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Molecular characterizations of *Toxoplasma gondii* among pregnant women attending antenatal care at central Gondar zone public hospital, Northwest Ethiopia

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ABSTRACT: ***Toxoplasma gondii*** **parasite that causes severe clinical problems such as congenital toxoplasmosis is a major public health problem that affects one-third of the world's populationand is also associatedwith a high socioeconomic impact for pregnant women. Molecular characterizations for genotyping using PCR for the detection of *T. gondii* genotyping *were* grouped into three subtypes, designated I, II, and III, based on polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP).This study aimed to determine molecular characterizations of *T. gondii* among pregnant women attending Anti-Natal Care (ANC) at Central Gondar Zone Public Hospitals (CGZPH), Northwest Ethiopia. Between 2022 and 2024, a cross-sectional study design was conducted to determine the molecular characterizations of *T. gondii* among pregnant women attending ANC at CGZPH. In this study, We determined the B1 and Surface antigen 2(SAG2) genotypes of *T. gondii* in pregnant women using PCR-RFLP. The genotyping data were coded for all genetic loci. Our research provides baseline information essential for planning and implementing control and prevention strategies, thereby enhancing the knowledge and epidemiology of toxoplasmosis. The study revealed that the *T. gondii* population in the Central Gondar Zone is predominantly represented by type II strains, which are most commonly associated with human toxoplasmosis. The use of PCR-RFLP at the SAG2 and B1 loci proved to be efficient method for rapid genotyping.**

KEYWORDS: ***Toxoplasma gondii,*** **Molecular characterization, Pregnant women, Ethiopia**

INTRODUCTION

Toxoplasma gondii is a global human zoonotic disease, affecting one-third of the global population. It is an obligate intracellular coccidian protozoan parasite, responsible for the development of toxoplasmosis [1]. Infecting warm-blooded species, it has a complex life cycle, undergoing a sexual phase in the definitive host and an asexual phase in intermediate hosts [2].

Molecular methods, such as PCR, are crucial for detecting and analyzing *T. gondii* DNA, aiding in diagnosis and understanding its epidemiology. These methods can identify *T. gondii* isolates and identify the source of infection. Genotyping of *T. gondii* isolates plays a key role in epidemiological studies in identification of the source of infection and correlation between genotype and disease and in studies of the population biology. Historically *T. gondii* was considered to be clonal with low genetic diversity and consists of three clonal lineages, However, recent studies have revealed a greater genetic diversity of *T. gondii*, particularly in isolates found in both animals and humans [3]. From the three main genotypes of *T. gondii*: I, II, and III. Among these, type II strains are more prevalent in humans and animals. However, type I strains exhibit the highest virulence and cause severe infections in humans. Type III is more common in birds[4]. The consequences of infection depend on parasite genotypes and host species. Infection can range from asymptomatic to severe acute toxoplasmosis, with symptoms ranging from mild flu-like symptoms to severe acute toxoplasmosis [5, 6].

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Severe toxoplasmic retinochoroiditis is often caused by mostly type I variants, causing symptoms like headaches, confusion, seizures, respiratory issues, and blurred vision in individuals with damaged immune systems. Latent toxoplasmosis, characterized by mild symptoms, causes lesions in various structures [7]. *Toxoplasma gondii* pathogenicity is determined by the strain's virulence, host species susceptibility, and host reaction. Type I isolates lethality towards out bred mice, while type II and III isolates have lower levels [5]. Type II and III lineages predominate and readily establish chronic infections in animals and humans, Primary infection in humans is subclinical, and during pregnancy, it can lead to miscarriage or congenital infection, causing complications like retinochoroiditis, neurological impairment, and fetal death [8, 9].

Congenital toxoplasmosis, a common human disease, is associated with geographically varying strains, with type I, atypical, or recombinant strains being the most common. Virulence occurs in immature fetuses and immunocompromised individuals, leading to asymptomatic infections [10].

Type II isolates are present in various congenital illnesses, necessitating early screening and genotyping detection for toxoplasmosis in pregnant women, with low, moderate, and high-risk factors observed [11]. This study investigates *T. gondii* molecular characteristics among pregnant women at Central Gondar Zone Public Hospitals, providing baseline information for control and prevention strategies and improving toxoplasmosis epidemiology.

MATERIALS AND METHODS

Study design and setting

Between 2022 and 2024, a cross-sectional study design was conducted to determine the molecular characteristics of *T. gondii* among pregnant women were positive for ELISA testing. After the enrolment of 301-positive toxoplasmosis pregnant women from attending ANC at Central Gondar Zone Public Hospitals the molecular characteristics were performed. This zone was home to nine public hospitals.

Sample collection and processing

Venous blood was collected from each study participant using standardized procedures. To obtain serum samples, the collected blood was centrifuged at 3000 revolutions per minute (rpm) for 5 minutes. The serum samples were then frozen at -70°C for long-term storage. During DNA extraction, the serum samples and extraction kits were stored at -20°C to ensure their preservation for PCR preparation.

DNA extraction

From the serum samples, DNA extraction was performed using molecular methods based on PCR for the specific detection or analysis of *T. gondii*. The process involved elution, centrifugation, and elution at ambient temperature (15-25°C). The samples were equilibrated, and buffer AE or distilled water was prepared. The QIAGEN protease was applied to the bottom of a micro centrifuge tube, and 200 µl of samples were added to the tube. The sample volume was adjusted based on the volume. The sample was then vortexes for 15 seconds, and the DNA was incubated at 56°C for 10 minutes. The DNA yield and purity were unaffected by full-speed centrifugation. To improve DNA yield, the QIAamp Mini spin column was placed in a new 2 ml collection tube and centrifuged at maximum speed for one minute. For samples containing less than 1 µg of DNA, elute in 50 µl of Buffer AE or water. DNA purity was evaluated using a Nano Drop ND1000 and stored at -20°C until use.

PCR

The process was carried out in two stages. Initially, the first stage involved utilizing PCR to pinpoint a specific DNA region through the amplification of the B1gene. The PCR master mix was prepared. To avoid contamination, we used negative control that contained all the matrix mix but not the DNA part. From the seronegative samples, we used one sample as a negative control and one positive control that constituted of DNA as part of *T. gondii* RH strain. The first cycle took 15 min at95°C to activate the enzymes. In this first cycle, pairs of primers (BIR1 5′ - CCTTGACGTAGGCAAGTACTC-3′ and B1F1 5′ - GGAACTGCATCCGTTCATGAG-3′) were used to apply PCR. It involved 40 repeated cycles with every cycle consisted of 3 steps: denaturation for 30 s at 95°C, annealing for 30 s at the same temperature (at similar temperature according to the type of the Primer used), and extension for 30 s at 72°C. At the end of these cycles, a final extension was done for 10 min at 72 °C. These steps were applied in both stages of the PCR and the nested-PCR. The second stage entailed a nested-PCR, which was performed to amplify the products of the first PCR cycle to obtain clear DNA. This was done by using 1 µl of the PCR products of the first cycle (diluted at 1:10) with the rest of PCR Master Mix contents plus the primers B1R2 5′ - ACGTATCCAACGTCAGTGAC-3′andB1F2 5′ TGCATAGGTTGCAGTCACTG-3′ at 60 °C for 30 s

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Genotype analysis

To conduct the genotype analysis, we first processed the positive samples identified using nested PCR for the *T. gondii* B1 gene. This was done to amplify the SAG2 (surface antigen of the intracellular *T. gondii*) gene at both the 3' and 5' ends. This two-step amplification process allowed us to thoroughly analyze the genetic material and identify specific genotypes of *T. gondii* present in the samples**.**We used a PCR reaction mix that contained 5µl of buffer PCR X 10 (100µlTrisHCL+15 µmolMgCl$_2$+500 µmolKCl) and added to it 1µmolMgCl2, 500µmol dNTPs, 5µmol from each unit of F and R primers Taq DNA polymerase enzyme, and 100 nanogram of genomic DNA. Initial stages involved PCR testing for 10 min at a temperature of 95 °C, followed by 40 cycles (each cycle composed of three steps: denaturation for 60 s at a temperature 95 °C, annealing for 60 s at a similar temperature of 65 °C according to the type of primer used, and extension for 60 s at a temperature of 72 °C). The primers used to amplify the 5′ end of the SAG2 gene were SAG2F4 5′-GACCTCGAACAGGAACAC-3′ and SAG2R4 5′ -CTGGAGCTTGTCCTTGTG-3′.The nested PCR test was performed by adding 1 µl (diluted 1/10), and a second amplification of 40 cycles was performed with the internal primers SAG2F15′-GAAATGTTTCAGGTTGCTGC-3′ and SAG2R25′-TAAGAGTACGGAGGCGAAG-3′ by using 1 ml of the diluted product as the template.Sau3AI restriction analysis of the 5′ amplification products from type I, II, and III strains. Products were resolved in 25% agarose gels stained with ethidium bromide at an annealing temperature of 65 °C, and the restriction fragments were analyzed by agarose gel electrophoresis. The amplified fragments were purified with Gene Clean and digested with Sau3AI endonuclease enzyme by adding 1.5 µl of SA buffer with 2 µl of Sau3AI l enzyme, 10 µl of the PCR product of 5′ end SAG2 genes, and 11.5 µl of distilled water. The digestion was conducted at a temperature of 37 °C for 3 hours. For the amplification of the 3′ end of the locus, it was similarly analyzed with the primers SAG2F35'-TCTGTTCTCCGAAGTGACTCC-3 and SAG2R35'AGACAAGAGGCTTCACTGAGG-3 for the initial amplifications and the internal primers SAG2F25'-ATTCTCATGCCTCCGCTTC-3 and SAG2R1 5'-CTTTACAAAGTCCAACGACG-3′ for the second round of amplification at an annealing temperature of 65°C65. The amplified fragments were purified with Gene Clean and digested with HhaI endonuclease enzyme by adding 1.5 µl of SL Buffer with 2 µl of HhaI enzyme, 10 µl of the PCR product of 3′ end SAG2, and 11.5 µl of distilled water. The digestion was conducted at a temperature of 37 °C for 3 hours. Products were resolved in 25% agarose gels stained with ethidium bromide at an annealing temperature of 65 °C, and the restriction fragments were analyzed by agarose gel electrophoresis using three types of *T.gondii genotypes as reference standards and as a positive control* by using polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP).

Data management and analysis

Data were checked for completeness before entering for analysis. Then, the data were coded and entered into SPSS (version 21). The data were described using frequencies and percentages. For these analyses, PCR-RFLP typing data was coded for all genetic loci. For a given locus, the presence or absence of DNA restriction fragments was coded.

RESULTS

Participants characteristics

This study examined the distribution of *T. gondii* genotypes (types I, II, and III) across different categories of the 301 pregnant woman samples analyzed,appears that we've provided a table with various categories and counts related to genotyping, age, residence, pregnancy stage, cat feces, dog presence, uncooked fruit or vegetables, and uncooked meat Table 1.

Genotyping of *T. gondii* among pregnant women

The current study examined the molecular characteristics of 301 *T. gondii*-positive samples from pregnant women who had previously tested positive on a *T. gondii* ELISA test. We used a nested-PCR technique targeting two different genomic regions, B1 and SAG2, to genotype the *T. gondii* strains present in the samples. Analysis of the positive samples from pregnant women revealed the following *T. gondii* genotypes: Type II strains were the most common, found in 178 (59%) of the 301 samples. Type III strains were detected in 87 (29%) of the samples. Type I strains were the least common, present in only 36 (12%) of the samples. Prior to genotyping, all 301 samples had tested positive for the *T. gondii* parasite through PCR analysis of human blood serum samples. This study provides insights into the distribution of *T. gondii* genotypes infecting pregnant women in the study population, with type II being the predominant strain detected.

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B1 Gene and the nested PCR

Based on the DNA gel electrophoresis, the first cycle of PCR amplification of the *T. gondii* B1 gene produced a DNA fragment of 196 base pairs (bp) in length. This corresponds to the expected size of the B1 gene fragment amplified by the PCR process. This indicates that the initial PCR step successfully amplified the target *T. gondii* (Figure 1).The nested-PCR test performed on the positive samples (second cycle) yielded the expected 97 base pair (bp) fragment. Based on this the nested-PCR step, which involves a second round of more targeted PCR amplification, successfully generated a 97 bp amplicon from the original positive samples. The nested-PCR technique is commonly used to increase the specificity and sensitivity of the genetic analysis, by using a set of inner primers that target a smaller region within the initially amplified sequence. This allows for more accurate identification and differentiation of the target organism of *T. gondii*. The fact that the nested-PCR produced the expected 97 bp fragment indicates that the protocol was effective in selectively amplifying the desired *T. gondii* genetic target from the positive samples. This 97 bp amplicon could then be further analyzed, such as through RFLP analysis, to determine the specific *T. gondii* genotype present in each sample (Figure 2).

Table 1. The distribution of *T. gondii* genotypes (Type I, Type II, and Type III) across different categories

Category		Genotyping			Total
		Type I	Type II	Type III	
Age	16-20	7	13	5	25
	21-25	6	42	16	64
	26-30	12	71	42	125
	31-35	6	35	12	53
	≥36	5	17	12	34
	Total	36	178	87	301
Residence	Rural	14	48	21	83
	Urban	22	130	66	218
	Total	36	178	87	301
Pregnancy stage	1st triminister	2	34	13	49
	2nd triminister	10	46	28	84
	3 rd triminister	24	98	46	168
	Total	36	178	87	301
Cat feces	Yes	14	75	40	129
	No	22	103	47	172
	Total	36	178	87	301
Dog present	Yes	15	80	36	131
	No	21	98	51	170
	Total	36	178	87	301
Uncooked fruit or vegetables	Yes	34	167	76	277
	No	2	11	11	24
	Total	36	178	87	301
Uncooked Meat	Yes	8	48	35	91
	No	28	130	52	210
	Total	36	178	87	301

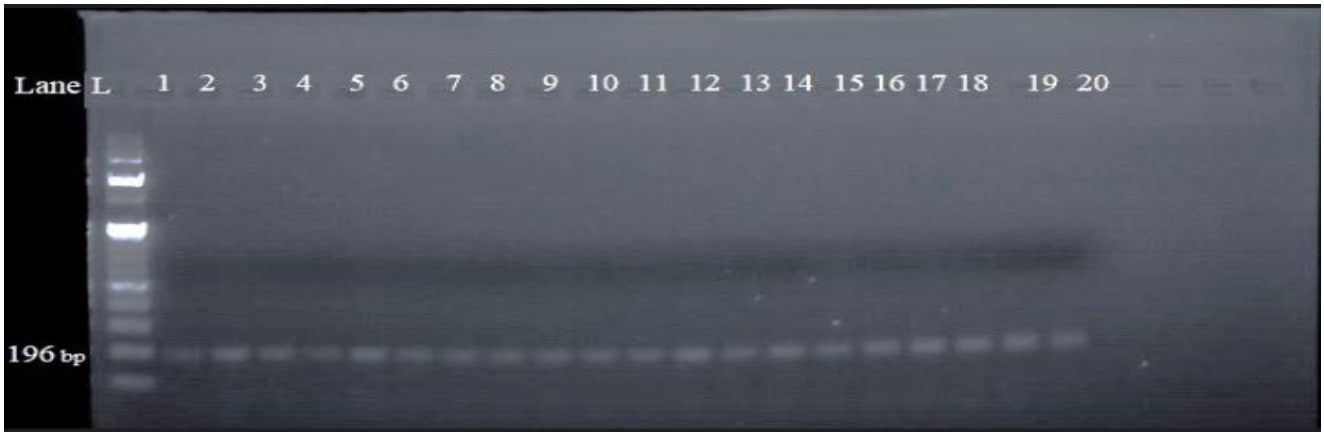

Figure 1. Amplification of *T. gondii* B1 gene Lane 1: Positive control. Lane 2-20: positive samples: Molecular weight marker 100 bp

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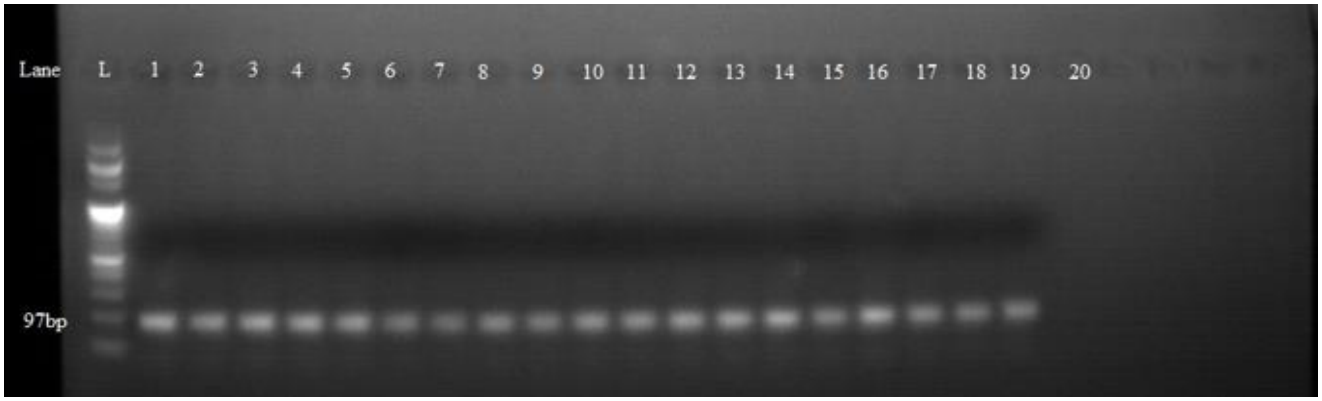

Figure 2. Nested PCR on B1 gene Lane 1: positive control. Lane 2-19 positive samples . Lane 20: negative control. Molecular weight marker 50 bp

SAG2 Gene and the nested PCR

The first amplification of the SAG2 locus, targeting both the 5' and 3' ends, in the *T. gondii* positive cases produced the expected 341 base pair (bp) fragment. The SAG2 gene is a commonly used genetic marker for the identification and genotyping of *T. gondii*. By amplifying specific regions within the SAG2 locus, we obtain DNA fragments that can be further analyzed to determine the *T. gondii* genotype present in the samples. In this case, the initial PCR amplification of the SAG2 locus, covering both the 5' and 3' ends, successfully generated the anticipated 341 bp fragment. This suggests that the PCR primers and conditions were able to efficiently target and amplify the desired SAG2 sequence from the *T. gondii*-positive samples.

The production of the expected 341 bp amplicon is an important first step in the genotyping process, as it confirms the presence of the *T. gondii* genetic material and provides the necessary DNA template for subsequent analysis, such as RFLP, to determine the specific *T. gondii* genotype (Figure 3).

For the confirmed *T. gondii* cases, the nested-PCR test (second cycle) targeting the SAG2 locus at both the 5' and 3' ends produced the expected fragment sizes of 241 bp and 221 bp, respectively. The nested-PCR technique is used to increase the specificity and sensitivity of the genetic analysis. In this case, the nested-PCR step was performed on the positive samples identified in the initial SAG2 locus amplification. The nested-PCR targeting the 5' end of the SAG2 locus yielded the expected 241 bp fragment, while the 3' end amplification produced the anticipated 221 bp fragment. These fragment sizes are consistent with the known sequence characteristics of the SAG2 gene in *T. gondii*. The successful generation of these expected fragment sizes from the nested-PCR confirms the presence of the specific *T. gondii* genetic targets and indicates that the nested-PCR primers and conditions were able to selectively amplify the desired regions of the SAG2 locus. This provides reliable genetic material for further analysis, such as RFLP, to determine the *T. gondii* genotype in the confirmed positive samples (Figures. 5–6).

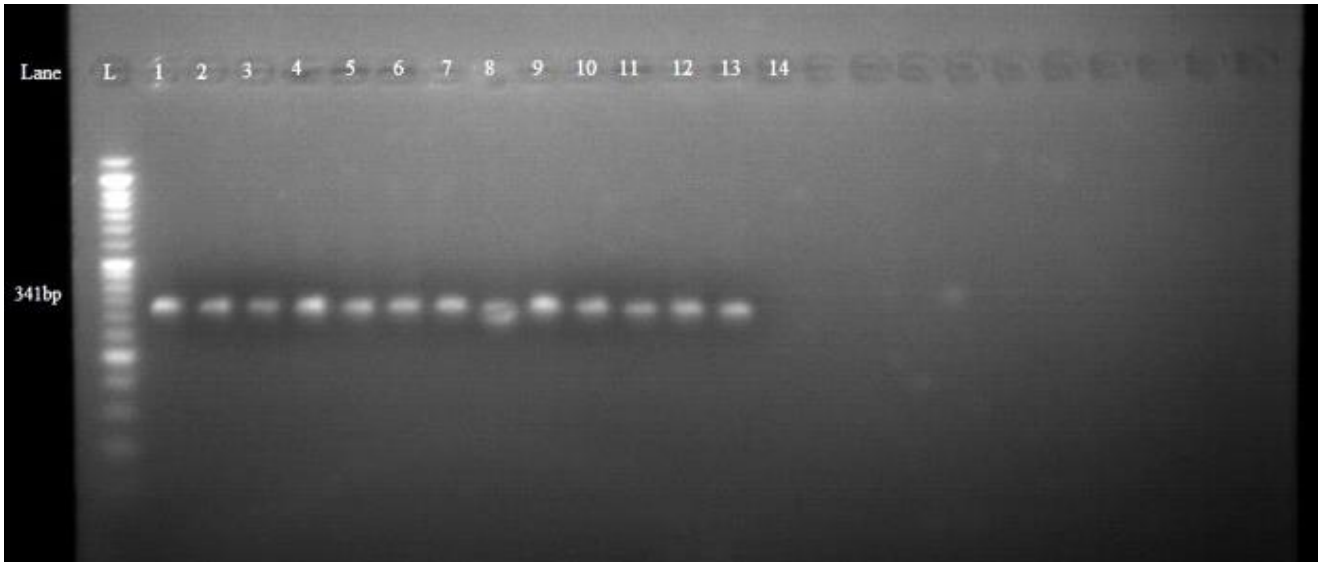

Figure 3. First amplification of the SAG2 locus in the 5'. Lane 1: positive control. Lane 2 - 13 positive samples Lane 14: negative control. Molecular weight marker 50-bp

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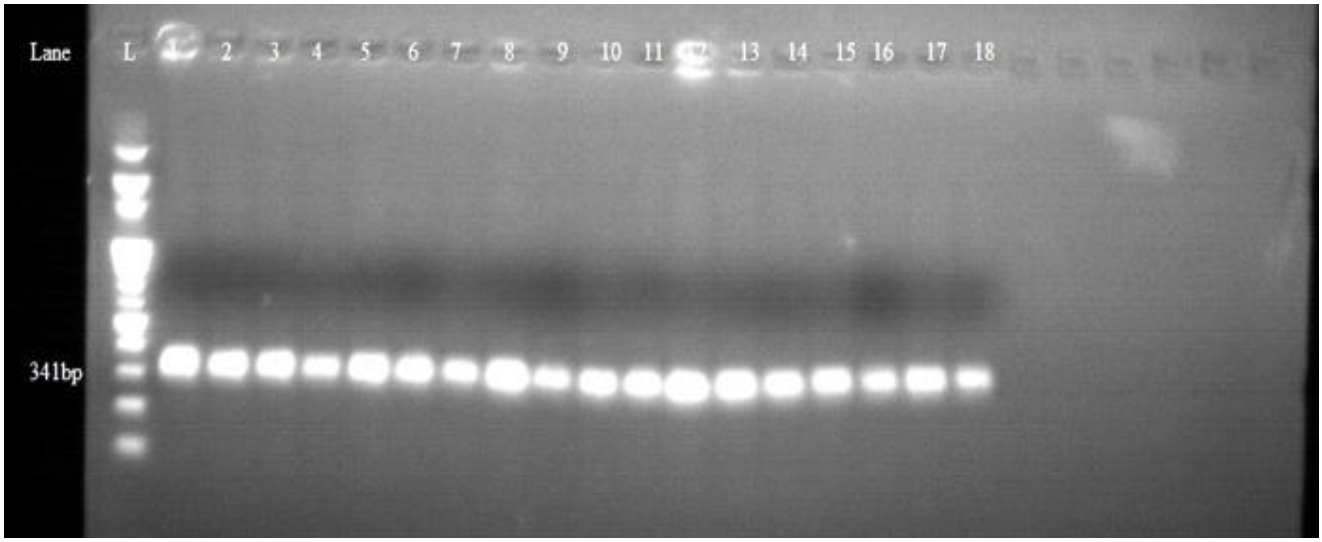

Figure 4. First amplification of the 3' ends of SAG2 locus. . Lane 1: positive control. Lane 2 – 18 positive samples Lane 20: negative control. Molecular weight marker 100-bp

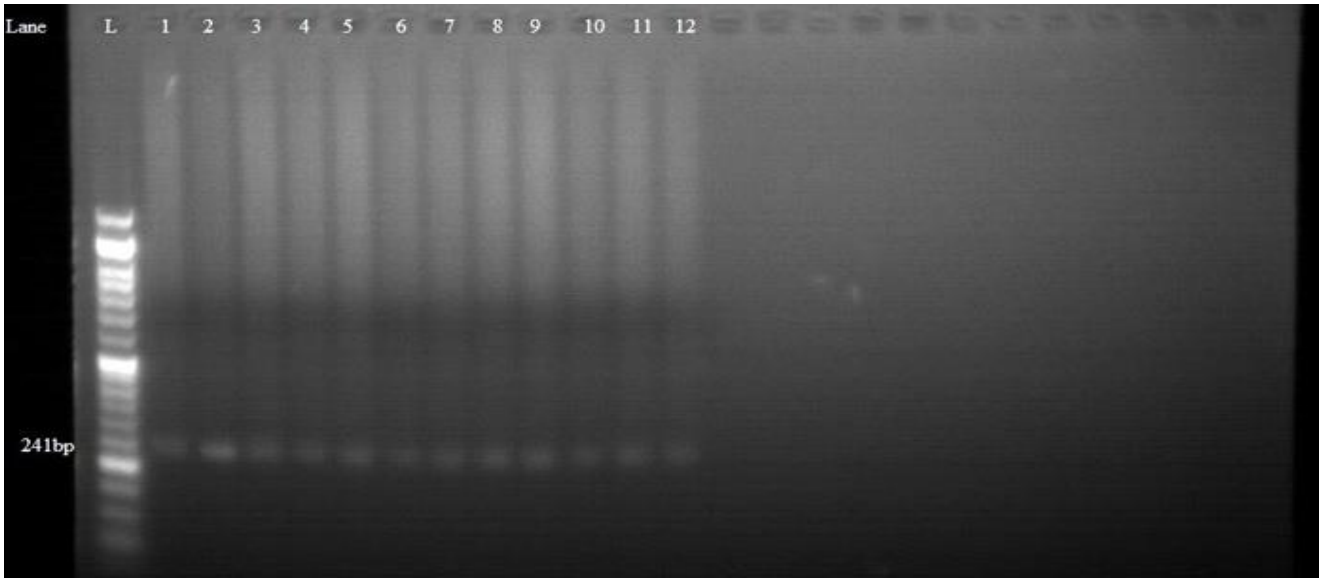

Figure 5. Second amplification of the 5'of SAG2 locus. Lane 1: positive control. Lane 2 – 12 positive samples. Molecular weight marker 50-bp

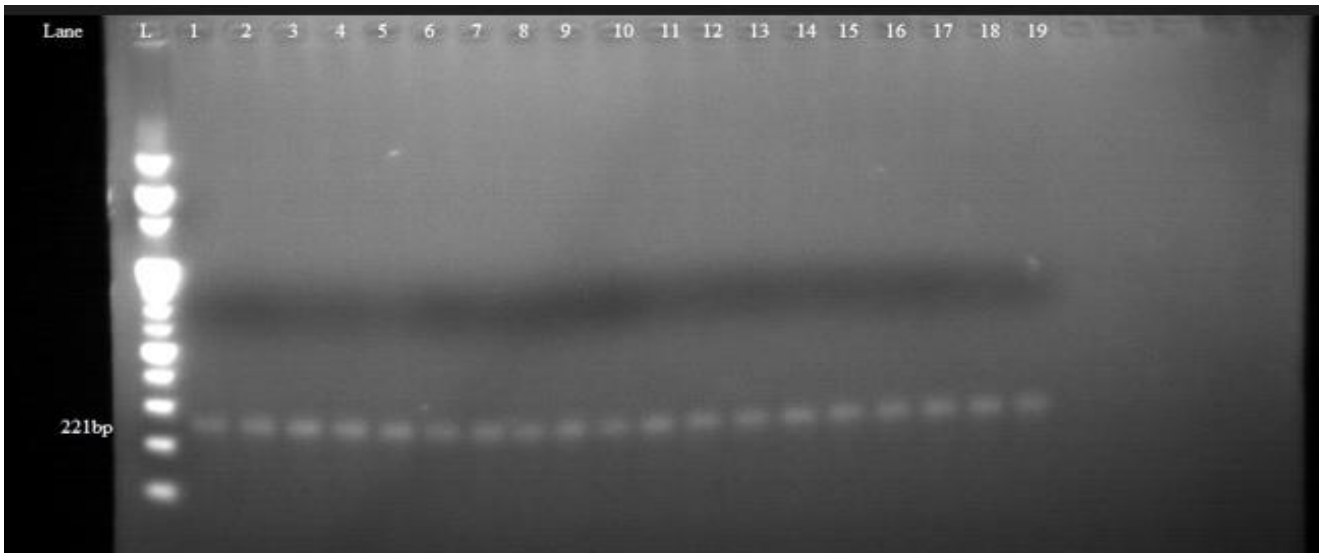

Figure 6. Second amplification of the 3' ends of SAG2 locus Lane 1: positive control. Lane 2 – 19 positive samples. Molecular weight marker 100 bp

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PCR-RFLP analysis

This genetic typing approach allowed the differentiation of the *T. gondii* populations present in the analyzed samples, providing insight into the distribution and prevalence of the different *T.gondii* genotypes. The genotyping was performed using PCR-RFLP, with the results determined through agarose gel electrophoresis. The samples analyzed were separated into distinct populations, which were then identified and classified into one of the three primary *T. gondii* lineages (Type I, Type II, and Type III) using PCR-RFLP analysis of the amplified B1 and SAG2 gene products. The genetic analysis workflow involved: Initial PCR amplification of the *T. gondii* B1 gene, producing a 196 bp fragment, nested-PCR of the B1 gene, yielding the expected 97 bp fragment. Amplification of the SAG2 locus, generating the anticipated 341 bp fragment at both the 5' and 3' ends nested-PCR of the SAG2 locus, resulting in the expected 241 bp and 221 bp fragments for the 5' and 3' ends, respectively. By analyzing the specific fragment sizes obtained from the PCR and nested-PCR steps, we able to classify the *T. gondii* strains present in the samples into the three primary lineages (Type I, Type II, and Type III) using PCR-RFLP techniques. The 3' end that was digested was characterized as type II (Figure 7) digested using the enzyme HhaI; however, we were able to determine that it is type II because the digestion process was apparent enough.

Toxoplasma gondii samples were further digested and classified into types I and III based on RFLP analysis. Specifically, the 5' end of the SAG2 locus was simply amplified and then digested using the restriction enzyme Sau3AI. This RFLP analysis of the 5' SAG2 region allowed differentiating between typesI and III *T. gondii* lineages. The use of RFLP analysis on the amplified SAG2 locus is a common technique for *T. gondii* genotyping. By digesting the PCR-amplified genetic material with specific restriction enzymes, Sau3AI in this case, the resulting fragment patterns can be used to assign the *T. gondii* samples to the appropriate lineage (types I, II, or III).

The RFLP analyses of the 5′ SAG2 region, the remaining samples were successfully classified as either type I or type III *T. gondii* strains. This genotyping information is valuable for understanding the distribution and prevalence of the different *T. gondii* genotypes within the studied population (Figure 8).

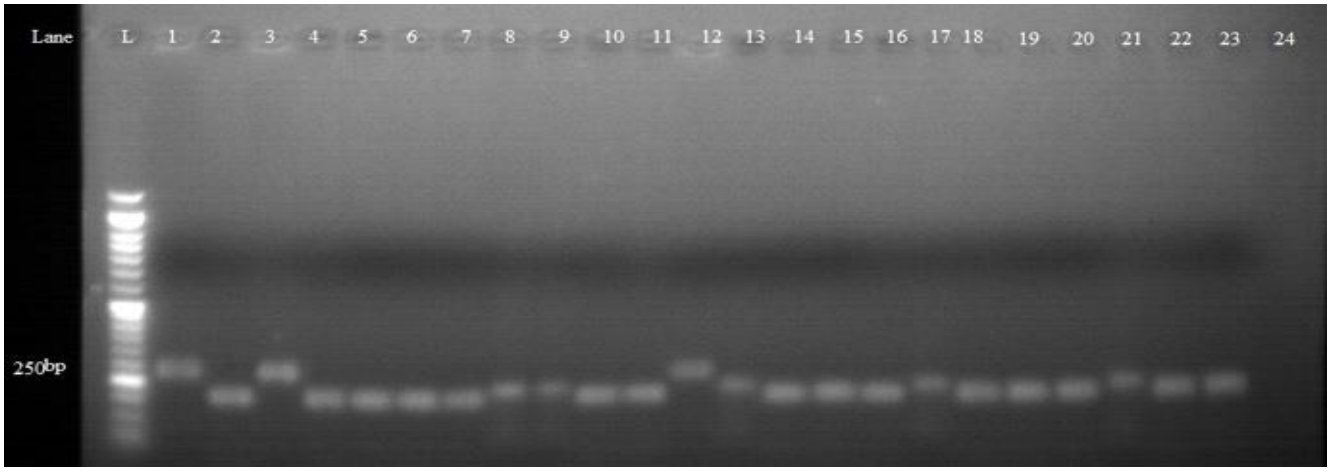

Figure 7. HhaI Restriction digestion of the 3' ends amplification products. Lane 1, 8, 9, 13, 17 and 21: type I. Lane 2,4–7, 10, 11,14,15,16,18,19,20,22,23: Type II. Lane 3, 12: Type III

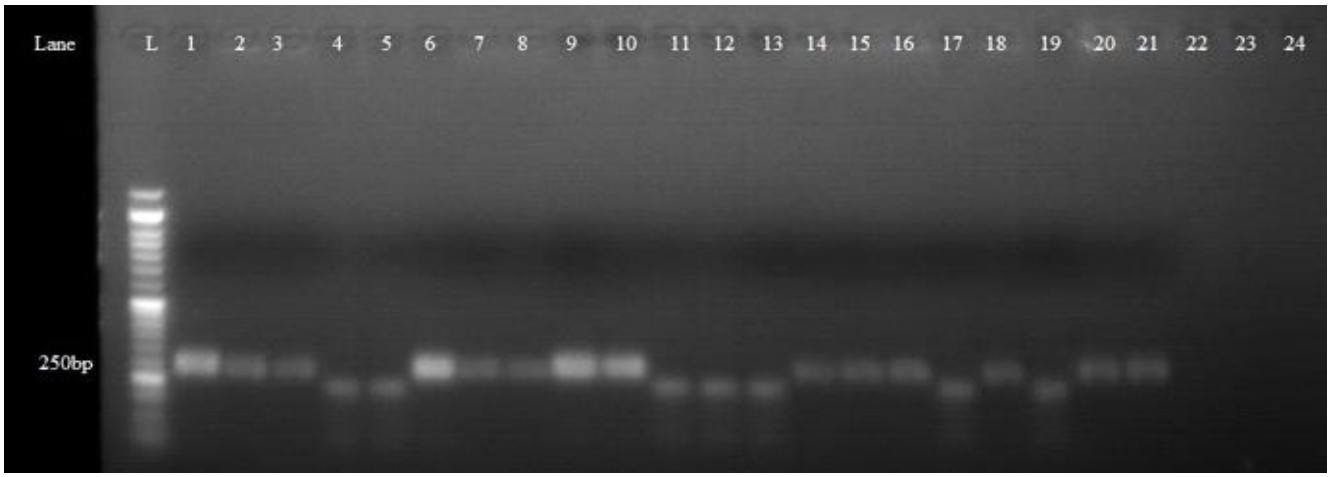

Figure 8. Sau3AI Restriction digestions of the 5'amplification products. Lane 1, 6, 9, 10: type I strain. Lane 2, 3, 7, 8, 14-16, 18, 20, 22, 23: Type II strain. Lane 4, 5, 11-13, 17, 19, 21: Type III strain.

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DISCUSSION

In our investigation, 301 *T. gondii*-positive samples from pregnant women were genotyped using PCR-RFLP. Our research revealed that type II strains were the most common, accounting for 178 (59%) of the samples, followed by type III infections in 87 (29%), and type I strains in just 36 (12%). Toxoplasmosis in pregnant women is frequently caused by immune suppression and the reactivation of tissue cysts, which can result in serious and life-threatening diseases such as congenital toxoplasmosis, toxoplasmic encephalitis and even death. In this study; we describe a rapid and effective methodology for detecting the genotype of *T. gondii* strains. Nested PCR amplification of the B1 and SAG2 genes, followed by RFLP analysis, enabled the assignment of all samples to one of three distinct *T. gondii* lineages. Type II strains of *T. gondii* were most commonly found in these samples, providing additional evidence that this genotype causes the bulk of toxoplasmosis in humans. PCR was previously utilized to detect *T. gondii* in clinical samples from toxoplasmosis patients [2]. According to various investigations, the *T. gondii* genotype varies across the world's continents. Type II strains of *T.gondii* are most common in human samples from America and Europe [12]. *Toxoplasma gondii* Type II strains have low pathogencity but significant cyst-forming ability [11].

Our investigation revealed that has a high percentage of *T. gondii* genotype type II (59%) In this study, we describe a rapid and effective methodology for detecting the genotype of *T. gondii* strains. By using nested PCR amplification of the B1 and SAG2 genes, followed by restriction fragment length polymorphism (RFLP) analysis, we were able to assign samples to one of three distinct *T. gondii* lineages: Type I, Type II, and Type III. Notably, Type II strains of *T. gondii* were found to be the most common in these samples, providing additional evidence that this genotype is responsible for causing the bulk of toxoplasmosis cases in humans. The use of PCR to detect *T. gondii* in clinical samples from toxoplasmosis patients had been employed previously, but this new approach offers improved capabilities. Most researchers have used the B1 or SAG1 genes for the detection of *T. gondii* [13, 14]; however, these loci are not sufficiently polymorphic to enable strain typing. Consequently, we chose to design a nested PCR assay based on the more polymorphic SAG2 gene. This gene is good for fast genotyping since it has several lineage-specific polymorphisms. The SAG2 gene encodes two distinct versions of the surface tachyzoite protein p22, which are recognized strain-specific monoclonal antibodies. Importantly, types I and III *T. gondii* strains share the same protein allele [15, 16].

In addition to the distinction between Type I, II, and III strains based on the SAG2 gene [5, 17], Prior research on 106 *T. gondii* strains using multilocus genotyping has revealed that this parasite has a highly clonal population structure, with only three primary lineages identified (types I, II, and III) [5, 10, 11] . Significantly, we found that there are only three distinct alleles present at the SAG2 gene locus, each corresponding to the Type I, II, and III strain types, respectively.

This limited allelic diversity at the SAG2 marker underscores the utility of targeting this polymorphic gene for rapid and effective genotyping of *T. gondii* isolates [18, 19].

As a result, based on the limited allelic diversity observed at the SAG2 gene, identifying the specific allele present provides high confidence in determining the *T. gondii* strain genotype [20]. In this investigation, the researchers first isolated parasites from blood samples obtained from patients. They then carried out nested PCR detection targeting the SAG2 locus on these same samples, confirming the presence of *T. gondii* in 301 cases of human toxoplasmosis. Significantly, Type II strains were discovered in nearly 178 (59%) of all cases, making them far more common than types I or III strains. This finding complements prior research indicating that Type II *T. gondii* strains are the genotype most commonly associated with human toxoplasmosis [21, 22]. Some type II strains of *T. gondii* can cause toxoplasmic encephalitis in infected individuals; these characteristics may result in a higher chronic cyst load, which could then favors the reactivation of the infection [23]. However, it remains unclear why type II strains are so frequently associated with human congenital toxoplasmosis, which is caused by primary infections in the pregnant mother that can then be transmitted across the placenta to the unborn fetus. The high prevalence of type II strains observed in human toxoplasmosis cases may simply reflect the predominance of these strain types among the parasite populations that are responsible for causing human infection. Previous studies have found that chronic Toxoplasma infections in both domestic and wild animal hosts are similarly distributed between types II and III strain types [12, 24].

The previous findings highlighted a shift in the understanding of *T. gondii* epidemiology. Historically, the Type I strain was believed to be the predominant genotype causing human disease. However, the newer studies with expanded strain sampling have revealed that type II strains are in fact much more common in human toxoplasmosis cases. This suggests that food animals, such as livestock raised for human consumption, may be serving as a major reservoir for the Type II strains that are infecting people [25]. Chronic Toxoplasma infections are widespread in many agricultural animals, and the prevalence of the Type II genotype in these animal hosts appears

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to correlate with its increasing incidence in human populations[26]. The epidemiological relationship is likely driven by opportunities for cross-transmission, such as through consumption of undercooked meat or inadvertent ingestion of oocyst shed in animal feces. As our food production and distribution systems have become more industrialized and globalised, the potential for zoonotic transmission of these parasites has likely increased. Understanding this dynamic is important for improving public health interventions and food safety measures to limit human exposure to the more virulent Type II strains of *T. gondii*. Further research is still needed to fully elucidate the complex ecology and transmission pathways involved [27].

The nested SAG2 PCR technique described is highly sensitive and allows for the rapid, unambiguous determination of a *T. gondii* parasite genotype through RFLP analysis. As a result, this method should be applicable for detecting toxoplasmosis in primary clinical samples [13, 28]. Another difference exists in the genotyping of *T. gondii*. Furthermore, assumptions about *T. gondii* global evolutions have sparked a worldwide effort to examine genetic variation within this fascinating creature vary Asian and African countries show low genetic diversity. However North and South America exhibit a wide diversity of *T. gondii* strains. It has been described as a parasite with little genetic variation and a clonal population pattern [3, 29]. However, molecular analysis has shown that *T. gondii* isolates exhibit substantial genotypic diversity, particularly in North America and Europe, where the majority of isolates fall into three predominant lineages known as Types I, II, and III. This worldwide effort to examine the genetic variation within *T. gondii* has challenged prior assumptions about its global evolution. The identification of these marked genotyping differences is a significant development in our understanding of this fascinating parasite [30].

In our analysis, the Type II genotype was the most common *T. gondii* strain identified. This aligns with the findings from most other studies, which suggest the high prevalence of Type II strains in humans is due to their widespread occurrence in animal reservoirs [6, 31]. Genotyping can provide important insights into the origins and sources of Toxoplasma infections. This data help researchers better understand the etiology and epidemiology of *T. gondii*, as well as guide the development of preventive measures. It is well established that Type II strains account for the majority of human toxoplasmosis cases examined in North America and Europe [32, 33]. However, the use of PCR-RFLP genotyping approaches has rarely uncovered recombinant *T. gondii* strains that reflect combinations of the three clonal lineages (around 5% of isolates) [5, 34, 35]. These recombinant genotypes, sometimes described as "atypical," "unusual," "non-archetypal," or "exotic" strains, display diverse mixes of the classical allelic types [8, 9]. While most *T.gondii* genotypes appear to be geographically localized, some strains have been found across multiple continents and are closely related; suggesting the recent widespread dissemination of certain pandemic genotypes. This underscores the complex global epidemiology of this ubiquitous parasite [10, 11, 35].

CONCLUSIONS AND RECOMMENDATIONS

The study conducted in Central Gondar presents the first data on *T. gondii* genotyping among pregnant women, revealing a predominance of Type II strains associated with human toxoplasmosis. These findings could have important public health implications, as the potential for drug resistance or immune evasion in these predominant lineages is a concern that warrants further investigation. This study provides valuable epidemiological information on *T. gondii* identification and genotyping, which can serve as a foundation for future molecular studies in this area. To build upon these initial findings, further large-scale genotyping studies with subsequent gene sequencing are recommended. This would allow confirmation of the current results and help detect any potential genetic diversity within the *T. gondii* populations in the study region. Expanding our understanding of the circulating *T. gondii* genotypes, particularly in vulnerable populations like pregnant women, is crucial. This information can inform public health strategies for the prevention, diagnosis, and management of toxoplasmosis, a disease with significant implications for maternal and fetal health. Continued research efforts in this area are warranted to fully elucidate the epidemiological patterns and clinical relevance of *T.gondii* genotypic diversity.

DECLARATIONS

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Correspondence and requests for materials should be addressed to Eden Woldegerima; E-mail edengem14@gmail.com: ORCID: https://orcid.org/0009-0002-7704-6341 We authors declare that agree to its

publication after any amendments arising from the peer review, agree to the posting of the full text of this work on the web page of the journal and to the inclusions of references in databases available on the internet

Authors' contributions

EW Meressa wrote the proposal in addition to contributing to conceptualization, data curation, data collection (investigation), data analysis, validation, visualization, resources, software and manuscript drafting. MB Atanaw, MMB, MA Eshetu, FK Abebe, AE Admasu, ML Kebede, TS Melese and NB Tessemma were involved in the conceptualization proposal's design (drafting), data gathering, methodology, resources, validation, visualization, supervision, Writing review and editing and analysis. EW Meressa took part in the data preparation and analysis. All authors read and approved the final manuscript.

Ethics, approval, and consent to participate

Ethical clearance was obtained from the Research and Ethical Review Committee of the University of Gondar with a reference number of VP/RTT/05/280/2022. A permission letter was also provided. Each pregnant woman who participated in the study was informed about the study's purpose, methodology, expected benefit, and potential risks. Participants were also informed to their right to refuse participation or withdraw from the study at any time without any consequences. Because the study was cross-sectional study, participation did not entail any negative consequences for the study participants. Written informed consent was obtained from each participant after explaining the study details.To ensure confidentiality, anonymous typing was applied; participant names and other identifiers were not recorded on the questionnaires. The data collected was used solely for the study's purpose.Pregnant women who tested positive for toxoplasmosis were contacted by phone and referred to ANC (antenatal care) for treatment and better patient management.

Consent for publication

Not applicable.

Availability of data and materials

For the sake of maintaining patient confidentiality, the raw data will not be shared. Data supporting this research article are available from the corresponding author or first author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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List of abbreviations

ANC: Anti-Natal Care; AOR: Adjusted Odd Ratio; CNS: Central Nervous System; COR: Crude Odd Ratio; CT: Congenital Toxoplasmosis; ELISA: Enzyme-Linked Immuno Sorbent Assay; FAT: Immuno Fluorescence Assay Test; IgA: Immunoglobulin A; IgE: Immunoglobulin A; IgE: Immunoglobulin G; IgM: Immunoglobulin M; LAT: Latex Agglutination Test; nPCR: Nested PCR; PCR: Polymerase Chain Reaction; PCR-RFLP: Polymerase Chain Reaction Restriction Fragment Length Polymorphism; qPCR: quantitative real time PCR;RDT: Rapid Diagnostic Test; SAG: Surface antigen

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