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

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Received: 2 September 2021 / Accepted: 11 January 2022 / Published online: 22 January 2022 © Indian Society for Parasitology 2022

**Abstract** This study investigated the occurrence, isolation and virulence of *Toxoplasma gondii* in slaughtered domestic ruminants in Gondar, Northwest Ethiopia. Three hundred thirty-five blood samples (135 sheep, 50 goats, and 150 cattle) were purposefully collected from abattoirs and slaughterhouses. *T. gondii* antibodies were assessed using a commercial Toxo-Latex agglutination test. Tissue digestion with the pepsin enzyme was also performed on 39 heart muscles of seropositive animals, and viable *T. gondii* was isolated in white albino mice. As a result, the occurrence of *T. gondii* infection was 55.8%. *T. gondii* antibodies were found in 59.3% of cattle, 58% of goats, and 51.1% of sheep. The prevalence of *T. gondii* antibodies in sheep was significantly higher in females ($\chi^2 = 4.55$, $p = 0.033$) and adults ($\chi^2 = 7.57$, $p = 0.006$). Similarly, in cattle, the presence of *T. gondii* antibodies was associated with old groups ($\chi^2 = 7.81$, $p = 0.005$) and cross-breeds ($\chi^2 = 6.30$, $p = 0.012$). The overall viable *T. gondii* isolates in bioassayed mice were 38.5%, and the parasites were isolated from sheep (8/16), cattle (3/14) and goats (4/9) samples, with the majority of these isolates (87.2%) being avirulent. In conclusion, the presence of *T. gondii* antibodies and a high proportion of viable *T. gondii* in this study may indicate the parasite’s prevalence and zoonotic importance in the study area. To plan control strategies, more research on the genotype and transmission dynamics of this parasite is required. Public education about *T. gondii* transmission routes and control methods is critical for preventing *T. gondii* transmission.

**Keywords** Bioassay • Ruminants • Occurrence • Toxo-latex • *Toxoplasma gondii* • Virulence

**Introduction**

*Toxoplasma gondii* is an obligate intracellular parasite capable of infecting all warm-blooded vertebrates, including humans, mammals, and birds (Schlüter et al. 2014). It infects up to 30% of the global human population (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). Felines become infected by consuming tissues containing viable cysts from intermediate hosts (Dubey 2010; Robert-Gangneux and Darde 2012) or, less effectively, by consuming sporulated oocysts (Dubey 2006; Saadatnia and Golkar 2012; Cornelissen et al. 2014; Shapirro et al. 2019; Attias et al. 2020).

Ruminants are thought to be important in the epidemiology of T. gondii infection around the world (Tenter 2009; Dubey 2010). Consumption of infected meat from food animals is a direct source of infection for humans and cats. Toxoplasma infection is common in meat-producing animals in most parts of the world (Tenter 2009; Tonouhewa et al. 2017; Stelzer et al. 2019) and poses 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 (Yang et al. 2012; Al-Kappany et al. 2018). Pregnant women who become infected either have an abortion or have a congenital infection of the fetus. Hydrocephalus, intracranial calcification, and retinocchoroiditis can result from a congenital infection of foetuses (Schlüter et al. 2014; Tonouhewa et al. 2017; Hosseini et al. 2018).

T. gondii infection is common in Ethiopian sheep, goats, and cattle, according to several serological surveys, (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). Little is known, however, about the viability and virulence of T. gondii isolates found in the meat of such seropositive animals. Consequently, this study was conducted: (1) to estimate the prevalence of T. gondii antibodies in slaughtered sheep, goats, and cattle in Gondar City, Northwest Ethiopia; and (2) to evaluate the viability and virulence of a T. gondii isolate using a mouse bioassay.

Materials and methods

Study design and sampling technique

Blood and tissue samples were collected from slaughtered domestic ruminants at the Gondar ELFORA abattoir and four local slaughterhouses in Gondar city using a cross-sectional study design. From November 2018 to June 2019, an experimental follow-up in bioassayed mice was also carried out. For the study, 335 animals (135 sheep, 50 goats, and 150 cattle) were purposefully sampled. The animals’ ages were calculated by looking at their erupting permanent incisors (Taylor 1984; Awgichew and Abegaz 2008). Young, adult, and old were used to categorize and record the approximate ages of the animals sampled. Sheep and goats under the age of one year were classified as young, while those over the age of one year were classified as adults (Awgichew and Abegaz 2008). Cattle over the age of seven were considered old, while those aged four to seven were considered adults (Taylor 1984).

Sample collection and transportation

During exsanguination or intracardially at the slaughter line, 335 blood samples were obtained using plain sterile tubes, each containing roughly 5–10 ml whole blood. For microscopic analysis and bioassay in mice, 200 of the 335 blood samples had matching heart tissue samples (75 from cattle, 75 from sheep, and 50 from goats). Each tissue sample weighed between 50 and 60 g. The samples were labelled and sent to the Veterinary Parasitology Laboratory at the University of Gondar’s College of Veterinary Medicine and Animal Sciences in Gondar, Ethiopia, in a cool box.

Serological assay

To separate the sera, the blood samples were allowed to coagulate in a slant position for a few minutes before being centrifuged at 4000 rpm for 5 min. The serum was then decanted into 1.5 ml Eppendorf tubes. For microscopic analysis and bioassay in mice, 200 of the 335 samples had matching heart tissue samples (75 from cattle, 75 from sheep, and 50 from goats). Each tissue sample weighed between 50 and 60 g. The samples were labelled and sent to the Veterinary Parasitology Laboratory at the University of Gondar’s College of Veterinary Medicine and Animal Sciences in Gondar, Ethiopia, in a cool box.

Tissue digestion and Bioassay in mice

Tissue samples from seropositive animals were digested according to Dubey’s instructions (Dubey 1998). Tissue samples weighing 50 g were chopped and digested for 1 h at 37 °C in a pepsin acid solution (pH 1.1–1.2). After filtering, a one-time neutralization with 1.2% sodium bicarbonate solution (pH = 8.3) was done, followed by centrifugation. The tissue digests were then inspected microscopically at 10 × and 40 × magnification powers to confirm the presence of T. gondii freed bradyzoites and/or tissue cysts. Only the 39 microscopically positive digested...
tissue samples were inoculated subcutaneously into 5 mice per each sample (1 ml suspension per mouse) to determine the survivability and virulence of the cysts recovered. The samples were diluted in 5–10 ml of antibiotic saline solution (1000 IU/ml penicillin and 100 g streptomycin/ml in saline solution) before inoculation into mice. *T. gondii* was isolated from five to six weeks aged female white albino mice weighing 20–25 g. Approximately 5 ml aliquots of the suspension were saved and refrigerated at + 4 °C till they were injected the next day in the same animals (Beltrame et al. 2012). Mice were monitored for 49 days after injection to see if they developed clinical symptoms or died. After anaesthesia with diethyl ether, all survivors were euthanized with cervical dislocation at 49 days, and blood was taken through the heart puncture. The blood was allowed to coagulate for 3 to 4 h and centrifuged for 4 min at 4000 rpm before being pipetted the sera. On the same day, the sera were tested with the LAT for the presence of *T. gondii* antibodies.

To detect the cysts microscopically, the brain of the killed mouse was homogenized in 1 ml phosphate-buffered saline (pH = 7.2) using a mortar and pestle. The number of cysts in each mouse’s brain was calculated by translating the sum of cysts in 30 μl to the total homogenates of the brain (Goodwin et al. 2008; Fritz et al. 2012). If at least one *T. gondii* cyst was found in any injected mouse, the bioassay was judged to be positive or if the LAT reacts positively to at least the sera of the mouse (Dubey et al. 1995). The virulence of the parasite was classified based on mice mortality rate (1995). The virulence of the parasite was classified positively to at least the sera of the mouse (Dubey et al. 2003). The bioassay was judged to be positive or if the LAT reacts positively to at least the sera of the mouse (Dubey et al. 1995). The virulence of the parasite was classified positively to at least the sera of the mouse (Dubey et al. 1995).

**Results**

**Overall occurrence and associated risk factors of *Toxoplasma gondii* antibodies**

Antibodies to *T. gondii* had been found in 55.8% of slaughtered domestic ruminants (95% CI: 50.1–61.2). Cattle had 59.3% (95% CI: 51.4–67.3), goats had 50% (95% CI: 44.0–72.0), and sheep had 51.1% (95% CI: 43–60). Unfortunately, the difference was insignificant.

The prevalence of *T. gondii* antibodies in sheep was significantly higher in females ($\chi^2 = 4.55, p = 0.033$) and adults ($\chi^2 = 7.57, p = 0.006$). Similarly, the presence of *T. gondii* antibodies in cattle was associated with old groups ($\chi^2 = 7.81, p = 0.005$) and cross-breeds ($\chi^2 = 6.30, p = 0.012$) (Table 1). In goats, however, no association was found with either sex or age groups.

**Bioassay in mice**

In mice, 39 positive tissue samples containing *T. gondii* tissue cysts and/or liberated bradyzoites (Fig. 1) were bioassayed (14 from cattle, 16 from sheep, and 9 from goats). *T. gondii* isolates were found in 38.5% of bioassayed mice, and the parasites were found in sheep (8/16), cattle (3/14) and goats (4/9) samples. Table 2 provides a summary of viability data based on serological and cyst positivity in inoculated mice, whereas Table 3 provides detailed data for each tested isolate.

We used a total of 195 mice for seropositivity and virulence testing. However, we lost one mouse at the time of inoculum injection and twenty-nine mice during the follow-up period (post-infection). The seropositivity test and cyst detection were not performed on any lost mouse (due to autolysis). As a result, the overall seropositivity of *T. gondii* antibodies was found to be 16.4% (27/165) in experimentally infected surviving mice from all animal species. *T. gondii* antibody seropositivity was 18.2% (12/66), 16.7% (6/36) and 14.3% (9/63) in experimentally infected mice using inoculum from sheep, goat, and cattle, respectively. *T. gondii* tissue cysts were recovered in 21 experimentally infected mice. Figure 2 depicts some of the tissue cysts observed in inoculated mice.

The overall mean cyst detected and enumerated from experimentally infected mice brains was 162.57 ± 34.840 (mean ± SE) cysts per mouse brain. Overall, mean cyst counts in the brains of mice inoculated with goat heart homogenates (n = 278.1) were higher than those found in sheep (n = 129.57) and cattle (n = 104.0) samples in the bioassay (Table 4).
Virulence of isolates and clinical signs in mice

During the follow-up period, the majority of inoculated mice were asymptomatic. However, 29 mice died before the 49th day (7 from cattle, 13 from sheep, and 9 from goats). More specifically, 9 mice (5 from sheep inoculum and 4 from goat inoculum) died on the third and fourth days after inoculation, whereas 20 mice (8 from sheep, 7 from cattle, and 5 from goat inoculum) died after the fourth day post-infection (pi). In this study, the majority of the recovered isolates (87.2%) were avirulent to mice. One (1/8) highly virulent isolate from sheep inoculum (sp67) was suggested, and all inoculated mice (5/5) were killed on the third and fourth-day pi. Four intermediate virulent isolates were proposed (1/16 sheep, 1/14 cattle, and 2/9 goats) (Table 3). Death, ruffled, stiff, arched back, leg paralysis, tachypnoea, inappetence, rough hair coat, dullness, and neurological signs were observed in symptomatic mice.

Discussion

Occurrence of *T. gondii* antibodies in slaughtered domestic ruminants

*Toxoplasma gondii* antibodies were found to be present in 55.8% of slaughtered domestic ruminants. This finding is higher than the previously reported prevalence of 22.2% in Eastern Ethiopia (Tilahun et al. 2018) and 37% in Tunisia (Lahmar et al. 2015). This disparity could be attributed to the sample size, the age of population studies, and the methods used. Indeed, previous studies used ELISA and DAT techniques, which are less sensitive than the latex agglutination test used in this study.

*Toxoplasma gondii* antibody occurrences were found significantly higher in females and adults of sheep in this study. Previous studies (Ramzan et al. 2009; Gebremedhin et al. 2013, 2014a, b) found a higher prevalence of *T. gondii* antibodies in females and adults. It could be because females are kept for breeding purposes and live for a long time, giving them a higher chance of harbouring the parasite for the rest of their lives. It is also attributed to the increased likelihood of exposure to the source of infection as age increases, implying that the majority of sheep acquire the infection postnatally (Andrade et al. 2013; Opsteegh et al. 2016). In cattle, older and cross-bred animals tested positive for *T. gondii* at a higher rate than adult and local breed cattle. Older animals may have been more likely to be exposed to the infectious agent from various sources because they lived longer (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-bred cattle had a higher risk of *T. gondii* infection than local breeds; this could be attributed to the host’s genetic and immune status.

Bioassay in mice

The current study’s overall viable *T. gondii* isolation rate of 38.5% is lower than the report of Berhanu (2015), which reported 67.6%. It is also higher than 8.57% reported by Elfadaly et al. (2017) in domestic ruminants in Egypt.
The isolation rate of viable *T. gondii* in sheep in this study (50%) is comparable to the report of 57.45% from central Ethiopia (Gebremedhin et al. 2014a) and higher than the findings from France with isolation rates of 26.7% (Dumètre et al. 2006) and 32% from Egypt (Younis et al. 2015).

In contrast, the current result is lower than the report from the United States, which had an isolation rate of 77.9% (Dubey et al. 2008), but higher than the report from Brazil, which had an isolation rate of 19.5% (Ragozo et al. 2008) from MAT seropositive sheep. The current study’s isolation rates of viable *T. gondii* (44.4%) in goats are

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

| 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)                                        |
comparable to the reported isolation rates of 45.45% in central Ethiopia (Gebremedhin et al. 2014a) and 46.15% in Sao Paolo, Brazil (Ragozo et al. 2009). This, however, contradicts the finding of Berhanu (2015), who reported 75% from Eastern Ethiopia, 26% from Brazil, and 62.8% from the United States (Dubey et al. 2011). The differences in these reports could be attributed to the density of *T. gondii* in sheep and goat tissues, the type of tissue sampled, and the strain or genotype of *T. gondii*.

It has been extremely difficult to isolate viable *T. gondii* from cattle tissues. There have been only a few successful tissue cysts recoveries reported (Opsteegh et al. 2011). This

| 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                      |
| Sp36       | 0/5                        | 0/5                        | 0/5                           | All survived                      |
| Sp37       | 0/5                        | 0/5                        | 0/5                           | All survived                      |
| Sp38       | 0/5                        | 0/5                        | 0/5                           | All survived                      |
| Sp39       | 0/5                        | 0/5                        | 0/5                           | All survived                      |
| Sp40       | 0/5                        | 0/5                        | 0/5                           | All survived                      |
| Sp41       | 0/5                        | 0/5                        | 0/5                           | All survived                      |
| Sp42       | 0/5                        | 0/5                        | 0/5                           | All survived                      |
| Sp43       | 0/5                        | 0/5                        | 0/5                           | All survived                      |
| Sp44       | 0/5                        | 0/5                        | 0/5                           | All survived                      |
| Sp45       | 0/5                        | 0/5                        | 0/5                           | All survived                      |
| Sp46       | 0/5                        | 0/5                        | 0/5                           | All survived                      |
| Sp47       | 0/5                        | 0/5                        | 0/5                           | All survived                      |
| Sp48       | 0/5                        | 0/5                        | 0/5                           | All survived                      |
| Sp49       | 0/5                        | 0/5                        | 0/5                           | All survived                      |
| Sp50       | 0/5                        | 0/5                        | 0/5                           | All survived                      |
| Sp51       | 0/5                        | 0/5                        | 0/5                           | All survived                      |
| Sp52       | 0/5                        | 0/5                        | 0/5                           | All survived                      |
| Sp53       | 0/5                        | 0/5                        | 0/5                           | All survived                      |
| Sp54       | 0/5                        | 0/5                        | 0/5                           | All survived                      |
| Sp55       | 0/5                        | 0/5                        | 0/5                           | All survived                      |
| Sp56       | 0/5                        | 0/5                        | 0/5                           | All survived                      |
| Sp57       | 0/5                        | 0/5                        | 0/5                           | All survived                      |
| Sp58       | 0/5                        | 0/5                        | 0/5                           | All survived                      |
| Sp59       | 0/5                        | 0/5                        | 0/5                           | All survived                      |
| Sp60       | 0/5                        | 0/5                        | 0/5                           | All survived                      |
| Sp61       | 0/5                        | 0/5                        | 0/5                           | All survived                      |
| Sp62       | 0/5                        | 0/5                        | 0/5                           | All survived                      |
| Sp63       | 0/5                        | 0/5                        | 0/5                           | All survived                      |
| Sp64       | 0/5                        | 0/5                        | 0/5                           | All survived                      |
| Sp65       | 0/5                        | 0/5                        | 0/5                           | All survived                      |
| Sp66       | 0/5                        | 0/5                        | 0/5                           | All survived                      |
| Sp67       | 0/5                        | 0/5                        | 0/5                           | 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                      |
study in cattle on the isolation rate of viable *T. gondii* (21.43%) via bioassay in mice is comparable to Opsteegh et al. (2016), who demonstrated 3.3% of viable *T. gondii* from selected European countries. The current study’s findings, however, are higher than the reported isolation rates of 0% from Ethiopia in cattle (Berhanu 2015) and 0% from Egypt in cows (Elfadaly et al. 2017) via 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 study and previous reports could be attributed to the density of *T. gondii* in cattle tissues, the type of tissue sampled, the digestion method used, and the strain or genotype of *T. gondii* (Dubey 2010).

*Toxoplasma gondii* antibodies were found in 16.4% (27/165) of experimentally infected and surviving mice from all animal species. This finding is consistent with the findings of Gebremedhin et al. (2015), who found 19.03% seroprevalence in experimentally infected mice with pig heart homogenates. However, it is lower than the 30.58% seroprevalence reported by Zewdu et al. (2015). Furthermore, *T. gondii* tissue cysts were found in the brains of experimentally infected mice at a rate of 12.7%. It is lower than the report of Gebremedhin et al. (2015) and Zewdu et al. (2015), who reported cyst percentages of 19.5% and 28.82%, respectively. In this study, the mean cyst count per mouse brain was 162.57 (mean ± SE = 162.57 ± 34.84). This finding is comparable to a mean tissue cyst count of 157.2 from central Ethiopia (Gebremedhin et al. 2015). It is, however, lower than the 277.97 mean cyst count per brain from central Ethiopia (Gebremedhin et al. 2014a), the tissue cyst counts ranging from 297 to 1380 by Berenreiterová et al. (2011), and the mean number of tissue cysts of 600 by Fritz et al. (2012), but higher than the previous report by Tesfamariam (2013) in free-range chickens from Ada’a Liben, Central Ethiopia with the mean tissue cyst count of 57.4 per brain of a mouse.

*Toxoplasma gondii* cyst burden in mice is not only unassociated to inoculum dose and route, but also the parasite strain inoculated (Waree et al. 2007) or mouse
genotype (Brown et al. 1995; Waree et al. 2007). Furthermore, the number of live bradyzoites in the digested heart tissue of study animals inoculated into mice may contribute to the variation in 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, visual counting of brain cysts in mice with chronic toxoplasmosis may indicate an infectious burden (Bourguin et al. 1993).

Clinical signs and virulence of *T. gondii* isolate in mice

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

Based on mouse bioassay results, the majority (87.2%) of the recovered isolates were avirulent to white Swiss Albino mice. This virulence assessment agrees with previous Ethiopian reports (Gebremedhin et al. 2014a, 2015). One isolate (sp67) from sheep inoculum, on the other hand, was remarkably virulent, killing all inoculated mice (5/5) on the third and fourth days after infection. According to Pena et al. (2008), 100% of death within four weeks of infection indicates that *T. gondii* is highly virulent in mice. Furthermore, 4/5 and 2/5 of mice were killed within four weeks pi due to isolates from goat tissue samples inoculum code (Gt6 and Gt 28, respectively), and 2/5 of mice from sheep (sp26) and cattle (B65) sample inoculum died within four weeks pi. This could be an indication of *T. gondii* strains with intermediate virulence (Cook et al. 2000; Pena et al. 2008). Alternatively, *T. gondii* was isolated from a cow’s intestine homogenate (Dubey 1992). *T. gondii* isolates differ significantly in their virulence to outbred mice, according to Dubey et al. (2002, 2007). Avirulent strains were defined as having no mortality at any dose, whereas a “low-dose survivability” phenotype was defined as the time it took to survive after being injected with 100 parasites (Kyan et al. 2012). *T. gondii* virulence in mice has been linked to various factors, including parasite stage, route, dose, mouse type, host, and parasite strain (Dardé 2004; Zewdu et al. 2015).

In conclusion, this finding suggests that *T. gondii* infection is widespread in domestic ruminants slaughtered for human consumption. More importantly, the current findings revealed a high rate of viable *T. gondii* isolation from seropositive domestic ruminants slaughtered for human consumption. However, the majority of the viable isolates recovered were avirulent. Only one isolate from sheep inoculum was found to be virulent, with four intermediate virulent isolates from sheep, cattle, and goat inoculum. As a result, the findings of high seropositivity, detection of viable *T. gondii* tissue cysts with varying degrees of virulence, and currently increasing trends in beef, mutton, and goat meat consumption in the study area indicate the disease’s public importance, particularly in vulnerable populations. Butchers and slaughterhouse workers who handle *T. gondii*-infected carcasses and organs are also at risk of contracting toxoplasmosis. According to the study’s findings, public education about *T. gondii* transmission routes and control methods is critical for preventing *T. gondii* transmission to humans. To determine the genotype and population structure of *T. gondii* strains, more research with advanced diagnostic techniques is required.

Acknowledgements We would like to acknowledge the Office of Vice President for Research and Community Service, the University of Gondar for its financial support.

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.

Funding The Office of the Vice President for Research and Community Services, University of Gondar, was granted the study (Ref. No. O/V/P/RCS/05/1237/2018). The funding body played no role in the design of the study, collection, analysis, interpretation of the data, or in writing the manuscript or decision to submit the paper for publication.

Data availability 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.

Declarations

Conflict of interest The authors have declared that no competing interests exist.

Ethical approval 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).

Human and animal rights There is no defined law for blood and tissue sample collection in Ethiopia from slaughtered animals and so no consent was obligatory. In this study, we took blood and heart samples after having permission from slaughterhouses. Besides, we used mice for experimental analysis following the guideline on how to use laboratory animals and having permission from the animal welfare committee from the University of Gondar.
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