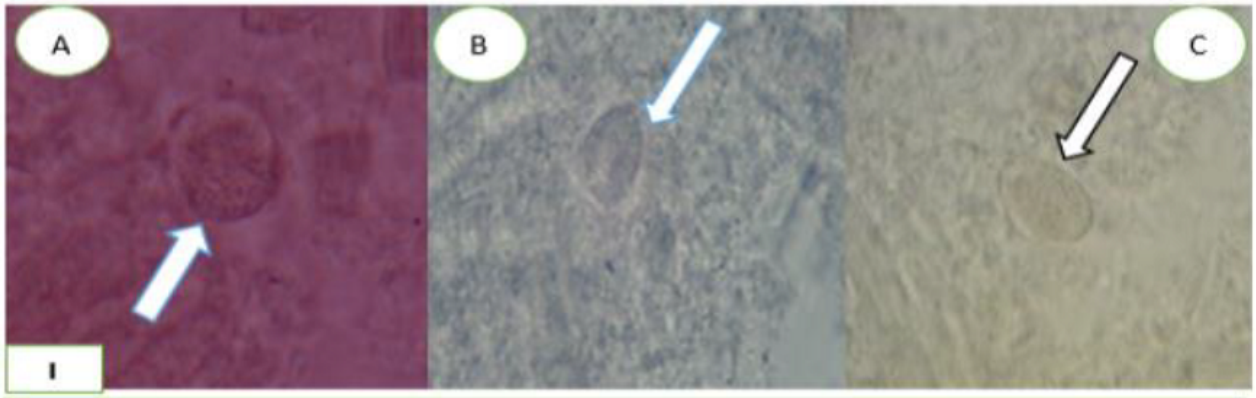
	B62	0/5	0/5	0/5	All survived
	B65	3/3	1/3	2/5	8(1), 9(1)
	B66	0/5	0/5	0/5	All survived
Goat	Gt2	2/5	2/5	0/5	All survived
	Gt4	2/5	0/5	0/5	All survived
	Gt6	1/1	1/1	4/5	3(2), 4(2)
	Gt8	0/5	0/5	0/5	All survived
	Gt25	0/5	0/5	0/5	All survived
	Gt28	1/3	2/3	2/5	17(1), 28(1)
	Gt33	0/4	0/4	1/5	16(1)
	Gt46	0/4	0/4	1/5	25(1)
	Gt48	0/5	0/5	0/5	All survived

Table 4

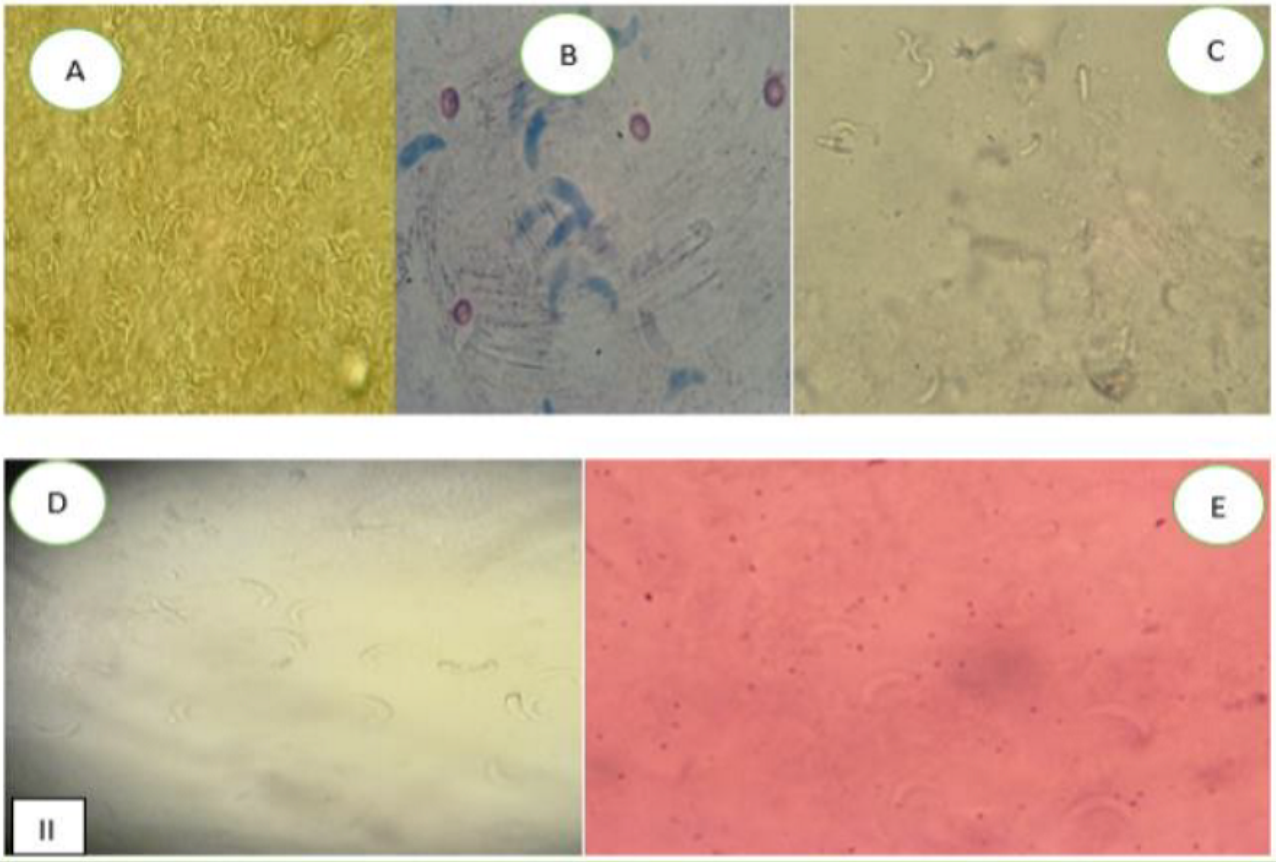
Sample sources	Bio-assayed samples per group of mice		No. cyst-positive mice	Mean cyst count	SE
	Seropositive (n)	Cyst positive (n)			
Sheep (n=16)	7	7	14/66	129.57	36.597
Goats (n=9)	4	3	5/36	278.40	93.801
Cattle (n=14)	3	2	2/63	104.00	21.000
Total	14	12	21/165	162.57	34.840

SE = standard error

Figures



T. gondii tissue cysts from pepsin digested animal heart tissue sample: **A**= from cattle; **B**= from goat; **C**=from sheep. **Note** the thin cyst wall (**arrow**) enclosing



Microscopically detected liberated bradyzoites of *T. gondii:* A= from cattle, B= Giemsa stained from cattle; C= from goat; D and E =from sheep

Figure 1

T. gondii tissue cysts (I) and liberated bradyzoites (II).

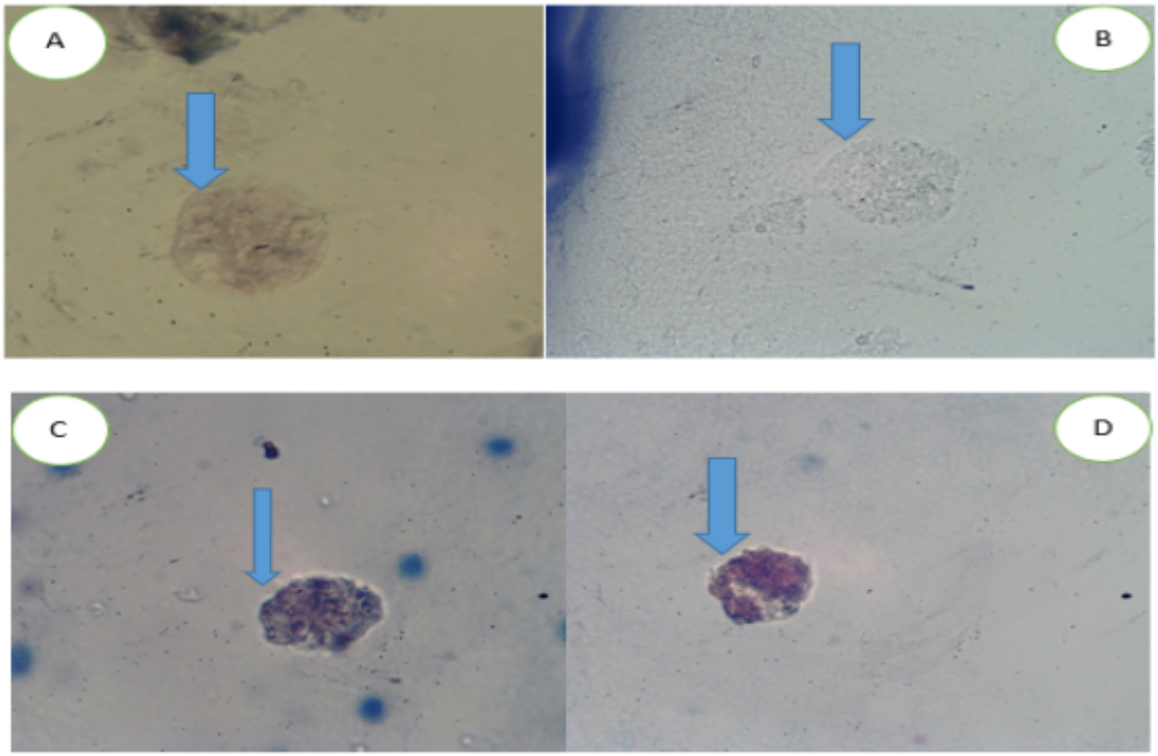


Figure 2

Tissue cysts of T. gondii isolated from the brain of mice: from sheep sample inoculum [A= unstained 40x], goat sample inoculum [B= unstained; C= impression smear 100x], and cattle [D impression 100x smear]. Note the thin cyst wall (arrow) enclosing bradyzoites.