Research Square

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Paired molecular testing of amniotic fluid and fetal ventricular cerebrospinal fluid for Toxoplasma gondii and cytomegalovirus in severe fetal hydrocephalus: a descriptive case series

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Abstract

Background

Severe fetal hydrocephalus has heterogeneous structural, genetic, hemorrhagic, and infectious causes. Amniotic-fluid molecular testing is established for suspected cytomegalovirus (CMV) and *Toxoplasma gondii* infection, whereas testing of fetal ventricular cerebrospinal fluid (CSF) obtained during cephalocentesis is rarely reported.

Methods

In this single-centre descriptive cross-sectional case series, nine pregnant women carrying fetuses with severe or progressive hydrocephalus underwent clinically indicated cephalocentesis at RSAB Harapan Kita, Jakarta, from March to July 2026. Paired amniotic-fluid and ventricular-CSF specimens underwent real-time PCR for *T. gondii* and CMV; maternal IgG, IgM, and IgG avidity were also assessed. Data were summarized descriptively, with exact binomial 95% confidence intervals for zero-detection proportions.

Results

Median maternal age was 31 years (26–36) and gestational age was 30.71 weeks (21.57–36.71). Neither pathogen was detected in amniotic fluid or ventricular CSF (0/9 for each pathogen in each compartment; exact 95% CI 0–33.6%). *T. gondii* IgG was positive in 5/9, IgM was negative in 9/9, and avidity was high in all five IgG-positive participants. CMV IgG was positive in 9/9, IgM was negative in 9/9, and avidity was high in 9/9. Median ventricular-CSF volume was 40 mL (30–50). Posterior ventricular width decreased from 35.35 to 25.61 mm immediately after aspiration (median decrease 8.21 mm, range 2.72–26.44), a descriptive anatomical observation. Delivery outcomes were available for seven pregnancies; all were live births by caesarean delivery, while two pregnancies remained ongoing.

Conclusions

In this small selected series, paired testing found no molecular evidence of *T. gondii* or CMV in either fetal compartment, and maternal serology did not support recent primary infection. A negative ventricular-CSF PCR should not be treated as a validated rule-out test. Prenatal infection testing should use amniotic fluid and be interpreted with maternal serology and fetal imaging; this series does not support cephalocentesis solely to detect infection.

Background

Fetal ventriculomegaly and hydrocephalus represent a spectrum of disorders characterized by abnormal enlargement of the cerebral ventricular system. Ventriculomegaly is conventionally recognized when the

atrium of the lateral ventricle measures at least 10 mm, and measurements of 15 mm or more are generally classified as severe. The term hydrocephalus is most appropriate when ventricular enlargement reflects disturbed cerebrospinal-fluid dynamics, often with progressive enlargement, pressure effects, or macrocephaly. The clinical importance lies less in ventricular size alone than in the underlying cause, associated cerebral abnormalities, progression over gestation, and the extent of preserved brain parenchyma [1–4].

The etiologic differential is broad. Structural causes include aqueductal obstruction, posterior-fossa abnormalities, neural-tube defects, and disorders of cortical or commissural development; hemorrhagic and destructive processes can also produce secondary ventricular enlargement. Genetic causes range from chromosomal abnormalities to copy-number and single-gene disorders, and contemporary reviews emphasize that a low-risk screening result for common aneuploidies does not exclude clinically important genetic disease [4, 5]. Accordingly, severe or progressive ventriculomegaly generally prompts detailed neurosonography, assessment for extracranial anomalies, consideration of fetal magnetic resonance imaging (MRI), and diagnostic genetic testing where appropriate [2–5].

Congenital infection is another important component of the work-up, particularly CMV and *Toxoplasma gondii* [2, 6]. CMV is a common congenital infection globally, with a meta-analysis of universal screening studies estimating a pooled birth prevalence of 0.67%, and a higher burden in low- and middle-income settings [8]. In a prospective urban birth cohort from a national referral hospital in Jakarta, congenital CMV infection was identified in 5.8% of screened neonates, providing locally relevant evidence that exposure and congenital infection are clinically important in Indonesia [7]. Fetal CMV can be associated with ventriculomegaly, periventricular abnormalities, calcifications, white-matter injury, and cortical malformations, although imaging findings are neither uniformly present nor pathognomonic [11–14]. Congenital toxoplasmosis may likewise involve the central nervous system, classically with hydrocephalus and intracranial calcification, but prenatal manifestations and diagnostic pathways are heterogeneous [15–17].

Maternal serology and fetal molecular testing answer different questions. For CMV, IgG, IgM, and IgG-avidity testing can support the classification of maternal infection, but isolated serologic patterns have important limitations, particularly for non-primary infection and for cases in which IgM is absent [9, 10]. For *T. gondii*, high IgG avidity is useful for excluding a recent primary infection when interpreted with gestational age and the assay-specific context, whereas low avidity is not by itself proof of recent acquisition [17, 18]. When fetal infection is suspected, nucleic-acid testing of amniotic fluid is the best-established prenatal molecular approach for both CMV and toxoplasmosis. A 2024 meta-analysis of congenital toxoplasmosis diagnostics reported pooled sensitivity and specificity of 85.1% and 99.7%, respectively, for amniotic-fluid PCR, underscoring both its high diagnostic value and the possibility of false-negative results [19].

Fetal ventricular CSF obtained during cephalocentesis is anatomically closer to the central nervous system than amniotic fluid, but its prenatal diagnostic performance for CMV and *T. gondii* has not been

established in large, validated cohorts. Moreover, cephalocentesis itself is a highly selected fetal procedure with limited contemporary evidence, and the literature on antenatal surgical interventions for hydrocephalus remains dominated by case reports and case series [21]. Direct comparison of paired amniotic-fluid and fetal ventricular CSF molecular results, interpreted together with maternal serology, therefore represents a clinically unusual dataset. This study aimed to describe the molecular detection profile of *T. gondii* and CMV in paired amniotic-fluid and ventricular CSF samples from fetuses with severe or progressive hydrocephalus and to contextualize those findings using maternal serology and available pregnancy outcomes.

Methods

Study design and setting

This was a single-centre descriptive cross-sectional study reported as a case series. Pregnant women carrying fetuses with severe or progressive hydrocephalus who underwent clinically indicated cephalocentesis were enrolled at RSAB Harapan Kita, Jakarta, Indonesia. Molecular testing was performed at Prodia Laboratory, Kramat Raya, Jakarta. Recruitment, sampling, and laboratory testing were conducted from March through July 2026. The study was designed to describe the infection-marker profile rather than to estimate population prevalence, test associations, or evaluate cephalocentesis efficacy.

Participants and eligibility

The target population comprised women in the second or third trimester whose fetuses had severe or progressive hydrocephalus or ventriculomegaly. Eligibility required a lateral ventricular atrial width of at least 15 mm or clinically meaningful progression on prenatal ultrasonography, referral for invasive fetal evaluation, and written consent. Major structural brain abnormalities judged at the screening evaluation to provide a clear primary non-infectious explanation, a high-risk non-invasive prenatal testing (NIPT) result for a major aneuploidy, procedural failure with compromised ventricular-fluid integrity, or an unusable laboratory specimen were exclusion criteria. Because cephalocentesis was performed infrequently, no formal power calculation was used; all consecutive eligible cases available during the study period were included. The final analytic sample comprised nine pregnancies.

Prenatal evaluation

All participants underwent ultrasound assessment of fetal anatomy and ventricular dimensions. NIPT had been performed as a screening test for major fetal aneuploidies, and all nine enrolled pregnancies had a low-risk result. NIPT was treated as a screening result rather than as a diagnostic exclusion of genetic disease. Maternal demographic and obstetric data, gestational age at sampling, fetal biometric measurements, posterior ventricular width, and available delivery and neonatal outcomes were recorded.

Amniocentesis and cephalocentesis

Amniocentesis and cephalocentesis were performed by fetomaternal clinicians using continuous real-time ultrasonographic guidance and aseptic technique. For amniocentesis, the maternal abdomen was prepared with antiseptic solution and a 20-gauge spinal needle was advanced transabdominally into a safe amniotic-fluid pocket while avoiding the fetus and, where feasible, the placenta. Amniotic fluid was collected into a sterile tube and submitted for molecular testing. The actual amniotic-fluid volume available in the study ranged from 30 to 50 mL.

Cephalocentesis was undertaken in pregnancies in which the procedure had a clinical indication related to the severe or progressive fetal hydrocephalus. Under real-time ultrasound guidance, an 18-gauge spinal needle was advanced transabdominally toward a posterior parietal entry point and into a lateral ventricle. Ventricular CSF was aspirated into sterile containers, with attention to avoiding major blood contamination. The actual CSF volume collected ranged from 30 to 50 mL. Posterior ventricular width, biparietal diameter, and head circumference were documented before and immediately after the procedure. These paired measurements were considered descriptive anatomical observations only; the study was not designed to establish therapeutic efficacy or neurological benefit.

Maternal serology and molecular testing

Maternal serologic testing comprised pathogen-specific IgG, IgM, and IgG-avidity assays for both CMV and *T. gondii*. Testing was performed using a chemiluminescent microparticle immunoassay on the ARCHITECT platform (Abbott). In the source laboratory documentation, the *T. gondii* IgM assay used P30 antigen, while IgG and avidity testing used recombinant antigens including P30/SAG1 and P35/GRA8. CMV IgM testing used a combination of AD169 strain lysate and recombinant antigen, whereas CMV IgG and avidity used AD169 lysate. Results were interpreted according to the laboratory's validated assay criteria. Maternal IgG positivity was treated as evidence of prior exposure; IgM and avidity were interpreted jointly and not as stand-alone proof of timing or fetal infection [9, 10, 17, 18].

DNA was extracted from paired amniotic-fluid and ventricular-CSF specimens. *T. gondii* DNA was assessed by real-time PCR targeting the multicopy 529-bp repetitive element (REP-529), a target with high analytic sensitivity in clinical molecular testing [20]. CMV DNA was assessed by the laboratory's validated real-time PCR protocol; the exact genomic target and commercial assay identifier were not documented in the supplied study source and therefore are not specified here. Results were classified as detected or not detected. Laboratory documentation indicated that internal controls were valid in all analyzed samples and that pre-analytical handling, including tube use, transport, centrifugation, and temperature control, followed the laboratory standard operating procedures. A not-detected result was interpreted as absence of detectable target DNA within the analytical and biological limits of the assay, not as absolute exclusion of infection.

Outcomes and statistical analysis

The primary descriptive outcomes were detection of *T. gondii* and CMV DNA in amniotic fluid and fetal ventricular CSF. Secondary outcomes included maternal IgG, IgM, and IgG-avidity profiles; aspirated fluid volumes; immediate pre- to post-procedure changes in fetal ventricular and cranial measurements; and available delivery and neonatal outcomes. Categorical variables are presented as counts and percentages. Numerical variables are summarized as median and range because of the small sample and heterogeneous gestational ages. No hypothesis testing of associations was planned. For molecular outcomes with zero detected events, two-sided 95% Clopper–Pearson exact binomial confidence intervals were calculated descriptively to make the uncertainty around 0/9 observations explicit.

Ethics approval

The study received ethics approval from the Health Research Ethics Committee of RSAB Harapan Kita (reference DP.04.03/D.XXXVI.19/23/2026; 2 April 2026). Written informed consent was obtained from participating women for study participation and the invasive procedures. Study data were handled confidentially and are reported without direct patient identifiers.

Results

Maternal and pregnancy characteristics

Nine pregnancies met the eligibility criteria and contributed paired amniotic-fluid and ventricular-CSF samples. Maternal age was a median of 31 years (range 26–36), and gestational age at sampling was 30.71 weeks (21.57–36.71). Three women were nulliparous, five were primiparous, and one was multiparous. All nine pregnancies had a low-risk NIPT result. Before cephalocentesis, posterior ventricular width was a median of 35.35 mm (16.01–56.80), demonstrating substantial heterogeneity in ventricular enlargement (Table 1).

Table 1
Maternal and pregnancy characteristics

Characteristic	Result
Maternal age, years; median (range)	31 (26–36)
Gestational age at sampling, weeks; median (range)	30.71 (21.57–36.71)
Nulliparous	3/9 (33.3%)
Primiparous	5/9 (55.6%)
Multiparous	1/9 (11.1%)
Low-risk NIPT	9/9 (100%)
Posterior ventricular width before cephalocentesis, mm; median (range)	35.35 (16.01–56.80)

NIPT, non-invasive prenatal testing. Percentages use the full cohort denominator unless otherwise specified.

Paired molecular findings

Neither study pathogen was detected in either fetal compartment. *T. gondii* DNA was not detected in amniotic fluid from 9/9 pregnancies and was not detected in ventricular CSF from 9/9 pregnancies. CMV DNA was likewise not detected in amniotic fluid from 9/9 pregnancies and was not detected in ventricular CSF from 9/9 pregnancies. For each zero-detection proportion, the exact two-sided 95% confidence interval was 0–33.6%, emphasizing that the small sample does not support a population-level claim of zero prevalence. All reported PCR internal controls met the validity criteria (Table 2).

Maternal serologic profiles

Maternal *T. gondii* IgG was positive in five of nine women (55.6%), while IgM was negative in all nine. Each of the five IgG-positive women had high IgG avidity. CMV IgG was positive in all nine women, CMV IgM was negative in all nine, and high CMV IgG avidity was recorded in all nine. Collectively, these profiles did not provide serologic support for recent primary maternal infection with either pathogen at the time of fetal sampling, although they cannot exclude every form or timing of infection, particularly non-primary CMV infection.

Table 2
Molecular and maternal serologic findings

Measure	*T. gondii*	CMV
Maternal IgG positive	5/9 (55.6%)	9/9 (100%)
Maternal IgM positive	0/9 (0%)	0/9 (0%)
High IgG avidity	5/5 IgG-positive (100%)	9/9 (100%)
Amniotic-fluid PCR detected	0/9 (0%); 95% CI 0–33.6%	0/9 (0%); 95% CI 0–33.6%
Ventricular-CSF PCR detected	0/9 (0%); 95% CI 0–33.6%	0/9 (0%); 95% CI 0–33.6%

CI, confidence interval; CMV, cytomegalovirus; CSF, cerebrospinal fluid; IgG, immunoglobulin G; IgM, immunoglobulin M; PCR, polymerase chain reaction. Exact 95% CIs are Clopper–Pearson intervals added descriptively from the supplied individual-level data.

Procedural measurements and interim outcomes

The median ventricular-CSF volume aspirated was 40 mL (range 30–50), while the median amniotic-fluid volume available for testing was 30 mL (30–50). Posterior ventricular width decreased in all nine fetuses immediately after CSF aspiration, from a median of 35.35 mm (16.01–56.80) to 25.61 mm (7.80–40.83). The median absolute decrease was 8.21 mm (2.72–26.44). Biparietal diameter decreased by a median of 2.30 mm (0.60–11.60), and head circumference decreased by 7.20 mm (3.80–18.50). These

measurements describe the immediate mechanical/anatomical response to fluid removal and were not analyzed as evidence of clinical efficacy.

Delivery outcomes were available for seven of nine pregnancies at the study data cutoff. All seven documented deliveries were by caesarean section and resulted in live births. Median birth weight among the seven neonates was 2895 g (range 2290–3900). Two pregnancies were still ongoing at the last recorded follow-up (Table 3). Long-term neonatal neurological outcomes and standardized postnatal confirmatory infection testing were not available for the full cohort.

Table 3
Procedural measurements and interim pregnancy outcomes

Measure	Result
Ventricular CSF volume, mL; median (range)	40 (30–50)
Amniotic-fluid volume, mL; median (range)	30 (30–50)
Posterior ventricular width before procedure, mm; median (range)	35.35 (16.01–56.80)
Posterior ventricular width after procedure, mm; median (range)	25.61 (7.80–40.83)
Absolute decrease in posterior ventricular width, mm; median (range)	8.21 (2.72–26.44)
Absolute decrease in biparietal diameter, mm; median (range)	2.30 (0.60–11.60)
Absolute decrease in head circumference, mm; median (range)	7.20 (3.80–18.50)
Delivery outcome available	7/9 (77.8%)
Live birth among documented deliveries	7/7 (100%)
Caesarean delivery among documented deliveries	7/7 (100%)
Birth weight, g; median (range)	2895 (2290–3900)
Pregnancies ongoing at data cutoff	2/9 (22.2%)

CSF, cerebrospinal fluid. Pre- and post-procedure changes are immediate descriptive measurements and should not be interpreted as estimates of treatment effect or neurological benefit.

Discussion

This study provides a rare paired description of molecular testing from amniotic fluid and fetal ventricular CSF in severe or progressive fetal hydrocephalus. Across nine pregnancies, neither *T. gondii* nor CMV DNA was detected in either fetal compartment, and maternal serology did not support recent primary infection at the time of sampling. The central interpretation is therefore narrower than simply concluding that infection was absent: within the timing, specimens, and analytical limits of the assays used, this series found no molecular evidence that these two pathogens explained the observed severe fetal hydrocephalus. Because zero detections among nine observations still have a two-sided exact

upper 95% confidence limit of 33.6%, the findings are best regarded as descriptive evidence from a highly selected clinical population rather than an estimate of prevalence.

The CMV findings warrant particular nuance. All women were CMV-IgG positive, IgM negative, and had high IgG avidity. In many clinical contexts, this pattern weighs against recent primary infection, but contemporary laboratory reviews emphasize that CMV serology cannot by itself exclude non-primary infection or every clinically relevant maternal-fetal transmission scenario [9, 10]. Maternal IgM may be absent, and avidity is assay-dependent. Consequently, the concordant absence of CMV DNA in amniotic fluid adds important fetal-level evidence to the maternal serology. Amniotic-fluid nucleic-acid testing is an established approach to suspected fetal CMV infection [9]. The simultaneous absence of CMV DNA in ventricular CSF is concordant with the amniotic-fluid result, but the incremental diagnostic value of fetal CSF remains uncertain because prenatal ventricular-fluid PCR has not been validated at the same scale as amniotic-fluid testing.

This distinction matters because severe ventriculomegaly can occur in congenital CMV, and neuroimaging abnormalities may include periventricular changes, ventricular enlargement, calcifications, white-matter injury, and disorders of cortical development [11–14]. MRI can add information beyond neurosonography in selected cases, including parenchymal and migrational abnormalities [12, 22]. Nevertheless, these imaging patterns overlap with other destructive and developmental processes. In a cohort selected for severe hydrocephalus rather than for confirmed CMV infection, negative molecular testing appropriately shifts attention toward other etiologies but does not identify which alternative mechanism is responsible.

The *T. gondii* results are similarly internally consistent. Five women were IgG positive, all nine were IgM negative, and the five IgG-positive women had high avidity. High *T. gondii* IgG avidity is particularly useful for arguing against recent primary acquisition when interpreted with gestational age and the characteristics of the specific assay [17, 18]. At the fetal level, *T. gondii* DNA was absent from all amniotic-fluid and ventricular-CSF specimens. A recent meta-analysis reported amniotic-fluid PCR sensitivity of 85.1% and specificity of 99.7% for congenital toxoplasmosis [19], which supports its diagnostic value but also shows why a negative result cannot be interpreted as perfectly exclusionary. Sampling relative to maternal infection, low organism burden, compartmental distribution, and analytical limits can all influence sensitivity. The use of the multicopy REP-529 target in this study is analytically favorable, and prior molecular work supports the sensitivity of REP-529-based real-time PCR, although strain-related sequence variability and laboratory-specific assay performance remain relevant [20].

The paired sampling strategy is the most distinctive feature of this series. Amniotic fluid is the better-established prenatal matrix for both pathogens, whereas fetal ventricular CSF offers direct access to the compartment affected by hydrocephalus. Anatomical proximity, however, should not be equated with proven diagnostic superiority. Ventricular fluid from a fetus with severe hydrocephalus may differ from normal CSF in cellularity, protein content, blood contamination, or other matrix characteristics that can influence extraction and amplification. Valid internal controls reduce concern for major amplification

failure or severe inhibition in the analyzed specimens, but they cannot eliminate biological false negatives [23]. In the absence of standardized prenatal CSF validation studies, a negative ventricular-CSF PCR should therefore be described as target DNA not detected in that specimen, not as a stand-alone rule-out test for systemic fetal infection.

The absence of evidence for CMV and *T. gondii* also highlights the broader etiologic problem in fetal hydrocephalus. Contemporary imaging and genetic literature emphasizes aqueductal obstruction, developmental brain anomalies, destructive lesions, copy-number variants, and monogenic disorders among the many alternatives [1, 4, 5]. All fetuses in this study had low-risk NIPT, but this only reduced the likelihood of the common aneuploidies included in the screening panel; it did not exclude chromosomal microdeletions or duplications, single-gene disorders, or many structural causes. This is especially important because some fetuses had additional central-nervous-system abnormalities on later imaging. The appropriate clinical inference from a negative infectious work-up is therefore not that the hydrocephalus was idiopathic, but that detailed neuroimaging and diagnostic genetic evaluation remain necessary components of etiologic assessment.

Cephalocentesis also requires careful interpretation. The immediate decrease in posterior ventricular width, biparietal diameter, and head circumference after aspiration is physiologically expected when ventricular fluid is removed. It should not be interpreted as evidence that the procedure improved brain development or long-term neurological outcome. A 2025 systematic review of fetal surgical interventions for hydrocephalus identified only 12 eligible reports, largely case reports and case series, and concluded that the quality of developmental-outcome evidence was poor [21]. The present study was neither designed nor powered to evaluate cephalocentesis safety or efficacy. Its contribution is diagnostic and descriptive: ventricular-CSF testing did not provide additional molecular detection beyond amniotic-fluid testing in this series. These findings do not support cephalocentesis solely to detect CMV or toxoplasmosis.

Several strengths support the internal descriptive value of the study. The same pregnancies contributed paired fetal specimens from two biological compartments, allowing direct assessment of concordance without introducing between-patient variability. Maternal serology included IgG, IgM, and avidity rather than a nonspecific 'TORCH' label, and the molecular dataset was linked to fetal imaging measurements and interim pregnancy outcomes. The source laboratory documentation also indicated valid internal PCR controls and standardized pre-analytical handling. These features strengthen confidence that the reported negative PCR results were technically interpretable, even though they cannot overcome biological or sampling limitations.

The limitations are substantial and should define the boundaries of interpretation. First, the cohort was very small, single-centre, and highly selected because cephalocentesis is uncommon; the study therefore cannot estimate the prevalence of infection among all fetuses with ventriculomegaly or hydrocephalus. Second, there was no comparator group and no inferential analysis, so associations between infection markers, imaging severity, or outcomes cannot be tested. Third, gestational age and fetal anatomy were

heterogeneous. Fourth, the timing of sampling relative to any putative maternal infection could not be standardized, which matters for molecular sensitivity. Fifth, the exact CMV PCR genomic target and commercial assay identifier were not documented in the source materials available for this manuscript and should be confirmed from the laboratory before submission. Sixth, standardized postnatal confirmatory testing for congenital CMV or toxoplasmosis and long-term neurodevelopmental follow-up were not available for all participants. Two pregnancies were ongoing at the data cutoff. Finally, the diagnostic accuracy of PCR in prenatal fetal ventricular CSF is not sufficiently established to infer sensitivity, specificity, or negative predictive value from these observations.

Despite these limitations, the findings have practical relevance for fetal-medicine diagnostic reasoning. Severe ventriculomegaly should not be presumed infectious on morphology alone, and maternal seropositivity should not be interpreted as fetal infection. Conversely, negative maternal IgM, high avidity, or a single negative molecular specimen should not be used in isolation. The most defensible pathway integrates detailed neurosonography and fetal MRI when indicated, diagnostic genetic testing appropriate to the phenotype, maternal infection history and serology, and appropriately timed molecular testing of validated fetal specimens [2–6, 9, 15]. For prenatal detection of CMV and T. gondii, PCR of amniotic fluid obtained by amniocentesis is the established molecular approach [9, 15, 17]. This series did not demonstrate an additional diagnostic benefit from ventricular-CSF testing and therefore does not support cephalocentesis solely for infection detection.

Conclusions

Among nine pregnancies complicated by severe or progressive fetal hydrocephalus, paired real-time PCR did not detect *T. gondii* or CMV DNA in either amniotic fluid or fetal ventricular CSF. Maternal serologic patterns were also not supportive of recent primary infection. These concordant findings reduce support for active primary CMV or *T. gondii* infection as the explanation for hydrocephalus in this series, but they do not establish a zero infection prevalence, exclude every biologic scenario of congenital infection, or validate fetal CSF as a stand-alone rule-out specimen.

Prenatal evaluation for T. gondii and CMV infection in fetal hydrocephalus can be performed using PCR of amniotic fluid obtained by amniocentesis, interpreted alongside maternal serology, fetal imaging, and diagnostic genetic evaluation as appropriate. Cephalocentesis is not required solely to detect these infections, as this series did not demonstrate an additional diagnostic benefit from ventricular-CSF testing. This finding does not establish equivalence between the two specimen types. Further assessment of fetal ventricular-CSF PCR requires larger prospective studies with standardized laboratory methods, postnatal confirmation, and long-term neurodevelopmental follow-up.

Abbreviations

CMV: cytomegalovirus

CSF: cerebrospinal fluid

IgG: immunoglobulin G

IgM: immunoglobulin M

MRI: magnetic resonance imaging

NIPT: non-invasive prenatal testing

PCR: polymerase chain reaction

REP-529: 529-bp repetitive element of *Toxoplasma gondii*

Declarations

Ethics approval and consent to participate

The study was approved by the Health Research Ethics Committee of RSAB Harapan Kita (DP.04.03/D.XXXVI.19/23/2026; 2 April 2026). Written informed consent was obtained from all participating women for study participation and the invasive procedures.

Consent for publication

Not applicable. No direct identifiers, participant images, or participant-level case narratives are presented in this manuscript.

Availability of data and materials

The de-identified datasets generated and/or analysed during the current study are not publicly available because they contain sensitive clinical information from a small cohort, but may be available from the corresponding author on reasonable request, subject to institutional approval and applicable confidentiality requirements.

Competing interests

[None declared.]

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Authors' contributions

AN conceived the study, collected and analysed the data, and drafted the manuscript. GA, YBS, NW, RM, DP, YP, MAFD contributed to study design and supervised the research. All authors reviewed and approved the final manuscript. DS is the guarantor.

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