UNIVERSIDADE FEDERAL DA BAHIA
PROGRAMA DE PÓS-GRADUAÇÃO EM FARMÁCIA

VICTOR OTERO MARTINEZ

IMPACTO DAS INFECÇÕES MATERNAS CRÔNICAS:

Associações entre a soropositividade materna para anticorpos IgG contra *Toxoplasma gondii* com restrição no crescimento fetal e efeitos no neurodesenvolvimento infantil

Salvador, BA
2024

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Tese apresentada ao Programa de Pós-graduação em Farmácia da Universidade Federal da Bahia como requisito parcial para obtenção do título de doutor.

Orientador: Prof. Dr. José Antônio Menezes Filho

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SUMÁRIO

APRESENTAÇÃO 8

HIPÓTESE 9

OBJETIVOS 9

Objetivo geral 9

Objetivos específicos 9

CAPÍTULO 1 10

REFERENCIAL TÉORICO - MANUSCRITO DE REVISÃO
Relações entre ativação imunológica materna, doenças infecciosas crônicas/latentes pré-natais e neurodesenvolvimento: Uma revisão sistemática

CAPÍTULO 2 31

Impact of chronic toxoplasmosis in pregnancy: association between maternal seropositivity for *Toxoplasma gondii* IgG antibodies and fetal growth restriction

CAPÍTULO 3 57

Impact of Chronic Toxoplasmosis in Pregnancy: Association between maternal IgG antibodies against *Toxoplasma gondii* and neurocognitive development effects

CONSIDERAÇÕES FINAIS 82

PERSPECTIVAS FUTURAS 83

ANEXOS

ANEXA A – Parecer do Comitê de Ética da Faculdade de Farmácia – Universidade Federal da Bahia 84

APÊNDICE B – Termo de Consentimento Livre esclarecido 90

APÊNDICE C – Termo de Assentimento 92

APÊNDICE D – Questionário sociodemográfico 94

APÊNDICE E – Artigo publicado no periódico *Parasitology Research* - Impact of chronic toxoplasmosis in pregnancy: association between maternal seropositivity for *Toxoplasma gondii* IgG antibodies and fetal growth restriction 97

APÊNDICE F – Artigo publicado no periódico *Neurotoxicology* - Impact of chronic toxoplasmosis in pregnancy: Association between maternal IgG antibodies against *T. gondii* and neurocognitive development effects 108

APRESENTAÇÃO

Este trabalho faz parte de um estudo amplo, denominado DSAN-12m (Determinantes socioambientais do neurodesenvolvimento de crianças aos 12 meses: Um estudo longitudinal no Recôncavo Baiano), cujo objetivo é investigar os determinantes socioambientais do neurodesenvolvimento de crianças aos 12 meses de idade em uma coorte de nascimentos no Recôncavo Baiano.

Na presente tese foram avaliados o estado da arte atual quanto às relações entre a ativação imunológica materna, doenças infecciosas crônicas/latentes pré-natais e o neurodesenvolvimento. Além disso, foram elaborados dois manuscritos originais, que visaram investigar o impacto de infecções durante o período gestacional, particularmente quando não há evidencias de doença aguda, no crescimento fetal ao nascimento e no neurodesenvolvimento infantil os 12 meses de idade.

Para melhor organização, o texto foi dividido em um manuscrito de revisão, que apresenta o referencial teórico e sistematiza as publicações sobre o tema da tese. Em seguida, são apresentados dois manuscritos originais, seguidos por considerações finais, a fim de manter a unidade do trabalho.

O primeiro manuscrito, intitulado "***Impact of chronic toxoplasmosis in pregnancy: association between maternal seropositivity for Toxoplasma gondii IgG antibodies and fetal growth restriction***", investigou as relações entre a prevalência de anticorpos específicos em gestantes e alterações no desenvolvimento fetal ao nascimento. Destaca-se que este manuscrito foi publicado no periódico *'Parasitology Research'* (ISSN: 1432-1955, Fator de impacto: 1,8) em dezembro de 2023 (APÊNDICE E).

O segundo manuscrito, com o título "***Impact of Chronic Toxoplasmosis in Pregnancy: Association between maternal IgG antibodies against Toxoplasma gondii and neurocognitive development effects***", explorou a associação entre a prevalência de anticorpos específicos em gestantes com alterações no neurodesenvolvimento de seus filhos aos 12 meses de idade. Esse manuscrito está formatado de acordo com as normas do periódico '*Parasitology Research'* e será submetido para publicação após as correções sugeridas pela banca.

Por fim, estão as considerações finais e as perspectivas futuras, integrando os resultados desta tese e dos demais fatores determinantes socioambientais que têm efeito adverso no neurodesenvolvimento infantil nesta coorte de nascimento realizada no Recôncavo Baiano, o estudo DSAN-12m.

HIPÓTESE

Dado a existência de um crescente corpo de evidências sobre a relação entre as infecções maternas crônicas/latentes e alterações no desenvolvimento infantil, levantamos a hipótese de que a soropositividade para anticorpos IgG específicos durante a gestação pode afetar o desenvolvimento infantil, com efeitos adversos tanto no crescimento fetal quanto no neurodesenvolvimento.

OBJETIVOS

Objetivo geral

Investigar o impacto das infecções crônicas/latentes durante a gestação no crescimento fetal e no neurodesenvolvimento infantil aos 12 meses de idade, considerando a exposição simultânea a diversos determinantes socioambientais, em uma coorte de nascimento no Recôncavo Baiano.

Objetivos específicos

- Revisar sistematicamente a literatura a respeito as relações entre ativação imunológica materna, doenças infecciosas crônicas/latentes pré-natais e o neurodesenvolvimento;
- Determinar a prevalência de soropositividade materna para anticorpos IgG contra *T. gondii* e citomegalovírus em gestantes de Nazaré e Aratuípe, Recôncavo Baiano;
- Verificar os fatores socioambientais associados à soropositividade materna para anticorpos IgG específicos;
- Avaliar a associação entre a soropositividade materna para anticorpos IgG específicos e restrição no crescimento fetal;
- Testar a associação entre a soropositividade materna para anticorpos IgG específicos e alterações no neurodesenvolvimento infantil aos 12 meses de idade.

Capítulo 1

REFERENCIAL TÉORICO - MANUSCRITO DE REVISÃO

Relações entre ativação imunológica materna, doenças infecciosas crônicas/latentes pré-natais e neurodesenvolvimento: Uma revisão sistemática

Relationships between maternal immune activation, chronic/latent prenatal infectious diseases, and neurodevelopment: A systematic review

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RESUMO

A hipótese da ativação imune materna sugere que a exposição a um meio imunológico materno alterado no útero afeta o neurodesenvolvimento fetal. Embora diversos agentes infecciosos estejam associados a um risco elevado de distúrbios específicos, evidências acumuladas sugerem que este processo, mesmo na ausência de um episódio infeccioso agudo, pode aumentar a suscetibilidade a uma ampla gama de patologias do sistema nervoso central (SNC). Este estudo teve como objetivo revisar sistematicamente a literatura científica sobre as associações entre a ativação imunológica materna induzida por agentes infecciosos, particularmente nos casos em que a doença aguda não está presente, e as alterações subsequentes no neurodesenvolvimento na população humana. Revisamos e resumimos os estudos publicados desde 1990. Os descritores utilizados foram relacionados às infecções maternas e a desfechos no neurodesenvolvimento ou doença mental; as principais bases de dados da literatura médica foram acessadas. Foram revisados 61 artigos, dos quais 13 (21,3%) discutiram a ativação do sistema imune materno mediada por infecções crônicas/latentes e a associação com alterações no neurodesenvolvimento e/ou doença mental. Estudos associam a presença de marcadores maternos, como anticorpos IgG anti-Herpes vírus tipo 2, ao aumento do risco de Transtorno do Espectro Autista. Além disso, há evidências de associações entre infecções maternas crônicas/latentes e risco aumentado de esquizofrenia, com destaque para Herpes vírus tipo 2 e *Toxoplasma gondii.* No entanto, a heterogeneidade dos estudos e a falta de investigações sobre a interação entre fatores genéticos e ambientais limitam a generalização dos resultados. A presente revisão sugere a importância de monitorar e intervir durante a gravidez em relação a infecções crônicas/latentes. Todavia, novos estudos longitudinais são necessários para entender melhor os mecanismos subjacentes e como diferentes patógenos influenciam o desenvolvimento neurológico, considerando também fatores genéticos e ambientais.

Palavras-chave: Infecção materna, neurodesenvolvimento, ativação imunológica materna, transtorno do espectro autista, esquizofrenia

ABSTRACT

The maternal immune activation hypothesis suggests that exposure to an altered maternal immune environment in utero affects fetal neurodevelopment. Although various infectious agents are associated with an increased risk of specific disorders, accumulated evidence indicates that this process, even in the absence of an acute infectious episode, can increase susceptibility to a wide range of central nervous system pathologies. This study aimed to systematically review the scientific literature on the associations between maternal immune activation induced by infectious agents, particularly in cases where acute illness is not present, and subsequent neurodevelopmental alterations in the human population. We reviewed and summarized studies published since 1990. The descriptors used were related to maternal infections and neurodevelopmental outcomes or mental illness; major medical literature databases were accessed. A total of 61 articles were reviewed, of which 13 (21.3%) discussed maternal immune system activation mediated by chronic/latent infections and its association with neurodevelopmental alterations and/or mental illness. Studies associate the presence of maternal markers, such as IgG antibodies against Herpes simplex virus type 2, with an increased risk of Autism Spectrum Disorder. Additionally, there is evidence of associations between chronic/latent maternal infections and an increased risk of schizophrenia, with particular emphasis on Herpes simplex virus type 2 and Toxoplasma gondii. However, the heterogeneity of the studies and the lack of investigations into the interaction between genetic and environmental factors limit the generalization of the results. This review suggests the importance of monitoring and intervening during pregnancy concerning chronic/latent infections. Nevertheless, new longitudinal studies are needed to better understand the underlying mechanisms and how different pathogens influence neurological development, also considering genetic and environmental factors.

Keywords: Maternal infection, neurodevelopment, maternal immune activation, autism spectrum disorder, schizophrenia.

INTRODUÇÂO

O neurodesenvolvimento (ND) durante o período pré-natal é um processo complexo, onde qualquer influência ambiental externa pode ser severamente prejudicial. As alterações no ambiente intrauterino no decorrer da gestação podem levar a prejuízos no desenvolvimento do cérebro fetal durante a etapa mais crítica de seu desenvolvimento. Mediadores imunológicos maternos conseguem atravessar a barreira placentária podendo alterar direta e indiretamente o ambiente uterino e, assim, influenciar o crescimento fetal e a neurogênese, traduzindo-se em consequências prejudiciais ao ND e à saúde mental futura da prole (Boulanger-Bertolus, Pancaro e Mashour, 2018; Han et al. 2021).

Ambientes insalubres com más condições sanitárias aumentam a exposição aos microrganismos, levando a infeções. Em razão da possibilidade de transmissão vertical e da virulência desta categoria de contaminantes, as gestantes são monitoradas durante o período pré-natal a fim de detectar precocemente possíveis infecções e implementar estratégias para limitar o impacto na saúde do recém-nascido (Miranda et al. 2012). Esses insultos ambientais durante o período gestacional podem levar a alterações imunológicas e epigenéticas e futuramente tornar a prole mais suscetível a alterações do ND (Kundakovic e Jarić, 2017).

A hipótese da ativação imune materna (MIA) propõe que a exposição a um meio imunológico materno alterado no útero afeta o ND fetal (Estes & Mcallister, 2016). As evidências sugerem que mediadores inflamatórios produzidos pela gestante em reposta à presença de um patógeno podem causar distúrbios do desenvolvimento neurológico no recém-nascido, como: distúrbios do espectro do autismo, comprometimento cognitivo, paralisia cerebral, epilepsia e esquizofrenia (ESQ) (Jiang et al. 2018).

Os primeiros indícios de que infecções maternas durante a gestação poderiam estar associadas a distúrbios do ND em crianças e adultos surgiram após a epidemia de rubéola em 1964, onde foi observado um aumento significativo nas incidências de transtorno do espectro autista (TEA) e de distúrbios comportamentais em indivíduos expostos durante o período pré-natal (Minakova e Warner, 2018). Houve um novo aumento na preocupação com transmissão materno-infantil de agentes infectocontagiosos, após o surto de vírus Zika no Brasil em meados de 2015 e o consequente aumento de bebês nascidos com microcefalia (Rasmussen, 2016). Além disso, uma ampla gama de infecções maternas, incluindo infecções virais, bacterianas e protozoárias, são evidentemente associadas a um maior risco de distúrbios neurológicos e neuropsiquiátricos na prole (Minakova e Warner, 2018). Os patógenos, como o TORCH (toxoplasmose, outros agentes, rubéola, citomegalovírus e herpes

simples) e o vírus Zika, podem infectar diretamente o feto através da transmissão vertical (Kovacs, 2020). No entanto, apesar destes agentes infecciosos estarem relacionados a um maior risco de distúrbios específicos, evidências crescentes sugerem que a MIA, mesmo na ausência de uma infecção aguda, pode aumentar o risco de um amplo espectro de doenças do sistema nervoso central (SNC) em humanos (Knuesel et al. 2014; Han et al. 2021).

Estudos realizados no contexto da pandemia da COVID-19 demostram que as respostas inflamatórias na placenta devido à infecção por SARS-CoV-2 durante a gravidez foram associadas à alterações do ND na primeira infância (Woods et al. 2022). Os bebês expostos pré-natalmente ao vírus exibiram pontuações mais baixas em habilidades motoras e comunicação em comparação com aqueles não expostos, reforçando a ideia de que a infecção materna pode ter efeitos duradouros no desenvolvimento infantil (Shook et al. 2022; Dubey et al. 2022).

Em modelos animais a administração de uma única citocina inflamatória em roedores grávidas é suficiente para induzir vários desfechos neurológicos na prole, como: déficits na memória e na flexibilidade cognitiva, alterações motoras, aumento da ansiedade e maior sensibilidade às anfetaminas (Jiang et al., 2018). Além de comprometer o ND, acredita-se que doenças infeciosas e a ativação a do sistema imune materno seja um dos principais fatores de risco para partos prematuros. O aumento da produção de citocinas pró-inflamatórias tem sido associado à ativação uterina e nascimentos pré-termo (Cappelletti et al. 2016). Os estudos pré-clínicos e as evidências epidemiológicas sugerem que a infecção pré-natal e a MIA subsequente podem resultar em alterações comportamentais na prole adulta.

Como relatado, muitas exposições diferentes foram associadas com alterações do ND na prole, indicando que a hipótese da ativação imunológica materna, e não patógenos específicos, está relacionada à modificações negativas no ND (Canetta e Brow, 2012). A hipótese da MIA é reforçada pelo crescente número de evidências sugerindo que marcadores imunológicos comuns a uma grande variedade de patógenos está ligada às doenças do SNC na prole adulta. Um estudo de coorte de nascimentos que investigou diversos patógenos e sua possível relação com transtornos do ND demonstrou que a presença de anticorpos IgG anti-Herpes simplex vírus-2 (HSV-2) estava associada a um risco aumentado de TEA na prole masculina, enquanto que, para presença de anticorpos para Herpes simplex vírus-1 (HSV-1), rubéola, *Toxoplasma gondii (T. gondii)* e citomegalovírus (CMV) não foi encontrada essa relação (Mahic et al. 2017).

A MIA pode explicar como diferentes patógenos podem conferir riscos semelhantes aos descendentes. Conhecer os determinantes associados às alterações no

neurodesenvolvimento humano favorece a identificação precoce e possibilita a criação de medidas de prevenção mais eficazes. Portanto, o objetivo deste estudo foi revisar sistematicamente a literatura científica que investigou as associações entre a ativação imunológica materna induzida por agentes infecciosos, particularmente nos casos em que a doença aguda não está presente, e as alterações subsequentes no ND na população humana.

METODOLOGIA

Realizamos uma revisão da literatura científica nas bases de dados Medline (National Library of Medicine, Bethesda, Maryland, EUA), Scopus (Elsevier, Oxford, Reino Unido), Scientific Electronic Library Online (São Paulo, Brasil), LILACS (Literatura Latino-Americana e do Caribe em Ciências da Saúde, São Paulo, Brasil) e Web of Science (Thomson Reuters Scientific, Clarivate Analytics, Boston, EUA). As buscas incluíram artigos publicados desde 1990 até março de 2024; os seguintes descritores foram utilizados: infecção materna/*maternal infection*; infecção pré-natal/*prenatal infection*; neurodesenvolvimento/*neurodevelopment*; cognição/*cognition*; ativação imunológica materna/*maternal immune activation*; esquizofrenia/*schizophrenia;* transtorno do espectro autista/*autism spectrum disorder*; psicoses/*psychoses*. Apenas artigos completos de pesquisa foram revisados, incluindo estudos epidemiológicos em humanos cuja avaliação esteja relacionada a alguma alteração no neurodesenvolvimento em crianças de qualquer idade. Quatro tabelas foram criadas para tabular as informações encontradas. As Tabelas 1, 2 e 3 apresentam um resumo esquemático dos estudos, mostrando suas características gerais, como país de origem, desenho do estudo, população de interesse, tamanho da amostra e exposição investigada. As Tabelas 4, 5 e 6 resumem os critérios diagnósticos; fonte, tipo e momento da amostra de exposição; ajuste para fatores de confusão, e os principais achados de cada estudo.

RESULTADOS

Foram revisados 61 artigos publicados em língua inglesa desde a década de 1990 os quais reportam doenças infeciosas e alterações de ND. No entanto, apenas 13 (21,3%) atenderam aos critérios de inclusão e de fato discutiram a ativação do sistema imune materno mediado por infecções crônicas/latentes e a associação ou relação com alguma alteração no ND e/ou doença mental.

Para melhor sumarização dos estudos revisados foram criadas tabelas especificas para as relações entre infecções maternas crônicas/latentes com o TEA, com a ESQ e com os demais desfechos do ND.

As tabelas 1 e 4 reúnem informações a respeito das características dos estudos e seus principais achados sobre a associação entre infecções crônicas/latentes maternas e o TEA. Diversos estudos avaliados discutem se doenças infeciosas agudas pré-natais estão associadas ao desenvolvimento subsequente do TEA. No entanto, apenas três publicações discutem sobre uma possível associação entre infecções maternas crônicas/latentes com o TEA nos descendentes. O primeiro estudo revisado foi realizado na Noruega em 2017, envolvendo a Autism Birth Cohort, que incluiu mães, pais e filhos recrutados entre 1999 e 2008. Os resultados indicam que altos níveis de anticorpos IgG anti-HSV-2 no plasma materno estão associados a um risco elevado de TEA na prole do sexo masculino. Em contraponto, não foram evidenciadas associações significativas entre TEA e anticorpos IgG para *T. gondii*, vírus da rubéola, CMV ou HSV-1 (Mahic et al. 2017). Outro estudo, publicado no mesmo ano, investigou a relação entre anticorpos maternos contra *T. gondii* e as chances de autismo infantil na prole, evidenciando que níveis elevados de anticorpos IgM maternos contra *T. gondii* estavam associados a uma diminuição significativa das chances de autismo infantil, enquanto que, níveis baixos de anticorpos IgG maternos *contra T. gondii* foram associados ao aumento das chances de autismo na prole. Este fato indica que uma forte resposta imunitária ao *T. gondii* pode proteger contra o desenvolvimento do autismo em crianças, enquanto que, baixos níveis de IgG maternos específicos contra este patógeno pode ser um fator de risco (Spann et al., 2017).

Seguindo a mesma linha dos estudos anteriores, nos quais a exposição foi avaliada através da determinação dos níveis séricos de anticorpos IgG específicos (CMV e HSV-2) na gestante durante o período pré-natal, um estudo de coorte encontrou uma associação significativa entre a soropositividade materna para CMV e níveis mais altos de sintomas de TEA em crianças, em análise multivariada. Em contraste, o estudo não encontrou uma associação significativa entre soropositividade materna para o HSV-2 e sintomas de TEA em crianças (Slawinski et al. 2018).

As tabelas 2 e 5 reúnem informações a respeito das características dos estudos e seus principais achados sobre a associação entre infecções crônicas/latentes maternas e a ESQ. Foram incluídos nesta revisão 4 estudos que, de fato, avaliaram se a presença pré-natal de marcadores imunes específicos a patógenos está associada com maior a probabilidade de filhos diagnosticados com ESQ. O primeiro estudo foi publicado em 2001 e consistiu de um

caso-controle aninhado com 81 participantes, 54 controles e 27 indivíduos portadores de ESQ ou outros transtornos psicóticos. Dos 27 casos de psicose, 13 foram diagnosticados especificamente com esquizofrenia, sendo encontrada uma associação significativa entre níveis elevados de imunoglobulinas séricas maternas anti-HSV-2 no parto e o desenvolvimento subsequente de psicoses (incluindo ESQ) na prole. No entanto, nenhuma associação significativa foi encontrada entre os desfechos e anticorpos para *T. gondii*, rubéola, CMV ou HSV-1 (Buka et al. 2001).

Dois estudos encontram evidências de que a presença de anticorpos IgG anti-*T. gondii* está associado ao desenvolvimento de esquizofrenia subsequente. Em um estudo de caso-controle aninhado de uma grande coorte de nascimentos foram quantificados os níveis de IgG anti-*T. gondii*, usando o teste de corante Sabin-Feldman e os resultados indicam que filhos de mães com altos títulos de anticorpos (≥1/128) têm uma razão de chances ajustada de 2,61 (IC95% = 1,00 – 6,82) para desenvolver ESQ ou transtornos do espectro da esquizofrenia (Brown et al. 2005). Outra pesquisa, com 198 indivíduos diagnosticados com ESQ, verificou que existe uma associação significativa entre a exposição materna ao *T. gondii* e ao CMV e o risco de esquizofrenia na prole. O estudo usou amostras de sangue neonatal em papel filtro para quantificar os níveis de anticorpos direcionados contra os agentes infecciosos, o que permitiu aos pesquisadores avaliar a exposição materna durante a gravidez (Blomström et al. 2012).

O estudo de Cheslack-Postava e colaboradores, 2015 é o mais recente encontrado. A pesquisa apresentou o maior número de participantes em comparação com as anteriores, 963 casos com esquizofrenia (CID-10 F20) ou transtorno esquizoafetivo (CID-10 F25) e 963 controles pareados. Na análise não ajustada, anticorpos IgG anti-HSV-2 materno foram associados aos casos (OR = 1,33, IC95% = 1,03-1,72, p = 0,03), no entanto, após ajustes por covariáveis a associação deixou de ser significante (OR = 1,22, IC95% = 0,93-1,60, p = 0,14). As proporções de indivíduos soropositivos e os níveis médios de anticorpos contra *C. trachomatis* foram semelhantes para casos e controles (Cheslack-Postava et al. 2015).

As tabelas 3 e 6 reúnem informações a respeito das características dos estudos e seus principais achados sobre a associação entre infecções crônicas/latentes maternas e demais desfechos no ND. Foram incluídos nesta revisão seis estudos. O estudo de Buka e colaboradores (2001), previamente analisado por avaliar a ESQ como desfecho, também investigou a relação entre as exposições a anticorpos IgG específicos durante a gestação e outros desfechos no ND, especificamente transtorno bipolar, psicose breve e tipos não especificados de psicose. Os indivíduos cujos anticorpos maternos para HSV-2 excederam o

75º percentil da amostra de controle tiveram uma razão de chances de 3,4 para psicose; aqueles cujos anticorpos maternos excederam o 90º percentil tiveram uma razão de chances de 4,4. O mesmo grupo de pesquisa realizou um novo estudo com uma amostra maior e focado no risco de psicoses entre filhos de mães com evidência sorológica de infecção por HSV-2. Neste levantamento, a infecção materna por HSV-2 aumentou significativamente o risco de desenvolvimento de psicoses (OR= 1,6; IC95% = 1,1-2,3) (Buka et al. 2008).

Estudo realizado por Xiao et al. (2009) investigou a relação entre a infecção por diferentes genótipos de *T. gondii* e o risco de psicose em filhos adultos de mães que apresentaram padrões sorológicos consistentes com infecção por este parasita. Foi demonstrado que os filhos de mães com um padrão sorológico consistente com infecção por *Toxoplasma* tipo I apresentava risco significativamente maior de desenvolvimento de psicoses em comparação com as mães soronegativas (OR = 1,94; IC95% = 1,08 - 3,46; p = 0,03). O risco foi particularmente elevado para psicoses afetivas (OR = 5.24; IC95% = 1.67 - 16.5; p = 0.005). No entanto, não foram encontradas associações entre anticorpos maternos para os genótipos (tipos II e III) e risco de psicoses na prole (Xiao et al. 2009).

Outra pesquisa previamente revisada investigou a relação entre a exposição materna crônica/latente a *T. gondii*, CMV, HSV-1 e HSV-2 e o risco de psicoses não afetivas, incluindo ESQ, nos filhos. Não foram encontradas associações entre a exposição materna aos agentes infecciosos e outras psicoses não afetivas (Blomström et al. 2012). Em 2015, o mesmo grupo de pesquisa publicou um novo estudo que analisou amostras de sangue seco neonatal de 199 casos de psicose não afetiva e 525 controles pareados, com foco em indivíduos nascidos na Suécia entre 1975 e 1985. A pesquisa encontrou um risco relativo aumentado para psicose não afetiva associada à infecção materna por *T. gondii* (RR = 2,1, IC95% = 1,1 – 4,0), mas foi significativo apenas entre neonatos com baixos níveis de proteínas de fase aguda (Blomström et al. 2015). Um estudo de caso controle oriundo de uma coorte de nascimentos examinou se anticorpos maternos IgG anti-*T. gondii* estavam relacionados às alterações na cognição infantil e a transtorno bipolar nos filhos. A cognição foi avaliada nas idades de 5 e 9-11 anos através do Peabody Picture Vocabulary Test e das matrizes coloridas de Raven. O transtorno bipolar foi identificado a partir de base de dados. A IgG materna para *T. gondii* não foi associada ao risco de transtorno bipolar ou à cognição infantil (Freedman et al. 2016).

DISCUSSÃO

O presente estudo revisou sistematicamente a literatura sobre a ativação imune materna induzida por infecções crônicas/latentes durante o período pré-natal e suas possíveis associações com alterações no neurodesenvolvimento da prole. A relação entre a MIA e o ND tem sido um tema de interesse crescente na pesquisa científica. A evidência acumulada sugere que a exposição a mediadores inflamatórios durante a gestação pode ter consequências duradouras, afetando não apenas o desenvolvimento neurológico, mas também a saúde mental das futuras crianças. A sistematização dos estudos apresentados permitiu a identificar que as infecções maternas, mesmo na ausência de manifestações clínicas agudas, podem desempenhar um papel significativo na predisposição para transtornos neuropsiquiátricos, como transtorno do espectro autista e esquizofrenia.

Os mecanismos pelos quais a MIA influencia o ND são complexos e multifatoriais. Estudos demonstraram que mediadores inflamatórios, como citocinas, podem atravessar a barreira placentária e alterar o ambiente intrauterino, prejudicando a neurogênese e o desenvolvimento cerebral. Por exemplo, a pesquisa de Jiang et al. (2018) aponta que a exposição a citocinas inflamatórias em modelos animais resultou em déficits cognitivos e comportamentais significativos na prole. Essa linha de investigação é corroborada por dados epidemiológicos que associam infecções maternas a uma variedade de distúrbios neurológicos, incluindo TEA e ESQ (Kundakovic e Jarić, 2017; Boulanger-Bertolus, Pancaro e Mashour, 2018). Além disso, estudo recente sugere que recém-nascidos de mães soropositivas para anticorpos IgG anti-*T. gondii* possuem maior probabilidade de nascer pequenos para a idade gestacional ($p = 0,035$). Esse achado sugere que a infecção materna crônica por *T. gondii* também pode contribuir para maiores taxas de recém-nascidos com restrição de crescimento (Martinez et al. 2024).

Dentre os estudos que encontraram associações significativas entre infecções maternas e TEA está a pesquisa de Mahic et al. (2017) que destacou altos níveis de anticorpos IgG anti-HSV-2 no plasma materno como um fator de risco para o desenvolvimento de TEA em filhos do sexo masculino. Por outro lado, Spann et al. (2017) demonstraram que níveis elevados de anticorpos IgM contra *T. gondii* estavam associados a uma diminuição das chances de autismo, sugerindo uma relação protetora. Slawinski et al. (2018) não encontraram associações significativas entre a soropositividade para CMV e sintomas de TEA, o que destaca a variabilidade dos efeitos das infecções maternas e sugere que fatores como o tipo de

patógeno, o momento da infecção e a resposta imunológica materna podem desempenhar papéis críticos.

No que diz respeito à ESQ, todos os artigos revisados relatam alguma associação significativa entre as infecções crônicas maternas e a ESQ nos filhos. Buka et al. (2001) e Brown et al. (2005) encontraram associações significativas entre anticorpos maternos e o risco de desenvolvimento de esquizofrenia, particularmente em relação ao HSV-2 e *T. gondii*, respectivamente. Cheslack-Postava et al. (2015) em uma amostra mais abrangente demostram que anticorpos IgG anti-HSV-2 materno estavam associados aos casos de ESQ (OR = 1,33; IC95% = 1,03-1,72), no entanto, essa associação foi significativa apenas para o modelo não ajustado por covariáveis confundidoras.

Quanto aos demais desfechos avaliados, os estudos de Buka e colaboradores (2001, 2008) encontraram associações significativas entre a infecção materna por HSV-2 e o desenvolvimento de psicoses nos filhos. No primeiro estudo, foi observada uma razão de chances de 3,4 para o desenvolvimento de psicose em indivíduos com anticorpos maternos para HSV-2 acima do 75º percentil, aumentando para 4,4 quando os anticorpos estavam acima do 90º percentil. O estudo subsequente, com uma amostra mais substancial, confirmou que a infecção materna por HSV-2 aumentou o risco de psicoses (OR = 1,6; IC95% = 1,1-2,3). O estudo de Xiao et al. (2009) mostrou que filhos de mães com infecção por *T. gondii* tipo I apresentaram um risco significativamente maior de desenvolver psicoses (OR = 1,94; IC95% = 1,08 - 3,46), com um risco ainda mais elevado para psicoses afetivas (OR = 5.24; IC95% = 1,67 - 16,5). Este fato pode indicar que cepas específicas ativam de forma diferente o sistema imune materno. Em contraste, outros estudos não encontraram associações significativas entre infecções maternas e desfechos neuropsiquiátricos. Blomström et al. (2012) não observaram associações entre a exposição materna a *T. gondii*, CMV, HSV-1 e HSV-2 e o risco de psicoses não afetivas. Freedman et al. (2016) também não encontraram associação entre anticorpos maternos IgG anti-*T. gondii* e o risco de transtorno bipolar ou alterações na cognição infantil, indicando que a presença de anticorpos não necessariamente se traduz em desfechos clínicos adversos nos filhos.

Embora esta revisão da literatura tenha proporcionado uma visão abrangente sobre as associações entre infecções maternas e alterações no ND, várias limitações devem ser consideradas. A heterogeneidade dos estudos em termos metodológicos, populações analisadas, e tipos de infecções investigadas dificultam a generalização dos resultados. Além disso, a maioria dos estudos revisados não explorou a interação entre fatores genéticos e ambientais que podem influenciar a suscetibilidade a desfechos negativos no ND. Diante do

limitado número de trabalhos publicados e a ausência de estudos com a população brasileira, os autores deste artigo realizaram um estudo de coorte nascimentos que buscou investigar a associação entre a prevalência de anticorpos específicos em gestantes (*T. gondii* e CMV) em gestantes participantes do estudo DSAN-12m (Determinantes do Neurodesenvolvimento Socioambiental aos 12 Meses - Uma Coorte de Nascimentos no Recôncavo Baiano) realizado em dois municípios da região do Recôncavo Baiano, no Brasil, com alterações do neurodesenvolvimento de seus filhos aos 12 meses de idade (CAPÍTULO 3).

Todavia, os dados compilados nesta revisão sistemática reforçam a hipótese de que a MIA, induzida por infecções crônicas/latentes durante a gestação, está associada a um maior risco de transtornos neuropsiquiátricos na prole. Essa evidência reflete a importância da monitorização e intervenção durante a gravidez no que concerne às exposições a agentes infecciosos. A identificação precoce de infecções maternas e a implementação de estratégias para mitigar a inflamação podem ser cruciais para melhorar os desfechos negativos no ND infantil. Novos estudos longitudinais que acompanhem a saúde mental e o desenvolvimento cognitivo de crianças expostas a diferentes perfis de infecção materna são essenciais para construir um entendimento mais abrangente sobre essa relação. Pesquisas futuras devem se concentrar em entender melhor os mecanismos subjacentes à MIA e como diferentes patógenos podem influenciar o desenvolvimento neurológico de maneiras distintas. Além disso, é fundamental investigar a interação entre fatores genéticos e ambientais que podem modificar a resposta imunológica materna e, consequentemente, o risco de distúrbios no ND infantil.

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Tabela 1. Características dos estudos sobre a associação entre infecções maternas e transtorno do espectro autista, publicados entre 2017 e 2018.

Estudo	País	Desenho do estudo	n de controles	n de casos de TEA	Exposição investigada
Mahic et al. 2017	Noruega	Coorte de nascimento.	464	442	Anticorpos IgG maternos contra *T. gondii*, vírus da rubéola, CMV, HSV-1 e HSV-2.
Spann et al. 2017	China	Estudo de caso-controle aninhado.	874	874	Anticorpos IgG e IgM maternos contra *T. gondii*. Avidez de IgG.
Slawinski et al. 2018	EUA	Coorte longitudinal.	-	82	Soropositividade materna para CMV e HSV-2.

Tabela 2. Características dos estudos sobre a associação entre infecções maternas e esquizofrenia, publicados entre 2001 e 2015.

Estudo	País	Desenho do estudo	n de controles	n de casos de ESQ	Exposição investigada
Buka et al. 2001	EUA	Estudo de caso-controle aninhado.	54	13	Anticorpos IgG maternos contra CMV, vírus da rubéola, *T. gondii*, parvovírus humano B19, HSV-1, HSV-2 e *Chlamydia trachomatis.*
Brown et al. 2005	EUA	Estudo de caso-controle aninhado.	123	63	Anticorpos IgG maternos contra *T. gondii.*
Blomström et al. 2012	Suécia	Estudo de caso-controle.	524	198	Anticorpos IgG maternos contra T. *gondii*, CMV, HSV-1 ou HSV-2.
Cheslack-Postava et al. 2015	Vários países	Estudo de caso-controle aninhado.	963	963	Anticorpos IgG maternos contra HSV-2 e *Chlamydia trachomatis.*

Tabela 3. Características dos estudos sobre a associação entre infecções maternas e alterações no neurodesenvolvimento, publicados entre 2001 e 2015.

Estudo	País	Desenho do estudo	n de controles	n de casos	Exposição investigada
Buka et al. 2001	EUA	Estudo de caso-controle aninhado.	54	27	Anticorpos IgG maternos contra CMV, vírus da rubéola, *T. gondii*, parvovírus humano B19, HSV-1, HSV-2 e *Chlamydia trachomatis.*
Buka et al. 2008	EUA	Estudo de caso-controle aninhado.	554	200	Anticorpos IgG maternos contra HSV-2.
Xiao et al. 2009	EUA	Estudo de caso-controle aninhado.	618	219	Anticorpos maternos contra diferentes genótipos de *T. gondii.*
Blomström et al. 2012	Suécia	Estudo de caso-controle.	524	198	Anticorpos IgG maternos contra *T. gondii*, CMV, HSV-1 ou HSV-2.
Blomström et al. 2015	Suécia	Estudo de caso-controle.	525	199	Anticorpos IgG maternos contra *T. gondii*, CMV, HSV-1 ou HSV-2.
Freedman et al. 2016	EUA	Estudo de caso-controle.	255	85	Anticorpos IgG maternos contra *T. gondii.*

Tabela 4. Perfil dos resultados relatados em estudos sobre a associação entre infecções maternas e transtorno do espectro autista, publicados entre 2017 e 2018

Estudo	Critérios diagnósticos	Fonte, tipo e momento da amostra de exposição	Ajuste para covariáveis confundidoras	Principais resultados
Mahic et al. 2017	Crianças com TEA foram identificadas por meio de triagem com questionário maernos. Encaminhamentos profissionais e parentais de participantes suspeitos de ter TEA. Vínculos com o Registro de Pacientes Norueguês.	Amostras de sangue materno foram coletadas na metade da gravidez (cerca da 18ª semana). Os níveis de anticorpos IgG contra *T. gondii*, vírus da rubéola, CMV, HSV-1 e HSV-2 no plasma foram medidos usando o sistema de teste Zeus AtheNA Multi-Lyte ToRCH IgG Plus.	Idade materna no parto, tabagismo materno durante a gravidez, paridade e nível de escolaridade materna.	Altos níveis de anticorpos IgG contra HSV-2 no plasma materno na metade da gravidez foram associados a um aumento do risco de TEA em filhos do sexo masculino, quando ajustado para paridade e ano de nascimento da criança.
Spann et al. 2017	Registro de alta hospitalar e ambulatorial da Finlândia.	Soros maternos foram prospectivamente analisados a partir de um biobanco nacional.	Sexo da prole, idade materna, idade paternal, número de partos anteriores, status socioeconômico, idade gestacional, peso ao nascer e semana gestacional da coleta de sangue.	Baixos níveis de anticorpos IgG maternos contra *T. gondii* foram associados a uma maior probabilidade de TEA na prole.
Slawinski et al. 2018	Escala de Responsividade Social versão 2 (SRS-2).	Soro materno coletado durante a primeira consulta pré-natal.	Idade materna, etnia, nível educacional, paridade e sexo da criança.	Crianças cujas mães eram soropositivas para CMV apresentaram escores na SRS-2 de 3,6 a 4,2 pontos mais altos em comparação com aquelas cujas mães eram soronegativas. Não houve associação significativa entre soropositividade para HSV-2 e sintomas de TEA nas crianças.

Tabela 5. Perfil dos resultados reportados em estudos sobre a associação entre infecções maternas e esquizofrenia, publicados entre 2001 e 2015.

Estudo	Critérios diagnósticos	Fonte, tipo e momento da amostra de exposição	Ajuste para covariáveis confundidoras	Principais resultados
Buka et al. 2001	DSM-IV*	Amostras de sangue que foram obtidas das mães no final da gravidez. As amostras foram analisadas para imunoglobulinas totais de classe específica e anticorpos específicos.	Doença mental materna, ganho de peso, tabagismo durante a gravidez e renda familiar, nível educacional e ocupação no nascimento.	Os filhos de mães com níveis elevados de IgG total e IgM contra HSV-2 apresentaram maior risco de desenvolver ESQ. Não foram encontradas associações significativas entre ESQ e anticorpos contra patógenos como *T. gondii*, rubéola, CMV ou HSV-1.
Brown et al. 2005	DSM-IV	Amostras de soro materno foram obtidas durante a gravidez.	Idade materna, etnia materna, status socioeconômico materno e idade gestacional da amostra de soro.	A razão de chances ajustada para ESQ/transtornos do espectro esquizofrênico para prole de mães com altos títulos de anticorpos IgG contra *T. gondii* foi de 2,61 (IC 95% = 1,00–6,82).
Blomström et al. 2012	DSM-IV, registros de CID-9 e −10*	Os níveis específicos de IgG em amostras de sangue seco neonatal arquivadas de indivíduos e foram determinados por imunoensaios.	Idade materna e migração.	Os níveis de IgG direcionados a *T. gondii*, correspondentes à exposição materna, foram associados a ESQ subsequente (OR = 2,1, IC 95% 1,0–4,5), assim como os níveis de IgG direcionados a CMV (OR = 2,2, IC 95% 1,0–5,1), mas não a HSV-1 ou HSV-2.
Cheslack-Postava et al. 2015	DSM-V ou registro de CID-10	Imunoensaios mediram anticorpos de classe IgG contra HSV-2 em soro de amostras maternas coletadas durante a gravidez.	Idade paternal, província de nascimento e diagnóstico de ESQ dos pais.	A soropositividade materna para IgG contra HSV-2 foi marginalmente mais alta entre os casos do que entre os controles (OR = 1,22, IC = 0,93–1,60;). As proporções de sujeitos soropositivos e os níveis médios de anticorpos contra *C. trachomatis* foram semelhantes para casos e controles.

* Manual Diagnóstico e Estatístico de Transtornos Mentais

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Tabela 6. Perfil dos resultados reportados em estudos sobre a associação entre infecções maternas e alterações no neurodesenvolvimento, publicados entre 2001 e 2015.

Estudo	Critérios diagnósticos	Fonte, tipo e momento da amostra de exposição	Ajuste para covariáveis confundidoras	Principais resultados
Buka et al. 2001	DSM-IV*	Amostras de sangue que foram obtidas das mães no final da gravidez. As amostras foram analisadas para imunoglobulinas totais de classe específica e anticorpos específicos.	Doença mental materna, ganho de peso, tabagismo durante a gravidez, renda familiar, nível educacional e ocupação no nascimento.	Os filhos de mães com níveis elevados de IgG e IgM totais de imunoglobulinas apresenta maior risco de desenvolver doenças psicóticas na idade adulta. Não foram encontradas associações significativas entre psicose e anticorpos maternos contra *T. gondii*, rubéola, CMV ou HSV-1.
Buka et al. 2008	DSM-IV	Amostras de soro materno foram coletadas no final da gravidez.	Educação materna e tratamento parental para transtornos mentais.	Os filhos de mães com evidência sorológica de infecção por HSV-2 tiveram um risco significativamente aumentado para o desenvolvimento de psicoses (OR = 1,6; IC95% = 1,1–2,3). Esse risco foi particularmente elevado entre mulheres com alta frequência de atividade sexual durante a gravidez (OR = 2,6; IC95% = 1,4–4,6).
Xiao et al. 2009	DSM-IV, registros de CID-9 and −10*	Anticorpos maternos contra *T. gondii* foram medidos no final da gravidez, visando polipeptídeos específicos dos genótipos de T. *gondii* (GRA5, GRA6 e GRA7).	Ano de nascimento, sexo e etnia dos filhos.	Mães com um padrão sorológico da infecção crônica por *T. gondii* do tipo I tiveram um risco significativamente aumentado de ter filhos que desenvolveram psicose, com uma razão de chances de 1,94 (IC95% = 1,08–3,46). O risco foi particularmente pronunciado para psicoses afetivas, com uma razão de chances de 5,24 (IC95% = 1,67–16,5).
Blomström et al. 2012	DSM-IV, registros de CID-9 and −10*	Os níveis específicos de IgG em amostras de sangue seco neonatal arquivadas foram determinados por imunoensaios.	Idade materna e migração.	Não houve associações entre qualquer um dos agentes infecciosos e psicoses não afetivas.

Blomström et al. 2015	Registros de CID-9 and −10*	Os níveis específicos de IgG em amostras de sangue seco neonatal arquivadas foram determinados por imunoensaios.	Não ajustado.	Psicose não afetiva foi associada à infecção materna por *T. gondii* OR = 2,1 (IC95% = 1,1 – 4,0) ou CMV OR = 1,7 (IC 95% = 0,9 – 3,3) apenas entre neonatos com baixos níveis de proteínas de fase aguda.
Freedman et al. 2016	Vínculos com bases de dados, Teste de Vocabulário por Imagens de Peabody (PPVT) e Matrizes de Raven (Raven).	Soros maternos coletados durante o terceiro trimestre de gravidez. A exposição específica investigada foi a presença de anticorpos IgG contra *T. gondii*.	Escores brutos foram padronizados e as idades combinadas.	O título de anticorpos IgG maternos contra *T. gondii* não foi associado a um aumento do risco de transtorno bipolar ou mudanças na cognição infantil.

* Manual Diagnóstico e Estatístico de Transtornos Mentais

** Classificação Internacional de Doenças

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Capítulo 2

Neste capítulo apresento o artigo "***Impact of chronic toxoplasmosis in pregnancy: association between maternal seropositivity for Toxoplasma gondii IgG antibodies and fetal growth restriction***" que explora a relação entre a infecção crônica por *T. gondii* em gestantes e o crescimento fetal, destacando a prevalência de anticorpos IgG e suas implicações para a saúde neonatal.

A hipótese central do estudo é que a infecção crônica por *T. gondii* em mulheres grávidas está associada a restrições no crescimento fetal, resultando em um aumento do risco de recém-nascidos serem classificados como pequenos para a idade gestacional. Foram avaliados a prevalência de anticorpos IgG contra *T. gondii* em gestantes e as possíveis associações entre a soropositividade materna para *T. gondii* e o crescimento fetal, considerando fatores socioeconômicos e demográficos. Este estudo foi realizado como uma coorte de nascimentos, envolvendo gestantes e seus recém-nascidos. As participantes foram recrutadas em dois municípios do Recôncavo Baiano, Brasil, e acompanhadas até o parto.

Os resultados do estudo sugerem que a infecção crônica por *T. gondii* em gestantes está significativamente associada a restrições no crescimento fetal, ressaltando a importância de monitorar a saúde materna durante a gestação para prevenir complicações no desenvolvimento neonatal. Os achados enfatizam a necessidade de intervenções direcionadas a populações vulneráveis, especialmente em áreas com alta prevalência de infecções.

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Impact of chronic toxoplasmosis in pregnancy: association between maternal seropositivity for *Toxoplasma gondii* IgG antibodies and fetal growth restriction (APÊNDICE E)

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Statements and Declarations

Competing Interests

The authors declare that there is no conflict of interest.

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Highlights

- Chronic toxoplasmosis in pregnant women is related to socioeconomic vulnerability.

- Newborns of pregnant women seropositive for anti-*Toxoplasma gondii* antibodies are more likely to exhibit intrauterine growth restriction.

- There is an association between maternal chronic infection with *T. gondii* and birth weight adequacy.

ABSTRACT

Insults caused by acute infections during the gestational period on fetal development are known; however, new evidence suggests that chronic infectious diseases can also impact the maternal immune status and lead to negative consequences for the neonate. This study investigated the association between the prevalence of specific antibodies in pregnant women and alterations in fetal development at birth. A follow-up study evaluated women during the gestational period and their respective newborns at delivery time. The pregnant women were tested for the presence of antibodies to infectious agents: *Toxoplasma gondii* (*T. gondii*), cytomegalovirus (CMV), syphilis, human immunodeficiency virus (HIV), Hepatitis B, and C. Semi-structured questionnaires were administered to the pregnant women at the time of recruitment after obtaining informed consent. Detailed information about the newborns was extracted from medical records. The seroprevalence of chronic *T. gondii* infection, as determined by the presence of IgG antibodies against the protozoan, was found to be 56.2%, while the overall prevalence of CMV IgG antibodies was 96.3%. Non-primiparous pregnant women from socio-economic classes less affluent groups and skilled working-class individuals had higher chances of testing positive for specific *T. gondii* IgG antibodies. Newborns classified as small for gestational age represented 12.9% of the total. Those born to mothers seropositive for anti-*T. gondii* IgG antibodies were 9.4 times more likely to be born small for gestational age ($p = 0.035$). The results suggest that chronic *T. gondii* infection may contribute to higher rates of newborns with growth restriction. These findings add to a growing body of evidence regarding the impact of chronic infectious diseases on intrauterine fetal development.

Keywords: Small for gestational age, toxoplasmosis, pregnancy, serology, birth cohort.

INTRODUCTION

Changes in the intrauterine environment during gestation can lead to impairments in fetal development. Maternal immune mediators can cross the placental barrier and directly or indirectly alter the uterine environment, thus influencing fetal growth and neurogenesis, resulting in detrimental consequences for the future development and mental health of the offspring (Boulanger-Bertolus et al. 2018; HAN et al. 2021). Genetic alterations and environmental exposures such as smoking, toxic metals, pesticides, alcohol, medications, and uterine infections are important causes of congenital disabilities. However, most structural, behavioral, functional, and metabolic disorders present at birth have no known genetic or environmental causes (Verma 2021).

Unhealthy environments with poor sanitation conditions increase exposure to microorganisms, leading to infections. Due to the possibility of vertical transmission and the virulence of this category of agents, pregnant women are monitored during the prenatal period to early detect potential infections and implement strategies to limit the impact on the newborn's health (Miranda et al. 2012). These environmental insults during gestation can result in immunological and epigenetic changes, making the offspring more susceptible to various insults in the future (Cattane et al. 2020).

Intrauterine growth restriction and preterm birth are the leading causes of perinatal mortality found in almost 10% of pregnancies (Nardozza et al. 2017). The INTERGROWTH-21st study developed a standardized fetal growth curve for international use. For this purpose, pregnant women from different ethnicities and regions were followed in a multiethnic and multicenter population-based cohort study, including countries such as Brazil, England, Oman, Italy, the United States, China, India, and Kenya (Villar et al. 2014). According to this classification, newborns (NBs) can be classified as small for gestational age (SGA), appropriate for gestational age (AGA), and large for gestational age (LGA) (Papageorguiou et al. 2018).

During the gestational period, there are dynamic changes in maternal and fetal immune responses that depend on the stage of pregnancy or development (Kollmann et al. 2017; Pazos et al. 2012). Despite the well-known deleterious effects of certain congenital infections, many pathogens fail to reach the fetus due to the powerful physical barrier provided by the placenta that protects the developing baby (Arora et al. 2017). One possible explanation for the mechanisms involved in fetal development disorders associated with different pathogens, even without vertical transmission, is that cytokines originating from

maternal inflammatory processes can cross the placenta and affect crucial stages of fetal development (Zhou 2012).

In addition to compromising neurodevelopment, infectious diseases, and maternal immune system activation are believed to be one of the main risk factors for preterm births. The increased production of pro-inflammatory cytokines has been associated with uterine activation and preterm births (Cappelletti et al. 2016). Studies suggest a relationship between maternal immune activation and disturbances in neonatal development. Maternal chronic infection with human immunodeficiency virus 1 (HIV-1) and hepatitis B has been shown to lead to higher concentrations of cytokines in umbilical cord blood and altered fetal immune responses, indicating that maternal immune responses can impact the fetus (Bunders et al. 2014; Hong et al. 2015).

The "TORCH" pathogens encompass a variety of infectious agents known to cause congenital disabilities. They include protozoa (*Toxoplasma gondii*), bacteria (*Treponema pallidum*), and viruses (rubella, cytomegalovirus, human herpesvirus, HIV, enteroviruses, parvovirus B19, and Zika virus) (Coyne and Lazear 2016). In addition to congenital disabilities, infections by these pathogens can cause various complications in pregnancy, including miscarriage, fetal growth restriction, and preterm birth (Cappelletti et al. 2016; Pereira et al. 2014). There has been increased concern about maternal-infant transmission of infectious agents after the Zika virus outbreak in Brazil in mid-2015 and the subsequent increase in babies born with microcephaly. A recent study revealed important information about the influence of this virus on infant neurodevelopment, even in neonates with average head size. The neurological development of 29 infants without microcephaly but with in-utero exposure to the virus was evaluated using the Bayley Neurodevelopmental Assessment scale, and 34% showed some form of impairment (Faiçal et al. 2019).

Animal models of these infections indicate that pregnancy complications can be mediated by the immune response induced by the pathogen (Yockey and Iwasaki 2018). Regardless of whether clinically detectable infection is present or not, elevated concentrations of cytokines, including interleukin (IL)-6, IL-1, IL-8, and tumor necrosis factor (TNF) in amniotic fluid or cervicovaginal region, are predictive factors for prematurity (Agrawal and Hirsch 2012).

Maternal immune status can indeed affect fetal development. In the case of "TORCH" infections, some common neurological defects observed include microcephaly, cranial calcifications, ventriculomegaly, ocular defects, and sensorineural hearing loss (Coyne and Lazear 2016). Many of these presentations, such as microcephaly, are similar across

pathogens, including cytomegalovirus, rubella, *Toxoplasma gondii*, and Zika virus (Devakumar et al. 2018). The similar clinical presentation across a variety of viral and protozoal pathogens suggests the presence of a common mechanism.

Understanding the determinants associated with alterations in human development facilitates early identification and enables the creation of more effective preventive measures. In this regard, Horta and Wehrmeister (2017) emphasize the importance of cohort studies as they provide an opportunity to evaluate the consequences of exposures occurring at different stages of the life cycle, allowing the identification of critical periods and the possibility of measuring the cumulative effect of various exposures.

While several studies show an association between congenital infections and various health issues in NBs, few studies have investigated whether immune status against certain specific agents can silently impact fetal development. Therefore, this study aimed to investigate the association between the prevalence of specific antibodies in pregnant women who participated in the DSAN cohort study (Determinants of Socioenvironmental Neurodevelopment at 12 Months -A Birth Cohort in the Recôncavo Baiano) conducted in two municipalities in the Recôncavo Baiano region of Brazil, with alterations in fetal development at birth.

METHODS

Study Design and Population

This cohort study evaluated pregnant women and their respective children at birth. Pregnant women up to 24 weeks of gestation were invited to participate in the study. The project was conducted in collaboration with the primary care network in the municipalities of Nazaré and Aratuípe, Bahia, Brazil. The pregnant women were investigated for antibodies against infectious agents, including *T. gondii*, cytomegalovirus, syphilis, HIV, Hepatitis B, and C.

Participants were fully informed about the study's objectives and methods, providing informed consent by signing the Free and Informed Consent Form (FICF). In the case of participants under 18 years of age, their legal guardian was consulted, and both the guardian and the underage participant signed the FICF and Assent Form, respectively. The Ethics Committee of the Federal University of Bahia approved the study under protocol number 3.246.555 on April 5, 2019. The project is partially funded by the Research for SUS Program

(PPSUS) of CNPq/FAPESB, Call notice no. 2/2020, request number 4393/2020, and by the CNPq Universal Call, process No. 421550/2018-0.

Data Collection Procedures

Biological samples

A trained phlebotomist, used a standard venipucture technique, collected blood from the cubital vein using vacuum tubes. A gel separator tube without anticoagulant (BD Vacutainer® SST™ - Yellow) was used for serum sample centrifugation at 3,000 rotations per minute for 10 minutes; all samples were adequately labeled, packaged, and transported to the Faculty of Pharmacy at the Federal University of Bahia (UFBA). The serum samples were transferred to 5-mL tubes and kept at -20°C (freezer) until processing.

Serological tests were performed using a chemiluminescence microparticle immunoassay to search for IgG and IgM against *Toxoplasma gondii* and cytomegalovirus infections (Abbot®, São Paulo - Brazil) using the Architect i1000SR automation equipment (Abbot®, São Paulo - Brazil). Specimens with concentration values ≥ 3.0 IU/mL are considered reactive for IgG antibodies to *T. gondii*, indicating chronic infection. For CMV, samples with concentration values ≥ 6.0 AU/mL are also considered reactive for IgG antibodies.

Immunochromatographic tests designed for diagnostic purposes were used to determine the presence of total antibodies against HIV, *Treponema pallidum*, and hepatitis C, following the same methodology for detecting hepatitis B surface antigen (Abbot®, São Paulo - Brazil).

Socioeconomic data, newborn assessment, and fetal anthropometry

Semi-structured questionnaires were administered to pregnant women during recruitment and after signing the informed consent form. The Interviewers conducted the questionnaire on socioeconomic status (based on family possession and income), general cultural habits, educational level, occupational history, work during pregnancy, and other evaluations. The socioeconomic level, stratified into five categories from A to E, was defined based on the criteria of the Brazilian Association of Population Studies (Brasil, 2008).

Detailed information about the newborn at birth, including sex, ethnicity, birth weight, delivery method, and gestational age at birth, were extracted from medical records. The APGAR score and fetal anthropometric data (considered here as the child's birth outcome) were also collected from the medical records to assess possible associations with the investigated biomarkers.

The INTERGROWTH-21st Project fetal growth curve (VILLAR et al. 2014; PAPAGEORGUIOU et al. 2018) was applied to estimate the adequacy of birth weight for gestational age. The newbornes (NBs) were classified as small for gestational age (SGA) when the birth weight (in grams) for completed gestational age was below the 10th percentile and large for gestational age (LGA) when equal to or above the 90th percentile. The remaining NBs were classified as appropriate for gestational age (AGA).

Data analysis

After data tabulation, frequency, central tendency, and dispersion measures were analyzed. The choice between parametric and non-parametric tests was based on the Kolmogorov-Smirnov normality test. The Binomial test for a proportion was used to obtain confidence intervals for the proportions. The Student's t-test was applied to compare the means of parametric variables. Binary logistic regression analysis was used to identify risk factors associated with *T. gondii* infection and CMV infection. To investigate possible associations between seropositivity for anti-*T. gondii* IgG and seropositivity for anti-CMV IgG with birth-related data, multivariate logistic regression modeling was performed. The odds ratio (OR) was obtained by exponentiating the Beta obtained in the multivariate model. The model was adjusted for maternal height and maternal weight during the prenatal period. The accepted level of statistical significance for all tests was $p < 0.05$. Descriptive and comparison statistical analyses were performed using SPSS® version 26.0 for Windows, and graphs were generated using GraphPad Prism® version 8.0.2.

RESULTS

Figure 1 presents the flowchart detailing the recruitment of study participants. The study included 136 pregnant women with an average age of 26.8 years ± 0.5 years. Of the total, 26 (41.6%) resided in the municipality of Aratuípe. Most self-identified as Afro-descendants (94.7%), and 38.2% were in their first pregnancy. Most were classified as belonging to social classes D-E (51.2%). Most pregnant women had completed middle and/or high school (73.0%), while 2.3% reported being illiterate. There was a frequency of 17.4% of reported previous miscarriages. Regarding their homes, a significant portion did not have access to potable water (18.2%), and 40.2% did not reside on a paved street (Table 1).

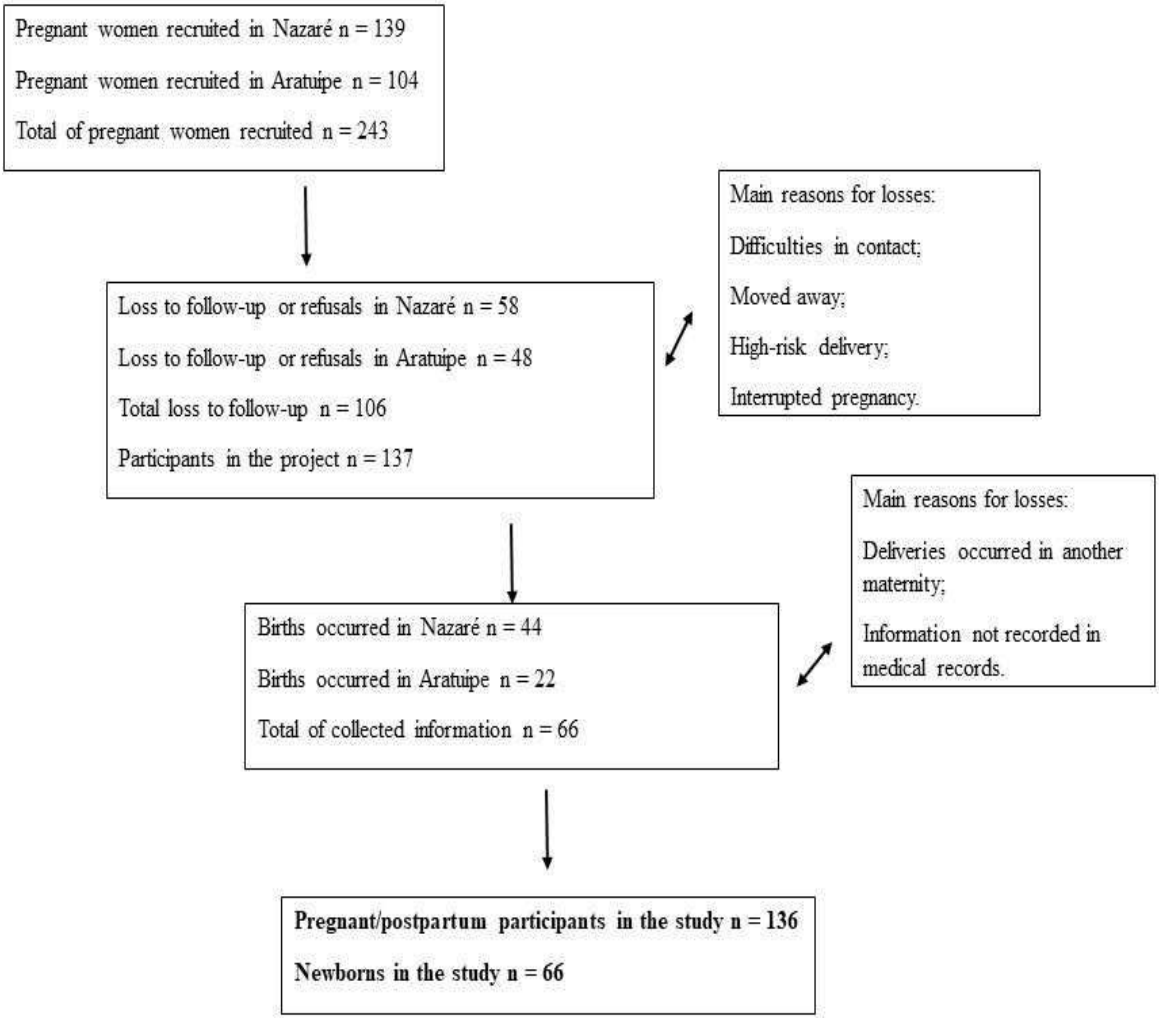

Fig 1. Recruitment flowchart.

The overall seroprevalence found for chronic *T. gondii* infection was 56.2% (95% CI, 47.8% - 64.2%), and for anti-CMV IgG antibodies, it was 96.3% (95% CI, 91.7% - 98.4%). Three pregnant women tested positive for anti-*T. pallidum* antibodies and one pregnant woman tested positive for antibodies against the hepatitis C virus (Figure 2). No pregnant woman tested positive for anti-HIV antibodies or the presence of the hepatitis B surface antigen.

In order to investigate a possible association between the categorized composite scores of Bayley-III and IgG anti-*T. gondii* antibody positivity, a multivariate logistic regression analysis was performed, crude odds ratio was used. A significant association ($p = 0.049$) was observed between the number of pregnancies and *T. gondii* infection, with non-primiparous women being more likely to have chronic *T. gondii* infection. Pregnant women and/or their family members classified as belonging to social classes D-E and C2 had a higher likelihood (3.19; 95% CI, 1.2 - 8.47) of testing positive for IgG anti-*T. gondii* antibodies. The data are presented in Table 2. Notably, some risk factors related to *T. gondii* infection, such as being older age and consuming untreated water, did not show a significant relationship with IgG anti-*T. gondii* antibody positivity (Table 2). No significant associations were found when investigating the relationship between the evaluated determinants and chronic CMV infection.

Table 3 describes the neonatal characteristics of the study participants. Information from 66 neonates was obtained. 54.5% of the NBs were male, and 86,7% were classified as African-Brazilians. Concerning the deliveries, 42.4% occurred via cesarean section, and the majority took place at Luís Argolo Maternity Hospital (61.2%) in the neighboring municipality of Santo Antônio de Jesus, Bahia. The gestational week at the delivery time had an average of 39.1 (±0.2) weeks. 12.9% of the NBs were classified as SGA and 11.3% as LGA. The overall mean APGAR score at one minute was 8.1 points.

Table 4 summarizes the results when comparing seropositivity for IgG anti-*T. gondii* and the characteristics of the NBs using multivariate logistic regression analysis, adjusted odds ratio was used. The variables of prenatal maternal weight and height were used to adjust the multivariate model. Associations between seropositivity for IgG anti-CMV and birth-related outcomes were also evaluated.

It was observed that NBs whose mothers had chronic *T. gondii* infection were 9.4 times more likely (95% CI, 1.18 - 88.1) of being born small for gestational age ($p = 0.035$). The other characteristics related to the NBs did not show a significant relationship with maternal seropositivity for IgG anti-*T. gondii* (Table 4).

There were no significant associations between the characteristics of the NBs and seropositivity for IgG anti-CMV.

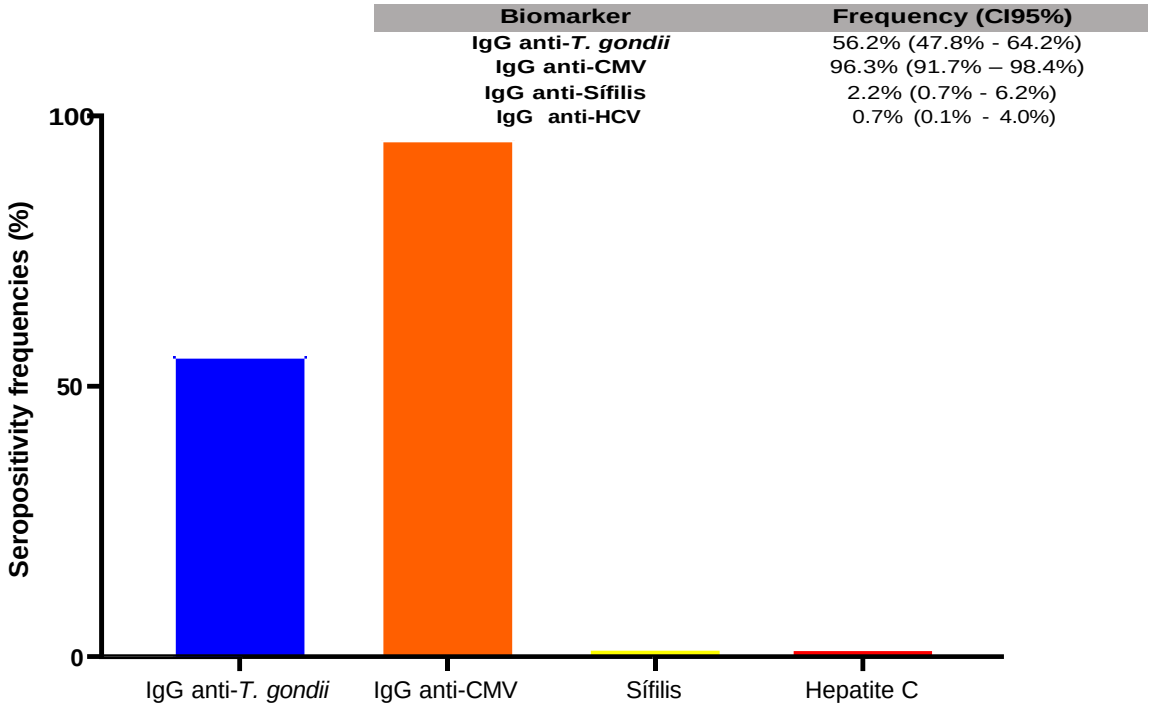

Fig 2. Prevalence of IgG antibodies against *T. gondii*, CMV, syphilis, and hepatitis C.

Table 1 – Sociodemographic characteristics of participating pregnant women in the study.

	TOTAL (n = 137)
	Mean (± SD)
Age (years)	26.8 (±0.5)
Minimum	15.0
Maximum	39.2
Variable	**n (%)**
Place of residence	
Nazaré	80 (58.4)
Aratuípe	57 (41.6)
Ethnicity	
White	4 (3.0)
African Brazilian	126 (94,7)
Yellow	3 (2.3)
Education	
Illiterate	3 (2.3)
Elementary I complete	19 (14.6)
Complete Elementary II	40 (30.1)
Medium complete	57 (42.9)
Graduated	14 (10.5)
Socioeconomic classification	
D-E	66 (51.2)
C2	41 (31.8)
C1	18 (14.0)
B2	3 (2.3)
B1	1 (0.7)
Previously pregnant?	
Yes	81 (61.8)
No	50 (38.2)
Previous miscarriage?	
Yes	23 (17.4)
No	109 (82.6)
Gestational planning?	
No	91 (68.9)
Yes	41 (31.1)
Have a cat as a pet?	
No	102 (77.9)
Yes	29 (22.1)
Does any family member's occupation involve activities in agriculture?	
Yes	55 (44.7)
No	68 (55.3)
Have they paved the house street?	
No	53 (40.2)
Yes	79 (59.8)
Residential water supply	
Artesian well	23 (18.2)
Piped	103 (81.8)
Water treatment	
Not treated	77 (59.2)
Treated (Filtered/boiled)	53 (40.8)

Table 2 - Socioenvironmental determinants of participating pregnant women being positive serology for IgG antibodies against *T. gondii* based on logistic regression models.

	IgG anti-*T. gondii* positive n (%)	IgG anti-*T. gondii* negative n (%)	OR (CI 95%)	p-value
Age range				0.260
15 to 26 years	36 (48.0)	33 (57.9)	Ref.	
27 years or older	39 (52.0)	24 (42.1)	1.49 (0.74 – 2.98)	
Ethnicity				0.934
Black	70 (94.6)	56 (94.9)	Ref.	
Nonblack	4 (5.4)	3 (5.1)	0.94 (0.20 – 4.36)	
Multiparous				0.049*
No	22 (30.6)	28 (47.5)	Ref.	
Yes	50(69.4)	31 (52.5)	2.05 (1.01 – 4.20)	
Previous miscarriage?				0.555
No	59 (80.8)	50 (84.7)	Ref.	
Yes	14 (19.2)	9 (15.3)	0.76 (0.30 – 1.91)	
Education				0.116
Complete high school or higher	35 (52.7)	36 (61.0)	Ref.	
Elementary school II or lower	39 (47.3)	23 (39.0)	0.57 (0.29 – 1.15)	
Socioeconomic classification				0.020*
C1/B/A	7 (9.9)	15 (25.9)	Ref.	
D-E/C2	64 (90.1)	43 (74.1)	3.19 (1.2 – 8.47)	
Have a pet?				0.638
No	31 (43.1)	23 (39.0)	Ref.	
Yes	41 (56.9)	36 (61.0)	1.18 (0.59 – 2.38)	
Does any family member's occupation involve activities in agriculture?				0.282
No	27 (40.3)	28 (50.0)	Ref.	
Yes	40 (59.7)	28 (50.0)	1.48 (0.72 – 3.03)	
Piped water?				0.643
Yes	57 (78.1)	48 (81.4)	Ref.	
No	16 (21.9)	11 (18.6)	0.82 (0.35 – 1.93)	
Water treatment				0.159
Treated	25 (34.7)	28 (48.3)	Ref.	
Not treated	47 (65.3)	30 (51.7)	1.39 (0.88 – 2.21)	

* Statistically significant association

Statistical tests: Univariate logistic regression.

Ref. - Reference group.

Table 3 – Sociodemographic and anthropometric characteristics of neonates included in the study.

	Total (n = 66)
Variable	**n (%)**
Sex	
Female	30 (45.5)
Male	36 (54.5)
Ethnicity	
White	6 (13.3)
African Brazilian	39 (86,7)
Type of delivery	
Vaginal	37 (56.1)
Cesarean	28 (42.4)
Residence	1 (1.5)
Place of birth	
Nazaré's Maternity	18 (26.9)
Luis Argolo Hospital	41 (61.2)
Other	8 (11.9)
Adequacy of weight for gestational age	
SGA	8 (12.9)
AGA	47 (75.8)
LGA	7 (11.3)
Variable	**Mean (± SD)**
Gestational age (weeks)	39.1 (±0.2)
Minimum	32.0
Maximum	41.0
Number of prenatal consultations performed	9.8 (±0.6)
Minimum	3
Maximum	17
Weight at birth (g)	3285.1 (±59.29)
Minimum	1744
Maximum	4900
Length at birth (cm)	47.8 (±0.4)
Minimum	32
Maximum	54
Head circumference (cm)	34.2 (±0.2)
Minimum	28
Maximum	38
Chest cage circumference (cm)	33.8 (±0.3)
Minimum	26
Maximum	40
Abdominal circumference (cm)	32.5 (±0.8)
Minimum	24
Maximum	39
APGAR scored in 1st minute	8.1 (±0.1)
Minimum	6
Maximum	9
APGAR scored at 5th minute	9.2 (±0.1)
Minimum	8
Maximum	10
Percentile	55.7 (±3.5)
Minimum	6.2
Maximum	99.5

Table 4 – Characteristics of newborns and positive maternal serology for IgG anti-*T. gondii*

	IgG anti-*T. gondii* positive n (%)	**IgG anti-*T. gondii* negative n (%)**	**OR (CI 95%)**	**p-value**
Gestational week of delivery				0.670
Term (37 weeks or more)	27 (90.0)	27 (93.1)	Ref.	
Preterm (up to 37 weeks)	3 (10.0)	2 (6.9)	0.67 (0.10 – 4.31)	
Weight at birth (g)				0.597
2501g or more	32 (94.1)	31 (96.9)	Ref.	
Up to 2500 g	2 (5.9)	1 (3.1)	0.52 (0.04 – 5.99)	
Length at birth (cm)				0.803
51cm or bigger	5 (16.1)	6 (18.8)	Ref.	
Up to 50 cm	26 (83.9)	26 (81.2)	1.21 (0.27 – 5.38)	
Brain perimeter (cm)				0.192
Bigger than 34 cm	17 (70.8)	16 (66.7)	Ref.	
Up to 33 cm	7 (29.2)	8 (33.3)	0.36 (0.07 – 1.68)	
Chest cage circumference (cm)				0.912
Bigger than 32 cm	18 (81.8)	19 (90.5)	Ref.	
Up to 31 cm	4 (18.2)	2 (9.5)	0.87 (0.08 – 9.26)	
Abdominal circumference (cm)				0.645
Bigger than 32 cm	6 (60.0)	5 (83.3)	Ref.	
Up to 31 cm	4 (40.0)	1 (16.7)	0.48 (0.02 – 10.65)	
Adequacy of weight for gestational age				0.035*
AGA/LGA	23 (76.7)	31 (96.9)	Ref.	
SGA	7 (23.3)	1 (3.1)	9.4 (1.18 – 88.1)	
APGAR scored in 1st minute				0.956
Seven or higher	23 (95.8)	20 (95.2)	Ref.	
Up to 6	1 (4.2)	1 (4.8)	0.92 (0.05 – 16.06)	

antibodies based on multivariate logistic regression models

* Statistically significant association

Statistical tests: Multivariate logistic regression.

Adjustment covariates - Maternal pre-natal weight and maternal pre-natal height.

Ref. - Reference Group

DISCUSSION

The study investigated the socio-environmental determinants associated with the presence of specific antibodies against teratogenic pathogens in pregnant women enrolled in the DSAN-12M birth cohort study being carried out in the Recôncavo Baiano region in Brazil. Additionally, these pregnant women were assessed for the potential influence of specific IgG antibodies on fetal development characteristics. The frequency of small-for-gestational-age NBs in the study population was associated with the prevalence of IgG antibodies against *T. gondii.*

The study participants generally exhibited socio-environmental characteristics that classify them as socioeconomically vulnerable to poor sanitary conditions. The seroprevalence for *T. gondii* infection was 56.2% (95% CI, 47.8% - 64.2%) among pregnant women. This frequency is relatively similar to results from other studies involving pregnant women in the Brazilian population. A study conducted in the State of Bahia, specifically in Ilhéus in the southern region of the state, with 726 pregnant women aged between 13 and 44 years, found a prevalence of 72.3% for IgG antibodies against *T. gondii*. The same study reported a frequency of 95.2% for IgG antibodies against CMV and 97.0% for antibodies against the rubella virus (Costa et al. 2018). Another study reports that *T. gondii* infection in pregnant women residing in the municipality of Ponta de Pedras, Marajó Archipelago, State of Pará, has a prevalence of 68.3%, with advanced age, contact with soil, and residing in urban areas being risk factors associated with seropositivity (Morais et al. 2020). A survey with spatial analysis of *Toxoplasma* infection in rural and peripheral neighborhoods of Juiz de Fora, Minas Gerais, showed that 44.4% exhibited seropositivity for IgG antibodies against *Toxoplasma gondii*, and this rate increased to 68.3% when considering only participants from rural areas (Antinarelli et al. 2021).

A study conducted between 2013 and 2017 assessed the prevalence of *T. gondii* among two distinct populations of women of childbearing age in Siena (Tuscany, central Italy) and Bari (Apulia, southern Italy), along with a group of pregnant women in Bari in 2016–2017. This study revealed a significantly higher seroprevalence of *T. gondii* in Bari compared to Siena (22.4% vs. 12.4%), with a noticeable age-related trend. Among the tested pregnant women, a low prevalence of *T. gondii* infection (13.8%) was observed (Fanigliulo et al., 2020). In a systematic review with a meta-analysis examining global, regional, and national seroprevalence of *T. gondii* in pregnant women, it was found that the global

seroprevalence among pregnant women stood at 38.4%, with a higher prevalence in Latin America and the Caribbean (Bigna et al., 2020).

The variation in toxoplasmosis prevalence can be explained by the influence of different factors, such as environmental conditions, cultural practices, dietary habits, and hygiene (Wilking et al. 2016). The seroprevalence found for toxoplasmosis in this study can be attributed to environmental factors, considering that the climate in the region of the Recôncavo Baiano, Brazil, is hot and humid, which favors the dissemination of the infection. Additionally, the low coverage of proper sanitation facilities contributes to waterborne contamination.

The prevalence found for CMV (96.3%) is consistent with that observed by other authors. A study with pregnant adolescents conducted in the northern region of Brazil found a frequency of 96.3% for anti-CMV IgG antibodies (Guerra et al., 2018). Considering the characteristics of the studied population, the high frequency of CMV antibodies may also be related to socioeconomic factors. Economically disadvantaged populations, lacking basic sanitation and housing conditions, are more susceptible to the virus's spread, leading to higher seroprevalence (Oliveira et al. 2002).

The high frequency of cesarean deliveries among the study's pregnant women (42.4%) stands out. The World Health Organization (WHO) recommends that the rate of cesarean sections should be between 10% and 15% of the total number of deliveries in a health service (World Health Organization 2018). New evidence suggests that babies born by cesarean section experience different hormonal, physical, and bacterial exposures and these changes may subtly impact neonatal physiology (Sandall et al. 2018).

The NBs participating in the study had an overall mean length of 47.8 cm at birth, and 12.9% were classified as SGA. Fetal growth restriction occurs when the fetus fails to achieve its intrinsic growth potential. It may be related to common mechanisms involving various factors (such as placental pathology, infections, and genetic constitution) (Gordijn et al. 2018). Infections during the prenatal period can activate inflammatory processes, leading to the release of cytokines, increased oxidative stress, and cellular apoptosis, which may impact placental and fetal growth (Kesavan and Devaskar 2019).

The present study found no associations between maternal IgG anti-CMV antibodies and neonatal characteristics. However, neonates of mothers seropositive for IgG anti-*T. gondii* antibodies were 9.4 times more likely to be born small for gestational age ($p = 0.035$). A meta-analysis study on perinatal outcomes in congenital *T. gondii* infection showed that the infected patient group had a 4.5 times higher chance of intrauterine growth restriction and a

4.9 times higher likelihood of fetal anomalies, such as ventriculomegaly, intracranial and hepatic calcifications, and ascites (Li et al. 2014). A large cohort study of 740 Hispanic women living in the United States found a frequency of 22.4% for IgG anti-*T. gondii* antibodies. Seropositive pregnant women had a higher incidence of babies born small for gestational age and an increased prevalence of spontaneous abortions and preterm births (Mutka et al. 2023).

A large-scale study evaluating the impact of chronic *T. gondii* infection on the history of spontaneous abortion, pregnancy complications, and neonatal outcomes found no differences between seropositive and seronegative pregnant women for APGAR at one minute ($p = 0.544$), gestational age at birth ($p = 0.491$), birth weight ($p = 0.257$), or birth length ($p = 0.232$) (Mocanu et al. 2022). In contrast, a study in the Czech Republic with 1,733 parturients showed a higher prevalence of preterm birth ($p = 0.019$) and low birth weight ($p = 0.006$) in women with chronic *T. gondii* infection. The odds of preterm birth were 1.7 times higher, and for low birth weight was 1.9 times higher in women with chronic toxoplasmosis (Hurt et al. 2022). This study corroborates with other evidence regarding the impact of *T. gondii* infection on fetal development when not in its acute form. Pregnant women with chronic toxoplasmosis show slower fetal development estimated at the sixteenth week of pregnancy and longer gestations (Flegr et al. 2005; Kaňková and Flegr 2007). In school-age children in the same region as the current study, a seroprevalence of 43.7% for *T. gondii* was observed, and there was a significant association with rule-breaking behavior (Martinez et al. 2020).

This study has some limitations. The main one is its small sample size, as the participants represent only 38.8% of the estimated sample size based on the birth rates in the two municipalities. However, it was possible to identify associations between chronic exposure to *T. gondii* and the investigated outcomes. The study had important strengths, such as the region's serological evaluation of different prevalent pathogens. Moreover, the study design allowed for assessing multiple exposures and outcomes and discerning the temporal relationships between pathogen exposure and fetal growth outcomes.

Indeed, several factors can significantly influence fetal growth, which is determined by the combination of several variables, such as socioeconomic status, genetic influence, environmental factors, nutritional status, etc. Given the challenges in evaluating all possible determinants that play a role in fetal growth, the results observed in this study suggest that maternal chronic infection by *T. gondii* may contribute to babies with smaller birth sizes. However, several other elements are involved in these effects.

CONCLUSIONS

The results of this study demonstrate that pregnant women residing in the municipalities of Nazaré and Aratuípe, Bahia, Brazil, have a high prevalence of chronic infection by *Toxoplasma gondii* and cytomegalovirus.

Multivariate regression analysis showed that chronic *T. gondii* infection in pregnant women may contribute to uterine growth restriction. The data found in this study suggest that chronic toxoplasmosis in pregnant women is associated with a higher frequency of small-for-gestational-age newborns. Associations between chronic toxoplasmosis and lower fetal growth have been identified, regardless of maternal weight and height. The present study found no relationships between CMV infections and birth characteristics.

It was observed that pregnant women with chronic infection by this parasite are 9.4 times more likely to have small-for-gestational-age babies. This finding confirms that chronic infections during the gestational period may be important determinants of intrauterine fetal development.

Declarations

Ethical Approval

The Ethics Committee of the Federal University of Bahia approved the study under protocol number 3.246.555 on April 5, 2019.

All participants involved in this study provided informed consent before their inclusion. They were provided with a detailed explanation of the study's purpose, procedures, potential risks, and benefits. Participants were assured of their confidentiality and the voluntary nature of their participation. This study was conducted in accordance with ethical guidelines and regulations to ensure the rights and well-being of all participants.

Additional headings

Not applicable.

Competing Interests

The authors declare that there is no conflict of interest.

Authors' contributions

Victor Otero Martinez: Conceived and designed the analysis, collected data, contributed data or analysis tools, performed the analysis, and wrote the paper.

Nathália Ribeiro dos Santos: Collected data and contributed to the analysis.

Homègnon Antonin Ferréol Bah: Collected data and contributed to the analysis.

Erival Amorim Gomes Junior: Collected data and contributed to the analysis.

Daisy Oliveira Costa: Collected data and contributed to the analysis.

José Antonio Menezes-Filho: Conceived and designed the analysis, collected data, contributed data or analysis tools, performed the analysis, and wrote the paper.

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Availability of data and materials

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Capítulo 3

Neste capítulo é apresentado o segundo manuscrito original intitulado "***Impact of Chronic Toxoplasmosis in Pregnancy: Association between maternal IgG antibodies against Toxoplasma gondii and neurocognitive development effects***" que aborda um estudo que avaliou as associações entre a soropositividade para anticorpos IgG anti-*T. gondii* e anti-citomegalovírus em gestantes e os desfechos neuropsicológicos em seus filhos aos 12 meses de idade

A hipótese central do estudo é que a presença de anticorpos IgG maternos específicos podem estar associadas a déficits no neurodesenvolvimento infantil, mesmo na ausência de transmissão direta do parasita. Os objetivos do estudo incluíram a investigação da prevalência de anticorpos IgG contra *T. gondii* e citomegalovírus em gestantes e a avalição da influência dessas exposições no desenvolvimento neuropsicológico de seus filhos aos 12 meses de idade. Trata-se de uma coorte de nascimentos, envolvendo a avaliação de mulheres grávidas e seus bebês aos 12 meses.

O desenvolvimento neuropsicológico das crianças foi avaliado utilizando as Escalas Bayley de Desenvolvimento Infantil e de Lactentes, terceira edição (Bayley-III), que mede várias áreas do desenvolvimento, incluindo cognição e linguagem.

Os resultados do estudo sugerem que a soropositividade materna para anticorpos IgG anti-*T. gondii* está significativamente associada a pontuações mais baixas na escala cognitiva da Bayley-III.

A relação causal entre a infecção materna crônica por *T. gondii* e o atraso no desenvolvimento cognitivo das crianças aos 12 meses não foi evidenciado anteriormente, o que pode nortear novas linhas de pesquisa.

Este estudo destaca a importância de monitorar a saúde materna durante a gestação e a necessidade de intervenções precoces para identificar e tratar possíveis atrasos no desenvolvimento neurocognitivo associados à infecção por *T. gondii*. Além de reforçar a relevância de estratégias de saúde pública focadas na prevenção de infecções durante a gravidez, visando melhorar os resultados de saúde para mães e filhos.

Impact of Chronic Toxoplasmosis in Pregnancy: Association between maternal IgG antibodies against *Toxoplasma gondii* and neurocognitive development effects

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Competing Interests

The authors declare that there is no conflict of interest.

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Highlights

- Maternal chronic *T. gondii* infection is linked to neurocognitive development at 12 months.
- Newborns of mothers with high IgG against *T. gondii* show lower cognitive scores.
- Maternal IgG anti-*T. gondii* may be linked to lower language scores in newborns.

ABSTRACT

Toxoplasmosis presents notable hazards in the context of pregnancy, impacting the health of the mother and the neurodevelopment of the fetus via immune reactions and possible vertical transmission. The maternal immune response from chronic *Toxoplasma* gondii *(T. gondii)* infection may negatively influence fetal neurodevelopment. This research evaluated the association between the seroprevalence of chronic *T. gondii* and cytomegalovirus infection in pregnant women and the neuropsychological development of their children at 12 months of age. A follow-up study evaluated women during the gestational period and their respective infants. The pregnant women were tested for the presence of antibodies to infectious agents: *T. gondii*, cytomegalovirus (CMV), syphilis, human immunodeficiency virus (HIV), hepatitis B and C. Semi-structured questionnaires were administered to the pregnant women at the time of recruitment after obtaining informed consent. Detailed information about the newborns was extracted from medical records. At 12±3 months of age, the infant's neurodevelopment was assessed using the Bayley-III Scales of Infant and Toddler Development by a trained specialist under the supervision of a neuropsychologist. The seroprevalence of IgG anti-*T. gondii* was 58.4%, and IgG anti-CMV antibodies was 96.4%. A statistically significant association was found between maternal IgG anti-T. gondii levels and lower scores on the Bayley-III cognition scale, with a non-standardized Beta coefficient of -0.078 (95% CI, -0.144 to -0.013), accounting for 35.1% of the variation in this outcome. These results suggest that chronic maternal *T. gondii* infection, even without vertical transmission, may be associated with subtle changes in the child's cognitive development. Therefore, monitoring and early intervention are essential to identify and address possible delays in childhood neurodevelopment related to chronic maternal toxoplasmosis.

Keywords: Toxoplasmosis; neurodevelopment; pregnancy, Serology; birth cohort

INTRODUCTION

Toxoplasmosis, caused by the parasite *Toxoplasma gondii*, is a significant concern during pregnancy due to the risk of vertical transmission and subsequent adverse outcomes for both the mother and the fetus (LI et al. 2014; Bigna et al. 2020). Maternal immune response, particularly IgG antibodies, is crucial role in combating the infection and influencing the risk of transmission to the fetus (Gómez-Chávez et al. 2019). Chronic toxoplasmosis in pregnancy has been associated with neurocognitive deficits and developmental abnormalities in offspring, highlighting the importance of understanding the relationship between maternal IgG antibodies against *T. gondii* and neurocognitive development (Freedman Et Al. 2016; Núñez et al. 2022).

Pregnant women exhibit increased vulnerability to specific infections and may manifest a more pronounced advancement of the illness in contrast to non-gravid individuals (ABU-RAYA et al. 2020). Such susceptibility can be attributed to the physiological and immunological alterations inherent to the gestational period (Deshmukh & Way, 2019). During the prenatal period, pregnant women require close monitoring to promptly identify potential infections and deploy strategies to restrict the impact on the infant's health (Miranda et al. 2012).

Many factors can influence childhood neurodevelopment, such as brain malformations, prematurity, infections, early childhood malnutrition, and maternal obesity (Oldenburg, O'shea & Fry, 2020). Perinatal inflammation plays a significant role in adverse neurodevelopmental outcomes, contributing to conditions like cerebral white matter damage, cerebral palsy, cognitive impairment, attention-deficit/hyperactivity disorder, and autism spectrum disorder (Jiang et al. 2018). Factors such as socioeconomic inequities, maternal obesity, infections, fetal growth restriction, neonatal sepsis, necrotizing enterocolitis, and prolonged mechanical ventilation are associated with perinatal inflammation (Han et al. 2021).

Maternal immune activation (MIA) significantly impacts fetal brain development, altering key processes and increasing the risk of neurodevelopmental disorders (Woods et al. 2023). Studies show that exposure to MIA during pregnancy can lead to changes in fetal brain cytokine balance, dysregulation of placental function, and altered epigenetic regulation of neurodevelopmental pathways, impacting neurogenesis, neuronal migration, and cortical lamination (Mcewan, Glazier & Hager, 2023). Activating of the maternal immune system could elucidate fetal developmental disorders linked to various pathogens, even without vertical transmission. Maternal inflammatory processes give rise to cytokines capable of

traversing the placental barrier and influencing critical stages of fetal development (Zhou, 2012).

Maternal chronic infection by *T. gondii* has been associated with the host's immune system modulation, resulting in MIA that could negatively impact fetal neurodevelopment (Núñez et al. 2022). This immune reaction can modify the environment within the uterus, potentially interrupting crucial processes involved in the fetus brain development (Hwang et al. 2018). Furthermore, chronic toxoplasmosis in pregnant women has been associated with adverse pregnancy outcomes, such as an increased occurrence of small-for-gestational-age (SGA) infants, which may have implications for neurocognitive advancement. (Martinez et al. 2024).

Detection of developmental delay in children at an early stage is essential in identifying those needing intensive monitoring and intervention services. The Bayley Scales of Infant and Toddler Development, Third Edition (Bayley-III), is frequently employed to evaluate the developmental advancements of young children. It assesses growth in five key areas: cognition, language, motor skills, social-emotional abilities, and adaptive behavior (Anderson & Burnett, 2017).

Maternal chronic infection with *T. gondii* might result in maternal immune activation, impacting fetal neurodevelopment and potentially leading to adverse outcomes. The timely identification of developmental delays through tools such as the Bayley-III is essential for prompt intervention. Therefore, this study aimed to investigate the associations between the prevalence of specific antibodies in pregnant women who participated in the DSAN cohort study (Determinants of Socioenvironmental Neurodevelopment at 12 Months -A Birth Cohort in the Recôncavo Baiano) conducted in two municipalities in the Recôncavo Baiano region of Brazil, with neurodevelopmental changes in their children at 12 months of age.

METHODS

Study Design and Population

This cohort study evaluated pregnant women and their respective babies at 12±3 months of age. Pregnant women up to 24 weeks of gestation were invited to participate in the study. The project was conducted in collaboration with the primary care network in Nazaré and Aratuípe, Bahia, Brazil. The pregnant women were investigated for antibodies against infectious agents, including *T. gondii*, cytomegalovirus, syphilis, HIV, Hepatitis B, and C.

Participants were fully informed about the study's objectives and methods, providing informed consent by signing the Free and Informed Consent Form (FICF). In the case of participants under 18, their legal guardian was consulted, and both the guardian and the underage participant signed the FICF and Assent Form, respectively. The Ethics Committee of the Federal University of Bahia approved the study under protocol number 3.246.555 on April 5, 2019. Complete study design and recruiting strategies are published elsewhere (Bah et al. 2023)

Data Collection Procedures

Biological samples

A trained phlebotomist used a standard venipuncture technique to collect blood from the cubital vein using vacuum tubes. A gel separator tube without anticoagulant (BD Vacutainer® SST™ - Yellow Cup; Nova Jersey, EUA) was used for serum sample centrifugation at 3,000 r.p.m. for 10 minutes. All samples were adequately labeled, packaged, and transported to the immunology laboratory of the Faculty of Pharmacy at the Federal University of Bahia (UFBA). The serum samples were transferred to 5-mL tubes and kept at -20^{o}C until processing.

Serological tests were performed using a chemiluminescence microparticle immunoassay to search for IgG and IgM against *Toxoplasma gondii* and cytomegalovirus infections (Abbot®, São Paulo - Brazil) using the Architect i1000SR automation equipment (Abbot®, São Paulo - Brazil). Specimens with concentration values ≥ 3.0 IU/mL are considered reactive for IgG antibodies to *T. gondii*, indicating chronic infection. For CMV,

samples with concentration values ≥ 6.0 AU/mL are also considered reactive for IgG antibodies.

Immunochromatographic tests designed for diagnostic purposes were used to determine the presence of total antibodies against HIV, *Treponema pallidum*, and hepatitis C, following the same methodology for detecting hepatitis B surface antigen (Abbot®, São Paulo - Brazil).

Socioeconomic data, newborn assessment, and anthropometry

Semi-structured questionnaires were administered to pregnant women during recruitment and after signing the informed consent form. The Interviewers conducted the questionnaire on socioeconomic status (based on family possession and income), general cultural habits, educational level, occupational history, and other evaluations. The socioeconomic level, stratified into five categories from A to E, was defined based on the criteria of the Brazilian Association of Population Studies (Brazil, 2008).

Detailed information about the newborn at birth, including sex, ethnicity, birth weight, delivery method, and gestational age at birth, were extracted from medical records. The anthropometric data was also collected from the medical records.

The INTERGROWTH-21st Project fetal growth curve (Villar et al. 2014; Papageorguiou et al. 2018) was applied to estimate the adequacy of birth weight for gestational age. The newborns (NBs) were classified as small for gestational age (SGA) when the birth weight (in grams) for completed gestational age was below the 10th percentile and large for gestational age (LGA) when equal to or above the 90th percentile. The remaining NBs were classified as appropriate for gestational age (AGA).

Neurodevelopmental assessment

At 12±3 months of age, the infant's neurodevelopment was assessed using the Bayley-III Scales of Infant and Toddler Development by a trained specialist (DCO) under the supervision of a neuropsychologist (NC). This instrument assesses five developmental parameters in children aged 1-42 months: cognition, language (receptive and expressive communication), motor skills, and socio-emotional and adaptive behavior. Each of these five subscales provides a composite score that indicates the child's performance level in that specific developmental domain. These composite scores have a mean of 100 and a standard

deviation of 15, allowing comparison of the assessed child's performance with normative population data (Del Rosario et al. 2020).

For categorical analysis of the Bayley-III composite scores, a cutoff point of 85 points was considered. Composite scores below 85 (1 standard deviation below the normative mean) indicate a risk for developmental delay in that specific domain (Johnson, Moore & Marlow, 2014).

Data analysis

After data tabulation, frequency, central tendency, and dispersion measurements were analyzed. The choice between parametric and non-parametric tests was based on the Kolmogorov-Smirnov normality test. The Binomial test for a proportion was used to obtain confidence intervals for the proportions. Multivariate logistic regression modeling was applied to investigate possible associations between anti-*T. gondii* IgG and anti-CMV IgG seropositivity with neuropsychological development scores from the Bayley III scale. The odds ratio (OR) was obtained by exponentiating the beta quotients obtained in the multivariate model. The model was adjusted for gestational week of birth and adequacy of birth weight for gestational age. Multivariate linear regression modelling by stepwise was used to evaluate the association between Bayley-III composite scores and maternal titers of anti-*T. gondii* IgG and anti-CMV IgG. The model was adjusted for gestational week at birth and adequacy of birth weight for gestational age (percentile). The adjusted R^2 was used to determine the percentage of variance in the data explained by the model, and the non-standardized beta coefficient was used to indicate the strength of the association between the predictor variables and the outcome variables. The accepted level of statistical significance for all tests was $p < 0.05$. Descriptive and comparison statistical analyses were performed using SPSS® version 26.0 for Windows (IBM Inc. Massachussets, USA).

RESULTS

Figure 1 presents the flowchart detailing the recruitment of study participants. The study included 137 pregnant women with an average age of 26.8 years ± 0.5 years. Of the total, 80 (58.4%) resided in Nazaré. Most self-identified as Afro-descendants (94.7%), and 38.2% were in their first pregnancy. Most were classified as belonging to the lower social classes D-E (51.2%). Most pregnant women had completed middle or high school (73.0%), while 2.3% reported being illiterate. 29 (22.1%) pregnant women reported having cats as pets. There was a frequency of 17.4% of reported previous miscarriages. Regarding their homes, a significant portion did not have access to potable water (18.2%), and 40.2% did not reside on a paved street (Table 1).

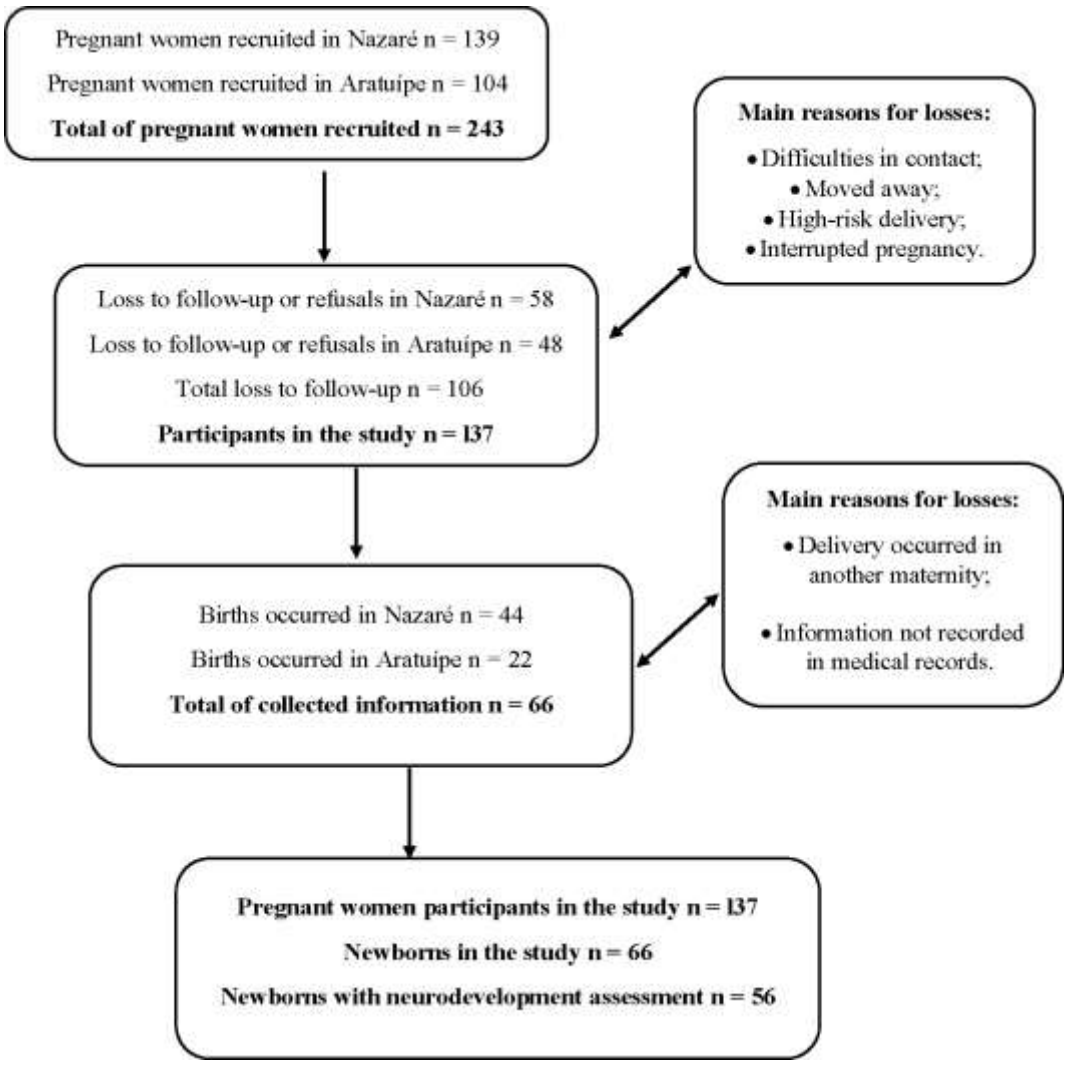

Fig 1. Recruitment flowchart.

Table 1 – Socioenvironmental determinants and serological status of pregnant women participating in the study.

Socioenvironmental data	**TOTAL (n = 137)**
	Mean (± SD)
Age (years)	26.8 (±0.5)
Minimum	15.0
Maximum	39.2
IgG anti-*T. gondii*	140.1 (±23.2)
Minimum	0
Maximum	1000
IgG anti-cytomegalovirus	110.9 (±8.7)
Minimum	0
Maximum	250
	n (%)
IgG positive	
anti-*T. gondii*	80 (58.4)
anti-cytomegalovirus	132 (96.4)
Place of residence	
Nazaré	80 (58.4)
Aratuípe	57 (41.6)
Ethnicity	
White	4 (3.0)
African Brazilian	126 (94,7)
Yellow	3 (2.3)
Education	
Illiterate	3 (2.3)
Elementary I complete	19 (14.6)
Complete Elementary II	40 (30.1)
Medium complete	57 (42.9)
Graduated	14 (10.5)
Socioeconomic classification	
D-E	66 (51.2)
C2	41 (31.8)
C1	18 (14.0)
B2	3 (2.3)
B1	1 (0.7)
Previously pregnant?	
Yes	81 (61.8)
No	50 (38.2)
Previous miscarriage?	
Yes	23 (17.4)
No	109 (82.6)
Gestational planning?	
No	91 (68.9)
Yes	41 (31.1)
Have a cat as pet?	
No	102 (77.9)
Yes	29 (22.1)
Residential water supply	
Artesian well	23 (18.2)
Piped	103 (81.8)
Water treatment	
Not treated	77 (59.2)
Treated (Filtered/boiled)	53 (40.8)
Have they paved the house street?	
No	53 (40.2)
Yes	79 (59.8)

The overall seroprevalence found for chronic *T. gondii* infection was 58.4% (95%-CI, 46.7% - 64.4%), and for anti-CMV IgG antibodies, it was 96.4% (95%-CI, 91.8% - 98.5%). (Table 1). Three pregnant women tested positive for anti-*T. pallidum* antibodies and one pregnant woman tested positive for antibodies against the hepatitis C virus. No pregnant woman tested positive for anti-HIV antibodies or the presence of the hepatitis B surface antigen.

Table 2 describes the neonatal characteristics of the study participants. Information from 66 neonates was obtained. 54.5% of the NBs were male, and 86.7% were classified as African-Brazilians. Regarding deliveries, 42.4% occurred via cesarean section, and most occurred at Luís Argolo Maternity Hospital (61.2%) in the neighboring municipality of Santo Antônio de Jesus, Bahia. The gestational week at the delivery time had an average of 39.1 (±0.2) weeks. 12.9% of the NBs were classified as SGA and 11.3% as LGA.

Table 2 – Sociodemographic and anthropometric characteristics of newborns included in the study.

	Total (n = 66)
Variable	**n (%)**
Sex	
Female	30 (45.5)
Male	36 (54.5)
Ethnicity	
White	6 (13.3)
African Brazilian	39 (86,7)
Type of delivery	
Vaginal	37 (56.1)
Cesarean	28 (42.4)
Residence	1 (1.5)
Place of birth	
Nazaré's Maternity	18 (26.9)
Luis Argolo Hospital	41 (61.2)
Other	8 (11.9)
Adequacy of weight for gestational age	
SGA	8 (12.9)
AGA	47 (75.8)
LGA	7 (11.3)
Variable	**Mean (± SD)**
Gestational age (weeks)	39.1 (±0.2)
Minimum	32.0
Maximum	41.0
Weight at birth (g)	3210.9 (±73.01)
Minimum	1744
Maximum	4192
Length at birth (cm)	47.8 (±0.4)
Minimum	32
Maximum	54
Percentile	55.7 (±3.5)
Minimum	6.2
Maximum	99.5

Upon reaching 12±3 months of age, the infants included in the study were assessed using the Bayley-III scales. The Mean (± SD) of composite scores is presented in Table 3. The socio-emotional scale had the highest mean score of 127.3 (±1.6), while the lowest was from the adaptive behavior scale with 93.8 (±1.7).

Table 3 – Composite scores of Bayley-III scales of infant included in the study.

Variable	Mean (± SD)
Cognition scale	111.6 (±1.7)
Minimum	80
Maximum	140
Language scale	105.1 (±1.9)
Minimum	77
Maximum	138
Motor scale	104.3 (±1.1)
Minimum	82
Maximum	118
Socio-emotional scale	127.3 (±1.6)
Minimum	95
Maximum	140
Adaptive behavior scale	93.8 (±1.7)
Minimum	66
Maximum	128

To investigate a possible association between the categorized composite scores of the Bayley-III (cut-off = 85 points) and the positivity for IgG anti-*T. gondii* or IgG anti-CMV antibodies, a multivariate logistic regression analysis was performed using the adjusted odds ratio. The variables gestational week of birth and adequacy of birth weight for gestational age were used to adjust the multivariate model. The categorized composite scores of the Bayley-III subscales did not show a significant relationship with maternal seropositivity for IgG anti-*T. gondii* (Table 4). There were no significant associations between the composite scores and maternal seropositivity for IgG anti-CMV.

Table 4 – Composite scores of Bayley-III and positive maternal serology for IgG anti-*T. gondii* antibodies based on multivariate logistic regression models

	IgG anti-*T. gondii* positive n (%)	IgG anti-*T. gondii* negative n (%)	OR (CI 95%)	p-value
Cognition scale				0.541
> 85 points	34 (94.4)	18 (90)	Ref.	
≤ 85 points	2 (5,6)	2 (10)	0.53 (0.07 – 4.08)	
Language scale				0.678
> 85 points	35 (97.2)	18 (90)	Ref.	
≤ 85 points	1 (2.8)	2 (10)	0.61 (0.06 – 6.36)	
Motor scale				**
> 85 points	35 (97.2)	20 (100)	**	
≤ 85 points	1 (2.8)	0		
Socio-emotional scale				**
> 85 points	36 (100)	20 (100)	**	
≤ 85 points	0	0		
Adaptive behavior scale				0.490
> 85 points	29 (82.9)	16 (80)	Ref.	
≤ 85 points	6 (17.1)	4 (20)	0.34 (0.02 – 7.24)	

* Statistically significant association
** Not calculated
Adjustment covariates – Gestational week of birth and adequacy of birth weight for gestational age.
Ref. - Reference Group

The results in Table 5 show the relationship between maternal IgG titers for anti-*T. gondii* antibodies and developmental outcomes in children, using composite scores from the Bayley-III assessment tool. Multivariate linear regression models were employed, considering adjustment covariates: gestational week of birth and birth weight adequacy (percentile). The results indicate a statistically significant association between maternal IgG titers and cognitive scale composite scores, with a non-standardized Beta coefficient of -0.078 (95%-CI, -0.144 – -0.013). This finding suggests that cognition scale composite scores decrease by approximately 0.08 points for every unit increase in maternal IgG titers. The adjusted R^2 suggests that maternal IgG titers explain 35.1% of the variation in cognition scale composite scores after adjustment for covariates.

The analysis also found a borderline association between maternal IgG titers and language scale composite scores, with a non-standardized Beta coefficient of -0.075 (95%-CI, -0.152 – 0.002), and a p-value of 0.056. Although not statistically significant at 0.05, the negative coefficient suggests elevated maternal IgG titers for anti-*T. gondii* may be associated with lower language scale composite scores. The associations with motor, socio-emotional, and adaptive behavior scale composite scores were not statistically significant (Table 5). There were no significant associations between the composite scores and maternal IgG titers for anti-CMV.

Table 5 – Composite scores of Bayley-III and maternal IgG titers for anti-*T. gondii* antibodies based on multivariate linear regression models.

	Adjusted R^2	Non-standardized Beta coefficients	CI-95%	p-value
Cognition scale	35.1%	-0.078	-0.144 – -0.013	0.025*
Language scale	14.4%	-0.075	-0.152 – 0.002	0.056
Motor scale	3.9%	-0.031	-0.071 – 0.010	0.122
Socio-emotional scale	4.7%	-0.014	-0.097 – 0.069	0.707
Adaptive behavior scale	14.4%	-0.031	-0.095 – 0.033	0.303

* Statistically significant association

Adjustment covariates – Gestational week of birth and adequacy of birth weight for gestational age (percentile).

DISCUSSION

The findings of this study provide evidence that chronic maternal infection with *T. gondii* may be associated with adverse outcomes in child neurodevelopment, particularly concerning cognitive function. Even though no significant association was observed between maternal IgG seropositivity for *T. gondii* and odds of low performance in neurodevelopment domains of the Bayley-III scales, the multivariate linear regression modeling revealed a statistically significant association between maternal anti-*T. gondii* IgG titers and cognitive composite scores. Furthermore, it was found that there is a potential link between elevated levels of maternal IgG to *T. gondii* and decreased composite scores on the language scale.

The pregnant women participating in the study demonstrated socio-environmental attributes that categorize them as socioeconomically challenged and living in inadequate sanitary conditions. The prevalence of *T. gondii* infection among pregnant women was 58.4% (95%-CI, 46.7% - 64.4%). This rate is quite comparable to findings reported in previous studies focusing on Brazilian pregnant women. The prevalence demonstrates notable variations across diverse regions, with reported rates ranging from 49.2% to 75.1% (Lopes et al. 2009; Ribeiro, Mutis & Fernandes. 2008). Various factors, including age, educational attainment, income level, animal exposure, and dietary practices impact the infection rate(Avelar et al. 2017; Messina et al. 2019; Antinarelli et al. 2020). Although acute infection is infrequent, the issue of vertical transmission continues to be a significant apprehension (Kohler et al. 2022).

The findings of the current study imply a populace characterized by a relatively elevated frequency of cesarean deliveries (42.4%) and a variety of neonatal sizes, including a significant number of SGA (12.9%) and LGA (11.3%) infants. Moreover, the racial/ethnic makeup of the study population suggests a predominantly Afro-Brazilian ethnical composition (86.7%). The rate of cesarean sections in Brazil ranks among the highest globally. Various determinants, such as socioeconomic variables, risk classification, and type of service (private or public), contribute to this occurrence (Dias et al., 2022).

At 12±3 months of age, the infants' neurodevelopment was assessed using the Bayley-III scales. The results suggest the infants had relatively socio-emotional solid development but lower scores on the adaptive behavior scale compared to the other domains assessed by the Bayley-III instrument. This finding could indicate potential challenges in the infants' ability to effectively adapt to their environment and engage in self-care behaviors at this stage of development. The cognitive, language, and motor scores averaged close to 100. It is

important to note that the Bayley-III scale has moderate predictive validity for cognitive outcomes in children. However, it may not identify all children at risk of future cognitive deficits (Spencer-Smith et al. 2015).

The present study found no associations between maternal anti-CMV IgG antibodies and composite scores of the Bayley-III. Nevertheless, the findings indicate that for each unit of maternal anti-*T. gondii* IgG titers increase, the composite scores of the cognitive scale decrease by approximately 0.08 points. This result suggests that higher levels of maternal IgG antibodies against *T. gondii*, indicative of chronic infection, may be associated with poorer cognitive performance in 12-month-old children. The adjusted R^2 suggests that 35.1% of the cognitive scale composite scores variation can be explained by maternal IgG titers and the adjustment covariates (gestational age at birth and birth weight adequacy). These findings corroborate with previous studies that have linked chronic maternal toxoplasmosis to neurodevelopmental deficits in children. MIA triggered by chronic *T. gon*dii infection may alter critical neurodevelopmental processes such as neurogenesis, neuronal migration, and cortical lamination, thereby increasing the risk of neurodevelopmental disorders (Bergdolt & Dunaevsky, 2019; Vlasova et al. 2021).

A study with animals that investigated the impact of MIA due to prenatal infection on offspring's psychiatric disorders, focusing on the association between *T. gondii* and schizophrenia, found that exposure to the parasite before gestation produced maternal pathogenic antibodies that can recognize fetal brain mimotopes and lead to neurochemical and behavioral alterations in the offspring (Núñez et al. 2022). In contrast, a study using a population-based birth cohort to investigate the association between maternal *T. gondii*, offspring bipolar disorder, and childhood cognition, assessed through the Peabody Picture Vocabulary Test and the Raven Matrices, found no significant associations between maternal IgG antibody titers and either offspring bipolar disorder or childhood cognition (Freedman et al 2016).

Although few studies directly investigated the relationship between chronic maternal infection by *T. gondii* and its effects on neurodevelopment, there is evidence of an association between the parasite and the leading psychiatric disorders, such as schizophrenia and bipolar disorder, highlighting its potential role as a risk factor in the development of these conditions (Nayeri et al. 2020; Xiao et al 2018). A study carried out a comprehensive review to synthesize existing preclinical and clinical evidence on the association between *T. gondii* infection and significant psychiatric disorders, suggesting that in addition to MIA, the parasite can induce dopaminergic signaling pathways in the brain, which can lead to psychotic episodes (Barros, Miranda and Teixeira, 2020).

While no substantial association was found between maternal anti-CMV IgG seropositivity and Bayley-III scores, it is essential to consider that maternal CMV infection could impact fetal neurodevelopment. Further studies are needed to elucidate maternal CMV infection's role in childhood neurodevelopment.

A limitation of this study is the relatively small sample size, especially assessing neonatal outcomes. Furthermore, neurocognitive assessment was performed only in a single visit at 12 months of age. Future studies with larger samples and longitudinal assessments of neurodevelopment at different ages are needed to confirm and deepen these findings. Another important consideration is that chronic maternal *T. gondii* infection does not always result in vertical transmission or adverse outcomes for the fetus. Factors such as the parasite's strain, parasite's load, the timing of infection during pregnancy, and the maternal immune response can influence the risk of transmission and fetal outcomes (LI et al. 2014).

Despite these limitations, this study contributes to understanding the potential association between chronic maternal *T. gondii* infection and adverse outcomes in child neurodevelopment. These findings reinforce the importance of monitoring and early detection of maternal infections during pregnancy to minimize adverse impacts on the child's development. Early identification of developmental delays using instruments such as the Bayley-III is essential for implementing intensive follow-up interventions and services. Furthermore, health education and preventive measures, such as washing fruits and vegetables properly and cooking meat adequately, can help reduce the incidence of toxoplasmosis during pregnancy.

CONCLUSIONS

In conclusion, this study suggests that chronic maternal *T. gondii* infection, even without vertical transmission, may be associated with adverse outcomes in child neurodevelopment, especially concerning cognition. These findings highlight the importance of monitoring and early detection of maternal infections during pregnancy to minimize adverse impacts on child development. Future studies with larger samples and longitudinal evaluations must confirm and deepen these results.

Declarations

Ethical Approval

The Ethics Committee of the Federal University of Bahia approved the study under protocol number 3.246.555 on April 5, 2019.

All participants involved in this study provided informed consent before their inclusion. They were provided with a detailed explanation of the study's purpose, procedures, potential risks, and benefits. Participants were assured of their confidentiality and the voluntary nature of their participation. This study was conducted in accordance with ethical guidelines and regulations to ensure the rights and well-being of all participants.

Additional headings

Not applicable.

Competing Interests

The authors declare that there is no conflict of interest.

Authors' contributions

Victor Otero Martinez: Conceived and designed the analysis, collected data, contributed data or analysis tools, performed the analysis, and wrote the draft.

Nathália Ribeiro dos Santos: Collected data and contributed to the analysis.

Homègnon Antonin Ferréol Bah: Collected data and contributed to the analysis.

Erival Amorim Gomes Junior: Collected data and contributed to the analysis.

Daisy Oliveira Costa: Collected data and contributed to the analysis.

José Antonio Menezes-Filho: Conceived and designed the analysis, collected data, contributed data or analysis tools, performed the analysis, and reviewed the draft.

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Availability of data and materials

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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CONSIDERAÇÕES FINAIS

Alguns dos resultados obtidos nesta pesquisa confirmam dados obtidos em outros estudos, contribuindo assim para a generalização do conhecimento a respeito do tema. Além disto, são apresentados resultados inéditos que podem nortear novas linhas de investigação.

Estudos epidemiológicos longitudinais com seres humanos, como este, corroboram para fortalecimento da hipótese de que infecções maternas, mesmo na ausência de doença aguda, estão associadas a desfechos negativos na prole. A partir dos resultados, foi possível identificar uma associação entre a infecção materna crônica por *T. gondii* e alterações no crescimento fetal e no desenvolvimento neurocognitivo aos 12 meses idade. Esses achados sugerem que a soropositividade materna para anticorpos IgG anti-*T. gondii* pode contribuir para a ocorrência de bebês pequenos para idade gestacional e com menor escores cognitivos mensurados pela escala Bayley-III. Desataca-se que o desenho do estudo, uma coorte de nascimentos, permite sugerir relações causais entre as exposições e os desfechos, já que atende a alguns dos preceitos de causalidade de Hill, sobretudo a temporalidade, já que avaliamos o perfil infeccioso materno e o desfecho no bebê aos 12 meses de idade.

A infecção por *T. gondii* apresenta-se como uma patologia negligenciada no âmbito da saúde pública brasileira, porém resultados como estes que avaliam as relações entre a infecção materna crônica assintomática e desfechos negativos nos respectivos filhos, podem sugerir um maior impacto deste parasito na saúde da população brasileira do que o postulado atualmente.

Apesar dos desfechos investigados possuírem múltiplas causas possíveis, os estudos em animais de experimentação e os estudos epidemiológicos, como este, sugerem que a infecção crônica pelo parasito deve ser considerada. Assim, medidas profiláticas que busquem reduzir a taxa de transmissão do *T. gondii*, ou ainda, o tratamento de indivíduos assintomáticos, podem ser medidas necessárias para reduzir a elevada frequência de alterações no desenvolvimento infantil.

PERSPECTIVAS FUTURAS

Os resultados aqui descritos nos fornecem informações base para aprofundarmos o nosso conhecimento a respeito da exposição a anticorpos IgG anti-*T. gondii* durante a fase gestacional, seus efeitos no crescimento fetal e no neurodesenvolvimento. Este estudo ainda prossegue através da:

- Marcação de um evento de saúde para apresentação dos dados gerais do projeto e possíveis encaminhamentos.
- Continuidade no acompanhamento das crianças para verificar novos desfechos negativos na saúde.
- Expansão do estudo de coorte de nascimentos para outras localidades.

ANEXOS

ANEXA A – Parecer do Comitê de Ética da Faculdade de Farmácia – Universidade Federal da Bahia

UFBA - FACULDADE DE FARMÁCIA DA UNIVERSIDADE FEDERAL DA BAHIA

Plataforma Brasil

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Determinantes socioambientais do neurodesenvolvimento de crianças aos 12 meses: Uma coorte de nascimento no Recôncavo Baiano

Pesquisador: José Antonio Menezes Filho

Área Temática:

Versão: 2

CAAE: 02036918.5.0000.8035

Instituição Proponente: Faculdade de Farmácia

Patrocinador Principal: Universidade Federal da Bahia - UFBA
MINISTERIO DA CIENCIA, TECNOLOGIA E INOVACAO

DADOS DO PARECER

Número do Parecer: 3.246.555

Apresentação do Projeto:

As iniquidades socioambientais têm preponderante papel na saúde das populações, especialmente para criança em formação. As desigualdades sanitárias e sociais impõem situações de vulnerabilidade que exacerbam as exposições desnecessárias, ao mesmo tempo em que tornam o organismo mais susceptível ás agressões externas. A exposição ainda na fase uterina a contaminantes químicos ou biológicos pode ter consequências para o resto da vida. Recentemente houve um surto no Nordeste brasileiro de microcefalia devido à infecção materna pelo vírus Zika. Populações em condições de vulnerabilidade podem estar expostas a múltiplos agentes concomitantemente, sendo seus efeitos observados nos indivíduos mais vulneráveis. A região do Recôncavo Baiano, ocupado desde o século XVI é eminentemente agrícola no passado, especialmente na cultura da cana de açúcar, e nas últimas décadas muitas indústrias têm se instalado, sobretudo ao redor da Baía de Todos os Santos. Atividades com grande uso de metais pesados, assim como agrotóxico têm sido reportadas em toda região. Nesse contexto, temos o objetivo de avaliar os determinantes socioambientais que podem interferir no neurodesenvolvimento das crianças. Metodologia: Trata-se de um estudo prospectivo, uma coorte de nascimento, no qual as gestantes no primeiro exame pré-natal serão convidadas a participar do estudo. Este se dará nos municípios de Aratuípe e Nazaré das Farinhas no Recôncavo Baiano. Estimamos a inclusão de 390 gestantes que após dar

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oconsentimento, obteremos informações sociodemográficas, antropométricas, amostras de sangue, urina, cabelo e unha dos pés para avaliar a exposição a agrotóxicos organoclorados, organofosforados e fungicidas (Mancozeb), a metais pesados (As, Hg, Mn e Pb) e sorologia para agentes infecciosos (vírus, bactérias e outros). Dados do parto e do neonato serão coletados de forma padronizada, assim como sangue do cordão umbilical. Visitas serão realizadas às casas das gestantes para avaliação dos níveis de metais na poeira depositada, assim como para avaliação do desempenho intelectual materno, usando a escala WASI. O neurodesempenho do bebê aos 12 meses será avaliado usando a escala de neurodesenvolvimento infantil Bayley III edição. Amostras de sangue, cabelo e unha dos pés da criança serão coletadas para avaliar a exposição corrente. Os dados serão analisados por métodos de regressão multivariados linear e não-linear para avaliar as associações entre as exposições e os desfechos no neurodesenvolvimento. Os resultados esperados desse estudo ajudarão as políticas públicas da saúde materno-infantil, conhecer as situações de exposição química que podem ter implicação direta na saúde fetal, assim como esclarecer como a transmissão vertical de infecções preveníveis que podem ter sequelas no recém-nascido

Objetivo da Pesquisa:

Objetivo Primário:

Investigar quais determinantes socioambientais tem efeito significativo no neurodesenvolvimento das crianças aos 12 meses de idade em uma coorte de nascimento no Recôncavo Baiano.

Objetivo Secundário:

• Avaliar a exposição ambiental das gestantes aos metais e aos agrotóxicos selecionados medindo seus níveis em matrizes ambientais (poeira doméstica) e biológicas (sangue,, urina, cabelo e unha dos pés) • Avaliar a exposição pré-natal dos neonatos aos metais e aos agrotóxicos, medindo seus níveis no sangue do cordão umbilical ao nascimento e em outras matrizes biológicas (cabelo e unha dos pés aos 12 meses após o nascimento). • Avaliar a possível exposição materno-fetal a agentes infeciosos através da avaliação do perfil

sorológico da mãe e do bebê aos 12
meses.• Descrever os determinantes socioambientais potencialmente associados de risco de maior exposição (socioeconômicas, demográficas,
hábitos culturais) aos contaminantes selecionados (metais, agrotóxicos, microrganismo). • Avaliar efeitos no crescimento fetal associados à exposição intra-uterina com dados antropométricos e escala Apgar coletados no momento do parto. • Avaliar
o neurodesenvolvimento das crianças,
e seus determinantes (socioeconômicas, demográficas e antropométricas), usando a Escala Bayley aos 12 meses de vida.• Investigar a associação
entre a soroprevalência para os diferentes marcadores de infecções potencialmente teratogênicas e as características do neurodesenvolvimento aos
12 meses de idade.• Investigar a associação entre a exposição individual in utero aos contaminantes selecionados e o neurodesenvolvimento aos 12
meses de idade, tendo em conta possíveis interação entre eles

Avaliação dos Riscos e Benefícios:

Riscos:

Os riscos físicos são mínimos, relativos à coleta de amostras biológicas. Possibilidade de hematoma no local da punção para coleta de sangue.

Porém, estas atividades já fazem parte da bateria de exames pré-natais que as voluntárias do estudo passarão.

Risco de constrangimento durante a aplicação de questionários para classificação socioeconômica ou sobre violência urbana. Para minimizar tais
impacto procuraremos usar do nosso melhor julgamento para abordar essas questões e que não implique em menosprezar as voluntárias do estudo.

Benefícios:

São inúmeros os benefícios direto para as voluntárias do estudo. Elas terão um diagnóstico mais abrangente de possíveis infecções antes do parto,
tendo assim a possibilidade de ser implementado tratamento, evitando a infeção vertical.
Ter avaliado a exposição a importantes agentes ambientais. Com tal conhecimento, mudanças de atitudes podem ser tomadas para diminuir a
exposição a contaminantes. Por exemplo, usar determinadas panelas de cerâmica vitrificadas para cozinhar e servir. Evitar ou mudar a origem de
determinados alimentos pois podem estar contaminados com metais ou agrotóxicos. Por fim, ter um diagnóstico do neurodesenvolvimento do recémnascido, que se indicado, medidas de melhor estimulação podem ser recomendadas para que a criança recupere o desenvolvimento adequado.

Comentários e Considerações sobre a Pesquisa:

O presente estudo possui relevância para a saúde pública, uma vez que abordará determinantes socioambientais e o neurodesenvolvimento de crianças até 12 anos no Recôncavo Baiano

Considerações sobre os Termos de apresentação obrigatória:

Todos atendidos.

Recomendações:

Recomendações anteriores atendidas nesta versão

Conclusões ou Pendências e Lista de Inadequações:

Nada consta a respeito de pendências

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BASICAS_DO_PROJETO_1224544.pdf	12/03/2019 11:39:24		Aceito
Brochura Pesquisa	Brochura_DSAN12Mv2.pdf	12/03/2019 11:28:47	José Antonio Menezes Filho	Aceito
TCLE / Termos de Assentimento / Justificativa de	Termo_de_AssentimentoV2.pdf	12/03/2019 11:26:46	José Antonio Menezes Filho	Aceito

Ausência	Termo_de_AssentimentoV2.pdf	12/03/2019 11:26:46	José Antonio Menezes Filho	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLEv2.pdf	12/03/2019 11:26:11	José Antonio Menezes Filho	Aceito
Projeto Detalhado / Brochura Investigador	Brochura_DSAN12M_v2.pdf	12/03/2019 11:12:13	José Antonio Menezes Filho	Aceito
Folha de Rosto	Folha_de_Rosto.pdf	30/10/2018 14:05:33	José Antonio Menezes Filho	Aceito
Orçamento	Orcamento.docx	29/10/2018 09:52:29	José Antonio Menezes Filho	Aceito
Declaração de Instituição e Infraestrutura	TermoAnuencia_de_Campo_Aratuipe.pdf	29/10/2018 09:43:43	José Antonio Menezes Filho	Aceito
Declaração de Instituição e Infraestrutura	Termo_de_Anuencia_de_Campo_Nazare.pdf	29/10/2018 09:43:21	José Antonio Menezes Filho	Aceito
Declaração de Instituição e Infraestrutura	TermodeConcessaoMaternidade.pdf	29/10/2018 09:41:29	José Antonio Menezes Filho	Aceito
Declaração de Pesquisadores	Termo_de_Confidencialidade_signed.pdf	29/10/2018 09:39:32	José Antonio Menezes Filho	Aceito
Declaração de Pesquisadores	Declaracao_de_Nao_Coleta.pdf	29/10/2018 09:37:37	José Antonio Menezes Filho	Aceito
Declaração de Instituição e Infraestrutura	Declaracao_Instituicao_Copart_Fiocruz.pdf	29/10/2018 09:36:29	José Antonio Menezes Filho	Aceito
Declaração de Instituição e Infraestrutura	Declaracao_Instituicao_Copart_UCSal.pdf	29/10/2018 09:34:39	José Antonio Menezes Filho	Aceito
Cronograma	Cronograma.docx	29/10/2018 09:20:42	José Antonio Menezes Filho	Aceito
Declaração de Pesquisadores	Termo_Compromisso_Pesquisador.pdf	29/10/2018 09:08:36	José Antonio Menezes Filho	Aceito
Declaração de Instituição e Infraestrutura	AutorizacaoInstProponente.pdf	29/10/2018 09:07:08	José Antonio Menezes Filho	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

UFBA - FACULDADE DE FARMÁCIA DA UNIVERSIDADE FEDERAL DA BAHIA

Continuação do Parecer: 3.248.555

SALVADOR, 05 de Abril de 2019

Assinado por:
Ana Leonor Pardo Campos Godoy
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APÊNDICE B – Termo de Consentimento Livre esclarecido


Universidade Federal da Bahia
Faculdade de Farmácia
Laboratório de Toxicologia


Termo de Consentimento Livre e Esclarecido

De acordo com as Normas da Resolução nº 466, do Conselho Nacional de Saúde de 12 de dezembro de 2012.

Título da Pesquisa: "Determinantes socioambientais do neurodesenvolvimento de crianças aos 12 meses: Uma coorte de nascimento no Recôncavo Baiano – (Estudo DSAN-12M)"

Investigador principal: Prof. Dr. José Antonio Menezes Filho.
Instituições participantes da pesquisa: Universidade Federal da Bahia; Universidade Católica do Salvador; Fundação Oswaldo Cruz.

Estamos convidando a senhora (ou sua filha)[1] como voluntária, para participar da pesquisa. Neste estudo pretendemos avaliar os fatores sociais e ambientais que podem alterar o desenvolvimento do sistema nervoso do bebê ainda na fase fetal. Pretendemos também avaliar a influência de agentes infecciosos (vírus, bactérias e outros) no neurodesenvolvimento da criança avaliado aos 12 meses de vida. A senhora não é obrigada a participar da pesquisa, e poderá se afastar dela a qualquer tempo, sem qualquer prejuízo de sua relação com os pesquisadores ou com as instituições locais de saúde (como a Unidade de Atenção Básica do seu município). Todas as informações pessoais serão sigilosas, os resultados de suas análises serão fornecidos unicamente a senhora, e sua identidade não será revelada em qualquer publicação resultante deste estudo. Os exames e procedimentos aplicados serão gratuitos, não havendo qualquer custo. A sua participação também será de forma voluntária, portanto não irá receber dinheiro por isso. **Antes de assinar este termo, a senhora deve entender as informações sobre a pesquisa e fazer todas as perguntas que achar necessário.**

O problema investigado são os possíveis impactos de condições externas às quais as gestantes estão expostas durante a gestão do bebê que pode interferir no desenvolvimento do sistema nervoso das crianças. As crianças serão avaliadas ao nascer e aos 12 meses de idade. Avaliaremos o impacto das desigualdades sociais que têm grande peso na saúde das populações, especialmente para criança ainda em formação. Por exemplo: acesso à água tratada, esgotamento sanitário, habitar perto de lixões, etc. Essas desigualdades tornam o organismo mais propenso às agressões externas. Além das consequências para a gestante, a exposição ainda na fase uterina a contaminantes químicos ou biológicos pode ter consequências para o resto da vida da criança. Recentemente, houve um surto no Nordeste brasileiro de microcefalia devido à infecção materna pelo vírus Zika. Populações em condições de vulnerabilidade podem estar expostas a múltiplos agentes ao mesmo tempo, sendo seus efeitos observados nos indivíduos mais vulneráveis. A região do Recôncavo Baiano, desde mais de quatro séculos é produtora de várias culturas (cana de açúcar especialmente) e nas últimas décadas muitas indústrias têm se instalado, sobretudo ao redor da Baía de Todos os Santos. Atividades com grande uso de metais pesados, assim como agrotóxico tem sido reportadas em toda região.

O cérebro é um órgão alvo comum para os metais pesados, os agrotóxicos, e alguns microrganismos cujas ações podem afetar diferentes funções de acordo com o elemento em questão, embora algumas áreas (por exemplo, auditiva, visual, motora, memória e comportamento) possam ser prejudicadas por todos mencionados acima. Durante a gravidez, dada as demandas do feto por minerais e outros nutrientes para o seu crescimento, muitos contaminantes têm absorção facilitada, atravessando a placenta mais facilmente e a barreira que protege o Sistema Nervoso ainda em formação. Nesse contexto, temos o objetivo de avaliar os determinantes socioambientais que podem interferir no neurodesenvolvimento das crianças ainda na fase uterina.

Serão convidadas as gestantes no primeiro exame pré-natal dos municípios de Aratuípe e Nazaré das Farinhas. Pretendemos medir os níveis de metais pesados (chumbo, arsênico, cadmio, mercúrio e manganês) na poeira depositada nas casas de cada participante. Para tal fim, pedimos autorização para colocar pequenos amostradores descartáveis no quarto onde dorme a gestante. Será necessário coletar amostras de sangue, urina, cabelo da região da nuca e unha dos pés das gestantes para analisarmos a exposição a agrotóxicos organoclorados, herbicidas e fungicidas (Mancozeb), a metais pesados (As, Hg, Mn e Pb), níveis de cortisol no cabelo e sorologia para agentes infecciosos (vírus, bactérias e outros) da gestante. No momento do parto, será coletado sangue do cordão umbilical, uma fração do tecido placentário (ambos pelas equipes de obstetrícias das maternidades) e os dados do parto e do bebê serão acessados a partir do prontuário médico. Após parto, em momento oportuno, será coletado uma amostra do

[1] No caso da gestante ser menor que 18 anos.

Universidade Federal da Bahia
Faculdade de Farmácia
Laboratório de Toxicologia

leite materno (colostro) pela equipe de obstetrícia das maternidades. Visitas serão realizadas às casas das gestantes para avaliação do desempenho intelectual materno antes do parto e avaliação do neurodesenvolvimento do bebê aos 12 meses pela equipe da neuropsicologia, usando testes adequados. Amostras de cabelo da região da nuca, unha dos pés e de sangue da criança serão coletadas aos 12 meses de idade para avaliar a exposição extrauterina aos contaminantes ambientais. Amostras do leite materno serão coletadas nas visitas após o nascimento. Após avaliação, qualquer desvio da normalidade, será imediatamente informado e os pais encaminhados ao Programa de Saúde da Família do Município correspondente com todos os resultados dos exames para serem devidamente acompanhados. A coleta será feita com material descartável, à vácuo na veia cubital no braço por profissional extremamente habilitado para coleta em crianças pequenas. No momento da punção a criança sentirá dor passageira, podendo ocorrer eventualmente, em alguns casos, leve hematoma. Caso ocorra, será aplicado um gel para ajudar a reabsorção. No local será colocado curativo adesivo. Uma pequena mecha de cabelo (cerca de 1 cm) será coletada na região da nuca e acondicionada em sacos de coleta. Será usada tesoura cirúrgica de ponta redonda, sendo higienizada entre os voluntários. Aparas das unhas dos dedos dos pés serão coletas com auxílio de cortador de unha (tipo Clipper com receptáculo) e acondicionados em sacos de amostragem devidamente identificados com código e iniciais do participante. Todos os protocolos serão aplicados (a depender do momento) nas consultas pré-natais, maternidade ou nas casas da participante, não havendo nenhuma despesa para a senhora. As amostras receberão um código que estará relacionado à identificação de cada dupla mãe-bebê, sendo de conhecimento somente da coordenação do projeto. As amostras serão armazenadas devidamente no Laboratório de Toxicologia (FF/UFBA) e poderão ser utilizadas na pesquisa para avaliar a co-exposição a outros metais.

A gestante ou seu responsável responderá a questionários durante entrevistas sobre dados socioeconômicos gerais, frequência alimentar, violência urbana, etc.. Usaremos do nosso melhor julgamento para não constrangê-las com questões sobre posse para classificação socioeconômica. Psicólogos ou estudantes de psicologia aplicarão testes específicos para avaliar o desenvolvimento do sistema nervoso da criança, assim como questionário específico sobre alguns hábitos dele e da família. Estas informações são muito importantes como diagnóstico e para o sucesso desta pesquisa. Tais resultados ajudarão o Município a desenvolver medidas psicopedagógicas para resgatar possíveis funções que tenham sido comprometidas nas crianças, ter melhor controle das condições sanitárias e da contaminação ambiental, assim definir políticas públicas que visem a promover a saúde materno-infantil.

O Termo é emitido em duas vias, sendo uma das vias deste termo com nosso contato para a senhora. Pode tirar suas dúvidas sobre o projeto e sua participação, agora ou a qualquer momento. Eventuais danos decorrentes da pesquisa poderão ser passíveis de indenização.

Em caso de dúvida, procurar o pesquisador ou CEP nos endereços:

- Laboratório de Toxicologia – Faculdade de Farmácia-UFBA Av. Barão de Jeremoabo s/n Campus de Ondina, 40115-179, Salvador, Bahia. Tel.: (71)3283-6960
- Comitê de Ética em Pesquisa (CEP): Escola de Enfermagem-UFBA Campus Universitário do Canela, Salvador, Bahia. Tel. (71) 3283-7615

_____
Pesquisador

Declaro que fui devidamente explicada dos objetivos, riscos e benefícios da minha participação (ou da minha filha) na pesquisa. Fui informada que não receberei dinheiro por participar como voluntária, que será mantido o sigilo das minhas informações e que poderei a qualquer momento me retirar, juntamente com meu filho ou filha da pesquisa sem qualquer constrangimento.

_____
Participante da Pesquisa/Responsável Legal

APÊNDICE C – Termo de Assentimento

Universidade Federal da Bahia
Instituto de Saúde Coletiva
Faculdade de Farmácia (LabTox)

Termo de Assentimento

De acordo com as Normas da Resolução nº 466, do Conselho Nacional de Saúde de 12 de dezembro de 2012.

Você está sendo convidado(a) como voluntário(a) a participar da pesquisa "**Determinantes socioambientais do neurodesenvolvimento de crianças aos 12 meses: Uma coorte de nascimento no Recôncavo Baiano – (Estudo DSAN-12M)**". Neste estudo pretendemos avaliar os fatores sociais e ambientais que podem alterar o desenvolvimento do sistema nervoso do seu futuro filho.

O motivo que nos leva a estudar esse assunto é que as desigualdades sociais, o estresse pré-natal, as infecções e outros contaminantes ambientais (como metais pesados e agrotóxicos) podem alterar o desenvolvimento do sistema nervoso do seu futuro filho. Os cientistas já sabem que estes fatores podem causar danos (as vezes irreversíveis) no desenvolvimento do feto, da crianças cujas consequências atrapalham a qualidade de vida adulta e na velhice.

Para este estudo adotaremos os seguintes procedimentos: primeiro os seus pais ou responsáveis darão autorização, depois vamos fazer entrevista com vocês sobre os hábitos gerais, condições de moradia, sobre problemas na sua comunidade para avaliar os parâmetros de desigualdades. Vamos fazer exames sorológicos (sobre Zika, Dengue e Chikungunya), de sangue, de urina, de cabelo, das unhas e do leite materno para saber quais são as concentrações dos contaminantes no seu organismo e do seu futuro filho. Coletaremos também alguns dados das suas consultas pré-natais. Depois uma equipe de fisioterapeuta e psicólogos vai aplicar diversos testes para avaliar o desenvolvimento do sistema nervoso do seu filho e a sua capacidade de raciocínio, atenção e memória; como se fossem jogos de quebra-cabeça.

Você e seus pais não terão nenhum custo ou despesas, nem receberá dinheiro para participar. Você será esclarecido(a) de qualquer coisa que desejar e estará livre para participar ou recusar-se. O seu responsável poderá retirar o consentimento ou interromper a sua participação a qualquer momento. Você participa se quiser e a recusa em participar não tem problema e você continua nossa amiga e pode colaborar de outra forma com nossa equipe. A sua identificação só nós do projeto saberemos, isso se chama confidencialidade. Seu nome não aparecerá em nenhuma publicação. Este estudo apresenta risco mínimo, ou seja, na hora da coleta de sangue, você não pode puxar o braço, pois pode formar um hematoma, a dor de uma picada de agulha passa rápido. Caso ocorra, colocaremos um gel para acelerar a reabosorção.

Os resultados estarão à sua disposição quando finalizado. Seu nome ou o material que indique sua participação não será liberado sem o seu consentimento e permissão do seu responsável. Os dados e instrumentos utilizados na pesquisa ficarão arquivados com o pesquisador responsável por um período de 5 anos, e após esse tempo serão destruídos. Este termo de assentimento foi impresso em duas vias, sendo que uma cópia será arquivada pelo pesquisador responsável, e a outra será entregue a você.

Em caso de dúvida, procurar o pesquisador ou CEP nos endereços:

- Laboratório de Toxicologia – Faculdade de Farmácia-UFBA Av. Barão de Jeremoabo s/n Campus de Ondina, 40115-179, Salvador, Bahia. Tel.: 3283-6960

Universidade Federal da Bahia
Instituto de Saúde Coletiva
Faculdade de Farmácia (LabTox)

• Comitê de Ética em Pesquisa: Escola de Enfermagem-UFBA Campus Universitário do Canela, Salvador, Bahia. Tel. 3283-7615

_____
Pesquisador

Declaro que fui devidamente explicado sobre os objetivos, riscos e benefícios da minha participação na pesquisa. Fui informado(a) que não receberei dinheiro por participar como voluntário(a), que será mantido o sigilo das minhas informações e que poderei a qualquer momento me retirar da pesquisa sem qualquer constrangimento.

_____
Voluntário(a)

APÊNDICE D – Questionário sociodemográfico

Universidade Federal da Bahia
Faculdade de Farmácia
Laboratório de Toxicologia

Questionário Socioeconômico – Projeto DSAN-12

Entrevistador:	Data da Entrevista: _/_/_	Horário de início:
Nome completo do responsável:		
Nome completo da gestante: Documento de identidade (RG/CT/CNH):		
Apelido:		
Código(s) da participante:		
Coleta de amostras: ( ) Sangue ( ) Cabelo ( ) Unha ( ) Urina ?????		
Endereço completo (com CEP):		
Telefone para contato:		
Coordenadas geográficas (GPS): O: _____ S:_____		

IDENTIFICAÇÃO				
1. Sexo	( ) masculino	( ) feminino		
2. Data de nascimento	__/__/__	Idade:___ anos.		
3. Etnia	( ) branca	( ) negra	( ) Parda	( ) outras

4. Quem preenche este questionário? ( ) A gestante ( ) Companheiro ( ) Pai ou Mãe ( ) Outro:_____

5. A Sra mora com quem? ( ) Marido ( ) Pais ( )Só com a Mãe ( ) Só com o Pai ( ) Outro:_____

6. Quem está cuidando de você atualmente? ( ) Marido ( ) Pais ( )Só com a Mãe

7. Qual é seu estado civil: ( ) Solteira ( ) Casada legalmente ( ) União estável (>6meses)

( )Viúva ( ) Separada ou divorciada

8. Há quantos anos que mora neste domicilio? : .....

É própria ( ) ou alugada ( )?

9. A Sra já engravidou quantas vezes? .....

10. Tem quantos filhos vivos?.....

11. Quantas pessoas moram na sua casa (incluindo você mesmo)? ___ adulto(s) ___ criança(s)

12. Quantos cômodos têm na sua casa? ___ quarto(s) ___ sala(s) ___ cozinha(s)

13. Quais e quantos destes itens a sua família possui?					
TV	( )0	( )1	( )2	( )3	( )4 ou mais
Rádio	( )0	( )1	( )2	( )3	( )4 ou mais
Banheiro	( )0	( )1	( )2	( )3	( )4 ou mais
Carro/automóvel	( )0	( )1	( )2	( )3	( )4 ou mais
Motocicleta	( )0	( )1	( )2	( )3	( )4 ou mais
Empregado mensalista	( )0	( )1	( )2	( )3	( )4 ou mais
Máquina de lavar	( )0	( )1	( )2	( )3	( )4 ou mais
Secadora roupa	( )0	( )1	( )2	( )3	( )4 ou mais

Universidade Federal da Bahia

Faculdade de Farmácia

Laboratório de Toxicologia

Lava louca	( )0	( )1	( )2	( )3	( )4 ou mais
Videocassete e/ou DVD	( )0	( )1	( )2	( )3	( )4 ou mais
Microcomputador	( )0	( )1	( )2	( )3	( )4 ou mais
Geladeira	( )0	( )1	( )2	( )3	( )4 ou mais
Freezer (independente ou parte dageladeira duplex)	( )0	( )1	( )2	( )3	( )4 ou mais
Micro-Ondas	( )0	( )1	( )2	( )3	( )4 ou mais

14. A água utilizada neste domicílio é proveniente de

( ) Encanada	( ) Outro meio Qual?_____

15. Considerando o trecho da rua do seu domicílio, você diria que a rua é:

( ) Asfaltada/Pavimentada	( ) Terra/Cascalho

16. Grau de escolaridade da Sra	18. Grau de escolaridade do PAI do seu filho
( ) Analfabeto/ Fundamental I incompleto (1ª a 4ª série) ( ) Ensino Fundamental I completo/ Fundamental II incompleto ( ) Ensino Fundamental II/ Médio (colegial) incompleto ( ) Ensino Médio completo (regular ou técnico)/ Superior incompleto ( ) Ensino superior completo (faculdade)	( ) Analfabeto/Fundamental I incompleto (1ª a 4ª série) ( ) Ensino Fundamental I completo/Fundamental II incompleto ( ) Ensino Fundamental II/ Médio (colegial) incompleto ( ) Ensino Médio completo (regular ou técnico)/ Superior incompleto ( ) Ensino superior completo (faculdade)
17. Profissão da MÃE (atual ou passada) _____ (Título do cargo)	19. Profissão do PAI (atual ou passada) _____ (Título do cargo)

20. O trabalho de alguém entre vocês dois envolve atividades em olaria:: ( ) Pai ( ) Mae ( ) Membro da Família Qual?_____;

21. Está envolvida na distribuição de cerâmicas? ( ) Não ( ) Sim

22. Quantas pessoas colaboram na renda familiar total? _____

23. A sua família recebe bolsa-família?

24. Renda mensal total da família em salário mínimo (valor aproximado não incluído a bolsa familiar) : .....Reais

( ) Até meio	( ) Entre meio e 1	( ) Entre 1 e 2	( ) Entre 2 e 5	( ) Acima de 5

25. A água utilizada neste domicílio é proveniente de

( ) Encanada	( ) Outro meio Qual?_____

26. Considerando o trecho da rua do seu domicílio, você diria que a rua é:

( ) Asfaltada/Pavimentada	( ) Terra/Cascalho

27. Cozinha					
Quem cozinha em casa:	( ) A Sra	( ) Parceiro	( ) Mãe	( ) Outras	
Tipos de copos	( ) Metal	( ) Plastic	( ) Vidro	( ) Ceramica	_____ (Dethales)
Tipos de travessa, panelas, pratos,	( ) Metal	( ) Plastic	( ) Vidro	( ) Ceramica vitrificada	_____

Universidade Federal da Bahia
Faculdade de Farmácia
Laboratório de Toxicologia

Utensilo de cozinha (Garfos, Colher)	( ) Metal ( ) Plastic ( ) Vidro ( ) Outro _____ (Dethales)
Container para agua	( ) Metal ( ) Plastic ( ) Vidro ( ) Ceramica _____ (Dethales)
Fontes de água potável	( ) Torneira ( ) Outro _____ (Dethales)
Origem dos ingredientes para cozinhar	( ) Ingredientes local ( ) Mercearia ( ) Supermercado _____ (Dethales)

28. Há quantos anos a criança mora nesta localidade? ____ anos.

29. A Sra já foi hospitalizada na sua vida? ( ) Sim ____ vezes. ( ) Não

Qual foi o motivo?.....

30. A criança teve/tem algum problema de saúde? ( ) Sim Qual?_____ ( ) Não

31. A criança toma algum medicamento? ( ) Sim Qual?_____ ( ) Não

32. Comentários que ache válido sobre sua gestação ou desenvolvimento da criança
_____

33. A Sra fuma? (cigarro e/ou maconha)

( ) Nunca	( ) Às vezes	( ) Frequentemente	( ) Sempre
Número de cigarros/semana:			

34. A mãe consuma bebida alcóolica?

( ) Nunca	( )Às vezes	( ) Frequentemente	( ) Sempre
Número de latas (350 mL) ou doses/semana:			

35. A mãe consuma qualquer tipos de drogas (cocaína, cola de sapateiro, crack...)?

( ) Nunca	( )Às vezes	( ) Frequentemente	( ) Sempre
Qual:			

Tabagismo no domicílio (ou em outro ambiente que a Sra costuma passar muito tempo)				
36. Quem fuma em casa:	( )Ninguém (se NINGUÉM, ENCERRE O QUESTIONÁRIO)	( ) Parceiro	( ) Mãe ou Pai	( ) Outras
37. Quantos cigarros seu parceiro fuma /dia	_____ cigarros		( ) NS/NR	
38. Quantas horas por dia o parente costuma ficar em cas com você	_____ horas		( ) NS/NR	
39. O PAI da criança fuma quantos cigarros/dia	_____cigarros		( )NS/NR	
40. Quantas horas por dia o Pai costuma ficar em casa com o filho	_____ horas		( )NS/NR	

Data final da entrevista: __/__/__	**Horário de Término:** ____ hs

Encerre o questionário e agradeça a participação.

Existe alguma observação a ser feita por parte do entrevistador?

APÊNDICE E – Artigo publicado no periódico *Parasitology Research* - Impact of chronic toxoplasmosis in pregnancy: association between maternal seropositivity for *Toxoplasma gondii* IgG antibodiesand fetal growth restriction

Parasitology Research (2024) 123:25
https://doi.org/10.1007/s00436-023-08068-y

RESEARCH

Impact of chronic toxoplasmosis in pregnancy: association between maternal seropositivity for *Toxoplasma gondii* IgG antibodies and fetal growth restriction

Victor Otero Martinez[1] · Nathália Ribeiro dos Santos[1,2] · Homègnon Antonin Ferréol Bah[2,3] · Erival Amorim Gomes Junior[2] · Daisy Oliveira Costa[1,2] · José Antonio Menezes-Filho[1,2,3]

Received: 18 August 2023 / Accepted: 20 October 2023

Abstract
Insults caused by acute infections during the gestational period on fetal development are known; however, new evidence suggests that chronic infectious diseases can also impact the maternal immune status and lead to negative consequences for the neonate. This study investigated the association between the prevalence of specific antibodies in pregnant women and alterations in fetal development at birth. A follow-up study evaluated women during the gestational period and their respective newborns at delivery time. The pregnant women were tested for the presence of antibodies to infectious agents: *Toxoplasma gondii* (*T. gondii*), cytomegalovirus (CMV), syphilis, human immunodeficiency virus (HIV), hepatitis B and C. Semi-structured questionnaires were administered to the pregnant women at the time of recruitment after obtaining informed consent. Detailed information about the newborns was extracted from medical records. The seroprevalence of chronic *T. gondii* infection, as determined by the presence of IgG antibodies against the protozoan, was found to be 56.2%, while the overall prevalence of CMV IgG antibodies was 96.3%. Non-primiparous pregnant women from socio-economic classes, less affluent groups, and skilled working-class individuals had higher chances of testing positive for specific *T. gondii* IgG antibodies. Newborns classified as small for gestational age represented 12.9% of the total. Those born to mothers seropositive for anti-*T. gondii* IgG antibodies were 9.4 times more likely to be born small for gestational age ($p = 0.035$). The results suggest that chronic *T. gondii* infection may contribute to higher rates of newborns with growth restriction. These findings add to a growing body of evidence regarding the impact of chronic infectious diseases on intrauterine fetal development.

Keywords Small for gestational age · Toxoplasmosis · Pregnancy · Serology · Birth cohort

Section Editor: Xing-Quan Zhu

Highlights
- Chronic toxoplasmosis in pregnant women is related to socioeconomic vulnerability.
- Newborns of pregnant women seropositive for anti-*Toxoplasma gondii* antibodies are more likely to exhibit intrauterine growth restriction.
- There is an association between maternal chronic infection with *T. gondii* and birth weight adequacy.

✉ Victor Otero Martinez
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Introduction

Changes in the intrauterine environment during gestation can lead to impairments in fetal development. Maternal immune mediators can cross the placental barrier and directly or indirectly alter the uterine environment, thus influencing fetal growth and neurogenesis, resulting in detrimental consequences for the future development and mental health of the offspring (Boulanger-Bertolus et al. 2018; HAN et al. 2021). Genetic alterations and environmental exposures such as smoking, toxic metals, pesticides, alcohol, medications, and uterine infections are important causes of congenital disabilities. However, most structural, behavioral, functional, and metabolic disorders present at birth have no known genetic or environmental causes (Verma 2021).

Unhealthy environments with poor sanitation conditions increase exposure to microorganisms, leading to infections.

Published online: 11 December 2023

Due to the possibility of vertical transmission and the virulence of this category of agents, pregnant women are monitored during the prenatal period to early detect potential infections and implement strategies to limit the impact on the newborn's health (Miranda et al. 2012). These environmental insults during gestation can result in immunological and epigenetic changes, making the offspring more susceptible to various insults in the future (Cattane et al. 2020).

Intrauterine growth restriction and preterm birth are the leading causes of perinatal mortality found in almost 10% of pregnancies (Nardozza et al. 2017). The INTERGROWTH-21st study developed a standardized fetal growth curve for international use. For this purpose, pregnant women from different ethnicities and regions were followed in a multiethnic and multicenter population-based cohort study, including countries such as Brazil, England, Oman, Italy, the USA, China, India, and Kenya (Villar et al. 2014). According to this classification, newborns (NBs) can be classified as small for gestational age (SGA), appropriate for gestational age (AGA), and large for gestational age (LGA) (Papageorgiou et al. 2018).

During the gestational period, there are dynamic changes in maternal and fetal immune responses that depend on the stage of pregnancy or development (Kollmann et al. 2017; Pazos et al. 2012). Despite the well-known deleterious effects of certain congenital infections, many pathogens fail to reach the fetus due to the powerful physical barrier provided by the placenta that protects the developing baby (Arora et al. 2017). One possible explanation for the mechanisms involved in fetal development disorders associated with different pathogens, even without vertical transmission, is that cytokines originating from maternal inflammatory processes can cross the placenta and affect crucial stages of fetal development (Zhou 2012).

In addition to compromising neurodevelopment, infectious diseases and maternal immune system activation are believed to be one of the main risk factors for preterm births. The increased production of pro-inflammatory cytokines has been associated with uterine activation and preterm births (Cappelletti et al. 2016). Studies suggest a relationship between maternal immune activation and disturbances in neonatal development. Maternal chronic infection with human immunodeficiency virus 1 (HIV-1) and hepatitis B has been shown to lead to higher concentrations of cytokines in umbilical cord blood and altered fetal immune responses, indicating that maternal immune responses can impact the fetus (Bunders et al. 2014; Hong et al. 2015).

The "TORCH" pathogens encompass a variety of infectious agents known to cause congenital disabilities. They include protozoa (*Toxoplasma gondii*), bacteria (*Treponema pallidum*), and viruses (rubella, cytomegalovirus, human herpesvirus, HIV, enteroviruses, parvovirus B19, and Zika virus) (Coyne and Lazear 2016). In addition to congenital disabilities, infections by these pathogens can cause various complications in pregnancy, including miscarriage, fetal growth restriction, and preterm birth (Cappelletti et al. 2016; Pereira et al. 2014). There has been increased concern about maternal-infant transmission of infectious agents after the Zika virus outbreak in Brazil in mid-2015 and the subsequent increase in babies born with microcephaly. A recent study revealed important information about the influence of this virus on infant neurodevelopment, even in neonates with average head size. The neurological development of 29 infants without microcephaly but with in utero exposure to the virus was evaluated using the Bayley Neurodevelopmental Assessment scale, and 34% showed some form of impairment (Faiçal et al. 2019).

Animal models of these infections indicate that pregnancy complications can be mediated by the immune response induced by the pathogen (Yockey and Iwasaki 2018). Regardless of whether clinically detectable infection is present or not, elevated concentrations of cytokines, including interleukin (IL)-6, IL-1, IL-8, and tumor necrosis factor (TNF) in amniotic fluid or cervicovaginal region, are predictive factors for prematurity (Agrawal and Hirsch 2012).

Maternal immune status can indeed affect fetal development. In the case of "TORCH" infections, some common neurological defects observed include microcephaly, cranial calcifications, ventriculomegaly, ocular defects, and sensorineural hearing loss (Coyne and Lazear 2016). Many of these presentations, such as microcephaly, are similar across pathogens, including cytomegalovirus, rubella, *Toxoplasma gondii*, and Zika virus (Devakumar et al. 2018). The similar clinical presentation across a variety of viral and protozoal pathogens suggests the presence of a common mechanism.

Understanding the determinants associated with alterations in human development facilitates early identification and enables the creation of more effective preventive measures. In this regard, Horta and Wehrmeister (2017) emphasize the importance of cohort studies as they provide an opportunity to evaluate the consequences of exposures occurring at different stages of the life cycle, allowing the identification of critical periods and the possibility of measuring the cumulative effect of various exposures.

While several studies show an association between congenital infections and various health issues in NBs, few studies have investigated whether immune status against certain specific agents can silently impact fetal development. Therefore, this study aimed to investigate the association between the prevalence of specific antibodies in pregnant women who participated in the DSAN cohort study (Determinants of Socioenvironmental Neurodevelopment at 12 Months -A Birth Cohort in the Recôncavo Baiano) conducted in two municipalities in the Recôncavo Baiano region of Brazil, with alterations in fetal development at birth.

Methods

Study design and population

This cohort study evaluated pregnant women and their respective children at birth. Pregnant women up to 24 weeks of gestation were invited to participate in the study. The project was conducted in collaboration with the primary care network in the municipalities of Nazaré and Aratuípe, Bahia, Brazil. The pregnant women were investigated for antibodies against infectious agents, including *T. gondii*, cytomegalovirus, syphilis, HIV, and hepatitis B and C.

Participants were fully informed about the study's objectives and methods, providing informed consent by signing the Free and Informed Consent Form (FICF). In the case of participants under 18 years of age, their legal guardian was consulted, and both the guardian and the underage participant signed the FICF and Assent Form, respectively. The Ethics Committee of the Federal University of Bahia approved the study under protocol number 3.246.555 on April 5, 2019. The project is partially funded by the Research for SUS Program (PPSUS) of CNPq/FAPESB, Call notice no. 2/2020, request number 4393/2020, and by the CNPq Universal Call, process No. 421550/2018–0.

Data collection procedures

Biological samples

A trained phlebotomist used a standard venipucture technique, collected blood from the cubital vein using vacuum tubes. A gel separator tube without anticoagulant (BD Vacutainer® SST™-Yellow) was used for serum sample centrifugation at 3000 rotations per minute for 10 min; all samples were adequately labeled, packaged, and transported to the Faculty of Pharmacy at the Federal University of Bahia (UFBA). The serum samples were transferred to 5-mL tubes and kept at − 20°C (freezer) until processing.

Serological tests were performed using a chemiluminescence microparticle immunoassay to search for IgG and IgM against *Toxoplasma gondii* and cytomegalovirus infections (Abbot®, São Paulo, Brazil) using the Architect i1000SR automation equipment (Abbot®, São Paulo, Brazil). Specimens with concentration values ≥ 3.0 IU/mL are considered reactive for IgG antibodies to *T. gondii*, indicating chronic infection. For CMV, samples with concentration values ≥ 6.0 AU/mL are also considered reactive for IgG antibodies.

Immunochromatographic tests designed for diagnostic purposes were used to determine the presence of total antibodies against HIV, *Treponema pallidum*, and hepatitis C, following the same methodology for detecting hepatitis B surface antigen (Abbot®, São Paulo, Brazil).

Socioeconomic data, newborn assessment, and fetal anthropometry

Semi-structured questionnaires were administered to pregnant women during recruitment and after signing the informed consent form. The interviewers conducted the questionnaire on socioeconomic status (based on family possession and income), general cultural habits, educational level, occupational history, work during pregnancy, and other evaluations. The socioeconomic level, stratified into five categories from A to E, was defined based on the criteria of the Brazilian Association of Population Studies (Brasil 2008).

Detailed information about the newborn at birth, including sex, ethnicity, birth weight, delivery method, and gestational age at birth, were extracted from medical records. The APGAR score and fetal anthropometric data (considered here as the child's birth outcome) were also collected from the medical records to assess possible associations with the investigated biomarkers.

The INTERGROWTH-21st Project fetal growth curve (VILLAR et al. 2014; PAPAGEORGIOU et al. 2018) was applied to estimate the adequacy of birth weight for gestational age. The newborns (NBs) were classified as small for gestational age (SGA) when the birth weight (in grams) for completed gestational age was below the 10th percentile and large for gestational age (LGA) when equal to or above the 90th percentile. The remaining NBs were classified as appropriate for gestational age (AGA).

Data analysis

After data tabulation, frequency, central tendency, and dispersion measures were analyzed. The choice between parametric and non-parametric tests was based on the Kolmogorov–Smirnov normality test. The binomial test for a proportion was used to obtain confidence intervals for the proportions. The Student's *t*-test was applied to compare the means of parametric variables. Binary logistic regression analysis was used to identify risk factors associated with *T. gondii* infection and CMV infection. To investigate possible associations between seropositivity for anti-*T. gondii* IgG and seropositivity for anti-CMV IgG with birth-related data, multivariate logistic regression modeling was performed. The odds ratio (OR) was obtained by exponentiating the beta obtained in the multivariate model. The model was adjusted for maternal height and maternal weight during the prenatal period. The accepted level of statistical significance for all tests was $p < 0.05$. Descriptive and comparison statistical

analyses were performed using SPSS® version 26.0 for Windows, and graphs were generated using GraphPad Prism® version 8.0.2.

Results

Figure 1 presents the flowchart detailing the recruitment of study participants. The study included 136 pregnant women with an average age of 26.8 years ± 0.5 years. Of the total, 26 (41.6%) resided in the municipality of Aratuípe. Most self-identified as Afro-descendants (94.7%), and 38.2% were in their first pregnancy. Most were classified as belonging to social classes D-E (51.2%). Most pregnant women had completed middle and/or high school (73.0%), while 2.3% reported being illiterate. There was a frequency of 17.4% of reported previous miscarriages. Regarding their homes, a significant portion did not have access to potable water (18.2%), and 40.2% did not reside on a paved street (Table 1).

The overall seroprevalence found for chronic *T. gondii* infection was 56.2% (95% CI, 47.8–64.2%), and for anti-CMV IgG antibodies, it was 96.3% (95% CI, 91.7–98.4%). Three pregnant women tested positive for anti-*T. pallidum* antibodies and one pregnant woman tested positive for antibodies against the hepatitis C virus (Fig. 2). No pregnant woman tested positive for anti-HIV antibodies or the presence of the hepatitis B surface antigen.

In order to investigate a possible association between the socioenvironmental determinants and IgG anti-*T. gondii* antibody positivity, a univariate logistic regression analysis was performed, and crude odds ratio was used. A significant association ($p = 0.049$) was observed between the number of pregnancies and *T. gondii* infection, with non-primiparous women being more likely to have chronic *T. gondii* infection. Pregnant women and/or their family members classified as belonging to social classes D-E and C2 had a higher likelihood (3.19; 95% CI, 1.2–8.47) of testing positive for IgG anti-*T. gondii* antibodies. The data are presented in Table 2. Notably, some risk factors related to *T. gondii* infection, such as being older age and consuming untreated water, did not show a significant relationship with IgG anti-*T. gondii* antibody positivity (Table 2). No significant associations were found when investigating the relationship between the evaluated determinants and chronic CMV infection.

Table 3 describes the neonatal characteristics of the study participants. Information from 66 neonates was obtained. 54.5% of the NBs were male, and 86.7% were classified

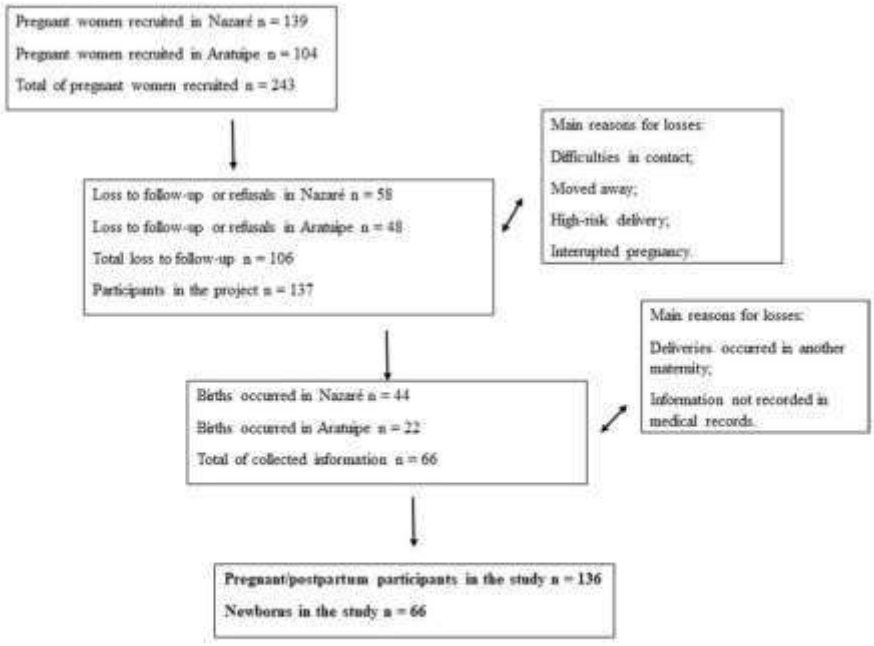

Fig. 1 Recruitment flowchart

Table 1 Sociodemographic characteristics of participating pregnant women in the study

	TOTAL ($n=137$) Mean (±SD)
Age (years)	26.8 (±0.5)
Minimum	15.0
Maximum	39.2
Variable	n (%)
Place of residence	
Nazaré	80 (58.4)
Aratuípe	57 (41.6)
Ethnicity	
White	4 (3.0)
African Brazilian	126 (94.7)
Yellow	3 (2.3)
Education	
Illiterate	3 (2.3)
Elementary I complete	19 (14.6)
Complete elementary II	40 (30.1)
Medium complete	57 (42.9)
Graduated	14 (10.5)
Socioeconomic classification	
D-E	66 (51.2)
C2	41 (31.8)
C1	18 (14.0)
B2	3 (2.3)
B1	1 (0.7)
Previously pregnant?	
Yes	81 (61.8)
No	50 (38.2)
Previous miscarriage?	
Yes	23 (17.4)
No	109 (82.6)
Gestational planning?	
No	91 (68.9)
Yes	41 (31.1)
Have a cat as a pet?	
No	102 (77.9)
Yes	29 (22.1)
Does any family member's occupation involve activities in agriculture?	
Yes	55 (44.7)
No	68 (55.3)
Have they paved the house street?	
No	53 (40.2)
Yes	79 (59.8)
Residential water supply	
Artesian well	23 (18.2)
Piped	103 (81.8)
Water treatment	
Not treated	77 (59.2)
Treated (filtered/boiled)	53 (40.8)

as African-Brazilians. Concerning the deliveries, 42.4% occurred via cesarean section, and the majority took place at Luís Argolo Maternity Hospital (61.2%) in the neighboring municipality of Santo Antônio de Jesus, Bahia. The gestational week at the delivery time had an average of 39.1 (±0.2) weeks. 12.9% of the NBs were classified as SGA and 11.3% as LGA. The overall mean APGAR score at 1 min was 8.1 points.

Table 4 summarizes the results when comparing seropositivity for IgG anti-*T. gondii* and the characteristics of the NBs using multivariate logistic regression analysis; adjusted odds ratio was used. The variables of prenatal maternal weight and height were used to adjust the multivariate model. Associations between seropositivity for IgG anti-CMV and birth-related outcomes were also evaluated.

It was observed that NBs whose mothers had chronic *T. gondii* infection were 9.4 times more likely (95% CI, 1.18–88.1) of being born small for gestational age ($p=0.035$). The other characteristics related to the NBs did not show a significant relationship with maternal seropositivity for IgG anti-*T. gondii* (Table 4).

There were no significant associations between the characteristics of the NBs and seropositivity for IgG anti-CMV.

Discussion

The study investigated the socio-environmental determinants associated with the presence of specific antibodies against teratogenic pathogens in pregnant women enrolled in the DSAN-12 M birth cohort study being carried out in the Recôncavo Baiano region in Brazil. Additionally, these pregnant women were assessed for the potential influence of specific IgG antibodies on fetal development characteristics. The frequency of small-for-gestational-age NBs in the study population was associated with the prevalence of IgG antibodies against *T. gondii*.

The study participants generally exhibited socio-environmental characteristics that classify them as socioeconomically vulnerable to poor sanitary conditions. The seroprevalence for *T. gondii* infection was 56.2% (95% CI, 47.8–64.2%) among pregnant women. This frequency is relatively similar to results from other studies involving pregnant women in the Brazilian population. A study conducted in the State of Bahia, specifically in Ilhéus in the southern region of the state, with 726 pregnant women aged between 13 and 44 years, found a prevalence of 72.3% for IgG antibodies against *T. gondii*. The same study reported a frequency of 95.2% for IgG antibodies against CMV and 97.0% for antibodies against the rubella virus (Costa et al. 2018). Another study reports that *T. gondii* infection in pregnant women residing in the municipality of Ponta de Pedras, Marajó Archipelago, State of Pará, has a prevalence

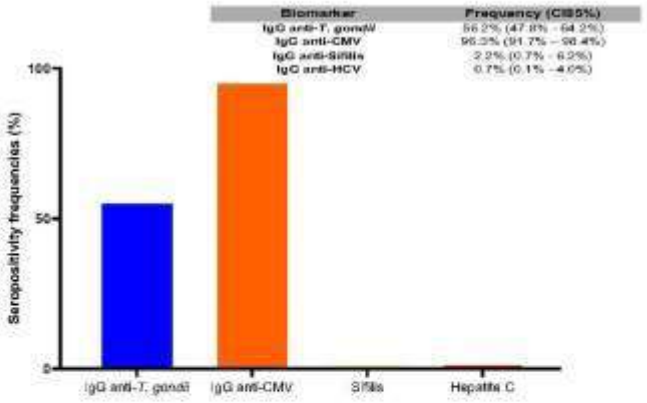

Fig. 2 Prevalence of IgG antibodies against *T. gondii*, CMV, syphilis, and hepatitis C

Table 2 Socioenvironmental determinants of participating pregnant women being positive serology for IgG antibodies against *T. gondii* based on logistic regression models

	IgG anti-*T. gondii* positive *n* (%)	IgG anti-*T. gondii* negative *n* (%)	OR (CI 95%)	*p*-value
Age range				0.269
15 to 26 years	36 (48.0)	33 (57.9)	Ref	
27 years or older	39 (52.0)	24 (42.1)	1.49 (0.74–2.98)	
Ethnicity				0.934
Black	70 (94.6)	56 (94.9)	Ref	
Non-Black	4 (5.4)	3 (5.1)	0.94 (0.20–4.36)	
Multiparous				0.049*
No	22 (30.6)	28 (47.5)	Ref	
Yes	50(69.4)	31 (52.5)	2.05 (1.01–4.20)	
Previous miscarriage?				0.555
No	59 (80.8)	50 (84.7)	Ref	
Yes	14 (19.2)	9 (15.3)	0.76 (0.30–1.91)	
Education				0.116
Complete high school or higher	35 (52.7)	36 (61.0)	Ref	
Elementary school II or lower	39 (47.3)	23 (39.0)	0.57 (0.28–1.15)	
Socioeconomic classification				0.020*
C1/B/A	7 (9.9)	15 (25.9)	Ref	
D-E/C2	64 (90.1)	43 (74.1)	3.19 (1.2–8.47)	
Have a pet?				0.638
No	31 (43.1)	23 (39.0)	Ref	
Yes	41 (56.9)	36 (61.0)	1.18 (0.59–2.38)	
Does any family member's occupation involve activities in agriculture?				0.282
No	27 (40.3)	28 (50.0)	Ref	
Yes	40 (59.7)	28 (50.0)	1.48 (0.72–3.05)	
Piped water?				0.643
Yes	57 (78.1)	48 (81.4)	Ref	
No	16 (21.9)	11 (18.6)	0.83 (0.35–1.95)	
Water treatment				0.159
Treated	25 (34.7)	28 (48.3)	Ref	
Not treated	47 (65.3)	30 (51.7)	1.59 (0.88–2.21)	

*Statistically significant association

Statistical tests: univariate logistic regression

Ref. reference group

Table 3 Sociodemographic and anthropometric characteristics of neonates included in the study

	Total (n = 66)
Variable	n (%)
Sex	
Female	30 (45.5)
Male	36 (54.5)
Ethnicity	
White	6 (13.3)
African Brazilian	39 (86.7)
Type of delivery	
Vaginal	37 (56.1)
Cesarean	28 (42.4)
Residence	1 (1.5)
Place of birth	
Nazaré's Maternity	18 (26.9)
Luis Argolo Hospital	41 (61.2)
Other	8 (11.9)
Adequacy of weight for gestational age	
SGA	8 (12.9)
AGA	47 (75.8)
LGA	7 (11.3)
Variable	Mean (± SD)
Gestational age (weeks)	39.1 (±0.2)
Minimum	32.0
Maximum	41.0
Number of prenatal consultations performed	9.8 (±0.6)
Minimum	3
Maximum	17
Weight at birth (g)	3285.1 (±59.29)
Minimum	1744
Maximum	4900
Length at birth (cm)	47.8 (±0.4)
Minimum	32
Maximum	54
Head circumference (cm)	34.2 (±0.2)
Minimum	28
Maximum	38
Chest cage circumference (cm)	33.8 (±0.3)
Minimum	26
Maximum	40
Abdominal circumference (cm)	32.5 (±0.8)
Minimum	24
Maximum	39
APGAR scored in 1st minute	8.1 (±0.1)
Minimum	6
Maximum	9
APGAR scored at 5th minute	9.2 (±0.1)
Minimum	8
Maximum	10
Percentile	55.7 (±3.5)
Minimum	6.2
Maximum	99.5

of 68.3%, with advanced age, contact with soil, and residing in urban areas being risk factors associated with seropositivity (Morais et al. 2020). A survey with spatial analysis of *Toxoplasma* infection in rural and peripheral neighborhoods of Juiz de Fora, Minas Gerais, showed that 44.4% exhibited seropositivity for IgG antibodies against *Toxoplasma gondii*, and this rate increased to 68.3% when considering only participants from rural areas (Antinarelli et al. 2021).

A study conducted between 2013 and 2017 assessed the prevalence of *T. gondii* among two distinct populations of women of childbearing age in Siena (Tuscany, central Italy) and Bari (Apulia, southern Italy), along with a group of pregnant women in Bari in 2016–2017. This study revealed a significantly higher seroprevalence of *T. gondii* in Bari compared to Siena (22.4% vs. 12.4%), with a noticeable age-related trend. Among the tested pregnant women, a low prevalence of *T. gondii* infection (13.8%) was observed (Fangliulo et al. 2020). In a systematic review with a meta-analysis examining global, regional, and national seroprevalence of *T. gondii* in pregnant women, it was found that the global seroprevalence among pregnant women stood at 38.4%, with a higher prevalence in Latin America and the Caribbean (Bigna et al. 2020).

The variation in toxoplasmosis prevalence can be explained by the influence of different factors, such as environmental conditions, cultural practices, dietary habits, and hygiene (Wilking et al. 2016). The seroprevalence found for toxoplasmosis in this study can be attributed to environmental factors, considering that the climate in the region of the Recôncavo Baiano, Brazil, is hot and humid, which favors the dissemination of the infection. Additionally, the low coverage of proper sanitation facilities contributes to waterborne contamination.

The prevalence found for CMV (96.3%) is consistent with that observed by other authors. A study with pregnant adolescents conducted in the northern region of Brazil found a frequency of 96.3% for anti-CMV IgG antibodies (Guerra et al. 2018). Considering the characteristics of the studied population, the high frequency of CMV antibodies may also be related to socioeconomic factors. Economically disadvantaged populations, lacking basic sanitation and housing conditions, are more susceptible to the virus' spread, leading to higher seroprevalence (Oliveira et al. 2002).

The high frequency of cesarean deliveries among the study's pregnant women (42.4%) stands out. The World Health Organization (WHO) recommends that the rate of cesarean sections should be between 10 and 15% of the total number of deliveries in a health service (World Health Organization 2018). New evidence suggests that babies born by cesarean section experience different hormonal, physical, and bacterial exposures and these changes may subtly impact neonatal physiology (Sandall et al. 2018).

Table 4 Characteristics of newborns and positive maternal serology for IgG anti-*T. gondii* antibodies based on multivariate logistic regression models

	IgG anti-*T. gondii* positive *n* (%)	IgG anti-*T. gondii* negative *n* (%)	OR (CI 95%)	*p*-value
Gestational week of delivery				0.670
Term (37 weeks or more)	27 (90.0)	27 (93.1)	Ref	
Preterm (up to 37 weeks)	3 (10.0)	2 (6.9)	0.67 (0.10–4.31)	
Weight at birth (g)				0.597
2501 g or more	32 (94.1)	31 (96.9)	Ref	
Up to 2500 g	2 (5.9)	1 (3.1)	0.52 (0.04–5.99)	
Length at birth (cm)				0.803
51 cm or bigger	5 (16.1)	6 (18.8)	Ref	
Up to 50 cm	26 (83.9)	26 (81.2)	1.21 (0.27–5.38)	
Brain perimeter (cm)				0.192
Bigger than 34 cm	17 (70.8)	16 (66.7)	Ref	
Up to 33 cm	7 (29.2)	8 (33.3)	0.36 (0.07–1.68)	
Chest cage circumference (cm)				0.912
Bigger than 32 cm	18 (81.8)	19 (90.5)	Ref	
Up to 31 cm	4 (18.2)	2 (9.5)	0.87 (0.08–9.26)	
Abdominal circumference (cm)				0.645
Bigger than 32 cm	6 (60.0)	5 (83.3)	Ref	
Up to 31 cm	4 (40.0)	1 (16.7)	0.48 (0.02–10.65)	
Adequacy of weight for gestational age				0.035*
AGA/LGA	23 (76.7)	31 (96.9)	Ref	
SGA	7 (23.3)	1 (3.1)	9.4 (1.18–88.1)	
APGAR scored in 1st minute				0.956
Seven or higher	23 (95.8)	20 (95.2)	Ref	
Up to 6	1 (4.2)	1 (4.8)	0.92 (0.05–16.06)	

*Statistically significant association

Statistical tests: multivariate logistic regression

Adjustment covariates—maternal pre-natal weight and maternal pre-natal height

Ref. reference group

The NBs participating in the study had an overall mean length of 47.8 cm at birth, and 12.9% were classified as SGA. Fetal growth restriction occurs when the fetus fails to achieve its intrinsic growth potential. It may be related to common mechanisms involving various factors (such as placental pathology, infections, and genetic constitution) (Gordijn et al. 2018). Infections during the prenatal period can activate inflammatory processes, leading to the release of cytokines, increased oxidative stress, and cellular apoptosis, which may impact placental and fetal growth (Kesavan and Devaskar 2019).

The present study found no associations between maternal IgG anti-CMV antibodies and neonatal characteristics. However, neonates of mothers seropositive for IgG anti-*T. gondii* antibodies were 9,4 times more likely to be born small for gestational age ($p=0.035$). A meta-analysis study on perinatal outcomes in congenital *T. gondii* infection showed that the infected patient group had a 4.5 times higher chance of intrauterine growth restriction and a 4.9 times higher likelihood of fetal anomalies, such as ventriculomegaly, intracranial and hepatic calcifications, and ascites (Li et al. 2014). A large cohort study of 740 Hispanic women living in the USA found a frequency of 22.4% for IgG anti-*T. gondii* antibodies. Seropositive pregnant women had a higher incidence of babies born small for gestational age and an increased prevalence of spontaneous abortions and preterm births (Mutka et al. 2023).

A large-scale study evaluating the impact of chronic *T. gondii* infection on the history of spontaneous abortion, pregnancy complications, and neonatal outcomes found no differences between seropositive and seronegative pregnant

women for APGAR at 1 min ($p=0.544$), gestational age at birth ($p=0.491$), birth weight ($p=0.257$), or birth length ($p=0.232$) (Mocanu et al. 2022). In contrast, a study in the Czech Republic with 1733 parturients showed a higher prevalence of preterm birth ($p=0.019$) and low birth weight ($p=0.006$) in women with chronic *T. gondii* infection. The odds of preterm birth were 1.7 times higher, and for low birth weight was 1.9 times higher in women with chronic toxoplasmosis (Hurt et al. 2022). This study corroborates with other evidence regarding the impact of *T. gondii* infection on fetal development when not in its acute form. Pregnant women with chronic toxoplasmosis show slower fetal development estimated at the sixteenth week of pregnancy and longer gestations (Flegr et al. 2005; Kaňková and Flegr 2007). In school-age children in the same region as the current study, a seroprevalence of 43.7% for *T. gondii* was observed, and there was a significant association with rule-breaking behavior (Martinez et al. 2020).

This study has some limitations. The main one is its small sample size, as the participants represent only 38.8% of the estimated sample size based on the birth rates in the two municipalities. However, it was possible to identify associations between chronic exposure to *T. gondii* and the investigated outcomes. The study had important strengths, such as the region's serological evaluation of different prevalent pathogens. Moreover, the study design allowed for assessing multiple exposures and outcomes and discerning the temporal relationships between pathogen exposure and fetal growth outcomes.

Indeed, several factors can significantly influence fetal growth, which is determined by the combination of several variables, such as socioeconomic status, genetic influence, environmental factors, nutritional status, etc. Given the challenges in evaluating all possible determinants that play a role in fetal growth, the results observed in this study suggest that maternal chronic infection by *T. gondii* may contribute to babies with smaller birth sizes. However, several other elements are involved in these effects.

Conclusions

The results of this study demonstrate that pregnant women residing in the municipalities of Nazaré and Aratuípe, Bahia, Brazil, have a high prevalence of chronic infection by *Toxoplasma gondii* and cytomegalovirus.

Multivariate regression analysis showed that chronic *T. gondii* infection in pregnant women may contribute to uterine growth restriction. The data found in this study suggest that chronic toxoplasmosis in pregnant women is associated with a higher frequency of small-for-gestational-age newborns. Associations between chronic toxoplasmosis and lower fetal growth have been identified, regardless of maternal weight and height. The present study found no relationships between CMV infections and birth characteristics.

It was observed that pregnant women with chronic infection by this parasite are 9.4 times more likely to have small-for-gestational-age babies. This finding confirms that chronic infections during the gestational period may be important determinants of intrauterine fetal development.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical approval The Ethics Committee of the Federal University of Bahia approved the study under protocol number 3.246.555 on April 5, 2019.

All participants involved in this study provided informed consent before their inclusion. They were provided with a detailed explanation of the study's purpose, procedures, potential risks, and benefits. Participants were assured of their confidentiality and the voluntary nature of their participation. This study was conducted in accordance with ethical guidelines and regulations to ensure the rights and well-being of all participants.

Competing interests The authors declare no competing interests.

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APÊNDICE F – Artigo publicado no periódico *Neurotoxicology* - Impact of chronic toxoplasmosis in pregnancy: Association between maternal IgG antibodies against T. gondii and neurocognitive development effects

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Impact of chronic toxoplasmosis in pregnancy: Association between maternal IgG antibodies against *T. gondii* and neurocognitive development effects

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ABSTRACT

Toxoplasmosis presents notable hazards in the context of pregnancy, impacting the health of the mother and the neurodevelopment of the fetus via immune reactions and possible vertical transmission. The maternal immune response from chronic *Toxoplasma* gondii *(T. gondii)* infection may negatively influence fetal neurodevelopment. This research evaluated the association between the seroprevalence of chronic *T. gondii* and cytomegalovirus infection in pregnant women and the neuropsychological development of their children at 12 months of age. A follow-up study evaluated women during the gestational period and their respective infants. The pregnant women were tested for the presence of antibodies to infectious agents: *T. gondii*, cytomegalovirus (CMV), syphilis, human immunodeficiency virus (HIV), hepatitis B and C. Detailed information about the newborns was extracted from medical records. At 12 ± 3 months of age, the infant's neurodevelopment was assessed using the Bayley-III Scales of Infant and Toddler Development by a trained specialist under the supervision of a neuropsychologist. A statistically significant association was found between maternal IgG anti-*T. gondii* levels and lower scores on the Bayley-III cognition scale, with a non-standardized β-coefficient of −0.078 (95 %-CI: −0.144 to −0.013), accounting for 35.1 % of the variation in this outcome. These results suggest that chronic maternal *T. gondii* infection, even without vertical transmission, may be associated with subtle changes in the child's cognitive development. Therefore, monitoring and early intervention are essential to identify and address possible delays in childhood neurodevelopment related to chronic maternal toxoplasmosis.

1. Introduction

Toxoplasmosis, caused by the parasite *Toxoplasma gondii*, is a significant concern during pregnancy due to the risk of vertical transmission and subsequent adverse outcomes for both the mother and the fetus (Li et al., 2014; Bigna et al., 2020). Maternal immune response, particularly IgG antibodies, is crucial role in combating the infection and influencing the risk of transmission to the fetus (Gómez-Chávez et al., 2019). Chronic toxoplasmosis in pregnancy has been associated with neurocognitive deficits and developmental abnormalities in offspring, highlighting the importance of understanding the relationship between maternal IgG antibodies against *T. gondii* and neurocognitive development (Freedman et al., 2016; Núñez et al., 2022).

Pregnant women exhibit increased vulnerability to specific infections and may manifest a more pronounced advancement of the illness in contrast to non-gravid individuals (Abu-Raya et al., 2020). Such susceptibility can be attributed to the physiological and immunological alterations inherent to the gestational period (Deshmukh and

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Way, 2019). During the prenatal period, pregnant women require close monitoring to promptly identify potential infections and deploy strategies to restrict the impact on the infant's health (Miranda et al., 2012).

Many factors can influence childhood neurodevelopment, such as brain malformations, prematurity, infections, early childhood malnutrition, and maternal obesity (Oldenburg et al., 2020). Perinatal inflammation plays a significant role in adverse neurodevelopmental outcomes, contributing to conditions like cerebral white matter damage, cerebral palsy, cognitive impairment, attention-deficit/hyperactivity disorder, and autism spectrum disorder (Jiang et al., 2018). Factors such as socioeconomic inequities, maternal obesity, infections, fetal growth restriction, neonatal sepsis, necrotizing enterocolitis, and prolonged mechanical ventilation are associated with perinatal inflammation (Han et al., 2021).

Maternal immune activation (MIA) significantly impacts fetal brain development, altering key processes and increasing the risk of neurodevelopmental disorders (Woods et al., 2023). Studies show that exposure to MIA during pregnancy can lead to changes in fetal brain cytokine balance, dysregulation of placental function, and altered epigenetic regulation of neurodevelopmental pathways, impacting neurogenesis, neuronal migration, and cortical lamination (McEwan et al., 2023). Activating of the maternal immune system could elucidate fetal developmental disorders linked to various pathogens, even without vertical transmission. Maternal inflammatory processes give rise to cytokines capable of traversing the placental barrier and influencing critical stages of fetal development (Zhou, 2012).

Maternal chronic infection by *T. gondii* has been associated with the host's immune system modulation, resulting in MIA that could negatively impact fetal neurodevelopment (Núñez et al., 2022). This immune reaction can modify the environment within the uterus, potentially interrupting crucial processes involved in the fetus brain development (Hwang et al., 2018). Furthermore, chronic toxoplasmosis in pregnant women has been associated with adverse pregnancy outcomes, such as an increased occurrence of small-for-gestational-age (SGA) infants, which may have implications for neurocognitive advancement (Martinez et al., 2024).

The humoral immune response to *T. gondii* infection can activate the maternal immune system, impacting fetal neurodevelopment through complex mechanisms. This immune response can result in chronic MIA, characterized by the release of inflammatory cytokines that can cross the placenta and affect fetal brain development (Gómez-Chávez et al., 2019). Furthermore, pregestational exposure to *T. gondii* may lead to the formation of maternal antibodies that recognize fetal brain structures through molecular mimicry, resulting in neurochemical and behavioral dysfunctions in the offspring (Núñez et al., 2022). Study has shown that the seropositivity for anti-*T. gondii* IgG may be associated with behavioral problems in children, suggesting that maternal inflammation may interfere with critical processes, such as neurogenesis and neuronal migration, leading to alterations in neuropsychological development (Martinez et al., 2020).

Detection of developmental delay in children at an early stage is essential in identifying those needing intensive monitoring and intervention services. The Bayley Scales of Infant and Toddler Development, Third Edition (Bayley-III), is frequently employed to evaluate the developmental advancements of young children. It assesses growth in five key areas: cognition, language, motor skills, social-emotional abilities, and adaptive behavior (Anderson and Burnett, 2017).

Maternal chronic infection with *T. gondii* might result in maternal immune activation, impacting fetal neurodevelopment and potentially leading to adverse outcomes. The timely identification of developmental delays through tools such as the Bayley-III is essential for prompt intervention. Therefore, this study aimed to investigate the associations between the prevalence of specific antibodies in pregnant women who participated in the DGAN cohort study (Determinants of Socio-environmental Neurodevelopment at 12 Months -A Birth Cohort in the Recôncavo Baiano) conducted in two municipalities in the Recôncavo Baiano region of Brazil, with neurodevelopmental changes in their children at 12 months of age.

2. Methods

2.1. Study design and population

This cohort study evaluated pregnant women and their respective babies at 12 ± 3 months of age. Pregnant women up to 24 weeks of gestation were invited to participate in the study. The project was conducted in collaboration with the primary care network in Nazaré and Aratuípe, Bahia, Brazil. The pregnant women were investigated for antibodies against infectious agents, including *T. gondii*, cytomegalovirus, syphilis, HIV, Hepatitis B, and C.

Pregnant women were recruited during their first or second prenatal visit, conducted at Primary Health Care Units. All women who agreed to participate were included in the study. Pregnancies with multiple gestations and those with newborns classified as having experienced birth distress, as determined by the obstetric team, were excluded from the study.

Participants were fully informed about the study's objectives and methods, providing informed consent by signing the Free and Informed Consent Form (FICF). In the case of participants under 18, their legal guardian was consulted, and both the guardian and the underage participant signed the FICF and Assent Form, respectively. The Ethics Committee of the Federal University of Bahia approved the study under protocol number 3.246.555 on April 5, 2019. Complete study design and recruiting strategies are published elsewhere (Bah et al., 2023)

2.2. Data collection procedures

2.2.1. Biological samples

A trained phlebotomist used a standard venipuncture technique to collect blood from the cubital vein using vacuum tubes. A gel separator tube without anticoagulant (BD Vacutainer® SST™ - Yellow Cup; Nova Jersey, EUA) was used for serum sample centrifugation at 3000 r.p.m. for 10 min. All samples were adequately labeled, packaged, and transported to the immunology laboratory of the Faculty of Pharmacy at the Federal University of Bahia (UFBA). The serum samples were transferred to 5-mL tubes and kept at −20 °C until processing.

Serological tests were performed using a chemiluminescence microparticle immunoassay to search for IgG and IgM against *Toxoplasma gondii* and cytomegalovirus infections (Abbot®, São Paulo - Brazil) using the Architect i1000SR automation equipment (Abbot®, São Paulo - Brazil). Specimens with concentration values ≥ 3.0 IU/mL are considered reactive for IgG antibodies to *T. gondii*, indicating chronic infection. For CMV, samples with concentration values ≥ 6.0 AU/mL are also considered reactive for IgG antibodies.

Immunochromatographic tests designed for diagnostic purposes were used to determine the presence of total antibodies against HIV, *Treponema pallidum*, and hepatitis C, following the same methodology for detecting hepatitis B surface antigen (Abbot®, São Paulo - Brazil).

2.2.2. Socioeconomic data, newborn assessment, and anthropometry

Semi-structured questionnaires were administered to pregnant women during recruitment and after signing the informed consent form. The Interviewers conducted the questionnaire on socioeconomic status (based on family possession and income), general cultural habits, educational level, occupational history, and other evaluations. The socioeconomic level, stratified into five categories from A to E, was defined based on the criteria of the Brazilian Association of Population Studies (Brasil, 2008).

Detailed information about the newborn at birth, including sex, ethnicity, birth weight, delivery method, and gestational age at birth, were extracted from medical records. The anthropometric data was also collected from the medical records.

The INTERGROWTH-21st Project fetal growth curve (Villar et al., 2014) was applied to estimate the adequacy of birth weight for gestational age. The newborns (NBs) were classified as small for gestational age (SGA) when the birth weight (in grams) for completed gestational age was below the 10th percentile and large for gestational age (LGA) when equal to or above the 90th percentile. The remaining NBs were classified as appropriate for gestational age (AGA).

2.2.3. *Neurodevelopmental assessment*

At 12 ± 3 months of age, the infant's neurodevelopment was assessed using the Bayley-III Scales of Infant and Toddler Development by a trained specialist (DCO) under the supervision of a neuropsychologist (NC). This instrument assesses five developmental parameters in children aged 1–42 months: cognition, language (receptive and expressive communication), motor skills, and socio-emotional and adaptive behavior. Each of these five subscales provides a composite score that indicates the child's performance level in that specific developmental domain. These composite scores have a mean of 100 and a standard deviation of 15, allowing comparison of the assessed child's performance with normative population data (Del Rosario et al., 2021).

For categorical analysis of the Bayley-III composite scores, a cutoff point of 85 points was considered. Composite scores below 85 (1 standard deviation below the normative mean) indicate a risk for developmental delay in that specific domain (Johnson et al., 2014).

2.3. *Data analysis*

After data tabulation, frequency, central tendency, and dispersion measurements were analyzed. The choice between parametric and non-parametric tests was based on the Kolmogorov-Smirnov normality test. The Binomial test for a proportion was used to obtain confidence intervals for the proportions. Multivariate logistic regression modeling was applied to investigate possible associations between anti-*T. gondii* IgG and anti-CMV IgG seropositivity with neuropsychological development scores from the Bayley III scale. The odds ratio (OR) was obtained by exponentiating the beta quotients obtained in the multivariate model. The model was adjusted for gestational week of birth and adequacy of birth weight for gestational age. Multivariate linear regression modelling by stepwise was used to evaluate the association between Bayley-III composite scores and maternal titers of anti-*T. gondii* IgG and anti-CMV IgG. The model was adjusted for gestational week at birth and adequacy of birth weight for gestational age (percentile). The adjusted R^2 was used to determine the percentage of variance in the data explained by the model, and the non-standardized beta coefficient was used to indicate the strength of the association between the predictor variables and the outcome variables. The accepted level of statistical significance for all tests was $p < 0.05$. Descriptive and comparison statistical analyses were performed using SPSS® version 26.0 for Windows (IBM Inc. Massachussets, USA).

3. Results

Fig. 1 presents the flowchart detailing the recruitment of study participants. The study included 137 pregnant women with an average age of 26.8 years ± 0.5 years. Of the total, 80 (58.4 %) resided in Nazaré. Most self-identified as Afro-descendants (94.7 %), and 38.2 % were in

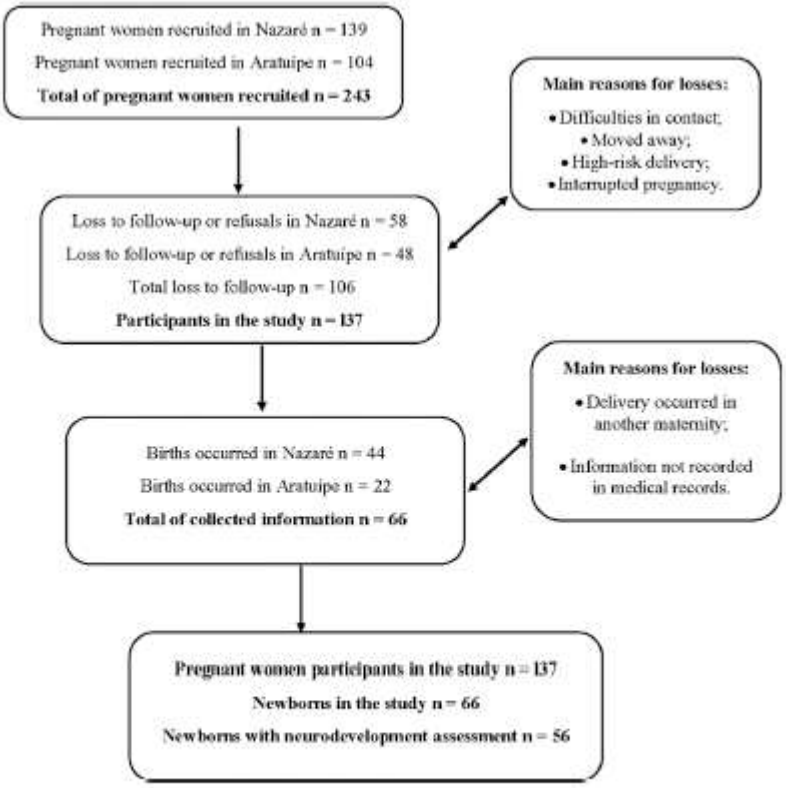

Fig. 1. Recruitment flowchart.

their first pregnancy. Most were classified as belonging to the lower social classes D-E (51.2 %). Most pregnant women had completed middle or high school (73.0 %), while 2.3 % reported being illiterate. 29 (22.1 %) pregnant women reported having cats as pets. There was a frequency of 17.4 % of reported previous miscarriages. Regarding their homes, a significant portion did not have access to potable water (18.2 %), and 40.2 % did not reside on a paved street (Table 1).

The overall seroprevalence found for chronic *T. gondii* infection was 53.4 % (95 %-CI, 46.7 % - 64.4 %), and for anti-CMV IgG antibodies, it was 96.4 % (95 %-CI, 91.8 % - 98.5 %) (Table 1). No participant tested positive for the presence of IgM antibodies against *T. gondii* and CMV. Three pregnant women tested positive for anti-*T. pallidum* antibodies and one pregnant woman tested positive for antibodies against the hepatitis C virus. No pregnant woman tested positive for anti-HIV antibodies or the presence of the hepatitis B surface antigen.

Table 1
Socioenvironmental determinants and serological status of pregnant women participating in the study.

Socioenvironmental data	TOTAL (n = 137)
	Mean (± SD)
Age (years)	26.8 (±0.5)
Minimum	15.0
Maximum	39.2
IgG anti-*T. gondii*	140.1 (±23.2)
Minimum	0
Maximum	1000
IgG anti-cytomegalovirus	110.9 (±8.7)
Minimum	0
Maximum	250
	n (%)
IgG positive	
anti-*T. gondii*	80 (58.4)
anti-cytomegalovirus	132 (96.4)
Place of residence	
Nazaré	80 (58.4)
Aratuípe	57 (41.6)
Ethnicity	
White	4 (3.0)
African Brazilian	126 (94,7)
Yellow	3 (2.3)
Education	
Illiterate	3 (2.3)
Elementary I complete	19 (14.6)
Complete Elementary II	40 (30.1)
Medium complete	57 (42.9)
Graduated	14 (10.5)
Socioeconomic classification	
D-E	66 (51.2)
C2	41 (31.8)
C1	18 (14.0)
B2	3 (2.3)
B1	1 (0.7)
Previously pregnant?	
Yes	81 (61.8)
No	50 (38.2)
Previous miscarriage?	
Yes	23 (17.4)
No	109 (82.6)
Gestational planning?	
No	91 (68.9)
Yes	41 (31.1)
Have a cat as pet?	
No	102 (77.9)
Yes	29 (22.1)
Residential water supply	
Artesian well	23 (18.2)
Piped	103 (81.8)
Water treatment	
Not treated	77 (59.2)
Treated (Filtered/boiled)	53 (40.8)
Have they paved the house street?	
No	53 (40.2)
Yes	79 (59.8)

Table 2 describes the neonatal characteristics of the study participants. Information from 66 neonates was obtained. 54.5 % of the NBs were male, and 86.7 % were classified as African-Brazilians. Regarding deliveries, 42.4 % occurred via cesarean section, and most occurred at Luís Argolo Maternity Hospital (61.2 %) in the neighboring municipality of Santo Antônio de Jesus, Bahia. The gestational week at the delivery time had an average of 39.1 (±0.2) weeks. Only two (3.4 %) of the births were preterm, occurring before the gestational age reached 36 weeks. 12.9 % of the NBs were classified as SGA and 11.3 % as LGA.

Upon reaching 12 ± 3 months of age, the infants included in the study were assessed using the Bayley-III scales. The Mean (± SD) of composite scores is presented in Table 3. The socio-emotional scale had the highest mean score of 127.3 (±1.6), while the lowest was from the adaptive behavior scale with 93.8 (±1.7).

To investigate a possible association between the categorized composite scores of the Bayley-III (cut-off = 85 points) and the positivity for IgG anti-*T. gondii* or IgG anti-CMV antibodies, a multivariate logistic regression analysis was performed using the adjusted odds ratio. The variables gestational week of birth and adequacy of birth weight for gestational age were used to adjust the multivariate model. The categorized composite scores of the Bayley-III subscales did not show a significant relationship with maternal seropositivity for IgG anti-*T. gondii* (Table 4). There were no significant associations between the composite scores and maternal seropositivity for IgG anti-CMV.

The results in Table 5 show the relationship between maternal IgG titers for anti-*T. gondii* antibodies and developmental outcomes in children, using composite scores from the Bayley-III assessment tool. Multivariate linear regression models were employed, considering adjustment covariates: gestational week of birth and birth weight adequacy (percentile). The results indicate a statistically significant

Table 2
Sociodemographic and anthropometric characteristics of newborns included in the study.

	Total (n = 66)
Variable	**n (%)**
Sex	
Female	30 (45.5)
Male	36 (54.5)
Ethnicity	
White	6 (13.3)
African Brazilian	39 (86,7)
Type of delivery	
Vaginal	37 (56.1)
Cesarean	28 (42.4)
Residence	1 (1.5)
Place of birth	
Nazaré's Maternity	18 (26.9)
Luis Argolo Hospital	41 (61.2)
Other	8 (11.9)
Adequacy of weight for gestational age	
SGA	8 (12.9)
AGA	47 (75.8)
LGA	7 (11.3)
Preterm birth (< 36 weeks)	
No	57 (96.6)
Yes	2 (3.4)
Variable	**Mean (± SD)**
Gestational age (weeks)	39.1 (±0.2)
Minimum	32.0
Maximum	41.0
Weight at birth (g)	3210.9 (±73.01)
Minimum	1744
Maximum	4192
Length at birth (cm)	47.8 (±0.4)
Minimum	32
Maximum	54
Percentile	55.7 (±3.5)
Minimum	6.2
Maximum	99.5

Table 3
Composite scores of Bayley-III scales of infant included in the study.

Variable	Mean (± SD)
Cognition scale	111.6 (±1.7)
Minimum	80
Maximum	140
Language scale	105.1 (±1.9)
Minimum	77
Maximum	138
Motor scale	104.3 (±1.1)
Minimum	82
Maximum	118
Socio-emotional scale	127.3 (±1.6)
Minimum	95
Maximum	140
Adaptive behavior scale	93.8 (±1.7)
Minimum	66
Maximum	128

* Statistically significant association
** Not calculated
Adjustment covariates – Gestational week of birth and adequacy of birth weight for gestational age.
Ref. - Reference Group

Table 4
Composite scores of Bayley-III and positive maternal serology for IgG anti-*T. gondii* antibodies based on multivariate logistic regression models.

	IgG anti-*T. gondii* positive n (%)	IgG anti-*T. gondii* negative n (%)	OR (CI 95 %)	p-value
Cognition scale				0.541
> 85 points	34 (94.4)	18 (90)	Ref.	
≤ 85 points	2 (5,6)	2 (10)	0.53 (0.07 – 4.08)	
Language scale				0.678
> 85 points	35 (97.2)	18 (90)	Ref.	
≤ 85 points	1 (2.8)	2 (10)	0.61 (0.06 – 6.96)	
Motor scale				**
> 85 points	35 (97.2)	20 (100)	**	
≤ 85 points	1 (2.8)	0		
Socio-emotional scale				**
> 85 points	36 (100)	20 (100)	**	
≤ 85 points	0	0		
Adaptive behavior scale				0.490
> 85 points	29 (82.9)	16 (80)	Ref.	
≤ 85 points	6 (17.1)	4 (20)	0.34 (0.02 – 7.24)	

association between maternal IgG titers and cognitive scale composite scores, with a non-standardized Beta coefficient of −0.078 (95 %-CI: −0.144 – −0.013). This finding suggests that cognition scale composite scores decrease by approximately 0.08 points for every unit increase in maternal IgG titers. The adjusted R^2 suggests that maternal IgG titers explain 35.1 % of the variation in cognition scale composite scores after adjustment for covariates.

The analysis also found a borderline association between maternal IgG titers and language scale composite scores, with a non-standardized Beta coefficient of −0.075 (95 %-CI: −0.152 – 0.002), and a p-value of 0.056. Although not statistically significant at 0.05, the negative coefficient suggests elevated maternal IgG titers for anti-*T. gondii* may be associated with lower language scale composite scores. The associations with motor, socio-emotional, and adaptive behavior scale composite

Table 5
Composite scores of Bayley-III and maternal IgG titers for anti-*T. gondii* antibodies based on multivariate linear regression models.

	Adjusted R^2	Non-standardized Beta coefficients	CI-95 %	p-value
Cognition scale	35.1 %	−0.078	−0.144 – −0.013	0.025 *
Language scale	14.4 %	−0.075	−0.152 – 0.002	0.056
Motor scale	3.9 %	−0.031	−0.071 – 0.010	0.122
Socio-emotional scale	4.7 %	−0.014	−0.097 – 0.069	0.707
Adaptive behavior scale	14.4 %	−0.031	−0.095 – 0.033	0.303

Adjustment covariates – Gestational week of birth and adequacy of birth weight for gestational age (percentile).
* Statistically significant association.

scores were not statistically significant (Table 5). There were no significant associations between the composite scores and maternal IgG titers for anti-CMV.

4. Discussion

The findings of this study provide evidence that chronic maternal infection with *T. gondii* may be associated with adverse outcomes in child neurodevelopment, particularly concerning cognitive function. Even though no significant association was observed between maternal IgG seropositivity for *T. gondii* and odds of low performance in neurodevelopment domains of the Bayley-III scales, the multivariate linear regression modeling revealed a statistically significant association between maternal anti-*T. gondii* IgG titers and cognitive composite scores. Furthermore, it was found that there is a potential link between elevated levels of maternal IgG to *T. gondii* and decreased composite scores on the language scale.

The pregnant women participating in the study demonstrated socio-environmental attributes that categorize them as socioeconomically challenged and living in inadequate sanitary conditions. The prevalence of *T. gondii* infection among pregnant women was 50.4 % (95 %-CI, 46.7 % - 64.4 %). This rate is quite comparable to findings reported in previous studies focusing on Brazilian pregnant women. The prevalence demonstrates notable variations across diverse regions, with reported rates ranging from 49.2 % to 75.1 % (Lopes et al., 2009; Ribeiro et al., 2005). Various factors, including age, educational attainment, income level, animal exposure, and dietary practices impact the infection rate (Avelar et al., 2017; Messina et al., 2019; Antinarelli et al., 2020). Although acute infection is infrequent, the issue of vertical transmission continues to be a significant apprehension (Kohler et al., 2022). In Brazil, the BrI strain of *T. gondii* is the most prevalent, frequently associated with outbreaks and infections in humans and animals. Although other clonal lineages, such as BrII, BrIII and BrIV, have also been identified, they are less common and present different levels of virulence (Carneiro et al., 2013; Brito et al., 2023).

The findings of the current study imply a populace characterized by a relatively elevated frequency of cesarean deliveries (42.4 %) and a variety of neonatal sizes, including a significant number of SGA (12.9 %) and LGA (11.3 %) infants. Moreover, the racial/ethnic makeup of the study population suggests a predominantly Afro-Brazilian ethnical composition (86.7 %). The rate of cesarean sections in Brazil ranks among the highest globally. Various determinants, such as socioeconomic variables, risk classification, and type of service (private or public), contribute to this occurrence (Diaz et al., 2022).

At 12 ± 3 months of age, the infants' neurodevelopment was assessed using the Bayley-III scales. The results suggest the infants had relatively socio-emotional solid development but lower scores on the adaptive behavior scale compared to the other domains assessed by the

Bayley-III instrument. This finding could indicate potential challenges in the infants' ability to effectively adapt to their environment and engage in self-care behaviors at this stage of development. The cognitive, language, and motor scores averaged close to 100. It is important to note that the Bayley-III scale has moderate predictive validity for cognitive outcomes in children. However, it may not identify all children at risk of future cognitive deficits (Spencer-Smith et al., 2015).

The present study found no associations between maternal anti-CMV IgG antibodies and composite scores of the Bayley-III. Nevertheless, the findings indicate that for each unit of maternal anti-*T. gondii* IgG titers increase, the composite scores of the cognitive scale decrease by approximately 0.06 points. This result suggests that higher levels of maternal IgG antibodies against *T. gondii*, indicative of chronic infection, may be associated with poorer cognitive performance in 12-month-old children. The adjusted R^2 suggests that 35.1 % of the cognitive scale composite scores variation can be explained by maternal IgG titers and the adjustment covariates (gestational age at birth and birth weight adequacy). These findings corroborate with previous studies that have linked chronic maternal toxoplasmosis to neurodevelopmental deficits in children. MIA triggered by chronic *T. gondii* infection may alter critical neurodevelopmental processes such as neurogenesis, neuronal migration, and cortical lamination, thereby increasing the risk of neurodevelopmental disorders (Bergdolt and Dunaevsky, 2019; Vlasova et al., 2021).

A study with animals that investigated the impact of MIA due to prenatal infection on offspring's psychiatric disorders, focusing on the association between *T. gondii* and schizophrenia, found that exposure to the parasite before gestation produced maternal pathogenic antibodies that can recognize fetal brain mimotopes and lead to neurochemical and behavioral alterations in the offspring (Núñez et al., 2022). In contrast, a study using a population-based birth cohort to investigate the association between maternal *T. gondii*, offspring bipolar disorder, and childhood cognition, assessed through the Peabody Picture Vocabulary Test and the Raven Matrices, found no significant associations between maternal IgG antibody titers and either offspring bipolar disorder or childhood cognition (Freedman et al., 2016).

Although few studies directly investigated the relationship between chronic maternal infection by *T. gondii* and its effects on neurodevelopment, there is evidence of an association between the parasite and the leading psychiatric disorders, such as schizophrenia and bipolar disorder, highlighting its potential role as a risk factor in the development of these conditions (Nayeri et al., 2020; Xiao et al., 2018). A study carried out a comprehensive review to synthesize existing preclinical and clinical evidence on the association between *T. gondii* infection and significant psychiatric disorders, suggesting that in addition to MIA, the parasite can induce dopaminergic signaling pathways in the brain, which can lead to psychotic episodes (Barroc et al., 2020).

While no substantial association was found between maternal anti-CMV IgG seropositivity and Bayley-III scores, it is essential to consider that maternal CMV infection could impact fetal neurodevelopment. Further studies are needed to elucidate maternal CMV infection's role in childhood neurodevelopment.

A limitation of this study is the relatively small sample size, especially assessing neonatal outcomes. Furthermore, neurocognitive assessment was performed only in a single visit at 12 months of age. Future studies with larger samples and longitudinal assessments of neurodevelopment at different ages are needed to confirm and deepen these findings. Another important consideration is that chronic maternal *T. gondii* infection does not always result in vertical transmission or adverse outcomes for the fetus. Factors such as the parasite's strain, parasite's load, the timing of infection during pregnancy, and the maternal immune response can influence the risk of transmission and fetal outcomes (Li et al., 2014).

Despite these limitations, this study contributes to understanding the potential association between chronic maternal *T. gondii* infection and adverse outcomes in child neurodevelopment. These findings reinforce the importance of monitoring and early detection of maternal infections during pregnancy to minimize adverse impacts on the child's development. Early identification of developmental delays using instruments such as the Bayley-III is essential for implementing intensive follow-up interventions and services. Furthermore, health education and preventive measures, such as washing fruits and vegetables properly and cooking meat adequately, can help reduce the incidence of toxoplasmosis during pregnancy.

5. Conclusions

In conclusion, this study suggests that chronic maternal *T. gondii* infection, even without vertical transmission, may be associated with adverse outcomes in child neurodevelopment, especially concerning cognition. These findings highlight the importance of monitoring and early detection of maternal infections during pregnancy to minimize adverse impacts on child development. Future studies with larger samples and longitudinal evaluations must confirm and deepen these results.

Ethical approval

The Ethics Committee of the Federal University of Bahia approved the study under protocol number 3.246.555 on April 5, 2019. All participants involved in this study provided informed consent before their inclusion. They were provided with a detailed explanation of the study's purpose, procedures, potential risks, and benefits. Participants were assured of their confidentiality and the voluntary nature of their participation. This study was conducted in accordance with ethical guidelines and regulations to ensure the rights and well-being of all participants.

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CRediT authorship contribution statement

Nara Cortes Andrade: Investigation, Methodology, Supervision, Validation. **Chrissie Ferreira de Carvalho:** Investigation, Methodology, Supervision, Validation. **José Antônio Menezes Filho:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Maria Isabel Santos Silveira Souza:** Writing – review & editing, Investigation. **Daisy Oliveira Costa:** Writing – review & editing, Investigation, Formal analysis, Data curation, Conceptualization. **Erival Amorim Gomes Junior:** Writing – review & editing, Investigation, Formal analysis, Data curation, Conceptualization. **Homègnon Antonin Ferréol Bahi:** Writing – review & editing, Investigation, Formal analysis, Conceptualization. **Nathália Ribeiro dos Santos:** Writing – review & editing, Formal analysis, Data curation, Conceptualization. **Victor Otero Martinez:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Jose Antonio Menezes Filho reports financial support was provided by CNPq-FAPESB. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data Availability

Data will be made available on request.

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