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RESEARCH ARTICLE

Serological and Molecular Detection of *Toxoplasma Gondii* among Pregnant Women Giving Birth at the University Of Gondar Specialized Hospital in Gondar Town, Northwest Ethiopia

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Abstract

Objective: Congenital toxoplasmosis can be presented when immunosuppressed women are reactivating of this infection acquired before pregnancy in addition to women recently exposed to this disease during pregnancy, which causes of fetal death, neonatal death or congenital defects. Women mainly get this infection by ingested improperly cooked or raw meat consisting of viable tissue cysts or by contaminated foods and water within sporulated oocysts or through the placenta. Thus, this study aimed to detect *Toxoplasma gondii* in pregnant women using serologically and molecularly at study area. **Methods:** A cross-sectional study was conducted from September 2019 to October 2020 by collecting a total of 200 umbilical cord blood and matching discharged placental tissue samples from systematic randomly selected pregnant women giving birth at University of Gondar specialized hospital. Latex agglutination test and nested polymerase chain reaction were used for the detections of this infection. **Findings:** The overall serological, first polymerase chain reaction and nested polymerase chain reaction findings of this study were 46%, 24% and 10%, respectively. The univariant analysis indicated that seropositivity of Toxoplasma gondii antibodies were also significantly influenced by eating raw meat, eating raw vegetables, drinking raw milk, presence of domestic cat contacts and source of water ($P \leq 0.05$). In the case of multivariate analysis drinking raw milk and source of water were also significantly associated to the seropositivity of Toxoplasma gondii. Moreover, there were also fair concordant between latex agglutination within first polymerase reaction (Kappa: 0.290) and

nested polymerase chain reaction tests of this parasite (Kappa: 0.231). **Novelty:** This nested polymerase chain reaction and the Toxo-latex agglutination tests had also fair agreements in pregnant women. Moreover, Consumption of raw meat, vegetables and milk; domestic cat contacts and source of water were the potential statically significant associated risk factors for Toxoplasma gondii seropositivity. **Conclusions**: Generally, these comparative tests confirmed the existence of this infection in pregnant women that played up a pool of public health risks for their embryo and infants. Thus, thoroughly cooking raw meats and vegetables; pasteurized raw milk; avoid domestic cat contacts and boiling of water will be the best prevention and control strategies of this infection. Further works on its genotyping of this pathogen will be also convinced.

Keywords: Latex agglutination; Nested polymerase chain reaction; Placental tissue; Pregnant women; Toxoplasma gondii; Umbilical cord blood

1 Introduction

Toxoplasmosis is a global public and veterinary importance of an obligate intracellular apicomplexan protozoan parasite, caused by *Toxoplasma gondii* (T. gondii) that affects almost all warm-blooded animals including humans and birds [1]. Domestic cats and wild felids are the only main final hosts, and the main role of transmission of this parasite by excreted resistant sporozoites in the environment. However, other non-felids of almost all animals and humans are used as an intermediate host in the transmission of *T. gondii* infection. Humans are primarily infected by ingested undercooked or raw meat consisting of viable tissue cysts or contaminated with food, water and environment with sporulated oocysts shed in the faces of infected cats or congenitally by tachyzoites [2]. Ingestion of cysts within contaminated raw fruits and vegetables can also carry this parasite. Nevertheless, on that point are also occasionally transmission of *T. gondii* infection occurs in the case of tachyzoites contained in organ and tissue transplants, blood transfusion, and drinking unpasteurized milk [3]. Moreover, research lab technicians who work within the contaminated blood are also exposed by accidental inoculations.

Immunocompetent people are asymptomatic in their entire life without reading a clinical sign, but *T. gondii* can induce serious complications and symptoms in immunosuppressed patients that are acquired during pregnancy causing as retinochoroiditis, lymphadenopathy, encephalitis, abortion and death [4]. Transplacental toxoplasmosis can be also occasionally presented when immunosuppressed women are reactivating of this infection acquired before pregnancy in addition to women recently exposed to *T. gondii* during pregnancy, which cause of fetal death, neonatal death or congenital defects. In the case of Human immunodeficiency virus (HIV) and cancer of immune depressed patients, *T. gondii* also the main cause of life-threatening diseases such as encephalitis or death due to the reactivating of latent infection [4].

The diagnosis of *T. gondii* has performed by microscopic, serological, bioassay and molecular methods, or by some combination of these above assays using by either finding this parasite or its deoxyribonucleic acid (DNA) or indirectly by detecting antibodies of different isotypes [5]. It is genotyping has also three common clonal lineages such as type I, II and III depending on their genetic multiform and also within atypical and recombinant species that are spread out in different geographical areas and hosts. Clonal types II and III are the most clonal types that infect both domestic animals and humans in Europe, North America and Africa [6]. While, type I lineage is more causes infection in immunocompromised patients and virulent in mice than type II and III clonal lineage [6,7].

Toxoplasmosis is the most worldwide public heath important parasitic disease among the world health organization (WHO) ranked foodborne diseases which 10.3 million cases have been reported between 2010 and 2015[8]. It is seroprevalence in cats that are found up to 100% in the world, which is the most important for the transmission of this parasite by shedding environmental resistance oocyst in the surroundings of animals and humans. Up to one–third of the human population is also chronically exposed and mostly occurred in humid and warmer areas within the endemicity rate of 10% to 70%. Among African nations, Ethiopia has also highly variable sero-prevalent of *T. gondii* from different agro-ecological areas within their regions in humans[9]. *T. gondii* infection in pregnant women has also reported from 20%–96.7% in different areas of the country[10–12].

The molecular diagnosis within the polymerase chain reaction (PCR) amplified a little quantity of minced dead or alive tissue cysts is the best sensitive; specific and rapidly detecting *T. gondii* at any point of infection. It does not depend on the immune response of the infected hosts which is unlike serological tests. These indicate more or less of the seronegative tests and seropositive tests are positive and negative by DNA detection techniques, respectively. This is imputable to the availability of getting this parasite from a low amount of minced tissue is difficult. Nevertheless, murine bioassays have been used more amount of live representative samples and sensitive than the molecular test for detecting this infection in tissue samples, but it has also expensive and unethically[5]. Therefore, on that point is no elucidating available report about the molecular detection, and its comparison within serological tests of T. gondii to confirm the presence of this infection in the illustration samples from Ethiopia, especially in the work region.

Toxoplasma gondii is the most prevalent and zoonotic significance disease in Ethiopia. It causes reproductive problems in humans, especially in immunosuppressed patients. Moreover, one- third of the human population of the globe is too affected by this parasite particularly in low-income African countries like Ethiopia[13]. There is a little seroprevalence of *T. gondii* reported in humans as easily as there is no previous story of molecular detection of *T. gondii* DNA in tissue samples directly from food animals and humans in Ethiopia, especially in the study area so far. However, the cultural habit of consuming raw or under-cooked meat and milk contain tissue cysts, and any contaminated water, vegetables and fruits within sporulated oocytes shed in the faces of infected cats; the disease is more harbors in tissue than blood for various times; the geographical and climatic change, and the management systems are the predispose factors for the occurrence of this disease in the study site[13]. Therefore, this study aimed to detect *T. gondii* by the joint of serological and molecular assays for confirming its presence in the representative samples. This study would have more conclusive results and forward appropriate prevention and control measures of this disease in animals and humans.

2 Methodology

2.1 Study setting, design and period

The study was conducted from September 2019 to October 2020 in Gondar town, which is the town of the Central Gondar Zone.

Currently, the town has one referral specialized hospital, University of Gondar comprehensive and specialized hospital which is a teaching as well as a referral hospital. It served more than five million peoples.

A cross-sectional study design was employed from September 2019 to October 2020 to determine the seroprevalence and detect DNA of *T. gondii* in pregnant women giving birth at the University of Gondar comprehensive and specialized hospital in Gondar town, Northwest Ethiopia.

2.2 Population and sample

The study subjects were pregnant women visiting and giving birth at University of Gondar comprehensive and specialized hospital during the study time. The sample sources were also influenced by their willingness and accessibility of the delivery women in the study time.

2.3 Sample size and sampling techniques

The sample size was determined by using single population proportion formula of[14]. N= P (1-P) (Z^2)/ d^2 which consists of 88.8% previous expected prevalence of *T. gondii* in Gondar town[15], 95% level of confidence interval (Z= 1.96), 5% desired absolute precision (d) and 30% contiguity of non-response rate. Thus, a total of 200 blood and matching tissue samples were collected from delivery pregnant women for the combined serological and molecular detection of *T. gondii* infection. Systematic random sampling technique was used with sampling interval of three to recruit the delivery pregnant mothers during the study period. The sampling interval consists every third delivery mother attending the hospital was enrolled in to the study until the calculated sample size was achieved during the study data collection period.

2.4 Data collection and laboratory methods

2.4.1 Questionnaire survey

A structured questionnaire format was also prepared and translates into local Amharic version to interview pregnant women to have information on the socio-demographic characteristics and some of the potential risk factors associated with *T. gondii* infection.

2.4.2 Sample collection methods and transportations

Following the interview, approximately 5 ml of umbilical cord whole blood in labeled plain tube during delivery and correspondingly 30 grams (g) of discharged placental tissue in labeled sterile plastic bags and scissors were collected in the delivery pregnant women in University of Gondar comprehensive and specialized hospital. Then, both samples were sent separately within a cold ice box to the Molecular Biology laboratory of the Department of Biotechnology for serological and molecular detection of *T. gondii* infection. The whole blood samples were left for a few hours at room temperature to allow clotting and centrifuged by 4000 RPM for 5 minutes to separate serum and it was placed in Eppendorf tube. Both samples of serum and tissues were stored at -20°C before being utilized for a long time.

2.4.3 Toxo-latex agglutination test from animals' blood

Toxo-latex agglutination test kit was used to assay the serum of the study subjects according to the manufacturer's recommendations (SPINREACT, S.A/S.A. U, GIRONA, SPAIN).

2.4.4 DNA extraction of T. gondii from animals' tissue

About 30 g of discharged placental tissue sample was collected for isolation of the target DNA. The tissue was cut into pieces by knives and crushed by pestle and mortar. It was also weighed 0.02 grams of minced tissues and retained in a labeled Eppendorf tube separately. Then, it was homogenized and digested by lysis buffer and proteinase K. The DNA was precipitated by 110 μl ethanol (96-100%). Then, the mixture was carefully applied to the column and centrifuged at 6000 x g (8000 RPM) for 1 minute. The columns were washed by centrifugation using the buffers WN solution at once time and working solution A (WA) at two times, according to the manufacturer's instruction Kit (Norgen Biotech crop, Thorold, Canada). Finally, the DNA was eluted from the column using 150 μl of the elution buffer B of the kit. The purity and concentration of the extracted DNA were determined by Nano-Drop, and agarose gel (1.5%) also runs to check the quality of the extracted DNA in 40 minutes. Finally, put under -20^{0}C freezer until conducted PCR.

2.4.5 Nested PCR detection of T. gondii DNA

Detection and amplification of *T. gondii* was conducted by using nested PCR assay targeting at the B1 gene. Tissue samples were determined to be PCR-positive for *T. gondii* if this gene is amplified. For specific amplification, designed primers were used, according to the sequence of the external and internal two pair of primers, respectively: TgF1: 5′-TGT TCT GTC CTA TCG CAA CG-3' and TgR1: 5′-ACG GAT GCA GTT CCT TTC TG-3' for first PCR (PCR1) and TgF2: 5′-TCT TCC CAG ACG TGG ATT TC-3'; TgR2: 5′-CTC GAC AAT ACG CTG CTT GA-3' for second PCR (nested PCR)[16].

Briefly, the PCR reaction was done in the same technique on the first and second amplification in a thermocycler by a final volume of 25 μl reaction mixture containing 5 μl of extracting DNA; 2 μl of 10x PCR buffer (without Mg2+); 0.5 μl (20 pmol) of each primer; 2 μl (25mM) MgCl2; 2.5 μl dNTPs mix (20 μM each); 0.3 μl (5U/μl) Taq DNA polymerase and up to 25 μl double distilled water. The PCR conditions included an initial denaturation at 95°C for 5 min; followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 53°C for 45 s, extension at 72°C for 1min, and a final elongation of 72°C for 10 minutes followed by holding at 4°C. However, 2μl of the first amplification products (PCR) as the template DNA and 55°C for 30 s of annealing temperature were employed in the second amplification reaction. The first (PCR1) and second PCR (nPCR) products were run by gel electrophoresis with a 1.5% agarose gel with 1×TAE buffer at 80 volts for up to an hour; and the DNA bands were visualized by staining with ethidium bromide in gel documentation system. By using 100 bp as DNA marker ladders, positive controls (RH strain DNA) and negative controls (nuclease-free water), the amplified fragments of the B1 gene by first PCR and nPCR were approximately at 580 bp and 531 bp, respectively[16].

2.4.6 Data analysis methods

Data was entered and analyzed using SPSS version 20. After data entry, the database was checked against the source documents for completeness. Descriptive statistics (frequencies and percentages) were computed. Moreover, univariate and multivariant logistic regression analysis was used to identify some of the potential risk factors associated with Toxoplasma infection. Variables

with P-value <0.25 by the univariate analysis were entered into multivariant model. The strength of associations was also calculated using odds ratio (OR) at 95% confidence interval (CI) and P-value $\leq$ 0.05 was seen as statistically significant. Agreements between the diagnostic tests were evaluated and interpreted by Dohoo et al. (2003) methods like as interpretation: < 0.2: slight agreement, 0.2–0.4: fair agreement, 0.4–0.6: moderate agreement, 0.6–0.8: substantial agreement, and > 0.8: almost perfect agreement.

2.4.7 Ethical considerations

The ethical clearance letter was also received from the Gynecology and Obstetrics Department of University of Gondar comprehensive and specialized hospital. Oral informed consent was also held from each examined participant before including in this study. This research aims and importance had been distinctly explained by the detective. There were no known risks or discomforts associated with specimen collection from these study women. Ultimately, the research project was sanctioned by the Institute of Biotechnology, Department of Medical Biotechnology. Finally, this project was also approved by the University of Gondar Research and Community Service Core Process Office.

3 Result and Discussion

3.1 Seroprevalence and associated risk factors of *T. gondii* in pregnant women

The seropositivity of *T. gondii* antibodies in pregnant women were 46% (95% CI: 32 – 58). The seropositivity *T. gondii* antibodies were significantly affected by consuming raw or undercooked meat (Crude Odds Ratio (COR): 0.190; P= 0.000); raw vegetables consumption (COR: 0.404; P: 0.005); raw milk consumption (COR: 0.233; P: 0.000); presence of domestic cat's contacts (COR: 0.472; P: 0.020) and source of water (COR: 0.251; P: 0.003). Further analysis using multivariate analysis drinking raw milk (AOR: 0.276; P: 0.018) and source of drinking water (AOR: 3.499; P: 0.002) were also significantly associated risk factors with the seropositivity of this disease. However, there were no other risk factors that influenced the seroprevalence of *T. gondii* antibodies in women giving birth at University of Gondar comprehensive and specialized hospital (Table 1).

Table 1. Results of bivariable and multivariable Binary logistic regression of factors associated with Seroprevalence of *T. gondii* among pregnant women at University of Gondar comprehensive and specialized hospital, Gondar, Ethiopia, 2022

Variables	Categories	N	Seroprevalence (%)	COR (95% CI)	P -value	AOR (95% CI)	P-value
Resident	Urban	152	64 (42.1)	1 1.925 (0.997-3.717)	0.051	1 0.562 (0.244-1.294)	0.176
	Rural	48	28 (58.3)				
Eating raw meat	No	156	84 (53.8)	1 0.190 (0.083-0.436)	0.000	1 2.393(0.678-8.447)	0.175
	Yes	44	8 (18.2)				
Eating raw vegetables	No	136	72 (52.9)	1 0.404 (0.216-0.756)	0.005	1 1.042 (0.422-2.572)	0.929
	Yes	64	20 (31.2)				
Drinking of raw milk	No	111	68 (61.3)	1 0.233 (0.128-0.427)	0.000	1 3.499 (1.575-7.764)	0.002
	Yes	89	24 (27)				
Domestic cats contacts	No	140	72 (51.4)	1 0.472 (0.251-0.887)	0.020	1 0.839 (0.329-2.137)	0.713
	Yes	60	20 (33.3)				
Contact to garden soils	No	157	77 (49)	1 0.557 (0.276-1.122)	0.101	1 1.031 (0.383-2.780)	0.951
	Yes	53	15 (34.9)				
Blood transfusion	No	153	76 (49.7)	1 0.523 (0.265-1.034)	0.062	1 1.607 (0.674-3.830)	0.285
	Yes	47	16 (34)				
History of abortion	No	142	61 (43)	1 1.525 (0.825-2.816)	0.178	1 0.510 (0.248-1.046)	0.066
	Yes	58	31 (53.4)				
Source of water	Pipe	134	56 (41.8)	1 0.251 (0.099-0.635)	0.003	1 0.276 (0.095-0.800)	0.018 0.035
	Well	39	16 (41.10)	0.243 (0.083-0.711)	0.010	0.266 (0.078-0.909)	
	River	27	20 (74.1)				
Total		200	92 (46)				

NB: N= Total number, COR= Crude odds ratio, AOR= Adjusted odds ratio, Cl= Confidence interval

3.2 The molecular detection of *T. gondii* in pregnant women

Besides the serological assay for anti-Toxoplasma antibodies, molecular assays using first PCR and nested PCR were performed to confirm the presence of *T. gondii* DNA specific to the B1 gene at 580 bp and 531 bp, respectively (Figure 4). The first PCR detection of DNA of *T. gondii* in pregnant women was estimated to be 24% (95% CI: 14 -36%). To undertake nested PCR with the first PCR product as the template, and a master mix was prepared to detect the DNA of *T. gondii* was found 10% (95% CI: 8 – 20%) (Figure 1).

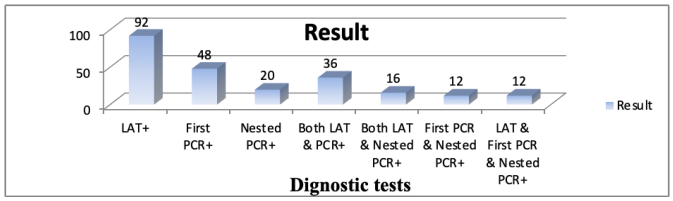

Fig 1. Detection of *T. gondii* among pregnant women giving birth at University of Gondar comprehensive and specialized hospital.

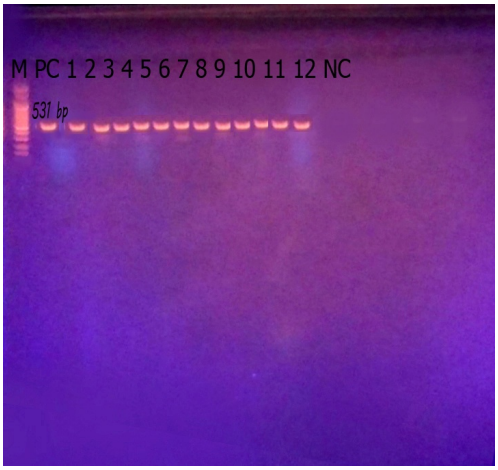

Fig 2. Genomic DNA purity determination by using 1.5%agarose gel electrophoresis (Note: M: 100 bp DNA marker: PC: positive control;first PCR positive of *T. gondii* DNA targeting at B1 gene (580bp) in pregnant women (lane 1-8) and NC: negativecontrol)

The overall LAT, first PCR and nested PCR detection of *T. gondii* infection were recorded as amount 92 (46%), 48 (24%) and 20 (10%) from the total 200 examined pregnant women, respectively. Among these findings, 36 (18%), 16 (16%) and 12 (14%) in women were both LAT and first PCR; LAT and nested PCR, and first PCR and nested PCR positive records by the statistical analysis methods, respectively. However, 56 LAT positive results were again negative in first PCR detection whereas 12 first PCR positive results were too negative by LAT of this infection. In 60 LAT positive results were returned negative by nested PCR (Table 2).

Table 2. Qualitative comparison of LAT and PCR tests results in pregnant women

LAT	First PCR			Nested PCR			First PCR and Nested PCR		
	-Ve	+Ve	Total	-Ve	+Ve	Total	-Ve	+Ve	Total
Negative	96	12	108	104	4	108	96	12	108
Positive	56	36	92	60	32	92	92	80	92
Total	152	48	200	164	36	200	188	12	200

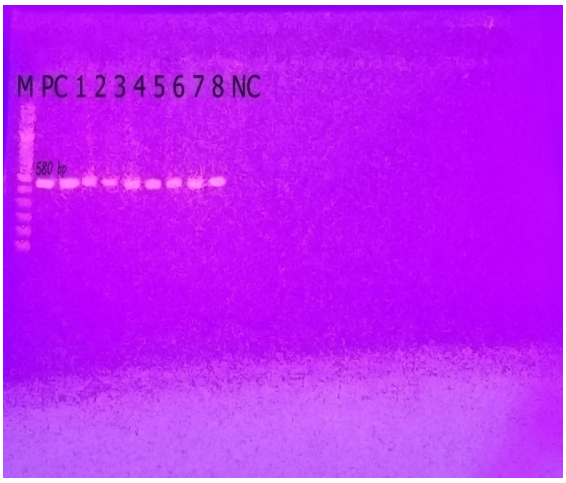

Fig 3. Genomic DNA purity determination by using 1.5%agarose gel electrophoresis (Note: M: 100 bp DNA marker: PC: positive control;nested PCR positive of *T. gondii* DNAtargeting at B1 gene (531bp) in pregnant women (lane 1- 12) and NC: negativecontrol)

3.3 The agreement results among LAT and PCR methods of *T. gondii* detections

The present diagnostic tests of *T. gondii* among pregnant women giving birth at University of Gondar comprehensive and specialized hospital were significantly fair correlation among LAT verses first PCR; LAT verses nested PCR and first PCR verses nested PCR were also fair concordant as indicated in Table 3.

Table 3. Agreement results among LAT, First PCR and nested PCR

Diagnostic tests	Pregnant women		
	Kappa	P- value	Interpretation
LAT vs first PCR	0.290	0.000	Fair agreements
LAT vs nested PCR	0.231	0.000	Fair agreements
First PCR vs nested PCR	0.247	0.000	Fair agreements

NB: LAT: Latex agglutination test; PCR: polymerase chain reaction; Kappa: Kappa value or coefficient.

The overall seroprevalence of *T. gondii* in pregnant women was found to be 46% (95% CI: 32-58). This finding was agreed within the early stories from Ethiopia [17]; Ghana [18]; Iran [19]; Libya [20]; Mexico [21]. Nevertheless, the present finding was also lower than other previous reports in Ethiopia [11] and in Saudi Arabia [22]. In contrast, it was also higher than the earlier various reports across the world [23,24]. These variations among reports might be attributed to the agro-ecological variation and climate change which are important for the survival and sporulation of oocysts in a given environment. Moreover, the culture and eating habits of humans, the presence of domestic cats, sample size and study methods, host factors, the virulence of this parasite, the type diagnostic test used and inadequate hygienic practices are also important factors that need to be considered [13,25].

Raw meat consuming women (COR= 0.190; P=0.000) were 0.81 times less exposed to *T. gondii* infection than those who did not consume. This coincides with the old reports in Ethiopia [11,26]. However, this also contradicts with the various reports from Ethiopia [24]. On the other hands, consuming unpasteurized milk was also 0.77 times (OR= 0.233; P= 0.000) less affected by this parasite than those who had not taken. This corroborates earlier report in Ethiopia. However, this contradicts other authors' findings in various countries from Ethiopia [23,24]. These claimed that consuming raw or undercooked meat (Kurite) is the master roots of *T. gondii* infection in humans, especially in pregnant women, and drank of unpasteurized milk might acquire Toxoplasma when the cows are asymptomatically affected by this parasite. Lack of personal hygiene during minced and preparing meat by knives as well as inadequate ranges of cooking the meat might also be acquiring this infection in pregnant women [13]. Not only pregnant women, it is also vertically transmitting to the young through the placenta.

Women consumed raw vegetables were 0.6 times less acquired *T. gondii* infection than those who didn't consumed. This finding constitutes the earlier reports from Ethiopia [12]. However, it was also contradicting the previous findings from Ethiopia, who stated that consumed raw vegetables were insignificant effect in seropositivity of women with *T. gondii* infection. This might be due to unwashed or uncooked vegetables and fruits, which may be contaminated with soil or cat faces, before consumption

had acquired this infection. Ingestion of cysts within contaminated raw fruits and vegetables can also carry this parasite [27].

Domestic cat contacts woman was 0.53 times less likely acquire *T. gondii* infection than those who hadn't cat contacts. This finding constitutes with Ethiopia [11,12]. However, it was also contradicting from Ethiopia [28]. This might reasonable to domestic cats and wild felids are the only main final hosts, and the main role of transmission of this parasite by excreted resistant sporozoites in the environment. Women who drank pipe water was 0.75 times less exposed the seropositivity of *T. gondii* compared to those who drank well and rivers source water. This finding constitutes with Ethiopia [12]. However, it was also contradicting the previous studies conducted from various region of Ethiopia [10,11]. Drinking of water contaminated with sporulated oocysts derived from the environment or (less frequently) directly from feline faeces could be transmit this infection.

The overall first PCR and nested PCR *T. gondii* DNA findings were found in 24% (95% CI: 14-36) and 10% (95%: CI 8-20) in pregnant women, respectively. These findings were lower than the present serological findings of 46% in pregnant women. These might be attributed to the molecular assay is the best sensitive and specific tests of any stages of this disease in the hosts. Whereas the serological tests lack sensitivity and specificity and it depends on the anti-Toxoplasma antibodies production following the infected hosts [29]. Moreover, the synergistic effect of the serological and molecular tests has more importance to confirm the presence of this parasite in biological samples. This also ascribed to the consumption of one cyst containing hundreds of bradyzoites is enough for a cat to be invaded which might also be acquiring this infection to their intermediate hosts [3].

The agreements of LAT verses first PCR findings (Kappa: 0.290); and first PCR verses nested PCR (Kappa: 0.247 were had fair agreements. These also attribute to the nested PCR technique is more sensitive and specific copies of the long DNA segments by applying two sets of primers than the conventional PCR, which are applied in two successive PCR within generating DNA products that have intended target site and non-specifically amplified DNA fragments in the first reaction rather than the second reaction [29]. These concordance results are influenced by the presence of false-positive results due to cross contamination. It is not totally removed risks in this best sensitive test of nested PCR for the diagnosis of *T. gondii* in practical investigations even if too performed at controlled areas. Moreover, nested PCR also takes more detailed knowledge of the target sequence of this disease [5].

Molecular findings of *T. gondii* DNA among pregnant women were also found in 24% in the first PCR and nested PCR (10%) of *T. gondii* targeting at B1 gene, which are varied from the previous molecular detection of *T. gondii* DNA in line with 10.5% - 28.6% [24,30]. However, these results were lower than the former reports of 50% in Libya [20]; 100% in placental tissue from Wasit province [31]; 41.7% (PCR), and 86.7% (nested PCR) in Iran [32]. On the other hand, these were also higher than the reports of 0.4% from Iran [35], and 6.74% in blood samples from Wasit province [32]. These variations of the above current and the previous reports might be attributed to the types of PCR and its targeting genes of *T. gondii* detection. These also due to the variation of study timing consists of IgM seropositivity in acute cases have a more PCR positive case in tachyzoites from blood samples, while, later leaving to placental tissue during the chronic phase also consists of IgG positivity cases that have more PCR positive *T. gondii* DNA from bradyzoite in women [31]. Moreover, These the present and above previous molecular findings speculated due to insufficient sample size; the low amount of minced placental tissue used; the cat density; lack of personal hygiene and management factors; the nature of this parasite occurred in the environment without desiccation, the culture of consuming raw or undercooking meat; the immune status of the women and the virulence nature of this parasite [13].

Finally, this study was limited by shortages of chemicals and reagents, and sampled small size population numbers, which might not a representative sample. This study did not also further genotyping of *T. gondii* in these positive study subjects due to lack of time and budget allocations, and the presence of COVID-19 pandemic.

4 Conclusion

The current synergistic of serological and molecular assays have confirmed the existence of *Toxoplasma gondii* occurrence in pregnant women giving birth at the University of Gondar comprehensive and specialized hospital in Gondar town. These highly seropositive women might be the risk for their fetus or newborns through placenta. Nevertheless, the nested polymerase chain reaction is the most precise diagnosis of Toxoplasma gondii at any stage of this disease than the first round of polymerase chain reaction and latex agglutination tests. This nested polymerase chain reaction and the Toxo-latex agglutination tests had also fair agreements in pregnant women. Moreover, Consumption of raw meat, vegetables and milk; domestic cat contacts and source of water were the potential statically significant associated risk factors for Toxoplasma gondii seropositivity.

5 Declarations

5.1 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).

5.2 Authors' contribution

TY: Conceptualizations of the study, Methodology, validation, Statistical analysis coordinates data collection. MI, TB, MD, ZS and NB performed the statistical analysis, software, and supervision. The author(s) read and approved the manuscript.

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