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Treatment outcomes for gestational toxoplasmosis in India with an emphasis on periconceptional infection in a prospective study

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Congenital toxoplasmosis (CT) may lead to severe foetal complications when *Toxoplasma gondii* infection is acquired during pregnancy. This prospective study—the first of its kind from India—investigated serological responses in 340 antenatal women, focusing on infection timing, particularly the periconceptional period, and assessed treatment outcomes in acute gestational toxoplasmosis. Diagnosis of gestational toxoplasmosis was based on combined IgM and IgG ELISA with IgG avidity testing to confirm acute infection; treatment of presumptive positive cases was initiated on spiramycin. Amniocentesis, being an invasive and risky technique, was done only in patients with abnormal ultrasound findings and positive serology. Confirmation of CT was done by positive quantitative PCR (qPCR) on amniotic fluid, and patients were switched to pyrimethamine and sulfadiazine. PCR was also used to test placental tissue from these patients at the time of delivery. All mothers and neonates were followed up for one year postpartum. Serological screening identified gestational toxoplasmosis in (35/340), 10.29% of participants, who were started on spiramycin. Of the 16 patients who underwent amniotic fluid PCR, 6 tested positive; therefore, 6/340(1.7%) had confirmed congenital toxoplasmosis. Out of these 4 (66.6%) pregnancies resulted in liveborn infants who remained asymptomatic with no clinical, serologic evidence of congenital toxoplasmosis during 12-month follow-up, while 2 (33.3%) pregnancies with PCR-confirmed CT ended in mid-trimester loss. Additionally, 2.05% acquired acute toxoplasmosis during the periconceptional period, identified through serology. These findings underscore the importance of early diagnosis and timely therapeutic intervention in gestational toxoplasmosis to reduce the risk of congenital transmission and improve foetal outcomes.

Keywords Acute gestational toxoplasmosis, Congenital transmission, Diagnostic standards, Maternal immune status, Periconceptual infection, Treatment

Acute toxoplasma infection during pregnancy is associated with adverse outcomes such as miscarriage, stillbirth, or congenital abnormalities in the newborn, including chorioretinitis, hydrocephalus, and intracranial calcifications[1]. Acute gestational toxoplasmosis is defined by serological evidence of acute *T. gondii* infection in a pregnant woman. Vertical transmission can result in congenital toxoplasmosis (CT), which is confirmed when *T. gondii* DNA is detected in amniotic fluid, or when parasite DNA or specific IgM is identified in neonates[2].

The risk of transplacental transmission and the severity of CT depend on several factors, including the gestational age at the time of maternal infection, the virulence of the parasite, the genotype involved, and the maternal immune response. Notably, the likelihood of transmission increases with advancing gestation, whereas disease severity is typically greater when infection occurs earlier in pregnancy. Therefore, accurately determining the timing of maternal infection is crucial in assessing foetal risk. Recent evidence suggests that infection during the periconceptual period (i.e., from four weeks before to four weeks after conception) may also result in CT[3].

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However, the implications of such timing on transmission risk remain poorly understood. Recently, Vimercati et al. proposed a modified Lebech classification, which estimates a 52% likelihood of maternal primary infection during the periconceptional period using both serology (avidity assay) and amniotic fluid PCR as diagnostic modalities[3].

Confirmation of CT can't solely rely on Ig M and Ig avidity serological results, as there are a few limitations. The detection of IgM antibodies may suggest a recent infection; however, IgM can remain detectable for prolonged periods, ranging from several months to even years, in about 9–27% of cases[4,5]. To improve diagnostic accuracy, IgG avidity testing is often used in conjunction with IgM detection to accurately distinguish between acute toxoplasmosis and chronic infections more reliably[6]. Global incidence and burden of CT vary across various geographical areas. In India, routine universal antenatal screening for congenital toxoplasmosis is not uniformly implemented. The percentage seropositivity for toxoplasmosis in antenatal women in India varies from 15 to 49.5%[7]. Research studies from India on the prevalence of gestational toxoplasmosis, the impact of treatment on congenital transmission, and the incidence rate of CT are very limited[8]. Diagnosis typically relies on serological testing (IgM/IgG ELISA and IgG avidity), while molecular testing, such as amniotic fluid (AF) PCR, is available only at very few select tertiary centres.

Once acute gestational toxoplasmosis is diagnosed, the recommendation is to start treatment with spiramycin. If *T. gondii* DNA is subsequently detected in amniotic fluid, indicating the certainty of foetal infection, therapy is typically changed to a combination of pyrimethamine and sulphonamides[9]. This study prospectively investigates the serological response patterns in pregnant women in relation to the timing of *T. gondii* infection, with a particular focus on the periconceptual period. It is also the first prospective study from India to evaluate treatment outcomes in cases of acute gestational toxoplasmosis and the rate of CT.

Materials and methods
Study design and setting
This prospective observational follow-up study was conducted from November 2021 to November 2023 at the Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, India, in collaboration between the Departments of Obstetrics & Gynaecology and Medical Parasitology.

Inclusion and exclusion criteria
The study included antenatal women presenting to the Department of Obstetrics & Gynaecology, PGIMER, Chandigarh (November 2021–October 2023).

Inclusion criteria.

- Pregnant women in their first or second trimester AND.
- Pregnant women with a history of one or more spontaneous abortions.

Exclusion criteria.

- Women with known causes of miscarriage (e.g., placenta previa, abruptio placentae, cord prolapse, or extreme prematurity).
- Pregnant women in the third trimester were excluded to ensure reliable assessment of treatment response.

Clinical and demographic data collection
Detailed history and physical examination were conducted at enrolment, assessing factors such as cervical lymphadenopathy, dietary habits, cat exposure, occupation, family history, and comorbidities (e.g., APLA, diabetes, thyroid disorders, hypertension).

Ethics approval
This study was approved by the Institutional Ethics Committee, PGIMER, Chandigarh (Ref No: PGI/IEC/2020/000509, dated 19th May 2020). Written informed consent was obtained from all participants and/or their legal guardians. All methods were performed in accordance with the relevant guidelines and regulations, including the ethical principles outlined in the Declaration of Helsinki.

Sample size Estimation
Using a prevalence-based formula:

$$n = Z^2 \times P \times (1 - P)/d^2$$

with a 10.1% estimated prevalence, the calculated minimum sample size was 160 patients per year[10].

Sample collection and laboratory processing
Two 3 mL blood samples were collected at enrolment: one in an EDTA tube for molecular testing and one in a plain tube for serology. Plain tubes were allowed to clot at room temperature and centrifuged to obtain serum, which was aliquoted and stored until testing for IgM, IgG, and IgG avidity. EDTA blood was centrifuged to isolate the buffy coat, enhancing parasite DNA yield by preventing coagulation; the buffy coat was stored at −20 °C until DNA extraction.

Amniotic fluid, when obtained, was collected directly into sterile containers without anticoagulant and processed for qPCR.

The following definitions were followed in our study[1,2,5,7].

Acute gestational toxoplasmosis:

 (i) IgM-positive with low/intermediate IgG avidity or recent IgG seroconversion.
 (ii) IgM-positive with high IgG avidity tested > 16 weeks of gestation.

Congenital toxoplasmosis:

- **Prenatal diagnosis**: Positive PCR for *T. gondii* DNA in amniotic fluid (considered the reference standard for confirming foetal infection).
- **Postnatal serology/molecular testing**:

 i. Neonate positive for anti-Toxoplasma IgM within the first 10 days of life, OR.
 ii. Persistently rising IgG titres during the first year of life, OR.
 iii. Neonatal CSF positive for *T. gondii* by PCR (if CSF sample available).

Serology

The serological testing, i.e., ELISA-IgM, IgG, and IgG avidity, was done manually on a 96-well ELISA plate for all the collected samples using the DIESSE Diagnostica Senese SpA (Italy) kit. IgG avidity testing was conducted only for patients who tested positive for IgG antibodies, as shown in Fig. 1.
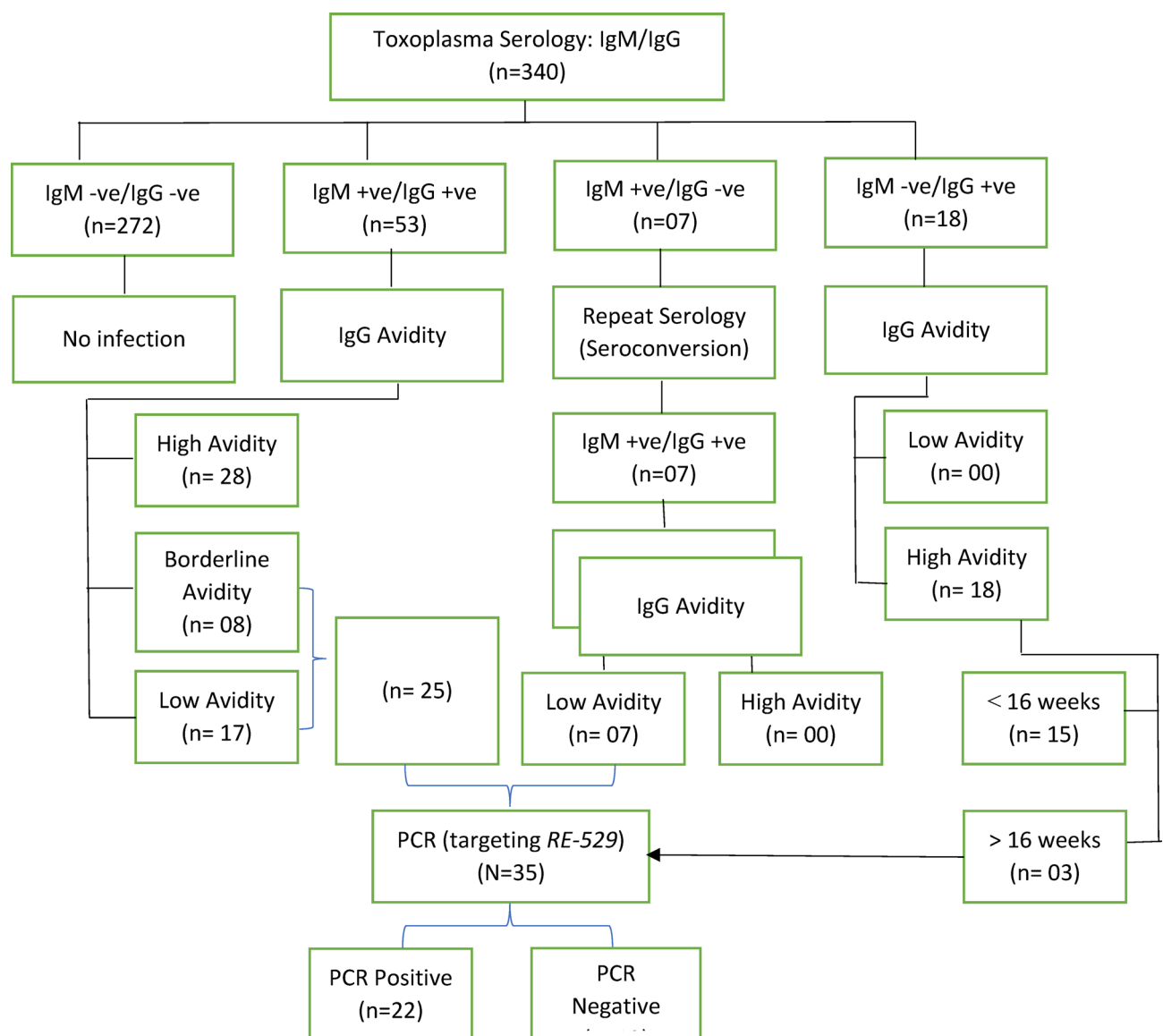

Fig. 1. Diagnostic algorithm for suspected gestational toxoplasmosis. The flowchart illustrates initial serological screening, avidity testing, and confirmatory PCR. Numbers (n) represent women tested at each step.

The patients were considered serology positive/ borderline/ negative based on the following values. (https://www.diesse.it/en/products/toxoplasma-enzywell/)

- **IgM (IU/ml)**: >1.1 Positive; 0.9–1.1 Borderline; <0.9 Negative (**kit Code**: 91041).
- **IgG (IU/ml)**: >1.3 Positive; 0.7–1.3 Borderline; <0.7 Negative (**kit Code**: 91040).
- **IgG avidity (IU/ml)**: <30% Low; 30–35% Borderline; >35% High ((**kit Code**: 91098).

Repeat samples were taken after 3–4 weeks in IgM-positive/IgG-negative cases to assess seroconversion.

DNA extraction

Maternal blood PCR (buffy coat) was performed on those patients with serological evidence of acute gestational toxoplasmosis.

- IgM positivity and low/ borderline IgG avidity.
- Ig M negative and IgG high avidity in more than 16 weeks of gestation (possible transmission in early part of pregnancy) as shown in Fig. 1[2].

DNA was extracted from all patient samples and from cultured *T. gondii* tachyzoites (*RH* strain) using the QIAamp DNA Blood Mini Kit. The *RH* strain, maintained in Swiss albino mice in the Department of Medical Parasitology, served as the standard reference. DNA quality and concentration were assessed using a nano-spectrophotometer before testing. The housekeeping gene GAPDH was amplified in all samples using conventional PCR to verify sample integrity. DNA extracted from the cultured strain was used as a positive control. All extracted DNA was stored at − 20 °C for future use.

Real-time PCR qPCR (maternal blood)

Amplification of the *RE-529* gene of *Toxoplasma gondii* was performed by real-time PCR using a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA). The assay employed the following primer and probe sequences: forward primer 5′-AGAGACACCGGAATGCGATCT-3′, reverse primer 5′-TTC GTCCAAGCCTCCGACT-3′, and a FAM-labelled probe 5′-TCGTGGTGATGGCGGAGAGAATTGA-3′, as described by Villar et al.[11]. Each 20 µL reaction contained 10 µL of iTaq™ Universal Probes Supermix (Bio-Rad Laboratories, Hercules, CA, USA), 0.3 µL each of forward and reverse primers, 0.5 µL of probe, 2 µL of template DNA, and 6.9 µL of nuclease-free water. Thermal cycling conditions consisted of initial activation at 94 °C for 3 min, followed by 40 cycles of denaturation at 94 °C for 30 s and annealing at 60 °C for 1 min. Each run included a positive control (DNA from *RH* strain tachyzoites) and a negative control (nuclease-free water) to validate results. Specificity and assay performance were confirmed by melting curve and temperature gradient analysis.

Treatment, amniocentesis, and follow-up

Patients with suspected acute gestational toxoplasmosis, based on the above-mentioned serological criteria, were commenced immediately on spiramycin (3×3 million IU/day). Treatment was administered in 3-week cycles separated by 1-week intervals, as per our hospital's local obstetric protocol, until 18 weeks of gestation.

Amniocentesis was done for patients with acute gestational toxoplasmosis (serological results) and abnormal foetal ultrasound (e.g., hepatosplenomegaly, hydrops, IUGR, placental calcification). This was performed after consent and counselling.

To ensure diagnostic accuracy and procedural safety, the procedure was performed in the second or early third trimester, at ≥ 18 weeks of gestation, and at least four weeks after the estimated time of maternal infection. Amniotic fluid was tested by qPCR targeting the *RE-529* gene.

Based on qPCR results, the following treatment was followed[2,7].

a) **Amniotic fluid qPCR-positive cases**: Treated with to pyrimethamine (Day 1: 50 mg; then 25 mg/day) and sulfadiazine (3 g/day for < 80 kg; 4 g/day for ≥ 80 kg) until delivery.
b) **Amniotic fluid qPCR-negative cases**: Continued spiramycin until term.

Patients who showed serological evidence of acute gestational toxoplasmosis but had no ultrasound findings suggestive of foetal abnormalities were continued on spiramycin therapy till the end of term (Fig. 2).

At the time of delivery, the following samples and were tested:

- **Placenta** (at delivery): This was only done for mothers with a positive PCR for amniotic fluid. It was subjected to qPCR targeting the 529 bp repetitive element of *T. gondii*.
- **Neonatal blood samples**, collected at birth, 1 month, 6 months, and 12 months, were tested for:
- **Serology**: Detection of anti-Toxoplasma IgM and IgG.
- **Molecular testing**: Real-time PCR for the *RE-529* gene from neonates' CSF (if available).

Sanger sequencing

A total of 10 samples positive on a PCR test were sent for Sanger sequencing to confirm results (maternal blood-positive: 09, Amniotic fluid: 01). Sequences were submitted to NCBI for accession numbers.

Statistical analysis

Data were analyzed using SPSS software version 16.0. Prevalence estimates for acute gestational toxoplasmosis, maternal blood PCR positivity, and amniotic fluid PCR positivity were calculated as proportions and expressed with 95% confidence intervals using the Wilson method.
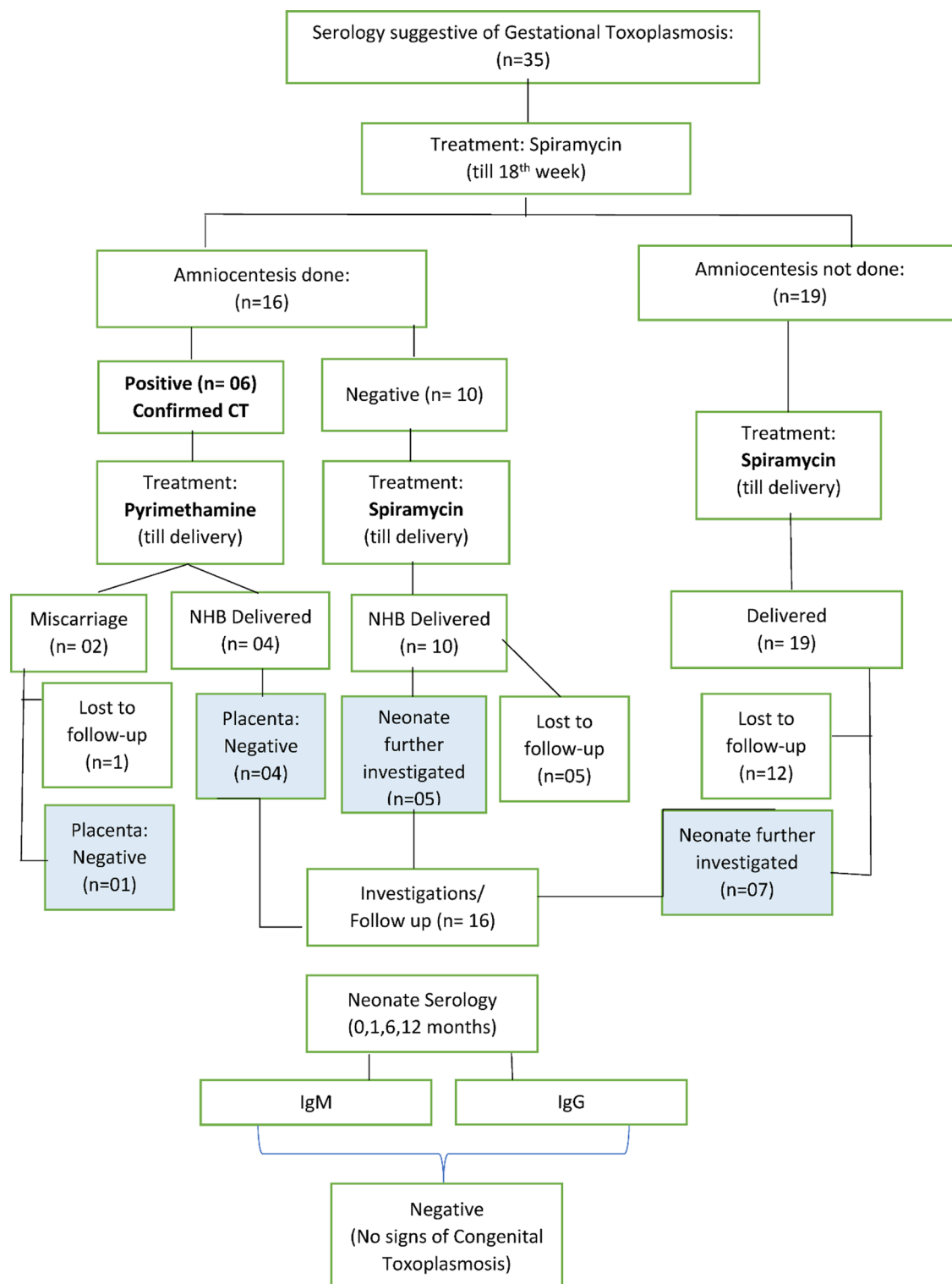
Fig. 2. Clinical management and pregnancy outcomes among women with serology suggestive of toxoplasmosis. Diagram includes maternal treatment (spiramycin, pyrimethamine), amniocentesis results, and neonatal follow-up at 0–12 months (NHB: Normal Healthy Baby; NFI: Neonate further Investigated).

Ig M	Ig G avidity	Proposed time of maternal infection
Positive	Low avidity	Acute infection
Positive	Intermediate avidity	Acute infection
Positive	Low avidity	Periconceptional
Negative	High avidity	Chronic infection

Table 1. Correlation of serological results with estimated time of maternal infection.

Parameter	n/N (%)	95% CI
Acute gestational toxoplasmosis	35/340 (10.3%)	7.5–14.0%
Maternal blood PCR positivity	22/340 (6.5%)	4.3–9.6%
Confirmed CT in cohort (overall prevalence)	6/340 (1.7%)	0.8–3.8%
Periconceptional transmission	7/340; (2.1%)	1.0–4.2%

Table 2. Prevalence of acute maternal toxoplasmosis and PCR positivity.

Glossary/definitions

"Terminology used in this study follows standard obstetric definitions:

– first trimester (0 to 12 weeks), second trimester (13–27 weeks), third trimester (28–40 weeks).
– Acute gestational toxoplasmosis refers to maternal infection in pregnancy, evidenced by IgM positivity with low/intermediate IgG avidity and/or documented seroconversion (considered if it occurred up to 20 weeks of gestation).
– Periconceptional period denotes – 4 weeks before and after conception.
– Low avidity: <30%, strongly suggestive of acute infection (acquired within the preceding 3–4 months).
– Intermediate avidity: 30–35%, considered indeterminate and requiring repeat sampling and correlation with clinical/ultrasound findings.
– High avidity: >35%, suggestive of chronic infection.
– Chronic/ Past infection is defined as IgM-negative, IgG-positive with high avidity (tested ≤16 weeks interpreted cautiously; >16 weeks consistent with past infection).
– Neonate refers to ≤28 days of life.
– **APLA** (antiphospholipid antibodies), **IUGR** (intrauterine growth restriction), **CSF** (cerebrospinal fluid), **qPCR** (quantitative PCR), **EDTA** (ethylenediaminetetraacetic acid).

Results

A total of 340 antenatal women were enrolled in the study. Of these, 147 (43.3%) were in the first trimester [21 between 1 and 4 weeks, 59 between 5 and 8 weeks, and 67 between 9 and 12 weeks], while the remaining 193 (56.7%) were in the second trimester [4 between 13 and 16 weeks, 83 between 17 and 20 weeks, and 106 between 21 and 24 weeks].

Age-wise distribution of patients enrolled was 276 (81.2%) women were between 20 and 35 years, and 64 (18.8%) were above 35. Only ten pregnant women (2.9%) had a history of contact with a cat, and 21 (6.17%) had cervical lymphadenopathy on physical examination.

Serology results

Among the 340 women tested: (Table 1; Fig. 1)

- 272 (80%) were negative for both IgM and IgG, indicating no prior exposure to *T. gondii*.
- 18 were IgM-negative, IgG-positive with high avidity. Of these, 3 were tested after 16 weeks' gestation and were considered possibly infected in early pregnancy. These 3 cases were included as acute gestational toxoplasmosis and underwent qPCR testing. The remaining 15 were considered chronic infections.

Of the 53 women positive for both IgM and IgG:

- 28 had high avidity IgG, suggesting past infection and likely persistent Ig M[5,12]
- 25 showed low or intermediate IgG avidity, consistent with recent infection, and were classified as acute gestational toxoplasmosis. These cases underwent maternal blood qPCR. (Fig. 1)
- An additional 7 women (7/340; 2.1%, 95% CI: 1.0–4.2) between 5-8th weeks of gestation were initially IgM-positive and IgG-negative. On repeat testing after 4 weeks, all showed IgG seroconversion with low avidity, confirming recent primary infection. Therefore, these serological results suggest periconceptional timing of maternal infection (Tables 1 and 2).

Acute gestational toxoplasmosis and maternal qPCR results

In total, 35 women (35/340; 10.3%, 95% CI: 7.5–14.0, Table 2) met the criteria for acute gestational 22/340; (6.5%, 95% CI: 4.3–9.6) Table 2 maternal blood samples.

A few samples were subjected to Sanger sequencing to confirm the results (for which the accession numbers are listed in Table 3).

Treatment, amniocentesis, follow-up results (Fig. 2)

All 35 diagnosed patients were initiated on spiramycin therapy (3×3 million IU/day for 21 days with 1-week breaks, until 18 weeks' gestation).

- **Third-trimester ultrasounds** were performed in all patients. Based on abnormal findings (e.g., IUGR, placental calcifications) for patients with positive acute gestational toxoplasmosis, **16 women** underwent amniocentesis. (Fig. 2)

    – **A total of 6 of 16 patients'** amniotic fluid samples tested positive by qPCR, confirming vertical transmission.
    – These patients were switched to pyrimethamine and sulfadiazine until delivery.
    – Of these 6 PCR-positive cases, two pregnancies (33.3%) ended in mid-trimester miscarriage.
    – The remaining four AF-PCR–positive cases resulted in live births. Therefore, 6 out of 340 (1.7%; 95% CI: 0.8–3.8, Table 2) pregnant women were confirmed to have CT in the entire cohort.

- **2 of the confirmed CT experienced miscarriages** at 6 months' gestation: (Table 4)

    – One patient had a history of uncontrolled gestational diabetes, who delivered outside the institute, experienced a miscarriage, and was subsequently lost to follow-up. She had started spiramycin at 14th weeks of gestation, and amniocentesis was performed six weeks later. Although maternal qPCR was positive, she had no history of contact with cats or lymphadenopathy at the time of presentation.
    – In the second case with miscarriage, placental PCR was negative, but foetal tissue could not be tested. (Fig. 2) She had started spiramycin at the 17th week of gestation, and amniocentesis was performed three weeks later.

- **4 patients (25%)** delivered liveborn infants with PCR-confirmed congenital toxoplasmosis. Although all infants remained clinically asymptomatic, placental PCRs were negative, and follow-up assessments of neonates (serology and PCR up to 12 months) did not show evidence of congenital toxoplasmosis (CT). Details are in Table 4.

The **remaining 19 patients** with no ultrasound abnormalities did not undergo amniocentesis. These, along with the **10 women with negative amniotic PCR**, continued on spiramycin therapy until term (Fig. 2).

In total, **29 patients** (19 without amniocentesis and 10 with negative amniotic PCR) delivered healthy infants. All liveborn infants of mothers with suspected acute infection underwent neonatal evaluation at birth, which included physical examination and serology. Follow-up was scheduled at approximately 1, 6, and 12 months of age with repeat clinical assessment and serology to exclude congenital toxoplasmosis. Among these 29 patient's complete follow-up data were available for 12 neonates, none of whom showed evidence of infection. The remaining infants were lost to follow-up. Cerebrospinal fluid samples were not obtained for any neonates.

Discussion

This prospective observational study conducted in North India found that 10.29% (95% CI: 7.5–14.0) of antenatal females serologically diagnosed with gestational toxoplasmosis. Treatment with spiramycin followed by pyrimethamine and sulfadiazine effectively reduced the rate of congenital toxoplasmosis to 1.7%. Additionally, through a thorough follow-up of pregnant females, our study highlighted that a measurable proportion, i.e., 2.05% of acute gestational toxoplasmosis occurs in the periconceptional window—a transmission period that is often overlooked in clinical practice. A structured diagnostic algorithm was used to diagnose gestational

S. No.	Sample	Parasite identified	Accession number
1.	Blood	*Toxoplasma gondii (RE-529 gene)*	PP497847.1
2.	Blood	*Toxoplasma gondii (RE-529 gene)*	OR513926.1
3.	Blood	*Toxoplasma gondii (RE-529 gene)*	PP497846.1
4.	Amniotic fluid	*Toxoplasma gondii (RE-529 gene)*	PP497845.1
5.	Blood	*Toxoplasma gondii (RE-529 gene)*	OR513925.1
6.	Blood	*Toxoplasma gondii (RE-529 gene)*	PP497844.1
7.	Blood	*Toxoplasma gondii (RE-529 gene)*	PP497843.1
8.	Blood	*Toxoplasma gondii (RE-529 gene)*	PP497842.1
9.	Blood	*Toxoplasma gondii (RE-529 gene)*	PP497841.1
10	Blood	*Toxoplasma gondii (RE-529 gene)*	PP497840.1

Table 3. Sanger sequencing data.

S. No.	Gestational Month of Enrolment	H/o Lymphadenopathy	H/o contact with cat	Co morbid	IgM	IgG	Maternal Blood PCR	Spiramycin started	Ultrasound indication for amniocentesis	Gestational age at amniocentesis	Status	Placental PCR
1.	Second month (7th week)	Present	Present	-	Positive	Positive; Low avidity	Positive	9th week	Placental calcification, growth restriction	18th week	Delivered healthy baby	Negative
2.	Fourth month (16th week)	-	-	-	Positive	Positive; Low avidity	Positive	17th week	Oligohydramnios	20th week	Miscarriage	Negative
3.	Fourth month (13th week)	-	-	Gestational diabetes, Fibroid	Positive	Positive; Low avidity	Positive	14th week	Placental calcification	20th week	Miscarriage	Lost to follow-up
4.	Fifth month (14th week)	-	-		Positive	Positive; Low avidity	Positive	15th week	Oligohydramnios, growth restriction	20th week	Delivered healthy baby	Negative
5.	Fifth month (15th week)	Present	-		Positive	Positive; Low avidity	Positive	15th week	Placental calcification, growth restriction	20th week	Delivered healthy baby	Negative
6.	Fifth month (14th week)	-	-		Positive	Positive; Low avidity	Positive	16th week	Oligohydramnios	20th week	Delivered healthy baby	Negative

Table 4. Clinical details of PCR-Positive amniocentesis cases: (Patients with a positive PCR result from amniotic fluid analysis).

toxoplasmosis (serology → avidity → selective amniotic fluid qPCR), combined with treatment to apparently reduce clinically congenital toxoplasmosis. Importantly, infants born to mothers confirmed to have CT who were treated remained clinically well at 12 months, highlighting the potential benefit of timely intervention.

Only a limited number of countries, like France, Austria, Belgium, Norway, and Italy, have established routine surveillance programs for congenital toxoplasmosis in their population of pregnant women and provide comprehensive and robust data. Additionally, no universally accepted guidelines exist for diagnosing, confirming, or clinically managing CT in antenatal patients, leading to an underestimation of its global prevalence. A recent systematic review reported a 3% risk of foetal loss or neonatal death in CT, often associated with ocular or cranial abnormalities[13]. Bridging these gaps in surveillance and standardization is essential to improve early detection, clinical management, and outcomes.

Among the 35 pregnant women with gestational toxoplasmosis, seven women (2.05%) had IgM positivity with low-avidity IgG and recent seroconversion, all occurring in the first trimester, suggestive of periconceptional transmission. Since periconceptional serological status was unknown, early IgG rise could reflect infection shortly before or just after conception. This is the first prospective Indian study documenting gestational toxoplasmosis likely due to periconceptional infection.

Periconceptional toxoplasmosis has been infrequently reported as a cause of CT. The first such case was described by Desmonts et al. in 1985[14]. Subsequent case reports suggest that parasite transmission may be delayed for weeks to months due to prenatal incubation. Simon et al. (France) documented a miscarriage linked to maternal infection occurring four months before conception, with PCR-confirmed *T. gondii* in foetal tissue, despite maternal immunocompetence[15]. They advocated a six-month interval before attempting conception after confirmed toxoplasmosis. Other studies (Wallon M, Garabedian C) estimate CT transmission during the periconceptional period at 3.3–3.8%[16,17].

For molecular detection, the multicopy *RE-529* gene (~200–300 repeats) was selected because of its higher analytical sensitivity. *T. gondii* genome has 200–300 copies of *RE 529* bp gene, and also its nucleotide sequence is more conserved as compared to other gene targets[9].

Of the 35 women diagnosed with acute gestational toxoplasmosis in our study, 22 had positive PCR results from maternal blood. Several studies, including those by Yamada H and Villar BB, have shown that maternal blood PCR has lower sensitivity and weaker correlation with CT compared to amniotic fluid PCR[11,18].

Molecular testing using amniotic fluid PCR is regarded as the reference gold standard for diagnosing congenital toxoplasmosis, typically performed at ≥18 weeks of gestation and at least four weeks after suspected maternal infection. Reported sensitivity ranges from 69.5 to 94.4% and specificity from 97.5 to 100%[19–21]. Variability in results can arise from differences in PCR methodology, timing of maternal infection and amniocentesis, parasite load, and gene targets[21,22]. Nonetheless, a negative AF-PCR does not completely exclude congenital infection, as diagnostic yield is influenced by methodology, parasite load, and timing of sampling[22,23]. In contrast, in our study, all amniotic fluid PCR-positive patients (except one, who was lost to follow-up) had negative placental tissue PCR. The lower sensitivity of the placenta tissue (≈58.9%) as compared to amniotic fluid has been reported by other authors[23].

In our study, 340 antenatal women were screened serologically for acute toxoplasmosis, and six (1.7%) patients were confirmed CT based on positive qPCR. The rate of CT varies geographically depending on the prevalence of T. gondii in that area, the patient profile, serotype involved, local transmission rates, type of sample, and molecular method used for diagnosis. In a recent study by Vimercati A et al. from Italy the rate of CT was 13.8% diagnosed by qPCR on amniotic fluid. Similarly, Diesel AA et al. from Brazil, 15% of antenatal mothers had CT.

In our cohort of antenatal patients, congenital infection was confirmed in six foetuses by AF-qPCR. Two of these patients had miscarriages. However, four of these infants remained clinically asymptomatic at 12 months under treatment and follow-up, underscoring that absence of symptoms does not exclude congenital infection. This may be attributed to effective treatment with spiramycin and possibly to regional strain variation of *T. gondii*.

Spiramycin, a macrolide antibiotic, has demonstrated efficacy in preventing vertical transmission by inhibiting the replication of tachyzoites in placental tissue. However, it cannot treat established foetal infections due to its limited placental permeability[24]. A study by Çakırca TD et al., in Turkey, reported CT in only 1% of treated mothers versus 30% in untreated cases[9]. Similarly, Mejia-Oquendo et al. (2021) from Colombia observed no CT among 1.43% of gestational toxoplasmosis cases treated with spiramycin[24]. In Italy, De Santis et al. (2024) found a CT rate of 3.4% in treated women compared to 7.7% in inadequately treated cases[25]. In Brazil, Gomes-Ferrari-Strang et al. (2023) noted a 6.5-fold increased CT risk in untreated mothers[26].

In 2019, a French expert group recommended initiating spiramycin as soon as toxoplasmosis is suspected during the first or second trimester. While spiramycin accumulates in the placenta and may prevent transmission, it does not treat established foetal infections. When amniotic fluid PCR is positive, treatment should be escalated to pyrimethamine plus sulfadiazine. If PCR is negative, spiramycin should be continued[16]. At our healthcare centre, spiramycin is prescribed in 3-week cycles separated by 1-week intervals, as per the local obstetric protocol, to improve maternal tolerability and compliance.

None of the pregnant women with congenital toxoplasmosis had a history of cat exposure, and among the remaining study participants, only 2.9% reported such exposure. This finding is significant, as in recent years, the epidemiology of toxoplasmosis transmission has changed with environmental contamination of soil, water, and sand by *Toxoplasma* oocysts emerging as the primary source of human infection[3].

Reliable diagnosis of acute toxoplasmosis depends on IgM and low-avidity IgG detection. However, current ELISA kits using crude tachyzoite antigens lack optimal sensitivity and specificity. Recombinant antigens such as SAG (surface antigen), GRA (dense granule antigen), and ROP (rhoptry protein) may improve accuracy, standardization, and reduce cross-reactivity, offering promise for future serodiagnostic[27].

In our study, amniocentesis is reserved for women with serological evidence of acute toxoplasmosis and a strong clinical or ultrasonographic suspicion of foetal infection, given the invasive nature of the procedure. Also, we have included only 1st and 2nd trimester women in our study. This allowed us to evaluate the effect for a few weeks. This would not be feasible if we had included 3rd trimester pregnant women. However, this approach may have led to many missed cases of CT cases, and this lacuna of our study.

Conclusion

Our findings provide important insights into the role of periconceptional maternal infection in the transmission of CT and highlight the protective effect of timely spiramycin treatment. We also recommend a six-month waiting period before conceiving after a confirmed Toxoplasma infection. This is the first Indian study to report periconceptional toxoplasmosis as a contributor to acute gestational infection, underscoring the need for early detection, treatment, and use of real-time PCR for amniotic fluid to reduce the burden of congenital toxoplasmosis. Given resource constraints and varied seroprevalence across India, we recommend risk-based first-trimester serological screening (with avidity) in tertiary and large secondary centres. More prospective studies need to be done since there is a scarcity of data from India regarding the incidence of CT and the effect of treatment on gestational toxoplasmosis.

Data availability

The datasets generated and/or analysed during the current study are available in the NCBI Nucleotide database repository, accessible at: https://www.ncbi.nlm.nih.gov/nuccore/?term=puja+garg+toxoplasma.

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Author contributions

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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