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Transmissão vetorial e sexual do vírus Zika e os efeitos clínicos e de desenvolvimento em crianças nascidas durante a epidemia em Salvador, Brasil

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SALVADOR

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“Pelos erros dos outros, o homem
sensato corrige os seus”

Oswaldo Cruz

AGUILAR TICONA, Juan Pablo. Transmissão vetorial e sexual do vírus Zika e os efeitos clínicos e de desenvolvimento em crianças nascidas durante a epidemia em Salvador, Brasil. 106 f. 2023. Tese (Doutorado) – Instituto de Saúde Coletiva, Universidade Federal da Bahia, Salvador, 2023.

1. RESUMO

Antecedentes: Em 2015, foi relatado o primeiro caso confirmado de infecção pelo vírus Zika no território continental de América, especificamente no Nordeste do Brasil. No entanto, os fatores que contribuem para a alta taxa de infecção e sua distribuição geográfica heterogênea ainda não estão claros. Além disso, o vírus Zika se diferencia de outros arbovírus devido à possibilidade de transmissão congênita e sexual, tornando crucial a avaliação das crianças expostas para o desenvolvimento de medidas preventivas que limitem o impacto no desenvolvimento infantil. **Objetivo:** Avaliar a transmissão do vírus Zika e os fatores de riscos em comunidades urbanas vulneráveis e seus efeitos clínicos e de desenvolvimento em crianças nascidas durante a epidemia de 2015-2016 em Salvador, Brasil. **Métodos:** Duas coortes foram avaliadas na cidade de Salvador, Brasil. A primeira, realizada na comunidade de Pau da Lima, teve como objetivo avaliar a transmissão do vírus Zika e os participantes foram acompanhados por meio de seguimentos semestrais antes e após o surto de 2015. Na segunda coorte, conduzida no Hospital Geral Roberto Santos (HGRS), foram avaliados desfechos do desenvolvimento infantil, incluindo crescimento antropométrico, neurodesenvolvimento, e desfechos clínicos neurológicos, oftalmológicos e auditivos aos 2 e 3 anos após o nascimento. A exposição ao vírus Zika foi determinada usando o ensaio imunológico IgG3 para o ZIKV na coorte de Pau da Lima e o Teste de Neutralização por Redução de Placa (PRNT) com amostras maternas de sangue coletadas no nascimento e durante o seguimento na coorte hospitalar do HGRS. **Resultados**: Os resultados deste estudo foram descritos em quatro artigos. 1) Na coorte de Pau da Lima, foi identificado que indivíduos com empregos informais e insegurança alimentar tiveram maior chance de soropositividade para o ZIKV, enquanto aqueles com alto índice socioeconômico tiveram menor chance. 2) Na mesma coorte, foi identificado que homens que se envolviam em relações sexuais casuais tinham mais chances de serem positivos para o ZIKV e mulheres com menos de 6 anos de escolaridade formal tinham duas vezes mais chances de ter um resultado positivo. 3) Na coorte HGRS, focou no neurodesenvolvimento de crianças sem microcefalia, constatando que crianças expostas ao ZIKV tiveram um risco maior de

comprometimento neurodesenvolvimental global. 4) Na mesma coorte, foi caracterizado o neurodesenvolvimento de crianças com microcefalia, observando-se atraso grave no neurodesenvolvimento das crianças com SCZ. Além disso, maiores escores na avaliação neurológica foram associados a melhores escores cognitivos e motores do Bayley-III, e maior circunferência da cabeça no acompanhamento foi associada a melhores escores cognitivos e motores. **Conclusão:** Em resumo, esses trabalhos oferecem informações sobre os fatores sociais e do comportamento associados à transmissão do ZIKV em comunidades vulneráveis, assim como do papel da exposição intrauterina e seu impacto no neurodesenvolvimento de crianças com e sem microcefalia. Consequentemente, é crucial destacar a necessidade de medidas além das focadas no controle do vetor que levem em consideração fatores sociais e comportamentais para minimizar o impacto da doença. Além disso, é importante realizar avaliações de longo prazo em crianças expostas ao ZIKV para permitir intervenções precoces e efetivas.

Palavras chaves: Vírus Zika; Neurodesenvolvimento; Desenvolvimento Infantil

AGUILAR TICONA, Vector and sexual transmission of the Zika virus and the clinical and developmental effects on children born during the epidemic in Salvador, Brazil. 106 p. 2023. Thesis (Doctorate) - Institute of Collective Health, Federal University of Bahia, Salvador, 2023. ABSTRACT

2. ABSTRACT

Background: In 2015, the first confirmed case of Zika virus infection in the continental territory of the Americas was reported, specifically in Northeast Brazil. However, the factors contributing to the high infection rate and its heterogeneous geographic distribution are still unclear. Additionally, Zika virus differs from other arboviruses due to the possibility of congenital and sexual transmission, making it crucial to evaluate exposed children for the development of preventive measures that limit the impact on child development. Objective: To evaluate Zika virus transmission and risk factors in vulnerable urban communities and its clinical and developmental effects in children born during the 2015-2016 epidemic in Salvador, Brazil. Methods: Two cohorts were evaluated in the city of Salvador, Brazil. The first, conducted in the community of Pau da Lima, aimed to evaluate Zika virus transmission and participants were followed through semi-annual follow-ups before and after the 2015 outbreak. In the second cohort, conducted at the Roberto Santos General Hospital (HGRS), infant developmental outcomes including anthropometric growth, neurodevelopment, and neurological, ophthalmological, and auditory clinical outcomes were evaluated at 2 and 3 years after birth. Exposure to Zika virus was determined using the IgG3 immunological assay for ZIKV in the Pau da Lima cohort and the Plaque Reduction Neutralization Test (PRNT) with maternal blood samples collected at birth and during follow-up in the HGRS hospital cohort. Results: The results of this study were described in four articles. 1) In the Pau da Lima cohort, it was identified that individuals with informal jobs and food insecurity had a higher chance of ZIKV seropositivity, while those with high socioeconomic status had a lower chance. 2) In the same cohort, it was identified that men who engaged in casual sexual relationships were more likely to test positive for ZIKV, and women with less than 6 years of formal education had twice the chance of a positive result. 3) In the HGRS cohort, the focus was on the neurodevelopment of children without microcephaly, finding that children exposed to ZIKV had a higher risk of overall neurodevelopmental impairment. 4) In the same cohort, the neurodevelopment of children with microcephaly was characterized, with severe developmental delays observed in children with SCZ. Additionally, higher

scores on the neurological evaluation were associated with better cognitive and motor scores on the Bayley-III, and larger head circumference at follow-up was associated with better cognitive and motor scores. Conclusion: In summary, these studies provide information on social and behavioral factors associated with ZIKV transmission in vulnerable communities, as well as the role of intrauterine exposure and its impact on the neurodevelopment of children with and without microcephaly. Consequently, it is crucial to highlight the need for measures beyond those focused on vector control and to take into account social and behavioral factors to minimize the impact of the disease. Additionally, it is important to conduct long-term evaluations of children exposed to ZIKV to allow for early and effective interventions.

Keywords: Zika Virus; Neurodevelopment; Child Development.

LISTAS DE ABREVIATURAS E SIGLAS

Bayley III	Escalas de desenvolvimento do bebê e da criança pequena 3er edição
CHIV	Vírus da Chikungunya
DENV	Vírus da Dengue
DP ou **SD**	Desvio Padrão ou "*Standard Desviation"*
FCI	Indicadores de cuidados familiares ou "*Family Care Indicators"*
HGRS	Hospital Geral Roberto Santos
HINE	*Hammersmith infant neurological examination*
MC	Microcefalia
EOA ou OEA	"*Otoacoustic emissions test*" ou Exame de otoemissão acústica
PPCT	Processo, Pessoa, Contexto e Tempo
PRNT	Teste de Neutralização por Redução de Placa ou "Plaque Reduction Neutralization Tests"
SCZ	Síndrome congênita da Zika
ZIKV	Vírus da Zika

SUMÁRIO

3. INTRODUÇÃO

O vírus Zika (ZIKV) é um arbovirus da família dos flavivírus que foi descoberto em 1947, quando foi isolado pela primeira vez em macacos Rhesus na floresta de Zika, em Uganda (BAUD; GUBLER; SCHAUB; LANTERI *et al.*, 2017). Ele é transmitido pelo mosquito *Aedes aegypti*, o mesmo vetor responsável pela transmissão da Dengue (DENV) e da Chikungunya (CHIKV) (RASMUSSEN; JAMIESON; HONEIN; PETERSEN, 2016). Embora o ZIKV tenha se espalhado silenciosamente por mais de 50 anos na África e na Ásia, ele se tornou responsável por uma série de epidemias na Micronésia, no Pacífico Sul e nas Américas no início do século XXI (IOOS; MALLET; LEPARC GOFFART; GAUTHIER *et al.*, 2014; MUSSO; GUBLER, 2016; RASMUSSEN; JAMIESON; HONEIN; PETERSEN, 2016). A maior parte dos casos de ZIKV tem uma apresentação assintomática ou associada a uma doença leve e autolimitada (BOEUF; DRUMMER; RICHARDS; SCOULLAR *et al.*, 2016), entretanto na última década estudos têm evidenciado que a infecção pelo ZIKV também pode causar alterações neurológicas em adultos (MUNOZ; BARRERAS; PARDO, 2016). Além disso, o acompanhamento de crianças nascidas durante a epidemia revelou que a infecção intrauterina pelo ZIKV está associada a uma série de problemas teratogênicos e do desenvolvimento, agrupados sob o termo Síndrome Congênita do Zika (SCZ) (MOORE; STAPLES; DOBYNS; PESSOA *et al.*, 2017).

Antes da chegada do vírus Zika nas ilhas Yap durante 2007 e no continente americano em 2014, acreditava-se que o vírus Zika se mantinha principalmente na natureza em um ciclo selvático. Embora anticorpos contra o vírus Zika tenham sido detectados em vários outros mamíferos não primatas e em roedores (WIKAN; SMITH, 2016), muitos dos casos de febre Zika relatados na Ásia e na África provavelmente representam casos de transmissão por transbordamento do ciclo selvático, nos quais os seres humanos se infectaram como hospedeiros acidentais. Mas durante as epidemias do século XXI, foi levantada a possibilidade de um ciclo de transmissão urbana (MUSSO; GUBLER, 2016). Além disso, outras formas de transmissão foram descritas, como a congênita, sexual e por contato com suor e urina, mas ainda não se sabe ao certo qual é o impacto dessas formas na dinâmica da epidemia e qual suas sequelas (BAUD; GUBLER; SCHAUB; LANTERI *et al.*, 2017).

A síndrome congênita do vírus da Zika (SCZ) é um conjunto de alterações resultantes das anomalias estruturais e deficiências funcionais secundárias ao dano nervoso

(MOORE; STAPLES; DOBYNS; PESSOA *et al.*, 2017; VAN DER LINDEN; FILHO; LINS; VAN DER LINDEN *et al.*, 2016; WEAVER; COSTA; GARCIA-BLANCO; KO *et al.*, 2016). As principais alterações descritas foram a microcefalia, lesões oftalmológicas, contraturas congênitas relacionadas com artrogriposes e pé torto, hipertonia precoce marcada e sintomas de afecção extrapiramidal (MOORE; STAPLES; DOBYNS; PESSOA *et al.*, 2017). Uma característica comum destas alterações iniciais associadas a SCZ, é que são problemas diagnosticados e evidentes ao nascimento (RICE; GALANG; ROTH; ELLINGTON *et al.*, 2018) e inclusive no período pré-gestacional mediante exames de imagem, como a ultrassonografia (PEREIRA; NIELSEN-SAINES; SPERLING; MAYKIN *et al.*, 2018). Novas pesquisas a partir da avaliação do desenvolvimento das crianças expostas intrauterinamente mostraram outras alterações relacionadas a SCZ, algumas observadas com menor frequência como a perda auditiva (PELOGGIA; ALI; NANDA; BAHAMONDES, 2018), convulsões (OLIVEIRA-FILHO; FELZEMBURGH; COSTA; NERY JR *et al.*, 2018) ou disfagia (MOORE; STAPLES; DOBYNS; PESSOA *et al.*, 2017). Enquanto outras mais sutis e subclínicas, como as alterações hormonais (VERAS GONÇALVES; MIRANDA-FILHO; ROCHA VILELA; RAMOS *et al.*, 2020). Sendo uma doença ainda pouco conhecida podemos inferir que os casos observados inicialmente sejam a manifestação das formas mais graves da doença (BAUD; GUBLER; SCHAUB; LANTERI *et al.*, 2017; OLIVEIRA MELO; MALINGER; XIMENES; SZEJNFELD *et al.*, 2016) e existe uma lacuna de informação sobre o desenvolvimento das crianças atingidas, sobretudo em crianças sem MC ou alguma alteração aparente, mas que foram expostas ao ZIKV intrauterinamente. Algumas pesquisas apontam atraso no neurodesenvolvimento, principalmente cognitivo e da linguagem nas crianças expostas ao ZIKV, porém normocefálicas (FAIÇAL; DE OLIVEIRA; OLIVEIRA; DE ALMEIDA *et al.*, 2019; LEE; COOPER; IWAMOTO; LASH *et al.*, 2019; NIELSEN-SAINES, K.; BRASIL, P.; KERIN, T.; VASCONCELOS, Z. *et al.*, 2019; OLIVEIRA MELO; MALINGER; XIMENES; SZEJNFELD *et al.*, 2016). Contrariamente, estudos como o de Grant et al., envolvendo crianças de dois anos sem microcefalia, descobriram que, embora tenham identificado 15,4% de problemas no neurodesenvolvimento em crianças expostas, uma proporção semelhante de alterações foi identificada no grupo controle de crianças não expostas (GRANT; FLÉCHELLES; TRESSIÈRES; DIALO *et al.*, 2021). Além disso, no estudo de Mulkey et al., foi observada uma tendência decrescente no desenvolvimento, mas ainda dentro dos parâmetros considerados normais (MULKEY; ARROYAVE-WESSEL; PEYTON; BULAS

et al., 2020). Tais resultados destacam a necessidade de ter um grupo de controle de qualidade para determinar se estas alterações são resultado da exposição ao ZIKV ou se elas são parte de outros problemas comuns na infância.

Estudos longitudinais são necessários para responder perguntas sobre transmissão e desfechos, pois permitem o acompanhamento de indivíduos ao longo do tempo, o que é essencial para avaliar mudanças na exposição, comportamento, fatores de risco e saúde em geral. Dessa forma, é possível identificar associações causais, elucidar mecanismos de ação e avaliar a eficácia de intervenções preventivas e terapêuticas. A coorte de Pau da Lima, que acompanha uma comunidade informal por mais de 20 anos em Salvador, Bahia, é uma oportunidade única para entender a transmissão de doenças infecciosas em um contexto real. Esse estudo longitudinal permitiu o acompanhamento e avaliação da evolução de diferentes doenças infecciosas, Chikungunya(ANJOS; MUGABE; MOREIRA; CARVALHO *et al.*, 2020), Leptospiroses(HAGAN; MORAGA; COSTA; CAPIAN *et al.*, 2016) e COVID-19 (FOFANA; NERY; AGUILAR TICONA; DE ANDRADE BELITARDO *et al.*, 2022). Além disso, o estudo permitiu a identificação de fatores de risco e a avaliação da eficácia das medidas de controle e prevenção e a contribuição para a formulação de políticas públicas de saúde.

Estudos como a coorte de Rio de Janeiro(NIELSEN-SAINES, KARIN; BRASIL, PATRÍCIA; KERIN, TARA; VASCONCELOS, ZILTON *et al.*, 2019) ou o seguimento de crianças em Pernambuco (ALVES; PAREDES; SILVA; MELLO *et al.*, 2018; DA SILVA; EICKMANN; DE ALENCAR XIMENES; MONTARROYOS *et al.*, 2020) que foram conduzidos na época da epidemia (2015-2016) permitiram acompanhar prospectivamente mulheres expostas para avaliar o efeito da transmissão congênita do vírus, os desfechos clínicos e de desenvolvimento em crianças nascidas destas mães. No Hospital Geral Roberto Santos (HGRS), em Salvador, vem se desenvolvendo um estudo de coorte hospitalar que inclui mães e suas crianças nascidas durante a epidemia. Este projeto apresenta uma oportunidade valiosa para determinar a incidência das manifestações clínicas, bem como caracterizar o desenvolvimento a longo prazo (2 – 3 anos) e as trajetórias de desenvolvimento das crianças expostas ao ZIKV intrauterinamente que nasceram durante a epidemia e que formam parte da coorte do HGRS.

4. EMBASAMENTO TEÓRICO

4.1. Zika Vírus

O vírus Zika (ZIKV) é um arbovírus ("*arthropod-borne virus"* que, em português, faz referência aos vírus que são transmitidos por artrópodes) que tem uma cadeia simples positiva de RNA, pertencente ao gênero *Flavivirus* da família *Flaviviridae* (IOOS; MALLET; LEPARC GOFFART; GAUTHIER *et al.*, 2014; MUSSO; GUBLER, 2016). filogeneticamente muito próximo do DENV e da Febre Amarela. Análises genômicas mostraram uma grande variedade de subclasses e as mais conhecidas pela sua relevância clínica são a Asiática e a Africana (IOOS; MALLET; LEPARC GOFFART; GAUTHIER *et al.*, 2014). Estudos de sequenciamento genômico de amostras brasileiras indicaram que o ZIKV que circulou na epidemia de 2015-2016 no Brasil pertencia à linhagem asiática, com uma homologia superior a 99,7%, mesma cepa responsável pelo surto da Polinésia Francesa em 2013 (BAUD; GUBLER; SCHAUB; LANTERI *et al.*, 2017; POLONIO; DE FREITAS; ZANLUQUI; PERON, 2017).

4.2. Transmissão do ZIKV

O ZIKV foi identificado primeiramente em macacos *Rhesus,* em 1947, na floresta que tinha o nome de Zika, em Uganda, e em mosquitos *Aedes africanus* em 1948 na mesma floresta (BAUD; GUBLER; SCHAUB; LANTERI *et al.*, 2017; WIKAN; SMITH, 2016). Posteriormente, o primeiro registro de um humano infectado pelo ZIKV foi feito na Nigéria em 1954 (BAUD; GUBLER; SCHAUB; LANTERI *et al.*, 2017). A partir desse momento o vírus circulou de forma silenciosa na África e Ásia durante mais de 50 anos e posteriormente emergiu nos surtos nas ilhas de Yap, em 2007, e na Polinésia Francesa em 2013 (BAUD; GUBLER; SCHAUB; LANTERI *et al.*, 2017). Até esse ponto, acreditava-se apenas na transmissão vetorial, mas durante os recentes surtos, outras formas de transmissão foram descritas.

4.2.1. Transmissão vetorial

O ciclo de transmissão vetorial envolve um hospedeiro/reservatório e um vetor, sendo que frequentemente os arbovírus necessitam de um hospedeiro/reservatório não-humano para se desenvolver (ciclo silvestre e enzoótico) (MUSSO; GUBLER, 2016; VASILAKIS; WEAVER, 2017). No entanto, o ZIKV, assim como o DENV ou o CHIKV,

se adaptou para ter um ciclo de transmissão direto entre mosquito e humano, dispensando a necessidade de um hospedeiro/reservatório não-humano (ciclo humano ou urbano). Esse ciclo é comum em ambientes urbanos e suburbanos (MUSSO; GUBLER, 2016; PETERSEN; JAMIESON; POWERS; HONEIN, 2016; VASILAKIS; WEAVER, 2017). O *Aedes aegypti* tem sido associado a quase todos os surtos urbanos conhecidos do vírus Zika. Embora o alcance de *Aedes albopictus* tenha uma maior extensão, o que pode aumentar as áreas de risco para surtos de Zika, durante a epidemia de 2015-2016 ele teve uma capacidade vetorial inerente menor do que *Aedes aegypti*. Isto pode ter sido porque não vive tão próximo aos humanos e não se alimenta preferencialmente deles (GREGORY; ODUYEBO; BRAULT; BROOKS *et al.*, 2017).

Entre 1992 e 2016, a soroprevalência do ZIKA em áreas endêmicas de Africa era inferior a 7,5% (HERRERA; CHANG; HAMEL; MBOUP *et al.*, 2017). Antes disso, há poucos registros da prevalência da doença. Entre eles, um estudo em Uganda em 1984 encontrou uma prevalência de 6% (RODHAIN; GONZALEZ; MERCIER; HELYNCK *et al.*, 1989), outro em Angola em 1960 identificou uma prevalência de 26% entre adultos (KOKERNOT; CASACA; WEINBREN; MCINTOSH, 1965) e o maior registro ocorreu em Mali entre 1964 e 1967, com soroprevalência de 53% (BRÈS, 1970). No entanto, durante esse período, foi difícil identificar se esses dados se deviam à transmissão silvestre ou urbana devido à falta de vigilância e à reatividade cruzada sorológica com outros flavivírus. Nos últimos anos, durante as epidemias no Pacífico e nas Américas, o ciclo de transmissão foi principalmente urbano e responsável por centenas de milhares de casos registrados. Na ilha de Yap, a soroprevalência chegou a 73% (DUFFY; CHEN; HANCOCK; POWERS *et al.*, 2009)e no epicentro da epidemia até 2016, no Brasil, foi identificada uma soroprevalência de 63% (NETTO; MOREIRA-SOTO; PEDROSO; HOSER *et al.*, 2017).

4.2.2. Transmissão não vetorial

Durante as recentes epidemias do ZIKV no início do século XXI, foi destacada a importância das diferentes vias de transmissão do vírus. Essas vias podem estar associadas ao prolongamento da epidemia e ao aumento da taxa de infecção. Nesta seção, discutiremos as evidências científicas que sustentam cada uma dessas vias.

Transmissão Vertical: A evidência de que existe transmissão vertical do vírus da ZIKV agora é extensa. A primeira delas, um estudo retrospectivo do surto da Polinésia

Francesa (CAUCHEMEZ; BESNARD; BOMPARD; DUB *et al.*, 2016). Entretanto, a transmissão intrauterina só foi confirmada durante o surto no Brasil (BAUD; GUBLER; SCHAUB; LANTERI *et al.*, 2017; MARINHO; ARAÚJO; PORTO; FERREIRA *et al.*, 2016; OLIVEIRA MELO; MALINGER; XIMENES; SZEJNFELD *et al.*, 2016), quando o RNA do vírus foi encontrado no líquido amniótico de mulheres com sintomas de infecção pelo ZIKV e em amostras de cérebros pertencentes a fetos produto de abortos. Estes achados confirmaram que o ZIKV pode atravessar a barreira placentária (CALVET; AGUIAR; MELO; SAMPAIO *et al.*, 2016; VAN DER EIJK; VAN GENDEREN; VERDIJK; REUSKEN *et al.*, 2016). Este tipo de transmissão não ocorre em todas as gestações e nem todos os fetos expostos apresentam sintomas clínicos de síndrome congênita do Zika (SCZ) (BAUD; GUBLER; SCHAUB; LANTERI *et al.*, 2017). Mais importante ainda, não se tem certeza sobre a real magnitude das taxas de transmissão.

Entre as sequelas associadas com a tranmsissiao congentia do ZIKV a mais grave destes foi a microcefalia que chegou a atingir a 2,8 crianças a cada 100.000 nascimentos durante o ano de 2015 (DE OLIVEIRA; CORTEZ-ESCALANTE; DE OLIVEIRA; CARMO *et al.*, 2016). Mas este achado só foi a ponta do iceberg e com o avanço das pesquisas novas alterações no nascimento e durante o desenvolvimento foram descritas.

Estes problemas congênitos, levaram o Ministério da Saúde de Brasil a declarar um estado de emergência sanitária nacional baseado nos 268 casos de microcefalia registrados no estado de Pernambuco (PEREIRA; DE LIMA; OLIVEIRA; DE FRANÇA *et al.*, 2017). Posteriormente, a Organização Mundial de Saúde realizaria uma medida similar, declarando o surto de ZIKV como um evento de saúde pública de interesse internacional de acordo com o Regulamento Sanitário Internacional (BUENO, 2017). A partir do ingresso do ZIKV no Brasil a doença foi avançando rapidamente pelo território americano, sendo que para o mês de março de 2017, 44 dos 57 países da América reportaram a transmissão autóctone do ZIKV (THÉZÉ; LI; DU PLESSIS; BOUQUET *et al.*, 2018) atingindo a mais de 4 milhões de pessoas. (BOEUF; DRUMMER; RICHARDS; SCOULLAR *et al.*, 2016; GYAWALI; BRADBURY; TAYLOR-ROBINSON, 2016).

Transmissão Sexual: O primeiro relato da transmissão por via sexual do ZIKV foi em 2008, quando um pesquisador infectou-se em Senegal e transmitiu o vírus para sua esposa ao retornar aos USA. A ideia da transmissão sexual foi proposta porque o pesquisador apresentou sintomas do ZIKV juntamente com hematospermia 4 dias após o retorno e, um

dia depois, sua esposa apresentou sintomas de infecção pelo ZIKV. Ela não tinha saído do país no último ano e tinha mantido relações sexuais com o esposo um dia após o retorno dele (FOY; KOBYLINSKI; FOY; BLITVICH *et al.*, 2011; MUSSO; ROCHE; ROBIN; NHAN *et al.*, 2015). Durante o surto na Polinésia Francesa, o ZIKV foi isolado no sêmen de pessoas infectadas com sintomas, alguns dos casos apresentaram hematospermia, o que demonstrava uma plausibilidade biológica da transmissão sexual (GRISCHOTT; PUHAN; HATZ; SCHLAGENHAUF, 2016; MUSSO; ROCHE; ROBIN; NHAN *et al.*, 2015). Relatos posteriores de casos em turistas relataram os mesmos passos dessa via de transmissão. Turistas expostos ao ZIKV que retornaram ao país de origem e mantiveram relações sexuais com as parceiras, que não haviam sido expostas anteriormente, apresentaram sintomas e provas sorológicas positivas para ZIKV (ARMSTRONG; HENNESSEY; ADAMS; CHERRY *et al.*, 2016; HILLS; RUSSELL; HENNESSEY; WILLIAMS *et al.*, 2016). Estudos adicionais comprovaram que o vírus está presente até 62 dias no sêmen das pessoas infectadas e tem a capacidade de se replicar (BARRY; PASCO; BABAK; SARAH *et al.*, 2016; GRISCHOTT; PUHAN; HATZ; SCHLAGENHAUF, 2016).

Amamentação: O ZIKV foi isolado no leite materno, mas a informação sobre a eficiência desta via de transmissão ainda não é suficiente (COLT; GARCIA-CASAL; PEÑA-ROSAS; FINKELSTEIN *et al.*, 2017). No momento, só se tem relatos de casos da transmissão na Polinésia Francesa e em New (AUBRY; TEISSIER; HUART; MERCERON *et al.*, 2017; IOOS; MALLET; LEPARC GOFFART; GAUTHIER *et al.*, 2014). Esses relatos descrevem mães que tinham sintomas relacionados ao ZIKV (febre e exantema cutâneo) até dois dias antes e três dias após do nascimento do filho e que foram confirmados por laboratório (AUBRY; TEISSIER; HUART; MERCERON *et al.*, 2017; IOOS; MALLET; LEPARC GOFFART; GAUTHIER *et al.*, 2014). Esses relatos descrevem mães que tinham sintomas relacionados ao ZIKV (febre e exantema cutâneo) até dois dias antes e três dias após do nascimento do filho e que foram confirmados por laboratório (AUBRY; TEISSIER; HUART; MERCERON *et al.*, 2017; IOOS; MALLET; LEPARC GOFFART; GAUTHIER *et al.*, 2014). Mães que amamentaram os neonatos que também apresentaram resultados sorológicos positivos e, em um só caso, se descreveu doença exantemática (DUPONT-ROUZEYROL; BIRON; O'CONNOR; HUGUON *et al.*, 2016).

Doadores de sangue: Na polinésia francesa assinalaram o potencial risco de uma transmissão em doadores de sangue; neles, identificou-se que 2,8% dos doadores eram assintomáticos e positivos para ZIKV (BIERLAIRE; MAUGUIN; BROULT; MUSSO, 2017). No Brasil, relatos de casos apontaram a provável transmissão do vírus por sangue de doadores infectados. Um relato de dos casos com seguimento antes e depois da doação de

plaquetas, mediante resultados dos testes moleculares, apontaram a infecção por ZIKV (BARJAS-CASTRO; ANGERAMI; CUNHA; SUZUKI *et al.*, 2016; BAUD; GUBLER; SCHAUB; LANTERI *et al.*, 2017; MOTTA; SPENCER; CORDEIRO DA SILVA; ARRUDA *et al.*, 2016).

Urina e saliva: O vírus também está presente na urina e na saliva das pessoas infectadas, mas isso não significa que seja viável para infectar (BONALDO; RIBEIRO; LIMA; DOS SANTOS *et al.*, 2016). Existe o temor de que, diante de uma doença declarada como uma emergência global, essas possíveis vias aumentem o risco de contrair a doença. Atualmente, alguns modelos em roedores, através de inoculação intranasal, mostraram um possível potencial de infecção, mas também indicam que o efeito diminui com a idade dos roedores (BAUD; GUBLER; SCHAUB; LANTERI *et al.*, 2017; DICK, 1952; GRISCHOTT; PUHAN; HATZ; SCHLAGENHAUF, 2016). Adicionalmente, a presença do vírus na urina foi útil para o diagnóstico tardio da infecção (GRISCHOTT; PUHAN; HATZ; SCHLAGENHAUF, 2016; ROZE; NAJIOULLAH; FERGE; APETSE *et al.*, 2016). Em comparação com os testes sorológicos, o ZIKV pode ser detectado por maior tempo na urina (BINGHAM; CONE; MOCK; HEBERLEIN-LARSON *et al.*, 2016; GRISCHOTT; PUHAN; HATZ; SCHLAGENHAUF, 2016). Um relatório indica que a presença do ZIKV na urina pode durar até 30 dias após o início dos sintomas (GOURINAT; O'CONNOR; CALVEZ; GOARANT *et al.*, 2015).

Lágrimas e suor: Isoladamente, há um relato de caso de uma pessoa que visitou um paciente americano que foi infectado durante uma viagem ao México. Ao retornar, o paciente apresentou resultados laboratoriais positivos para ZIKV, alta viremia e posteriormente faleceu. O visitante relatou ter tido contato com o paciente enquanto o ajudava a se acomodar na cama, sem luvas, e ao limpar os olhos dele. Após 5 dias da morte do primeiro paciente, o visitante também apresentou resultados laboratoriais positivos para ZIKV. Presume-se que a transmissão ocorreu por contato com fluidos corporais (como lágrimas ou suor), uma vez que não havia outra via possível de transmissão (SWAMINATHAN; SCHLABERG; LEWIS; HANSON *et al.*, 2016).

4.3. A doença do ZIKV

Inicialmente esta doença foi considerada leve e autolimitada, mas durante 2015 foram identificados os incrementos de transtornos neurológicos e de malformações

congênitas (CARDOSO; PAPLOSKI; KIKUTI; RODRIGUES *et al.*, 2015; COSTA; SARNO; KHOURI; DE PAULA FREITAS *et al.*, 2016; PETERSEN; JAMIESON; POWERS; HONEIN, 2016). Antes dos recentes surtos, a infecção pelo ZIKV estava associada geralmente com sintomas leves e autolimitados e na sua maior parte de forma assintomática. Na maior parte dos casos a doença aparece após um período de incubação de 3 a 12 dias (média de 7 dias) (DUFFY; CHEN; HANCOCK; POWERS *et al.*, 2009; IOOS; MALLET; LEPARC GOFFART; GAUTHIER *et al.*, 2014; PLOURDE; BLOCH, 2016).

4.3.1. Casos assintomáticos

Estudos sugerem que cerca de 80% das pessoas infectadas pelo ZIKV são assintomáticas. Essa afirmação é baseada em pesquisas do surto nas ilhas Yap, onde foram identificados 49 casos confirmados e 59 casos prováveis de infecção pelo ZIKV (DUFFY; CHEN; HANCOCK; POWERS *et al.*, 2009). A melhor forma de avaliar a prevalência de casos assintomáticos é por meio de estudos populacionais transversais, como o das ilhas Yap (HABY; PINART; ELIAS; REVEIZ, 2018). Outros estudos que empregaram essa metodologia foram o da Polinésia Francesa, que determinou uma prevalência de assintomáticos de 49% na população geral e de 29% dos participantes em idade escolar (AUBRY; TEISSIER; HUART; MERCERON *et al.*, 2017) e outro estudo em Porto Rico, que identificou que a prevalência de assintomáticos era de 57% (LOZIER; BURKE; LOPEZ; ACEVEDO *et al.*, 2018). No entanto, ainda não existe um consenso adequado sobre a prevalência de assintomáticos devido às diferentes metodologias empregadas e à alta heterogeneidade dos resultados. Uma revisão sistemática sobre os casos assintomáticos publicados encontrou que a prevalência de assintomáticos é de 61,8%, mas seu índice de heterogeneidade foi de 99% (IC 95%: 33,0–87,1%) (HABY; PINART; ELIAS; REVEIZ, 2018).

4.3.2. Formas leves

A doença causada pelo ZIKV tem início súbito e, embora o espectro de sintomas do ZIKV seja variado, os sintomas geralmente são moderados ou leves, autolimitados e pouco específicos, semelhantes aos sintomas de infecções por outros arbovírus (PLOURDE; BLOCH, 2016). A erupção cutânea (exantema maculopapular que se estende desde a cabeça até as extremidades) é o sintoma mais frequentemente relatado na literatura, com uma frequência superior a 90% (DUFFY; CHEN; HANCOCK; POWERS *et al.*, 2009). Em uma

coorte de pacientes adultos no Rio de Janeiro, mais de 97% dos participantes apresentaram erupção cutânea confirmada por laboratório (BRASIL; CALVET; SIQUEIRA; WAKIMOTO *et al.*, 2016). A erupção cutânea geralmente é acompanhada por febre abaixo de 38,5°C e tem duração de cerca de um dia. Outros sintomas como artralgia, artrite e conjuntivite também foram frequentemente relatados, acima de 50% de sua apresentação (DUFFY; CHEN; HANCOCK; POWERS *et al.*, 2009).

4.3.3. Formas graves

A febre geralmente é baixa e de curta duração, mas alguns casos podem apresentar febre alta (CALVET; SANTOS; SEQUEIRA, 2016). Outros sintomas, como linfadenopatia (WEITZEL; CORTES, 2016), dor abdominal severa (CARDONA-CARDONA; RODRÍGUEZ MORALES, 2016), trombocitopenia, e outros problemas de coagulação também foram relatados (KARIMI; GOORHUIS; SCHINKEL; CODRINGTON *et al.*, 2016; SINGH; ATANELOV; AABODI; KOO, 2016). As fatalidades atribuídas ao ZIKV são raras, excluindo perdas fetais em mulheres grávidas e recém-nascidos com grave doença congênita por ZIKV (CALVET; SANTOS; SEQUEIRA, 2016). No entanto, foram relatadas como casos clínicos algumas mortes relacionadas ao ZIKV no Brasil e na Colômbia (ORGANIZATION, 2016; SARMIENTO-OSPINA; VÁSQUEZ-SERNA; JIMENEZ-CANIZALES; VILLAMIL-GÓMEZ *et al.*, 2016), incluindo casos de trombocitopenia grave e vaso-oclusão desencadeada pela inflamação.

O ZIKV demonstrou ter uma grande afinidade pelo tecido neuronal, sendo responsável por uma série de doenças neurológicas agudas (DO ROSARIO; DE JESUS; VASILAKIS; FARIAS *et al.*, 2016; MOORE; STAPLES; DOBYNS; PESSOA *et al.*, 2017; MUNOZ; BARRERAS; PARDO, 2016). Como outros flavivírus, o ZIKV tem um efeito neurotrófico, possuindo habilidades neuroinvasivas e neurovirulentas (AWADH; CHUGHTAI; DYDA; SHEIKH *et al.*, 2017). Modelos *in vitro* e com roedores apoiam a presença dessa característica no ZIKV, e ele tem como alvo células neuronais progenitoras, astrócitos e neurônios que se encontram em qualquer fase de desenvolvimento (SCHWARTZ, 2017).

4.3.3.1. Síndrome de Guillain Barré e outras alterações na população adulta

Complicações neurológicas foram relatadas durante a fase aguda da infecção pelo vírus Zika, mais frequentemente a síndrome de Guillain Barré, um amplo espectro de neuropatias autoimunes agudas (DO ROSARIO; DE JESUS; VASILAKIS; FARIAS *et al.*, 2016; MUNOZ; BARRERAS; PARDO, 2016). Durante os surtos na Polinésia Francesa, nas Ilhas Yap e na epidemia na América, estudos epidemiológicos estimaram que 2 casos de GBS relatados podem ocorrer a cada 10.000 infecções pelo vírus Zika (MIER-Y-TERAN-ROMERO; DELOREY; SEJVAR; JOHANSSON, 2018). Outras complicações neurológicas como encefalite, meningoencefalite, cerebelite, encefalomielite disseminada aguda, mielopatia inflamatória e distúrbios dos nervos cranianos também foram descritas com menor frequência (MUNOZ; BARRERAS; PARDO, 2016). Casos de encefalites e mielites foram associados ao ZIKV quando foi determinada a presença deste vírus no líquido cefalorraquídeo. Mas a pouca frequência destas alterações (encefalites e mielites) dificulta a confirmação da sua relação com o ZIKV (MUNOZ; BARRERAS; PARDO, 2016) e a maior parte da sua evidência são relatos de casos.

4.3.3.2. Alterações congênitas, a síndrome congênita de Zika (SCZ)

A Organização Mundial da Saúde (OMS) considera as anomalias congênitas como câmbios estruturais ou funcionais consequentes de fatores ocorridos durante a vida intrauterina e que podem ser detectados durante a gravidez, no parto ou mais tarde na vida (WHO, 2014). Em geral, as anomalias congênitas apresentam gravidade heterogênea. Algumas são geralmente detectadas durante os primeiros dias após o nascimento, como o caso da microcefalia (VAN DER LINDEN; PESSOA; DOBYNS; BARKOVICH *et al.*, 2016) e em muitas vezes, são detectadas antes do nascimento, no período pré-natal, como aquelas detectadas através da ultrassonografia (PEREIRA; NIELSEN-SAINES; SPERLING; MAYKIN *et al.*, 2018). Porém algumas outras alterações menos graves também precisam de um seguimento prolongado para serem diagnosticadas com as alterações funcionais, principalmente em crianças expostas ao ZIKV intrauterinamente mas sem microcefalia (AGUILAR TICONA; NERY; LADINES-LIM; GAMBRAH *et al.*, 2021; MULKEY; ARROYAVE-WESSEL; PEYTON; BULAS *et al.*, 2020; NIELSEN-SAINES, K.; BRASIL, P.; KERIN, T.; VASCONCELOS, Z. *et al.*, 2019)

O SCZ é um conjunto amplo de alterações descritas em crianças infectadas pelo vírus através da via materno-fetal(MOORE; STAPLES; DOBYNS; PESSOA *et al.*, 2017). Sua definição ainda não é clara devido ao fato de que inicialmente foram as alterações estruturais mais graves e óbvias durante o nascimento que definiram o nome (VAN DER LINDEN; PESSOA; DOBYNS; BARKOVICH *et al.*, 2016), porém, novas alterações, em sua maioria funcionais, foram e ainda estão sendo descritas durante o seguimento das crianças afetadas. Um exemplo dos problemas de definição pode ser observado no estudo de crianças expostas intrauterinamente sem microcefalia, mas que apresentam algum atraso em seu desenvolvimento. No estudo de Mulkey et al., que apresentou um grupo de crianças menores de 18 meses com resultados anormais no neurodesenvolvimento, que foram expostas ao ZIKV intrauterinamente, os pesquisadores consideraram essas crianças como "sem SCZ") mas com alterações na neuroimagem, as quais consideram como "suspeita de SCZ" (MULKEY; ARROYAVE-WESSEL; PEYTON; BULAS *et al.*, 2020). Da mesma forma, outro termo empregado para caracterizar essas crianças pode ser encontrado no estudo de van der Linden et al., que acompanhou 13 crianças normocefálicas também expostas ao ZIKV intrauterinamente, as quais identificou como "infecção congênita do ZIKV" (VAN DER LINDEN; PESSOA; DOBYNS; BARKOVICH *et al.*, 2016).

No presente trabalho, para facilitar a compreensão das alterações relacionadas ao SCZ, as dividiremos entre alterações no nascimento, que na sua maior parte são estruturais e óbvias, e em alterações no seguimento, que na sua maior parte são funcionais e em alguns casos chegam a ser sutis.

Alterações no nascimento: As características clínicas da SCZ são consequência de danos neurológicos diretos e perda grave de volume intracraniano (MOORE; STAPLES; DOBYNS; PESSOA *et al.*, 2017). Os estudos iniciais do efeito da exposição intrauterina do ZIKV centraram-se no achado de microcefalia congênita, que é a mais grave e mais estudada (SCHULER-FACCINI; RIBEIRO; FEITOSA; HOROVITZ *et al.*, 2016). Se registraram 2,8 casos de microcefalia a cada 100.000 nascimentos durante o ano de 2015 em Brasil (DE OLIVEIRA; CORTEZ-ESCALANTE; DE OLIVEIRA; CARMO *et al.*, 2016) e numa metanálise identificou-se que 2,7% dos bebês nascidos vivos de mulheres infectadas pelo ZIKV poderiam nascer com microcefalia (COELHO; CROVELLA, 2017), similar aos 2,3% de prevalência reportados no estudo de coorte de Porto Rico (ELLINGTON; DEVINE; BERTOLLI; MARTINEZ QUIÑONES *et al.*, 2016).

Junto à microcefalia, outras alterações estruturais foram relacionadas à transmissão congênita do ZIKV. Estas alterações que inicialmente definiram o SCZ, incluíam: deficiência auditiva (PELOGGIA; ALI; NANDA; BAHAMONDES, 2018), que pode estar associada com hipoplasia do nervo vestíbulo-coclear e diversas alterações no sistema nervoso central relacionadas à função auditiva (DE CARVALHO LEAL; RAMOS; CALDAS NETO, 2019). Alterações oftalmológicas, em sua maior parte relacionadas com alterações da retina como cicatrização macular, mancha macular de pigmento, a atrofia coriorretiniana, e alterações no nervo óptico (DE PAULA FREITAS; VENTURA; MAIA; BELFORT, 2017; MARQUEZAN; VENTURA; SHEFFIELD; GOLDEN *et al.*, 2018) bem como contraturas congênitas e hipertonia marcada com sintomas de afecção extrapiramidal que são distintas de outras condições congênitas, também foram associadas com a infecção congênita por ZIKV (MOORE; STAPLES; DOBYNS; PESSOA *et al.*, 2017). Além disso, outras complicações graves, como calcificações intracranianas, hidrocefalia e malformações cerebrais também foram relatadas em bebês infectados pelo ZIKV durante a gravidez (MOORE; STAPLES; DOBYNS; PESSOA *et al.*, 2017; RICE; GALANG; ROTH; ELLINGTON *et al.*, 2018).

Alterações no seguimento: O espectro de alterações induzidas pelo ZIKV ainda apresenta muitas lacunas de conhecimento. Dentre as principais alterações no seguimento, encontram-se a microcefalia após o nascimento, atraso no neurodesenvolvimento (AGUILAR TICONA; NERY; LADINES-LIM; GAMBRAH *et al.*, 2021; LOPES MOREIRA; NIELSEN-SAINES; BRASIL; KERIN *et al.*, 2018; RICE; GALANG; ROTH; ELLINGTON *et al.*, 2018), alterações no crescimento antropométrico (MOURA DA SILVA; GANZ; SOUSA; DORIQUI *et al.*, 2016), epilepsia (OLIVEIRA-FILHO; FELZEMBURGH; COSTA; NERY JR *et al.*, 2018) e problemas hormonais (VERAS GONÇALVES; MIRANDA-FILHO; ROCHA VILELA; RAMOS *et al.*, 2020).

A microcefalia pós-natal relacionada ao ZIKV foi identificada pela primeira vez em Pernambuco. Treze lactantes com evidência laboratorial de infecção congênita pelo vírus Zika e com perímetro cefálico normal no momento do nascimento apresentaram diminuição do perímetro cefálico no seguimento durante os primeiros meses de vida. Isso pode estar relacionado à presença de danos neurológicos causados pelo ZIKV mesmo após o nascimento (ARAGAO; HOLANDA; BRAINER-LIMA; PETRIBU *et al.*, 2017; MELO; AGUIAR; AMORIM; ARRUDA *et al.*, 2016). Outras alterações relacionadas a essas

crianças são encontradas nas imagens de tomografias avaliadas, que apresentam alterações como ventriculomegalia e volume cerebral diminuído, polimicrogiria, paquigiria e calcificações subcorticais (ARAGAO; HOLANDA; BRAINER-LIMA; PETRIBU *et al.*, 2017; VAN DER LINDEN; PESSOA; DOBYNS; BARKOVICH *et al.*, 2016). No entanto, essas alterações ocorrem com menor frequência quando comparadas com crianças com microcefalia no nascimento, associadas ao ZIKV (ARAGAO; HOLANDA; BRAINER-LIMA; PETRIBU *et al.*, 2017).

Um dos desfechos da infecção congênita pelo ZIKV que requer acompanhamento cuidadoso é o neurodesenvolvimento das crianças. Os estudos que seguiram as crianças com microcefalia associada ao ZIKV identificaram que a maioria delas apresenta um grave atraso em todas as dimensões avaliadas, principalmente na cognição, linguagem e motricidade (ALVES; PAREDES; SILVA; MELLO *et al.*, 2018; CARVALHO; BRITES; MOCHIDA; VENTURA *et al.*, 2019; CARVALHO; VENTURA; TAGUCHI; BRANDI *et al.*, 2020). Estima-se que, aos 19 meses de idade, o desenvolvimento dessas crianças era equivalente ao de crianças de 2 meses (ALVES; PAREDES; SILVA; MELLO *et al.*, 2018), e espera-se que o atraso seja ainda mais evidente à medida que as crianças crescem. Embora os estudos iniciais tenham encontrado um maior atraso no desenvolvimento neurológico (ALVES; PAREDES; SILVA; MELLO *et al.*, 2018; CARVALHO; BRITES; MOCHIDA; VENTURA *et al.*, 2019; CARVALHO; VENTURA; TAGUCHI; BRANDI *et al.*, 2020; RICE; GALANG; ROTH; ELLINGTON *et al.*, 2018), outros estudos identificaram casos que se aproximam do neurodesenvolvimento normal (CARVALHO; VENTURA; TAGUCHI; BRANDI *et al.*, 2020; DE CARVALHO; BRITES; TAGUCHI; PINHO *et al.*, 2018). Esses achados heterogêneos sugerem uma variação nos resultados de desenvolvimento de crianças expostas ao ZIKV no útero. Dado que o neurodesenvolvimento é um processo dinâmico, é importante acompanhar essas crianças após os dois anos de idade para saber se essa heterogeneidade persiste ao longo do tempo.

Sobre as alterações associadas ao desenvolvimento das crianças expostas ao vírus Zika e sem microcefalia, essas são mais sutis. Essas alterações em crianças aparentemente saudáveis representam um desafio para o diagnóstico e o acompanhamento, especialmente porque a maioria (60%) das infectadas pelo vírus Zika foram assintomáticas. Consequentemente, alguns estudos de coorte selecionaram apenas casos sintomáticos ou casos positivos (EINSPIELER; UTSCH; BRASIL; PANVEQUIO AIZAWA *et al.*, 2019;

LOPES MOREIRA; NIELSEN-SAINES; BRASIL; KERIN *et al.*, 2018; NIELSEN-SAINES, K.; BRASIL, P.; KERIN, T.; VASCONCELOS, Z. *et al.*, 2019), omo ocorreu no estudo de coorte no Rio de Janeiro que incluiu 146 crianças, onde foi identificado que 28% delas apresentaram um atraso leve no neurodesenvolvimento(NIELSEN-SAINES, K.; BRASIL, P.; KERIN, T.; VASCONCELOS, Z. *et al.*, 2019). No entanto, o seguimento de coorte no Rio de Janeiro incluiu apenas crianças com exposição ao vírus Zika no útero, sem um grupo controle, o que limitou seus achados. Em contraste, um estudo de corte transversal em Guadalupe, Martinica e na Guiana Francesa registrou que 15% das crianças expostas ao vírus Zika intrauterinamente sem microcefalia apresentavam atraso no neurodesenvolvimento, mas também foi registrado que 25% das crianças não expostas apresentavam um grande atraso (GRANT; FLÉCHELLES; TRESSIÈRES; DIALO *et al.*, 2021). Esses achados parecem ser ainda mais sutis, como apresentado nos recentes estudos longitudinais na Colômbia e nos EUA. Embora não tenham encontrado atraso no desenvolvimento, os pesquisadores apresentaram a tendência decrescente do neurodesenvolvimento em crianças expostas ao vírus Zika. É importante ressaltar que as crianças avaliadas nessa pesquisa foram acompanhadas em um ou dois pontos no tempo, aos 4-8 meses e 9-18 meses; durante esse período, a maioria das avaliações são passivas em respeito às crianças (MULKEY; ARROYAVE-WESSEL; PEYTON; BULAS *et al.*, 2020). Diferentes resultados foram encontrados em crianças de 11 a 32 meses de idade, no coorte feito dentro de uma comunidade urbana em Salvador, Brasil, onde foram realizadas avaliações em dois estágios: uma triagem e, posteriormente, uma segunda avaliação em crianças que não passaram pela triagem com um exame mais completo. Os resultados do estudo de coorte em Salvador mostram um atraso cognitivo em 31% das crianças expostas intrauterinamente, sendo que 53% dessas alterações podem ser atribuídas ao vírus Zika quando comparadas a um grupo controle não exposto da mesma comunidade (AGUILAR TICONA; NERY; LADINES-LIM; GAMBRAH *et al.*, 2021).

Além do grande atraso em crianças com microcefalia associada ao vírus Zika, outras alterações entre crianças com vírus Zika foram a epilepsia, alterações hormonais e problemas no crescimento antropométrico. A epilepsia tem mostrado relação com baixas habilidades cognitivas, existindo uma relação inversamente proporcional entre as duas, e que tem sido identificada em 43% das crianças com microcefalia (ABDEL-SALAM; HALASZ; CZEIZEL, 2000; JAYATILAKE; OYEGUNLE; WAECHTER; LANDON *et al.*, 2020; OLIVEIRA-FILHO; FELZEMBURGH; COSTA; NERY JR *et al.*, 2018; VON DER

HAGEN; PIVARCSI; LIEBE; VON BERNUTH *et al.*, 2014). Alterações hormonais também podem ser observadas em crianças com microcefalia e têm relação com alterações cerebrais da linha média, como agenesia ou hipoplasia do corpo caloso, e também com alterações do nervo óptico (FERREIRA;; AGUILAR;; SILVEIRA-MATTOS;; ARRIAGA; *et al.*, 2021).

5. LACUNA NO CONHECIMENTO

5.1. Transmissão do vírus Zika e fatores de risco

Embora o papel das determinantes sociais e ambientais já tenha sido descrito em doenças como dengue ou febre amarela (DE THOISY; SILVA; SACCHETTO; DE SOUZA TRINDADE *et al.*, 2020; DO CARMO; SILVA JÚNIOR; PASTOR; DE SOUZA, 2020), ainda não está claro o papel dessas determinantes na alta taxa de ataque experimentada durante a epidemia de Zika em 2015 e sua particular distribuição espacial no território brasileiro. Essa falta de informações destaca a necessidade de explorar como características individuais, familiares e ambientais podem estar associadas à soropositividade para o vírus Zika, especialmente em populações vulneráveis, como as comunidades urbanas informais, que têm registrado uma maior carga de doenças infecciosas, como leptospirose (FELZEMBURGH; RIBEIRO; COSTA; REIS *et al.*, 2014; REIS; RIBEIRO; FELZEMBURGH; SANTANA *et al.*, 2008), DENV (KIKUTI; CUNHA; PAPLOSKI; KASPER *et al.*, 2015) e COVID-19 (FOFANA; NERY; AGUILAR TICONA; DE ANDRADE BELITARDO *et al.*, 2022), associadas ao seu baixo estatuto socioeconômico. Compreender esses aspectos pode fornecer informações valiosas sobre como fatores socioeconômicos e ambientais podem influenciar a exposição e transmissão de doenças infecciosas, incluindo o Zika vírus, e essas informações podem ser usadas para informar políticas de saúde pública.

Além disso, em áreas com transmissão contínua disseminada por mosquitos, é extremamente desafiador avaliar a contribuição da transmissão sexual para todas as infecções pelo vírus da Zika. Não se sabe como o risco de transmissão sexual de homens infectados se compara ao risco de uma picada de mosquito infectado, nem se a infecção através do contato sexual apresenta algum risco diferente para lesões fetais em comparação com a infecção por vetores. Estudos que investiguem diferenças nas assinaturas do RNA viral ou nas respostas imunológicas secretoras vaginais de mulheres infectadas por picadas de mosquito versus infecções por transmissão sexual podem ser úteis para caracterizar melhor a patogênese subsequente de rotas alternativas de infecção.

5.2. Avaliação do desenvolvimento infantil e da SCZ

A avaliação do desenvolvimento infantil no contexto da epidemia do ZIKV apresenta desafios importantes. Primeiro, há a necessidade de um adequado grupo de

controle (GRANT; FLÉCHELLES; TRESSIÈRES; DIALO *et al.*, 2021; VOUGA; POMAR; PANCHAUD; MUSSO *et al.*, 2019), no estudo de coorte realizado no Rio de Janeiro, por exemplo, que seguiu mães sintomáticas durante a gravidez e suas crianças, foi reportada uma prevalência de 35% de atraso na linguagem (NIELSEN-SAINES, K.; BRASIL, P.; KERIN, T.; VASCONCELOS, Z. *et al.*, 2019). Entretanto, sem um grupo de controle, não é possível analisar em que medida os mesmos resultados são encontrados em crianças não expostas ao ZIKV intrauterinamente. Caso as proporções de atraso do neurodesenvolvimento sejam similares entre a população geral e as crianças expostas, como mostram os resultados obtidos da coorte de Guadeloupe, Martinique, and French Guiana (GRANT; FLÉCHELLES; TRESSIÈRES; DIALO *et al.*, 2021), será necessário investigar outros fatores como determinantes deste atraso nesta população.

Segundo, é necessário um acompanhamento adequado do desenvolvimento infantil. Estudos longitudinais são necessários para avaliar o progresso do neurodesenvolvimento (MULKEY; DEBIASI, 2020). Uma medida única e a falta de evidência em estudos transversais não podem ser conclusivas quanto ao atraso ou ao desenvolvimento adequado. Como exemplo desta premissa, temos o estudo de Mulkey et al., onde identificou-se que as crianças sem microcefalia expostas intrauterinamente ao ZIKV não apresentam alterações no neurodesenvolvimento (MULKEY; ARROYAVE-WESSEL; PEYTON; BULAS *et al.*, 2020). Mas, aplicando uma análise longitudinal empregando modelos mistos, conseguiram avaliar as trajetórias de desenvolvimento das crianças. Infelizmente, muitas crianças expostas ao ZIKV aparentemente normais em seu nascimento não estão sendo acompanhadas longitudinalmente (MULLER; MULKEY, 2019).

Terceiro, é importante avaliar o contexto das crianças. A maior parte das pesquisas coloca o ZIKV como a causa necessária para o atraso no desenvolvimento, existindo pouca informação sobre fatores de risco que podem aumentar ou atenuar a probabilidade do atraso acontecer (MULKEY; ARROYAVE-WESSEL; PEYTON; BULAS *et al.*, 2020). Como exemplo desta premissa, temos o estudo de Emerson et al., que avaliou a contribuição de vários fatores no desenvolvimento de crianças menores de 5 anos de idade, incluindo fatores sociais como a renda e educação da mãe, fatores do contexto como acesso a água e saneamento, e os cuidados da família como a estimulação precoce. Eles obtiveram uma prevalência de atraso cognitivo de 10,1% das crianças, atribuindo de 2,5% a 42% aos fatores mencionados (EMERSON; SAVAGE; LLEWELLYN, 2018).

6. MODELO TEÓRICO

No modelo teórico do presente trabalho serão considerado as determinantes da saúde (FREITAS; SOARES; MOCELIN; LACERDA *et al.*, 2019) e o modelo bioecológico de Bronfenbrenner (SWICK; WILLIAMS, 2006) para entender a transmissão do ZIKV e o desenvolvimento infantil relacionados ao contexto dos participantes.

6.1. Determinantes sociais da saúde

A determinação social do estado de saúde foi estabelecida desde o início do século XIX, quando William P. Alison descreveu em 1820 a existência de uma estreita associação entre pobreza e doença e propôs a necessidade urgente de enfrentar as causas da miséria para evitar as epidemias da época. Um exemplo da importância dos determinantes sociais pode ser observado na disseminação heterogênea da epidemia de ZIKV no Brasil. Embora a doença tenha sido registrada na maioria dos estados brasileiros, o epicentro da doença foi na região Nordeste, onde também foram registradas as maiores taxas de anomalias congênitas (BRADY; OSGOOD-ZIMMERMAN; KASSEBAUM; RAY *et al.*, 2019; RODRIGUEZ-BARRAQUER; COSTA; NASCIMENTO; NERY *et al.*, 2019). Isto pode ser explicado a partir do papel de fatores sociais e ambientais que variam entre as regiões (ALI; GUGLIEMINI; HARBER; HARRISON *et al.*, 2017; BUTLER, 2016). Mas este não é um modelo único para ZIKV. Analogamente ele também vem se apresentando como explicação para outros arbovirus. Um deles é o DENV, um flavivírus, intimamente relacionado ao ZIKV, e para o qual estudos anteriores sugerem que determinantes sociais, como pobreza, etnia, moradia precária, falta de acesso ao saneamento básico e água potável explicam o impacto e distribuição da doença (HOTEZ; MURRAY; BUEKENS, 2014; MCMICHAEL, 2004).

Em relação ao desenvolvimento infantil, pode-se observar que as determinantes sociais da saúde foram pouco estudadas na literatura revisada. Isso pode ser justificado pelo fato de que as crianças foram avaliadas precocemente para minimizar o efeito do contexto no seu desenvolvimento. No entanto, se o objetivo for avaliar o desenvolvimento infantil a longo prazo, o contexto se tornará uma variável determinante na população em geral. Um estudo prévio com crianças em idade pré-escolar de países de baixa e média renda identificou que o atraso cognitivo pode ser atribuído à pobreza familiar, educação materna, acesso à

água potável e saneamento. A melhoria dessas determinantes sociais poderia levar a uma redução de 60% no atraso cognitivo (EMERSON; SAVAGE; LLEWELLYN, 2018).

A saúde pode ser analisada como um fenômeno complexo determinado pela interação entre vários fatores. Os determinantes sociais da saúde são as circunstâncias nas quais as pessoas nascem, crescem, vivem, trabalham e envelhecem, incluindo o sistema de saúde. Essas circunstâncias são o resultado da distribuição de dinheiro, poder e recursos nos níveis global, nacional e local, que por sua vez dependem das políticas adotadas.

6.2. Teoria Ecológica de Bronfenbrenner

A Teoria Ecológica de Bronfenbrenner propõe que o desenvolvimento dos indivíduos seja mediado pelos diferentes contextos nos quais eles crescem e se movem, influenciando consequentemente no seu desenvolvimento cognitivo, moral e relacional. Nesse sentido, o desenvolvimento infantil é moldado pelos diversos ambientes nos quais as crianças estão inseridas e pelas inter-relações entre eles. Segundo a teoria de Bronfenbrenner, os seres humanos não podem se desenvolver isoladamente (esse pensamento era uma crítica às análises comportamentais experimentais em laboratórios), mas dentro de um sistema de relacionamentos que incluem a família, a sociedade e o ambiente (DE PAULA FREITAS; VENTURA; MAIA; BELFORT, 2017; SWICK; WILLIAMS, 2006).

Uma forma de simplificar o modelo bioecológico para o entendimento funcional é através de uma estrutura que inclui quatro dimensões importantes: Processo, Pessoa, Contexto e Tempo (PPCT) (BENETTI; VIEIRA; CREPALDI; SCHNEIDER, 2013). Nesse sentido: a) O Processo refere-se à interação entre a criança e as pessoas, objeto e símbolos. Como exemplo, podemos ter ações como brincar com crianças, atividades entre crianças ou aprender novas habilidades como início do reconhecimento da criança em seu contexto, para posteriormente transformá-lo (BENETTI; VIEIRA; CREPALDI; SCHNEIDER, 2013); b) A Pessoa, que tem relação com a parte genética e biológica. Como exemplo, temos as variáveis de nível individual, como idade, sexo, deficiência, doença, etc. que podem ser vinculadas ao desenvolvimento, sendo ditos como recursos “passivos” com os quais as crianças interagem (BENETTI; VIEIRA; CREPALDI; SCHNEIDER, 2013);) O Contexto, que talvez seja o mais importante de todos os quatro componentes pela possibilidade de conceituar e projetar estudos sobre o desenvolvimento infantil. O contexto se refere aos

vários locais próximos ou distantes que têm a capacidade de modificar os processos, e eles incluem ambientes nos quais a criança está em constante interação, seja ela física ou socioeconômica. Por exemplo, quanto menos filhos um cuidador tiver, melhor ele será capaz de fornecer cuidados de qualidade, o que influencia no desenvolvimento positivo (BENETTI; VIEIRA; CREPALDI; SCHNEIDER, 2013); e d) O Tempo, que abrange os aspectos anteriores, adicionando-os como fatores de mudança. Um evento tem vários graus de impacto sobre o desenvolvimento, mas o impacto pode diminuir com o passar do tempo. Isso dependerá de um equilíbrio entre estabilidade e mudanças que podem ser impostas por condições externas ou partir da pessoa. Como exemplo, podemos mencionar uma doença debilitante dos pais, divórcio ou mudança de residência, que podem ter um impacto mais profundo nas crianças pequenas quando comparadas com as mais velhas (BENETTI; VIEIRA; CREPALDI; SCHNEIDER, 2013; MARTINS; SZYMANSKI, 2004).

No que diz respeito ao ZIKV, pouco se estudou a relação do contexto com o desenvolvimento das crianças afetadas intrauterinamente. Partindo da compreensão de que essas repercussões ocupacionais impactam e são impactadas pelos contextos nos quais a criança encontra-se inserida, compreende-se como necessária uma avaliação que contemple a complexidade da relação criança-ambiente, entendendo-se o ambiente para além de sua infraestrutura físicas, mas como o conjunto de relações e processos através dos quais a criança se desenvolve.

6.3. Modelo do projeto

Com base na revisão da literatura, nos conceitos sobre as determinantes sociais da saúde e sobre a teoria ecológica de Bronfenbrenner, o modelo proposto a seguir leva em consideração fatores individuais, sociais e ambientais.

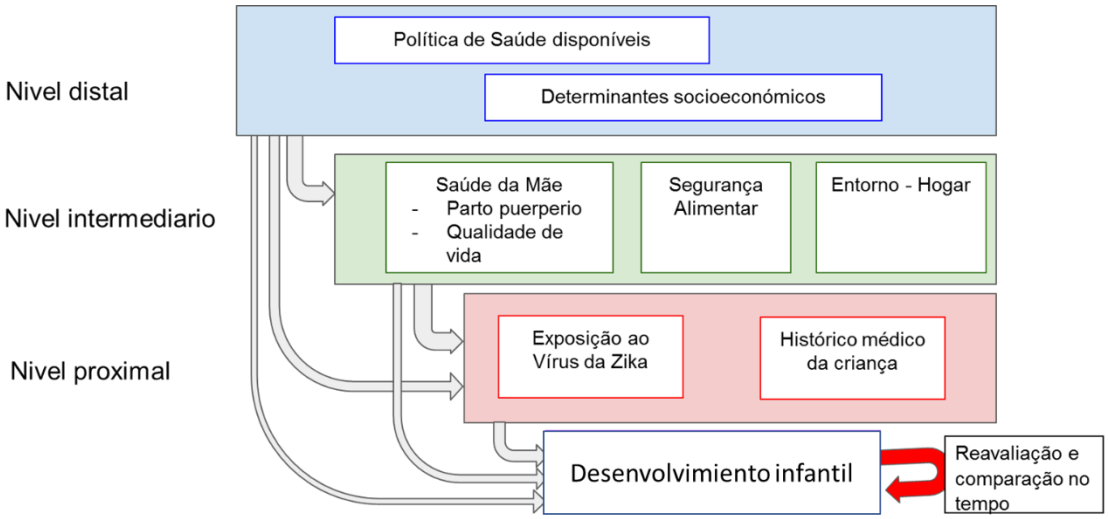

Figura 1. Modelo teórico do projeto.

7. OBJETIVOS

7.1. Objetivo geral

- Avaliar a transmissão do vírus Zika em comunidades urbanas vulneráveis e os efeitos clínicos e de desenvolvimento em crianças nascidas durante a epidemia em Salvador, Brasil.

7.2. Objetivos específicos

- **Objetivo 1.** Avaliar determinantes sociais e ambientais da transmissão vetorial por ZIKV em uma coorte de residentes de uma comunidade urbana informal. (Artigo 1)
- **Objetivo 2.** Caracterizar os comportamentos sexuais associados ao aumento do risco de infecção pelo ZIKV. (Artigo 2)
- **Objetivo 3.** Caracterizar o perfil das manifestações clínicas e do desenvolvimento aos 2 e 3 anos de vida de crianças com e sem microcefalia associada ao SCZ e determinar os fatores de riscos associados a atraso de cognição, comunicação/linguagem e motricidade. (Artigos 3 e 4)

8. MÉTODOS

Os métodos empregados para este trabalho são descritos em cada um dos artigos apresentados como resultados. De forma breve, descrevo a seguir os aspectos gerais relacionados à coorte de Pau da Lima e à coorte do Hospital Roberto Santos.

8.1. Coorte de Pau da Lima (Artigo 1 e 2)

O objetivo 1 e 2 deste trabalho está relacionado à transmissão do vírus Zika e aos fatores de risco associados. Para responder a este objetivo, realizamos a análise de uma coorte de participantes na comunidade de Pau da Lima. Desde 2003, esta coorte aberta investiga doenças de transmissão infecciosa, como leptospirose e infecções por arbovírus. O acompanhamento nesta coorte é realizado através de inquéritos semestrais, nos quais são aplicados questionários estruturados e coletadas amostras de sangue. Esta comunidade é um assentamento informal urbano em Salvador, caracterizado por três vales que cobrem uma área de 0,17 km2. Cerca de 85% dos habitantes moram em casas sem título de propriedade, e 50% têm uma renda domiciliar per capita de menos de US$ 1,25 por dia.

Os critérios de inclusão para os participantes desta coorte foram: morar em um domicílio dentro da área de estudo, dormir ≥3 noites por semana em uma residência selecionada para o estudo e ter ≥5 anos de idade. Moradores que se recusaram a participar foram excluídos.

A análise foi realizada empregando os soroinquéritos da coorte entre março e dezembro de 2015, período antes e após o pico da epidemia de Zika. Os fatores de risco associados ao vírus foram avaliados, incluindo fatores sociodemográficos e econômicos, ambientais, espaciais e comportamentos sexuais de risco. A exposição ao vírus Zika foi identificada por meio de um exame que avalia as respostas da imunoglobulina G3 (IgG3) à proteína NS1 do vírus (anti-ZIKV NS1 IgG3). A sensibilidade e especificidade deste teste foram comparadas ao teste de neutralização de placas (PRNT).

8.2. Coorte do Hospital Roberto Santos (Artigo 3 e 4)

O objetivo 3 foi realizado em uma coorte de mulheres e seus filhos no Hospital Geral Roberto Santos (HGRS). O HGRS é um hospital público localizado em Salvador, Bahia, que possui 640 leitos e atende a cidade de Salvador e outras cidades do estado da

Bahia. Além disso, o hospital possui um centro obstétrico que é responsável por 140 partos por mês e é referência para gestações de alto risco. Para o estudo, foram selecionadas mulheres grávidas e seus filhos que nasceram no centro obstétrico HGRS no período de 01 de outubro de 2015 até 31 de janeiro de 2016 e que residiam em Salvador, Brasil. Crianças com microcefalia associada ao Zika vírus, que foram acompanhadas no ambulatório de microcefalia do HGRS, também foram incluídas na análise dos desfechos clínicos. Crianças que não nasceram no HGRS ou que residiam na área metropolitana, mas que tiveram seu seguimento no HGRS, também foram incluídas na análise.

A infecção pelo vírus Zika foi determinada com base no Teste de Neutralização por Redução de Placa (PRNT), realizado nas amostras de soro coletadas no momento do nascimento e durante o acompanhamento das crianças aos 2 e 3 anos de idade. As amostras de soro foram consideradas positivas para o vírus Zika quando os títulos de anticorpos foram ≥ 20 para o ZIKV.

Os desfechos foram definidos em duas categorias: medidas antropométricas e avaliação neurológica e do neurodesenvolvimento. As medidas antropométricas registradas foram peso, comprimento e perímetro cefálico em dois momentos: no nascimento e no momento da visita domiciliar, aproximadamente aos 2 e 3 anos de vida. Os valores de referência das tabelas de crescimento do INTERGROWTH-21 e da OMS foram utilizados para a análise dessas medidas. Para a avaliação neurológica, foi utilizado o exame neurológico infantil Hammersmith (HINE), e a avaliação do neurodesenvolvimento foi realizada utilizando as escalas Bayley de desenvolvimento do bebê e da criança pequena, terceira edição (BAYLEY III). O HINE é composto por três seções que avaliam a função, postura, movimentos, tônus e reflexos dos nervos cranianos, a função motora por idade em relação aos marcos padrões no desenvolvimento infantil e o comportamento da criança em relação à consciência ou estado de alerta, estado emocional e orientação social. As escalas Bayley avaliam o desenvolvimento cognitivo, motor e socioemocional da criança.

9. ASPECTOS ÉTICOS

Coorte de Pau da Lima

Este estudo foi revisado e aprovado pelos Comitês de Revisão Institucionais da Universidade de Yale (1006006956) e da Fundação Oswaldo Cruz e do Ministério da Saúde do Brasil (45217415.4.0000.0040 e 55904616.4.0000.0040). Um consentimento informado por escrito foi obtido de todos os participantes antes da inclusão.

Coorte do Hospital Roberto Santos

O projeto foi aprovado pelo Comitê de Ética em Pesquisa em Seres Humanos do Hospital Geral Roberto Santos, através do Certificado de Apresentação para Apreciação Ética (CAAE) de número 5344216.1.1001.5028. Todas as mulheres que eram elegíveis e participaram do estudo leram e assinaram o Termo de Consentimento Livre e Esclarecido (TCLE) antes da realização das coletas de dados clínicos e amostras biológicas.

10. RESULTADOS

10.1. Artigo 1

Socio-economic, environmental and spatial factors associated with ZIKA virus transmission in an urban informal settlement located in Salvador, Brazil

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Abstract

Objective: To characterize the socio-economic, environmental, and spatial factors that contribute to the spread of the Zika virus (ZIKV) in an urban informal community.

Methods: We conducted a nested cross-sectional analysis in a cohort of 1,452 residents in Salvador, Brazil. We analyzed a serosurvey that was conducted from August to November 2015. Socioeconomic, family, and household environmental characteristics were collected through questionnaires from both the head of the family and individuals. ZIKV infection was determined using a validated IgG3 diagnostic test. We created a socioeconomic status index that included non-productive assets and sanitation characteristics using Principal Component Analysis. Then, we used an INLA model with random effects at the level of the participants' households to identify risk factors associated with ZIKV seroprevalence.

Results: Among 1,351 residents, we identified a ZIKV seroprevalence of 63.2%. In the bivariate analysis, we found that individual, family, and environmental factors were associated with a positive ZIKV IgG3 result. However, in the multivariable analysis including the spatial distribution, we identified that informal employment (aOR 1.50, 95% CI 1.05–2.14), food insecurity (aOR 1.35, 95% CI 1.04–1.75), and low socioeconomic status (aOR 0.87, 95% CI 0.77–0.98) were associated with a higher chance of having a positive IgG3 result. None of the environmental factors were associated in the final model with ZIKV seroprevalence.

Conclusions: Socioeconomic differences among individuals and families in slum communities are linked to the spread of ZIKV. Along with vector control measures, public health policies aimed at curbing ZIKV transmission should prioritize enhancing the living conditions in these communities and minimizing social inequalities.

Introduction

The Zika virus (ZKV) is a member of the Flaviviridae family and the Flavivirus genus that is primarily transmitted by mosquito bites [1, 2]. While the majority of cases result in mild, self-limiting illness, less than 20% of symptomatic cases exhibit mild symptoms such as fever, rash, headache, joint pain, red eyes, and muscle pain [2, 3]. For many years, ZIKV went unnoticed or was only sporadically reported with mild symptoms [4]. However, during the 2015 Brazilian ZIKV epidemic, researchers found that congenital transmission is associated with birth defects and developmental impairments [4, 5].

The transmission dynamics of ZIKV remain uncertain, particularly its ability to spread in immunologically naïve populations. In 2015, Brazil became the epicenter of the ZIKV epidemic with infection rates reaching 60%. [5, 6] The first laboratory-confirmed cases of ZIKV infection in Brazil were reported in April 2015 [6, 7], but the spread of ZIKV was not homogenous in all the Brazilian territories and the disease burden was highest in the northeast region [8]. This scenario is even more unclear in urban informal settlements where the burden of vector-borne diseases is higher due to poor living conditions, inadequate infrastructure and sanitation and low socioeconomic status.[9]. In Brazil, urban informal conditions accounted for 26.4% of the population in 2010, according to the United Nations [10].

The objective of this study was to characterize socio-economic, environmental, and spatial factors contribute to the spread of Zika virus (ZIKV) in an urban informal community during the 2015-2016 epidemic in Brazil. Socioeconomic and environmental factors have been found to play a significant role in the transmission of other arboviruses such as DENV and CHIKV in urban informal settlements in Brazil [11-13]. However, the ZIKV epidemic in Brazil due the high and rapid attack rate [14] differs from the endemic

situation of DENV and the high attack rate of CHIKV infection. Therefore, it is important to understand the specific factors contributing to ZIKV transmission to develop targeted public health interventions that can reduce the risk of ZIKV transmission in vulnerable communities.

Methods

Study design and setting

We conducted a serosurvey in Pau da Lima an urban informal settlement in Salvador, Northeast Brazil which was the epicenter of the 2015 Zika epidemic. This settlement spans an area of 0.17 km2 and is characterized by three valleys [15, 16]. Approximately 85% of the inhabitants are squatters who do not have legal ownership of their homes. Furthermore, half of the population has a median per capita household income of less than $1.25 per day [15]. This study is part of an ongoing open cohort study that was initiated in 2003 to investigate infectious diseases, such as leptospirosis[16] and arbovirus infections,[14] with bi-annual or annual follow-up. In October 2015, Pau da Lima reported a peak of ZIKV infection, with an attack rate of 70%.[17].

Participants

The study team conducted a census of the residents in the community of Pau da Lima by canvassing households in the study area. They recorded the number, age, and gender of the residents. Eligible individuals who were aged 2 years or older and slept at least 3 nights per week within the study area were invited to participate in household family/environmental and serological surveys. Participation required written informed consent, with parental consent being needed for minors under 18 years of age.

Zika virus exposure

Zika virus exposure was identified using an assay that measures the immunoglobulin G3 (IgG3) responses to the ZIKV NS1 protein (anti-ZIKV NS1 IgG3). When compared with plaque-reduction neutralization tests (PRNT), the sensitivity and specificity of the IgG3 assay were estimated to be 85% and 97%, respectively [17].

Risk factors

Individual factors

During the interview, participants provided their sociodemographic information, including age, sex, self-defined ethnicity, education, occupational status, and daily per capita household income. We stratified education into three categories (illiteracy, ≤ 6 years, and > 6 years) and compared the lower category with the other two categories. Occupational status was categorized into three groups: formal employment, which includes participants with a job that provides social benefits and a regular income; informal job, which includes those without social benefits and a regular income; and unemployed.

Family and environmental factors

We interviewed the head of each household to gather information about their family and home environment. To assess food insecurity, we used a two-item screening tool. Additionally, we recorded whether the household was owned or rented and whether they experienced any flooding issues during the rainy season. We also evaluated whether the backyard was covered with mud, bushes, or solid ground. We collected data on non-productive assets, such as televisions, DVD players, refrigerators, cars, motorcycles, freezers, washing machines, laptops, radios, and microwaves, to create a socioeconomic index.

The methods used to measure these environmental factors were previously described in the study by Maw Eyer et al. [18]. Briefly we obtained elevation data related to the bottom of the valley and X/Y coordinates for each household. Using a 2014 sewer system map and field observations, we estimated the distance to the nearest open sewer, including the main sewer at the bottom of the valley. We also measured the distance to the nearest trash accumulation point. To evaluate the surrounding environment of each household, we utilized a combination of satellite and aerial images to classify the land cover into three categories: vegetation, soil, and impervious land. Furthermore, we calculated the Topographic Wetness Index (TWI) based on elevation and coordinate data to assess flood risk. Higher TWI values indicate an increased potential for flooding due to flat terrain or the convergence of longer sloping areas.

Statistical analysis

Descriptive statistics

To summarize our results, we utilized descriptive statistics. Frequencies and percentages were used to characterize categorical factors, while means and standard deviations (SD) were used to characterize quantitative factors. Participants were grouped based on their ZIKV exposure status. To compare the risk factors between the groups, we employed the chi-squared test or Fisher's exact test for categorical variables and the t-test or Mann-Whitney U test for continuous variables.

Socioeconomic status index

Using Principal Component Analysis (PCA), we constructed a socioeconomic status (SES) index based on the work of Vyas S. et al [19] and the World Food Program guidelines [20]. PCA is a data reduction technique that uses uncorrelated principal

components to explain the variance and unobserved characteristics of the population, resulting in new meaningful variables and a reduced dataset dimensionality. We identified a set of indicators that reflect the SES of households or individuals, including income, education level, sanitation, and ownership of assets. We standardized the data by subtracting the mean from each indicator and dividing by the standard deviation to ensure each indicator was on the same scale and had the same weight in the analysis. We used the first principal components as the SES index, which explained a variance of at least 95%. We calculated the SES index score for each individual by multiplying the standardized values of each indicator by the corresponding loading coefficient from the PCA analysis and summing the results. A Kaiser-Maier-Olkin (KMO) was used to confirm the necessity of this analysis, a value of 0. was defined as the minimum acceptable value. Job status and food insecurity were excluded from the PCA analysis due to their important role in transmission, as demonstrated previously in the work of Nivison Nery et al [21] and Isabel Rodriguez-Barraquer et al. [14].

Spatial multivariable analysis

An QGIS version 3.4 software system database was constructed with georeferenced aerial photographs and topographic maps provided by the Company for Urban Development of the State of Bahia (CONDER). The study team identified households within the study site and marked their positions onto hard copy 1:1,500 scale maps which were then entered into the database.

We fitted a spatial model using Integrated Nested Laplace Approximation (INLA) and Stochastic Partial Differential Equation (SPDE) approach. For that we utilized the PrevMap package [22] to generate a semivariogram to assess the spatial dependence in the data. Next, we employed the SPDE approach to capture the spatial variability and

allow for flexibility in modeling the spatial correlation structure by constructing a mesh of stochastic finite elements. Finally, we created a scatter plot with points representing the study participants in the study area and superimposed it onto the diagram.

Variables with a potential association with the ZIKV exposure ($p \leq 0.10$) were considered in the initial model. Manual backward and forward selection methods were used to identify factors associated with the exposure considering DIC value for INLA. In this model a household level was considerate a random effect. We measured associations between ZIKV exposure and study factors by Odds Ratio (OR) with a 95% confidence interval (95% CI). Data information will be analyzed by using the statistical software R studio version 3.6.2.

Ethics considerations

This study was reviewed and approved by the Institutional Review Boards of Yale University (1006006956) and Oswaldo Cruz Foundation and Brazilian Ministry of Health (45217415.4.0000.0040 and 55904616.4.0000.0040). A written informed consent was obtained from all participants prior to the inclusion.

Results

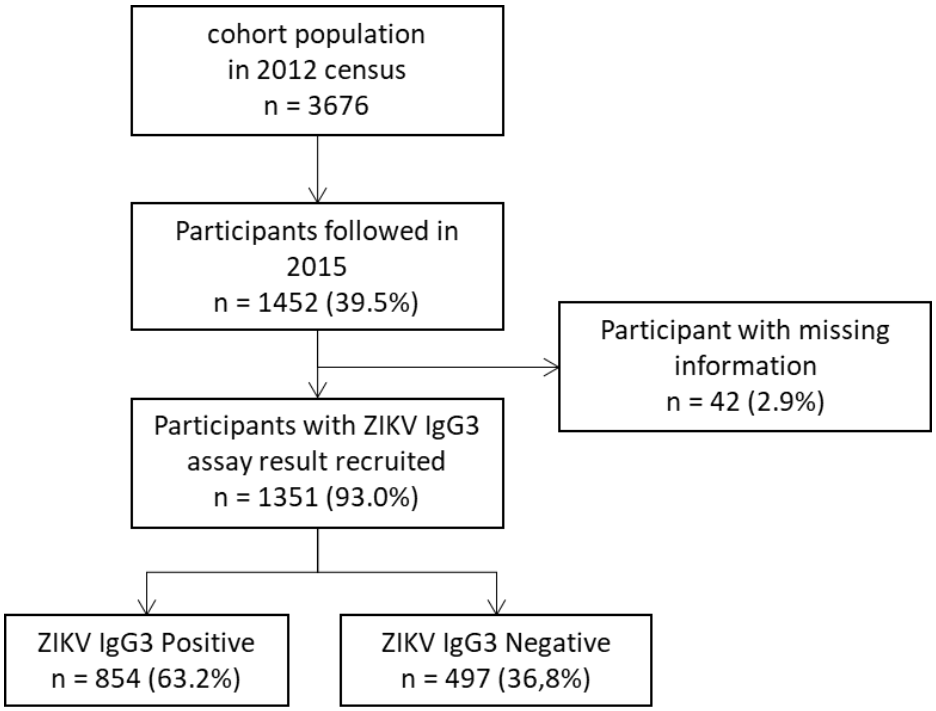

Fig 1. Study flowchart

In 2015, a total of 1452 residents were recruited from the study area, of which 1351 (93.0%) residents from 655 households were included in the study. Among these residents, 854 (63.2%) tested positive for ZIKV by IgG3 (Fig 1). The median age of the residents was 28 years (IQR 16 – 43), with 572 (42%) being male. Of the residents, 572 (42%) self-identified as black, 691 (51%) had at least 6 years of schooling, and only 248 (18%) reported having a formal job (Table 1).

Table 1. Evaluation of individual participant characteristics associated with IgG3 ZIKV results.

Characteristics	Cohort individuals	ZIKV IgG3 seropositive	ZIKV IgG3 seronegative	p value
	n (%) or median (IQR) N = 1351	n (%) or median (IQR) n = 854	n (%) or median (IQR) n = 497	
Age (median [IQR])	28 (16, 43)	29 (16, 43)	27 (16, 41)	0.240
Male	572 (42)	362 (42)	210 (42)	>0.999
Ethnicity				0.520

White	67 (5.0)	38 (4.4)	29 (5.8)	
Black	669 (50)	427 (50)	242 (49)	
mixed race	615 (46)	389 (46)	226 (45)	
Education < 6 years	691 (51)	454 (53)	237 (48)	0.059
Daily per capita household income (US$/day)	1.74 (1.00, 2.91)	1.60 (1.00, 2.81)	1.80 (1.05, 3.00)	0.860
Employment				0.014
Formal employment	248 (18)	143 (17)	105 (21)	
Informal employment	354 (26)	244 (29)	110 (22)	
Unemployed	749 (55)	467 (55)	282 (57)	

When we compared participants based on their SARS-CoV-2 IgG3 results, we observed that the proportion of individuals with formal employment was lower among seropositive individuals. In contrast, the proportion of participants with informal employment was significantly higher in the seropositive group compared to the seronegative group ($p = 0.014$) (Table 1). Regarding family and environmental factors, we found that the proportion of participants reporting food insecurity was higher among seropositives ($p \leq 0.010$), and the SES index was lower among seropositives, indicating a lower socioeconomic status. The variables included in the SES index are described in Table S1. Additionally, shorter distances to the sewer ($p = 0.012$) and a lower TWI ($p = 0.006$), indicating a lower risk of flooding, were associated with the seropositive status among participants when we evaluated environmental factors (Table 2).

Table 2. Evaluation of family and environmental characteristics associated with IgG3 ZIKV results.

Characteristics	Cohort individuals n (%) or median (IQR) N = 1351	ZIKV IgG3 seropositive n (%) or median (IQR) n = 854	ZIKV IgG3 seronegative n (%) or median (IQR) n = 497	p value
Vale				**0.017**
1	241 (18)	138 (16)	103 (21)	
2	548 (41)	338 (40)	210 (42)	
4	562 (42)	378 (44)	184 (37)	
Worried about food runs out and don t have money to buy more	645 (48)	431 (50)	214 (43)	**0.010**

Food runs out before to have money to buy more	511 (38)	350 (41)	161 (32)	**0.002**
Socio economic status index*	-0.30 (-0.85, 0.43)	-0.34 (-0.85, 0.30)	-0.19 (-0.60, 0.55)	**0.004**
Accumulated trash (10m of home)	496 (37)	327 (38)	169 (34)	0.13
Shortest distance from house to sewer in meters	10 (6, 17)	10 (6, 18)	9 (6, 17)	**0.012**
Shortest distance from house to main sewer at bottom of value in meters	27 (11, 45)	26 (11, 46)	27 (11, 42)	0.063
% of pixels in a 10m radius around house that were classified as vegetation	0.10 (0.00, 0.26)	0.10 (0.01, 0.26)	0.10 (0.00, 0.26)	0.98
% of pixels in a 10m radius around house that were classified as soil	0.25 (0.13, 0.38)	0.25 (0.13, 0.37)	0.26 (0.13, 0.40)	0.43
% of pixels in a 10m radius around house that were classified as impervious	0.58 (0.34, 0.81)	0.59 (0.34, 0.81)	0.56 (0.34, 0.83)	0.65
Topographic Wetness Index value in 10m buffer around house	10.79 (8.89, 13.69)	10.65 (8.74, 13.56)	11.35 (9.14, 13.94)	**0.006**
Number of persons in the household	4.00 (3.00, 5.00)	4.00 (3.00, 5.00)	4.00 (3.00, 5.00)	0.79

Finally, our multivariate analysis using the INLA model showed that having an informal job (aOR 1.50, 95% CI 1.05–2.14) and experiencing food insecurity (aOR 1.35, 95% CI 1.04–1.75) increased the likelihood of ZIKV seropositivity. Additionally, individuals with a higher SES index, indicating better socioeconomic status, were less likely to be ZIKV seropositive (aOR 0.87, 95% CI 0.77–0.98). In contrast, a low value in the Mean Topographic Wetness Index, indicating a low risk of flooding around the household, was found to be protective against seropositive status in the INLA base model that did not include the spatial component. However, this association was not statistically significant in the INLA model that included the spatial component (aOR 0.99, 95% CI 0.93–1.05) (Table 3). Finally, the spatial correlation was estimated to be 268 meters (95% CI 84–655 meters). These high values suggest the presence of spatial dependence that could not be fully explained by the variables used in the model.

Table 3. Multivariable analysis of risk factors associated with the ZIKV seroprevalence

	INLA BASE		INLA Spatial component	
Predictors	**aOR**	**CI**	**aOR**	**CI**
Shortest distance from house to main sewer at bottom of value in meters	1.01	(1 - 1.01)	1.01	(1 - 1.03)
Mean Topographic Wetness Index	**0.94**	**(0.9 - 0.98)**	0.99	(0.93 - 1.05)
Socio economic status index	**0.88**	**(0.78 - 0.98)**	**0.87**	**(0.77 - 0.98)**
Employment				
Formal employment	**1.49**	**(1.05 - 2.1)**	**1.50**	**(1.05 - 2.14)**
Unemployed	1.07	(0.79 - 1.44)	1.07	(0.79 - 1.47)
Worried about food runs out and don t have money to buy more	**1.45**	**(1.13 - 1.85)**	**1.35**	**(1.04 - 1.75)**
Receive government help by Brazil's Conditional Cash Transfer	1.21	(0.95 - 1.53)	1.16	(0.9 - 1.48)
Observations	1351			

The probability contour map (PCM) generated by the spatial model is shown in Fig 2. The red-dark shaded areas (indicating probabilities of at least 70%) represent regions with a high probability of ZIKV infection. Conversely, the pale white shaded areas (indicating probabilities of less than 40%) correspond to areas with a low probability of infection. The other areas shaded in red (with probabilities between 40% and 70%) can be considered as having high uncertainty regarding the likelihood of infection.

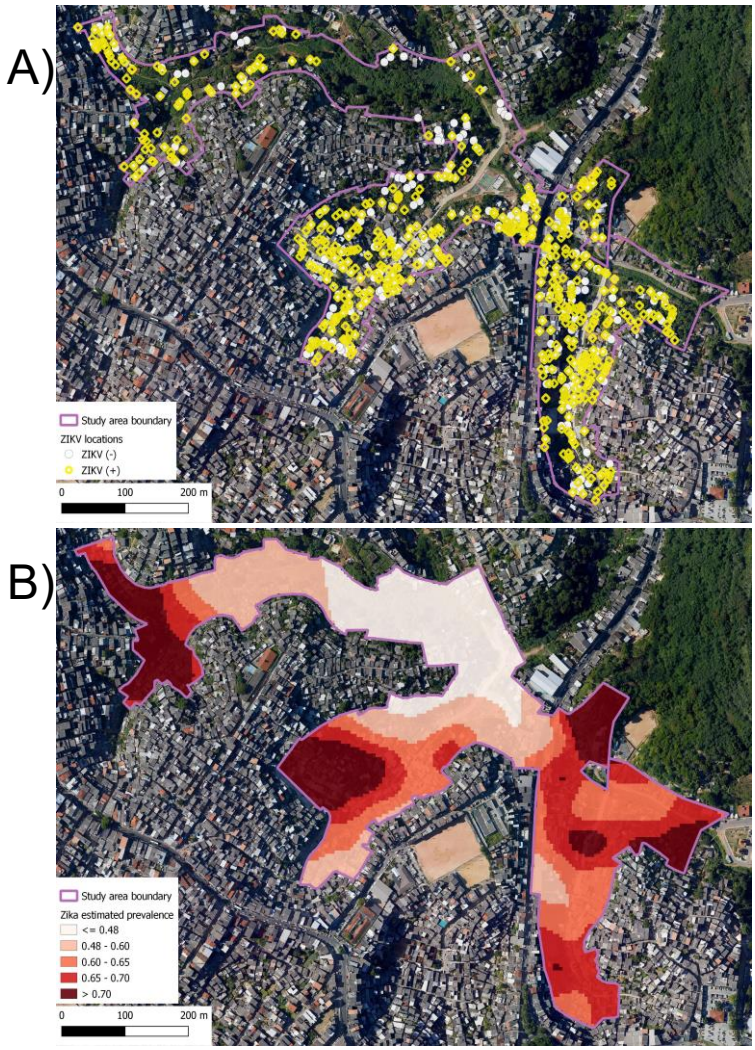

Fig. 2: Spatial Distribution of ZIKV Infections A) Distribution of households in the study site. B) A contour probability map indicating the seroprevalence probability of ZIKV in the study site.

Discussion

This study has shown that individual and family socioeconomic differences within slum communities are related to the transmission of ZIKV, increasing the likelihood of infection. Informal employment and food insecurity were identified as factors that increase the risk of ZIKV infection. Although we did not identify any environmental factors that were significantly associated with the infection, the spatial distribution of ZIKV infection in the community was heterogeneous. Furthermore, the high spatial

correlation (262 meters) identified in the model suggests the possibility of some spatial effects determining the ZIKV transmission.

In our study, we evaluated social determinants at both the family and individual levels, and found that socioeconomic differences within the community were associated with an increased risk of ZIKV infection. The family socioeconomic index calculated by our team and a informal employement status suggested that although this community had a socioeconomically vulnerable condition, there were differences among the evaluated families and individuals , and a poor socioeconomic situation is associated with the chance of ZIKV infection. This is in line with previous studies in this community that identified a poor social condition as being associated with the transmission of infections such as leptospirosis [23, 24], DENV [11] and COVID-19 [25]. These facotrs are part of the social determinants of health, which are critical factors that impact overall population health. They encompass both material living and working conditions, as well as social and environmental [26].

We also found that food insecurity is associeted with a increased risk of ZIKV infection, and two potential explanations for this association have been considered. Firstly, malnutrition resulting from an unbalanced or deficient diet in essential nutrients, resutl of the food insecurity, can compromise the immune system and increase susceptibility to infectious diseases [27, 28]. The correct consuption of micronitrientes as Zinc, Vitamin A and D has been associated with a low risk of DENV infection [27-29], and iron deficiency can also weaken the immune response, decreasing the immune cell activity and cytokine activity during an infection [29]. Secondly, it is important to note that the foood insecuryt may be intertwined with socioeconomic and environmental factors such as poverty, lack of access to clean water, and basic sanitation .

Arboviruses like ZIKV are known to be influenced by various environmental factors that affect the presence, abundance, and behavior of their vectors. These factors may include population density, inadequate housing, precarious water supply and waste disposal, high urban mobility, and climate variables such as temperature and rainfall. In our study site, these factors are present in the urban informal settlements. In our study site, which comprises urban informal settlements, these factors are present and contribute to the transmission of ZIKV. It's worth noting, however, that the impact of these factors on transmission may be consistent across our small-scale site of 0.17 km2, as the average travel distance of Aedes aegypti mosquitoes is 106 meters [30], allowing them to reach different regions within the study site. Despite these environmental factors, our study found heterogeneity in ZIKV transmission, as demonstrated by the spatial model used. This heterogeneity could be associated with population density, as areas without households had a lower predicted prevalence of ZIKV infection (<40%). Additionally, the high value of spatial correlation, along with the adjustment of the Topographic Wetness Index in the spatial model compared to the multilevel linear model, suggests that the spatial effect may be driven by other environmental/spatial factors..

Our study has some limitations. Firstly, the Pau da Lima cohort was primarily designed to evaluate the socioeconomic and environmental factors associated with Leptospirosis infection, and therefore, it was not specifically designed to address the ZIKV epidemic in the Americas or its potential sequelae. As a result, variables associated with arbovirus infection were not included during this period, such as the presence or abundance of vectors, risk behaviors related to hygiene in the community, and the type of water storage material used. Further studies are necessary to understand the effect of these variables at both individual and community levels. Secondly, as a prevalence survey, this study is limited to evaluating seroprevalence. However, as presented in the Rodriguez et al study,

the prevalence of ZIKV prior to the present survey was equal to 7% [14], indicating that the main transmission was registered in this survey. Furthermore, to reduce potential misclassification, we excluded participants with IgG3 ZIKV positive results. Finally, not all the variables included in the principal component analysis for the socioeconomic status are strongly and significantly correlated. Therefore, the application of the socioeconomic index could be limited to communities such as Pau da Lima, where non-productive assets, sanitation, and household characteristics are similar to this urban informal community.

Our study underscores the significance of tackling social determinants of health to reduce the threat of ZIKV transmission and enhance the well-being of susceptible populations. These determinants, such as substandard living conditions and inadequate access to food, can exacerbate exposure to disease-carrying mosquitoes. Along with vector control initiatives, there must be a focus on addressing social disparities and improving living conditions within communities. This includes implementing measures that can assist slum communities and prioritizing resources for those most in need.

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Table S1. Variables includes in the socioeconomic index analysis.

Characteristic	**ZIKV (+)**, N = 884[1]	**ZIKV (-)**, N = 527[1]	**p-value**[2]
Non-productive assets			
TV (No)	32 (3.7)	12 (2.4)	0.22
DVD (No)	249 (29)	140 (28)	0.63
Refrigerator (No)	48 (5.6)	13 (2.6)	**0.013**
Car (No)	821 (95)	472 (93)	0.06
Motorcycle (No)	828 (96)	476 (94)	0.052
Freezer (No)	804 (93)	463 (91)	0.16
washing machine (No)	401 (47)	205 (40)	**0.029**
Computer (No)	665 (77)	381 (75)	0.38
Radio (No)	235 (27)	116 (23)	0.08
Microwave oven (No)	554 (65)	308 (61)	0.16
Sanitation			
Without water piper system	41 (4.6)	33 (6.3)	0.23
Without water meter	796 (95)	459 (93)	0.22
Bathrooms without toilet	47 (5.5)	21 (4.2)	0.34
Household characteristics			
5 or more persons per household	163 (18.4)	78 (14.8)	**0.02**
Rent house (yes)	50 (5.8)	27 (5.3)	0.77
With Property title (No)	355 (44)	213 (44)	0.99
Household on hillside (Yes)	312 (35)	181 (34)	0.76
House access (mud / brushwood)	452 (51)	238 (45)	**0.033**
Wall material (Wood or similar material)	146 (17)	88 (17)	0.99

10.2. Artigo 2

The Journal of Infectious Diseases

BRIEF REPORT

Risk of Sexually Transmitted Zika Virus in a Cohort of Economically Disadvantaged Urban Residents

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To understand the disease burden of sexually transmitted Zika virus (ZIKV), we prospectively followed a cohort of 359 adult and adolescent residents of an urban community in Salvador, Brazil, through the 2015 ZIKV epidemic. Later, in 2017, we used a retrospective survey to associate sexual behavior during the epidemic with ZIKV infection as defined by immunoglobulin G3 NS1 enzyme-linked immunosorbent assay. We found that males who engaged in casual sexual encounters during the epidemic were more likely (adjusted odds ratio, 6.2 [95% confidence interval, 1.2–64.1]) to be ZIKV positive, suggesting that specific groups may be at increased risk of sexually transmitted infections.

Keywords. Zika; sexual transmission; sexual behavior.

Zika virus (ZIKV) is an arbovirus associated with congenital abnormalities [1]. Substantial evidence indicates that ZIKV can be transmitted sexually [2]. Current models support this finding and suggest that sexual transmission increases the disease burden; however, critical parameters of ZIKV infection in these models, such as sexual transmission rate, are not available [3–5].

To improve control of ZIKV and to better inform these models, it is therefore essential to understand how risky sexual behaviors, such as lack of condom use or having multiple sexual partners, are associated with the spread of sexually transmitted ZIKV infections. In this study, we characterize sexual behaviors associated with increased risk of ZIKV infection in a cohort of economically disadvantaged urban residents in Salvador, Brazil. We also compare infection rates between ZIKV and dengue virus (DENV) among participants to understand the ways in which differences in transmission methods may differentiate the spread of these 2 diseases.

METHODS

In a 2015 study, we characterized the attack rate (>70%) and transmission dynamics of ZIKV in a community in Salvador, Brazil, by performing multiple prospective serosurveys with 1453 individuals [6]. For the current study, we invited 443 (30.5%) randomly selected participants from this previous project. Among them, 84 (19.0%) were unable to participate, leaving 359 (81.0%) individuals who met our inclusion criteria of having participated in at least 2 serosurveys between March 2015 and October 2015 (73% of residents were infected by ZIKV during this period [6]), having available ZIKV seroconversion results, being >13 years old, and responding to our questionnaire on sexual behavior.

To quantify DENV exposure among our participants during the ZIKV epidemic, we had to account for their high likelihood of prior DENV exposure. Previous work by our team described the prevalence of 4 DENV serotypes within the nearby Pau da Lima community, with an annual incidence of 70.2 cases per 10 000 inhabitants [7]. Because this high rate of DENV infection was likely also present within our study community, the seroprevalence of total anti-DENV immunoglobulin G (IgG) antibodies would presumably be very high. Thus, to differentiate recent DENV infections from prior exposures, we used a previously described NS1 IgG3 assay [8]. Likewise, we developed our ZIKV IgG3 assay using this methodology as well [6, 8].

Participants provided their sociodemographic characteristics including sex, age, marital status, schooling, ethnicity, and clinical symptoms during 2015 serosurveys. Follow-up household visits and interviews were performed between February and April 2017 to collect data on sexual behavior during (August 2015–January 2016) and after (March–July 2016) the ZIKV epidemic. We used questions from the National Survey of Knowledge, Attitudes, and Practices of HIV and other Sexually Transmitted Infections (Pesquisa de Conhecimentos, Atitudes e Práticas na População Brasileira 2013) to evaluate the number of sexual partners, type of partner (fixed or casual), and condom usage (condom use in each sexual intercourse or condomless) [9]. Interviews were performed by trained health professionals who conducted them in a safe and comfortable

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place within the participants' own homes, to ensure the privacy and confidentiality of their answers and who used memory triggers to enhance recall.

We evaluated the association between risk factors (sexual behaviors) and ZIKV exposure (anti-ZIKV IgG3). We also evaluated DENV exposure as an alternative outcome for non–sexually transmitted flavivirus infection, to control for mosquito exposure as a confounding factor. Both the ZIKV and DENV IgG3 assays were previously validated in this cohort [6]. Relationships between outcomes and exposure factors were assessed using Pearson χ^2 test or Fisher exact test. To measure associations, we used odds ratios (ORs) and 95% confidence intervals (CIs). In cases where the evaluated frequency was equal to 0, we used ORs calculated with the Haldane–Anscombe correction. Multivariate analyses were performed to evaluate the relationship between ZIKV infection and the examined risk factors, and associations were calculated as adjusted ORs (aORs). Variables (including sociodemographic and sexual behavior factors) with $P < .10$ in bivariate analysis were included in the multivariate analysis. This was created using a forward-selection method using likelihood ratio as the criterion for adding significant variables to the model. We also compared intrahousehold relationships to test whether sexual relationships (eg, husband-wife) were more likely to exhibit concordance in ZIKV infection statuses than nonsexual relationships (eg, mother-daughter). To compare seroconcordance between sexual and nonsexual relationships, we used a generalized linear mixed model (GLMM) using a binomial distribution with the logit link function that included the household cluster effect as a random group intercept.

This study was reviewed and approved by the institutional review boards of Yale University (1006006956) and the Oswaldo Cruz Foundation and Brazilian Ministry of Health (45217415.4.0000.0040 and 55904616.4.0000.0040).

RESULTS

Among our 359 participants, 138 (38.4%) were male, 170 (47.4%) self-identified as black, mean age was 30 years (standard deviation, 16 years), and 274 (76.3%) reported having had sex at least once in their lifetime. Furthermore, 207 (57.7%) were ZIKV IgG3 positive. DENV IgG3 results were available for 302 individuals (84.1%), of whom 67 (22.2%) were positive (Supplementary Table 1).

Participants who reported having sex with >1 partner were more likely to be ZIKV positive (ZIKV positive, 8/113 [7.1%] vs ZIKV negative, 0/89 [0%]; $P = .02$) (Table 1). A marginal statistical difference was also identified between the proportion of participants who reported having had casual sex (ZIKV positive, 15/113 [13.3%] vs ZIKV negative, 5/88 [5.7%]; $P = .07$) (Table 1). Sex-stratified analysis found that males who reported interacting with >1 sexual partner were more likely to be ZIKV positive than males with a single sexual partner ($P = .04$). This analysis also found that males engaging in casual sexual relations during the epidemic were more likely to be ZIKV positive (OR, 5.8 [95% CI, 1.2–28.4]) and that of 12 males who reported engaging in casual sexual relations, 5 (41.7%) did not use condoms regularly (Table 1). After adjusting for schooling, the multivariate analysis confirmed this association between males engaging in casual sexual relations and ZIKV infections (aOR, 6.2 [95% CI, 1.2–64.1]). In the female group, we did not identify sexual behaviors associated with ZIKV, although having <6 years of formal schooling doubled the probability of a positive ZIKV result with marginal significance (aOR, 2.1 [95% CI, 1.0–4.6]; $P = .05$) (Table 2).

We analyzed sexual behavior during and after the epidemic and found no difference in sexual behaviors between these periods (Supplementary Table 2). Finally, in the household analysis, we were able to identify 165 pairwise relationships containing at least 1 individual infected with ZIKV with 78 (47.2%) seroconcordant and 87 (52.7%) serodiscordant pairwise relationships. We did not identify a statistically significant difference between sexual and nonsexual intrahousehold relationships in the GLMM analysis for the ZIKV seroconcordance (Supplementary Table 3).

DISCUSSION

In areas where mosquito-borne transmission is the main route of ZIKV transmission, determining the relative contribution of sexual transmission is challenging. Our findings, in a community of economically disadvantaged urban residents, suggest that sexual behaviors may be associated with ZIKV infection. This association was driven specifically by males engaging in risky sexual behavior, defined as lack of condom use, engagement with multiple sexual partners, and participation in casual sexual encounters. Among our study participants, males reported higher rates of risky sexual behaviors. Furthermore, among all members of our sample, engaging in casual sexual encounters increased the likelihood of testing positive for ZIKV 6-fold (Table 2). Although we only found a significant relationship between ZIKV infection and casual sex, we also found high rates of other risky behaviors in ZIKV-positive residents. Given the reports of sexual transmission of ZIKV, it is unsurprising that ZIKV infection is associated with these casual encounters, and our results are consistent with previous findings for other sexually transmitted infections and with current ZIKV models [3–5, 10]. While previous studies highlighted the role of infected males in sexual transmission because of higher viral persistence in genital secretions of males than in those of females [2], our findings also suggest an additional contribution of males to ZIKV transmission through risky sexual behaviors. These findings support the inclusion of gender and behavioral differences in mathematical models of ZIKV transmission [5, 11]. Recent household analyses in northeast Brazil [12] found that

Table 1. Sexual Behavior During the Zika Virus Epidemic, Stratified by Sex, in Pau da Lima Community, Salvador, Brazil

Sexual Behavior During the ZIKV Epidemic	ZIKV Positive	ZIKV Negative	*P* Value	OR (95% CI)
General population				
≤6 y of schooling	114/207 (55.1)	69/152 (45.4)	.07	1.5 (1–2.2)
Had sex (August 2015–January 2016)	113/207 (54.6)	89/152 (58.6)	.45	0.9 (.5–1.3)
>1 sexual partner	8/113 (7.1)	0/89 (0)	.02[a]	10.3 (1.1–606.2)[b]
Sex with fixed partners	100/112 (89.3)	83/89 (93.3)	.33	0.6 (.2–1.7)
Condomless sex with fixed partners	87/100 (87)	70/82 (85.4)	.75	1.5 (.5–2.7)
Sex with casual partners	15/113 (13.3)	5/88 (5.7)	.07	2.5 (.9–7.3)
Condomless sex with casual partners	8/15 (53.3)	1/5 (20)	.32[a]	4.6 (.4–51.1)
Self-report of Zika-like symptoms in sexual partner	17/112 (15.2)	10/88 (11.4)	.53	1.4 (.6–3.2)
Male group				
≤6 y of schooling	44/79 (55.7)	30/59 (50.8)	.57	1.2 (.6–2.4)
Had sex (August 2015–January 2016)	39/79 (49.4)	28/59 (47.5)	.82	1.1 (.5–2.1)
>1 sexual partner	6/39 (15.4)	0/28 (0)	.04[a]	7.7 (.7–483.1)[b]
Sex with fixed partners	29/38 (76.3)	25/28 (89.3)	.18	0.4 (.1–1.6)
Condomless sex with fixed partners	24/29 (82.8)	22/25 (88)	.71[a]	0.7 (.1–3.1)
Sex with casual partners	12/39 (30.8)	2/28 (7.1)	.02	5.8 (1.2–28.4)
Condomless sex with casual partners	5/12 (41.7)	0/2 (0)	.51[a]	2.2 (.1–201.4)[b]
Self-report of Zika-like symptoms in sexual partner	4/38 (10.5)	3/27 (11.1)	.99[a]	0.9 (.2–4.6)
Female group				
≤6 y of schooling	70/128 (54.7)	39/93 (41.9)	.61	1.6 (1–2.9)
Had sex (August 2015–January 2016)	74/128 (57.8)	61/93 (65.6)	.24	0.7 (.4–1.3)
>1 sexual partner	2/128 (1.6)	0/61 (0)	.50[a]	1.9 (.1–115.8)[b]
Sex with fixed partners	71/74 (95.9)	58/61 (95.1)	.99	1.2 (.2–6.3)
Condomless sex with fixed partners	63/71 (88.7)	48/57 (84.2)	.45	1.5 (.5–4.1)
Sex with casual partners	3/74 (4.1)	3/60 (5)	.99[a]	0.8 (.2–4.1)
Condomless sex with casual partners	3/3 (100)	1/3 (33.3)	.40[a]	12 (.3–556.7)[b]
Self-report of Zika-like symptoms in sexual partner	13/74 (17.6)	7/61 (11.5)	.32	1.6 (.6–4.4)

Data are presented as No. of positive answers/total No. of answers (%), unless otherwise indicated.

Abbreviations: CI, confidence interval; NA, not applicable; OR, odds ratio; ZIKV, Zika virus.

[a]Fisher exact test *P* value.

[b]OR calculated with the Haldane–Anscombe correction and Fisher exact test.

household members living with a seropositive patient had an increased risk of Zika and chikungunya infection and that this risk increases when the relationship between the individuals is sexual [12]. In contrast, our household analysis did not find a significant effect of the type of relationship. We think that this may be due to the fact that there are other environmental and social factors for individuals living in these economically disadvantaged communities, which may confound the effect of intrahousehold relationship type. This is also consistent with the high recorded incidence of ZIKV among participants in our study (57.7%).

Although the findings of our study are important, it is also essential to note its limitations. One limitation is the potential for recall bias, which can occur during retrospective reporting of individual behaviors. That said, we took careful steps to reduce recall bias, and interviews used specific memory triggers

Table 2. Risk Factors Associated With a Zika Virus Positive Result in Multivariate Analysis

Characteristics	ZIKV Positive	ZIKV Negative	*P* Value	OR (95% CI)	*P* Value	aOR (95% CI)
Female group[a]						
≤6 y of schooling	38/74 (51.4)	20/60 (30.3)	.03	2.1 (1.0–4.3)	.05	2.1 (1.0–4.6)
Sex with casual partners	3/74 (4.1)	3/60 (5)	.99[c]	0.8 (.2–4.1)	.99	0.8 (.1–6.5)
Male group[b]						
≤6 y of schooling	21/39 (53.8)	13/28 (53.8)	.73	1.3 (.5–3.5)	.46	1.7 (.5–5.5)
Sex with casual partners	12/39 (30.8)	2/28 (7.1)	.03[c]	5.8 (1.2–28.4)	.02	6.2 (1.2–64.1)

Data are presented as No. of positive answers/total No. of answers (%), unless otherwise indicated.

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; OR, odds ratio; ZIKV, Zika virus.

[a]One hundred thirty-four females who had sex during August 2015–January 2016.

[b]Sixty-seven males who had sex during August 2015–January 2016.

[c]Fisher exact test.

to enhance recall. In addition to recall bias, small sample size and large CIs were a further limiting factor in our study, which affects the generalizability of our findings. Notably, we did not find any association between sexual behavior and ZIKV infection in the female population. This may be due to limited sample size or to women underreporting this type of relationship [10]. Sample size also limited our ability to evaluate the relative contribution of sexual behavior in the context of other potential risk factors (eg, number of mosquito bites, resident movement). Finally, we note that our methodology identified correlational associations between variables; however, these relationships are not necessarily causative. As a result, it is possible that outside factors such as degree of risk aversion drive both an individual's likelihood of engaging in risky sexual behavior and their likelihood of taking precautions against mosquito-borne infections.

There were also many variables on which we were unable to collect data. For example, we were not able to consider the spatial distribution of ZIKV infection within our study. We also did not collect data on sexual preferences and orientation, although prior studies suggest the possibility of long-term disease persistence within communities of men who have sex with men, [2, 4], as well as other potential confounders such as sexual frequency or transactional sex. Notwithstanding, when we analyzed the same associations for DENV infection, they were not significant. Previously, a case report described an incidence of sexual transmission of dengue; however, this finding has not been replicated in epidemiological studies [13]. Thus, although ZIKV and DENV are ecologically similar arboviruses that share the same primary transmission vector, sexual transmission forms a relevant secondary transmission method in ZIKV infection that we do not find in DENV.

The World Health Organization advocates that persons of reproductive age receive information about the sexual transmission of ZIKV to improve parental planning. Our data suggest that the behaviors of individuals did not experience significant changes in the context of the epidemic. More studies to prospectively follow the change in the behavior before, during, and after epidemics are necessary to develop government policies and improve access to information about the disease in economically disadvantaged urban communities. One previous study that evaluated contraceptive sales in Brazil found an increase in demand for contraception that might be associated with the ZIKV epidemic [14], In contrast, however, a 2016 study of women of reproductive age in northeast Brazil found that despite high levels of knowledge about ZIKV and the congenital alterations, few women changed their attitudes about pregnancy and contraceptive use [15]. Similarly, we also did not find any difference in frequency of risky behaviors between the epidemic and postepidemic periods. Overall, long-term studies are necessary to evaluate changes in sexual behaviors despite ZIKV reduction rates.

This study provides the first glimpse of the population-level relationship between males reporting multiple partners or casual sex and ZIKV transmission, which should be further investigated to inform mathematical models and governmental recommendations pertaining to the risk of sexual ZIKV infection in vulnerable populations.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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10.3. Artigo 3

Clinical and developmental outcomes of children with in utero Zika virus exposure: a hospital-based cohort in Salvador, Brazil

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Abstract:

Key questions remain on the impact of Zika virus (ZIKV) exposure on long-term developmental outcomes, especially among infants who were exposed in utero but did not develop congenital Zika syndrome (CZS) at birth. To address these questions, we prospectively evaluated the clinical and developmental outcomes of a cohort of children with two years of age who were born during the Zika epidemic in Salvador, Brazil.

We enrolled a cohort of mothers and their newborn infants at a maternity hospital between Oct 01, 2015 and Jan 31, 2016. We selected children who did not have CZS or microcephaly at birth and performed anthropometric, auditory, ophthalmologic evaluations and Hammersmith Infant Neurological Examination (HINE) and Bayley Scales of Infant and Toddler Development-III (BSID-III) assessment during home and outpatient visits between Sep 11,2017 and Dec, 28, 2018. We ascertained ZIKV exposure in utero by assaying for neutralizing antibodies in sera from mothers during childbirth and evaluated the association with clinical and developmental outcomes.

Among 469 children enrolled at birth, 364 (78%) completed follow-up evaluations at a mean age of 26.9 (±4.0) months. Of the 364 children, 214 (58.8%) were born to mothers with serologic evidence of ZIKV exposure. High maternal age (OR=1.08; 95% CI 1.02-1.14) was associated with increased risk of global neurodevelopmental impairment. Of the 214 children with ZIKV exposure, 22 (10.2%) were identified to have neurodevelopmental impairment (31.8% cognitive, 45.5% language, and 22.7% motor domains). After adjusting for maternal age, ZIKV exposure was not associated with specific domain impairments but was associated with a higher risk (adjusted OR=2.36; 95% CI 1.05-5.80) for global neurodevelopmental impairment. The attributable fraction of neurodevelopmental impairment associated with ZIKV exposure was 36.0% (95% CI 3.4 to 68.6%). Significant associations were not observed between Zika exposure and anthropometric, auditory or ophthalmologic deficits.

Our study found that ZIKV exposure in utero imparts mild neurodevelopmental deficits, primarily associated with language acquisition, in a significant proportion of children without CZS by two years of life. Given the large numbers of infants that had inapparent ZIKV exposures during the pandemic, routine screening and intervention will be needed in affected regions of the Americas to mitigate these impacts on development, especially as these children enter schools.

Keywords: Zika virus, neurodevelopment, child development

Introduction

After the Zika virus (ZIKV) epidemic in Brazil in 2015, one of the main concerns related to ZIKV sequelae is developmental delays among children who were exposed to the virus in utero. Initially, ZIKV was associated with a large number of birth defects known as congenital Zika virus syndrome (CZV), with microcephaly being the most severe [1-3]. However, during follow-up of children exposed in utero but without microcephaly or other birth defects, researchers identified an association between in-utero exposure to ZIKV and mild neurodevelopmental delay [4-6]. Nevertheless, there is limited information available regarding the clinical and developmental outcomes of these children beyond their second year of life.

Children without microcephaly who were exposed to the ZIKV in utero have been found to develop alterations related to language and cognition [4, 6-8]. A base population cohort study conducted in Salvador, Brazil, the epicenter of the epidemic, identified that 50% of mild cognitive delays are attributable to ZIKV [4]. Research carried out in Rio de Janeiro and Salvador, Brazil revealed a higher likelihood of language impairments [5, 6]. Cognition and language are recognized as crucial abilities that undergo swift development in the early years of life. As consequence delays in these neurodevelopmental domains are associated with an increased risk of social and emotional impairments, compromising the overall quality of life and potentially affecting academic performance [5].

Our group carried out a cohort study in Salvador, Brazil, involving pregnant women and their children. It was estimated that the seroprevalence in this city was 63% until 2016, and from 2015 to 2016, 652 cases of Congenital Zika Syndrome (CZS) were reported [9]. In this context, CZS is just the visible part of the iceberg, and there is a possibility that minor alterations were not detected during the initial clinical evaluations. This research

aims to reveal the actual burden of ZIKV congenital transmission in a hospital cohort that includes normocephalic children without apparent abnormalities.

Methods

Ethical aspects

The project was approved by the ethics committee of the Hospital Geral Roberto Santos–Bahia (5344216.1.1001.5028) and the Institutional Review Boards of Yale University (1603017343). All women and their children, whose were eligible and participated in the study, provided signed consent for interviews, blood collection, and clinical evaluations.

Study design and setting

We conducted a hospital-based cohort study in Salvador, Brazil, which is the capital of Bahia state and the largest city in the northeast region of Brazil. According to the 2020 projection by IGBE the city's population is approximately 2.9 million [10]. In 2015, Salvador experienced the largest ZIKV epidemic, leading to a rise in the number of newborns with microcephaly due to congenital transmission [11]. Our research was conducted at Hospital Geral Roberto Santos (HGRS), which is the biggest tertiary referral health center in the state. The obstetric center of HGRS handles around 140 deliveries every month and provides care for expectant mothers with high-risk pregnancies from the surrounding area [12].

Participants

We enrolled pregnant women and their children residing in Salvador who delivered at the HGRS obstetric center between October 1, 2015, and January 31, 2016. Children with microcephaly associated with CZS had been described previously [1] and were not

included in this analysis. Children born to mothers with evidence of TORCHS infection during pregnancy were not included. After recruitment at birth, the children were followed up until they reached 2-3 years of age.

ZIKV intrauterine exposure

The Plaque Reduction Neutralization Test (PRNT) was used to determine ZIKV intrauterine exposure, as previously described in the work of Nery et al. [12]. The antibody titer for PRNT was defined based on a 50% reduction in the inhibition of the virus inoculum (PRNT50), and serum samples were considered positive for ZIKV if the antibody titers were $\geq$ 20. All tests were conducted at the Instituto de Ciências Biomédicas, Universidade de São Paulo - USP. Children with in utero ZIKV exposure were defined as those born to a mother with a positive PRNT, while children without in utero ZIKV exposure were defined as those born to a mother with negative results. PRNT was performed on serum samples obtained at birth and during mother-child follow-ups when the children were 2 to 3 years old. Nery et al. found a concordance of 93% between the PRNT results obtained at birth and those obtained during the follow-up sample collection [12].

Sociodemographic factors and maternal characteristics

A trained team of physicians and nurses collected data on sociodemographic factors and maternal characteristics during the postpartum period and when the child was two years old. The team used a structured questionnaire to gather information from participants about their demographic, socioeconomic, and clinical history, including any symptoms related to ZIKV during pregnancy. In addition to the questionnaire, the team also gathered information from the participants' medical records to supplement their responses.

Outcomes evaluation

Clinical and developmental assessments were conducted on the participants at the Microcephaly Outpatient Clinic at HGRS. A team of healthcare professionals, including physiotherapists, speech therapists, physicians (including specialists in ophthalmology and neurology), and nurses, conducted interviews to gather information about the mothers and children, and also conducted assessments on the children.

Anthropometric growth

Anthropometric measurements, including weight, length, and head circumference, were collected at birth and during the follow-up period. Reference values (Z-scores) from INTERGROWTH-21 [13] were used for analyzing the anthropometric data collected at birth, while the WHO parameters [14] were used for measurements collected during the follow-up period. Children with a head circumference at birth of less than -2 standard deviations were defined as having microcephaly and were excluded from the study.

Neurological and neurodevelopmental assessments

Neurological and neurodevelopmental assessments were performed using the Hammersmith Infant Neurological Examination (HINE), which is a clinical neurological exam. For this study, the first section of the HINE related to neurological assessment was used, which includes 26 items (score range between 0 – 78) [15, 16]. This section includes the evaluation of cranial nerve function, posture, movements, tone, and reflexes. In children over 18 months of age, an optimal HINE score is ≥74 [15, 16].

We used the Bayley Scales of Infant and Toddler Development, 3rd ed. (BSID-III), to evaluate the neurodevelopmental status of the children. The BSID-III is a validated tool

in Brazil for assessing children aged 16 days to 42 months [17]. We assessed the Cognitive, Language, and Motor domains twice. In the first evaluation, we employed the screening version of the BSID-III, and the results were adjusted for the child's age and categorized into three levels: at risk, emerging, and competent development. Children classified as at risk or emerging in at least one screening scale were re-evaluated using the complete BSID-III tool in the second assessment. The children were classified into three categories: severe neurodevelopmental delay (composite score ≤70 [≤-2 SD]), mild delay (71 to 85 [-2 SD to -1 SD]), or normal development (>85) [4, 18]. Those classified as competent in the BSID-III screening, defined as > -1 SD, were combined with children who underwent the complete Bayley protocol for the final analysis [4].

Neurosensory assessment

The neurosensory assessment comprised a range of examinations, including an external ocular examination, functional ocular examinations, ocular biomicroscopy, and indirect ophthalmoscopy with pupillary dilation. In addition, the visual function assessment involved evaluating several aspects, such as visual acuity, light sensitivity, ocular fixation, stability, object tracking, visual contact, social smile response, facial responses to visual stimuli, and facial expression imitation. Furthermore, the auditory evaluations were conducted using an adapted conditioned play audiometry test (Simonek hearing kit) to screen children up to 48 months of age [4, 19]. The test evaluated various reflexes, attention, location, and orientation in response to different auditory stimuli presented through objects with varying frequency levels ranging from 38.2 to 95.1 decibels.

Data Analysis

Electronic questionnaires were utilized for data collection, and the REDCap (Research Electronic Data Capture) system was used to store the responses on the server of the FIOCruz Bahia, Gonçalo Moniz Institute (IGM). RStudio software (version 4.2.2) was employed for statistical analyses.

We summarized the data using descriptive statistics. To evaluate in utero ZIKV exposure and covariates associated with clinical and developmental outcomes, we used the Chi-square test or Fisher's exact test for categorical variables. Quantitative data were compared using the t-test for normally distributed data and the Mann-Whitney test for non-normally distributed data. A p-value less than 0.05 was considered significant. Multivariate analysis was performed using binomial logistic regression. For model selection, variables with a p-value less than 0.20 were simultaneously entered into the multiple logistic regression model. The backward and forward elimination methods were used and compared to reach the most parsimonious model. The final model was summarized using odds ratios (OR) and their 95% confidence intervals (CI). Additionally, we calculated the attributable fraction (AF) of the final model measures to determine the proportion of neurodevelopmental impairments associated with the in utero ZIKV exposure using the package AF [20, 21].

Results

During October 2015 to January 2016, 685 pregnant women gave birth at the HGRS. Of these, 469 women and their newborns were recruited for our study at birth, which represents 68.4% of the total. Of these participants, 387 children (82.5%) completed our assessment when they were between two and three years old. We found that 207 out of the 387 normocephalic children (53%) had been exposed to the ZIKV virus while in utero,

while the remaining 150 out of 387 normocephalic children (39%) had not been exposed. (Fig 1).

Table 1 summarizes the sociodemographic characteristics of the study participants. The mean maternal age at delivery was 26 years (SD 7), with the majority of women self-identifying as black (180/357, 50%), mixed ethnicity (152/357, 43%), and having at least 9 years of education (257/357, 72%). The main maternal social determinant associated with ZIKV exposure during pregnancy was food insecurity, as indicated by reduced or restricted food intake due to lack of financial resources in the women's families, as well as low maternal age and education level ($p < 0.05$). In addition, symptoms reported during pregnancy, such as fever, rash, headache, and musculoskeletal pain, were significantly associated with a positive maternal ZIKV PRNT result ($p < 0.01$).

Clinical and developmental outcomes

The anthropometric assessment was completed in 349 (97.8%) of the children, and their results were within normal standards (±2 SD). However, children who were exposed to ZIKV in utero had a lower head circumference, with a mean of 0.55 SD (95% CI -0.16 to 1.11), compared to those who were unexposed, with a mean of 0.65 SD (95% CI 0.00 to 1.28). The difference in head circumference was marginally significant ($p=0.090$). We did not identify any neurological or neurosensory abnormalities associated with in utero exposure to ZIKV.

All 357 children included in the study completed the neurodevelopmental screening assessment. Of these, 29 (8.1%) were categorized as being at risk and 82 (23.0%) as emergent in at least one domain based on the BDS-III screening. In a second evaluation of children with at least one alteration in the screening using the complete BDS-III version, we identified that 25 (12.1%) of the children with in utero exposure to the Zika

virus (ZIKV) had a BDS-III score that was <1 SD. However, this difference was only marginally statistically significant (p=0.067) when compared to children who were not exposed to ZIKV in utero (Table 2).

In the bivariate analysis of factors associated with abnormal neurodevelopment, we found a positive correlation with maternal age (OR = 1.06, 95% CI 1.01 to 1.12), child age (OR = 0.87, 95% CI 0.78 to 0.97), low HINE neurological score (OR = 10.4, 95% CI 3.41 to 31.5), and ZIKV in utero exposure (OR = 2.15, 95% CI 1.01 to 5.01). The multivariate regression model revealed that ZIKV in utero exposure increased the likelihood of neurodevelopmental impairment (OR = 2.36; 95% CI 1.05 to 5.80) after adjusting for maternal age (OR = 1.06; 95% CI 1.01 to 1.12) and HINE result (OR = 10.4; 95% CI 3.41 to 31.5). Finally, we found that the attributable fraction of neurodevelopmental impairments associated with ZIKV is 36.0% (95% CI 3.4 to 68.6%).

Discussion

Our findings from a hospital cohort of women and their normocephalic children, from the epicenter of the ZIKV epidemic in Brazil in 2015, suggest that exposure of children in utero to ZIKV is associated with mild neurodevelopmental impairments. Congenital Zika Syndrome is associated with neurological alterations, with the most significant being microcephaly, retinal alterations, sensorineural hearing loss, and arthrogryposis [2, 22]. However, these alterations represent only the tip of a much broader spectrum of impairments associated with congenital ZIKV transmission. During follow-up of children exposed to ZIKV in utero, neurodevelopmental impairments were identified not only in children with CZS but also in normocephalic children [4-6, 23]. In our study, we found that normocephalic children exposed to ZIKV in utero may develop mild neurodevelopmental impairments mainly associated with language. Approximately one-

third of these impairments could be attributed to in-utero ZIKV infection (36% of the cohort).

According to our findings, language is the domain most affected, which is consistent with previous studies. In a cohort study conducted in Rio de Janeiro, 35% of children were affected by ZIKV [6]. Additionally, a systematic review showed that language is the most commonly affected domain in children exposed to ZIKV in utero, with a prevalence of 30% [24]. However, the main limitation of these studies is the absence of a control group to determine the proportion of alterations associated with the congenital transmission. Nevertheless, subsequent studies using control groups have identified that 54% of these impairments may be caused by ZIKV exposure in utero. Furthermore, we found that social vulnerabilities are linked to a higher risk of maternal infection, consistent with our previous study by Nery N. et al., which found that food insecurity and education were linked to transmission [12]. This is crucial because the context in which children are exposed to ZIKV in utero can influence their development. Although we did not identify any socioeconomic risk factors, further research is necessary to understand the impact on the development of these children.

There is a lack of clear understanding about factors associated with delays in child development beyond in utero ZIKV exposure. Results from a cohort study conducted in Rio de Janeiro suggest that smaller head circumference and auditory abnormalities in infants are linked to poor neurodevelopment in toddlers, particularly language impairments [23]. In our study, we found that small head circumference was marginally associated with neurodevelopmental problems, but it was excluded from the final model. On the other hand, higher maternal age and a low score on the HINE were associated with mild developmental impairments. Extreme maternal age, including advanced maternal

age (≥35 years) or teenagers (≤19 years), was linked to severe outcomes such as microcephaly [1, 25]. Also, HINE scores can predict cognitive and motor development in children with CZS-associated microcephaly [1, 26-28]. The is a simple neurological exam that does not require specialized materials. It is already used to evaluate the developmental and functional prognoses of children with cerebral palsy, and changes in their score values have been associated with either a worse or better developmental prognosis. [29, 30].

Our study had some limitations, first, despite multiple attempts, there was difficult to complete all the clinical and developmental assessment. As consequence we summarized the data in tables with different denominators and a subsample was selected to perform the multivariate model. Of this evaluation the most missing evaluation were the complete ophthalmological and auditory evaluations, thus possibly affecting the rate of defects that could have been identified. Additionally, the study only included pregnant women and children from Salvador, who delivered at a specific obstetric center during a specific time period, which further limits the generalizability of the findings to other locations or time periods. Lastly, although blood samples were not collected from all participants simultaneously, a strong correlation of 93% was observed between PRNT results obtained during delivery and those obtained at follow-up (2-3 years later). Additionally, PRNT results for the four DENV serotypes were only available for 23% of mothers, but there were no significant differences in DENV infection rates between ZIKV-positive and negative subjects [12].

Conclusion

To conclude, our assessments have identified anomalies in neurodevelopmental outcomes associated with in utero exposure to ZIKV, with a specific focus on language functioning.

Our results have important implications for practice and policy. The close monitoring of ZIKV prenatally exposed children is needed throughout childhood to allow for the early detection of developmental impairment and to inform subsequent specific clinical care (language, motor, or cognitive therapies). It is imperative to conduct larger studies involving prospective groups during both pre-school and school-age stages. Conducting methodical evaluations of these children using standardized measures is of utmost importance in identifying abnormal development at an early stage and implementing interventions to achieve optimal outcomes and prevent disabilities.

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Tables and figures

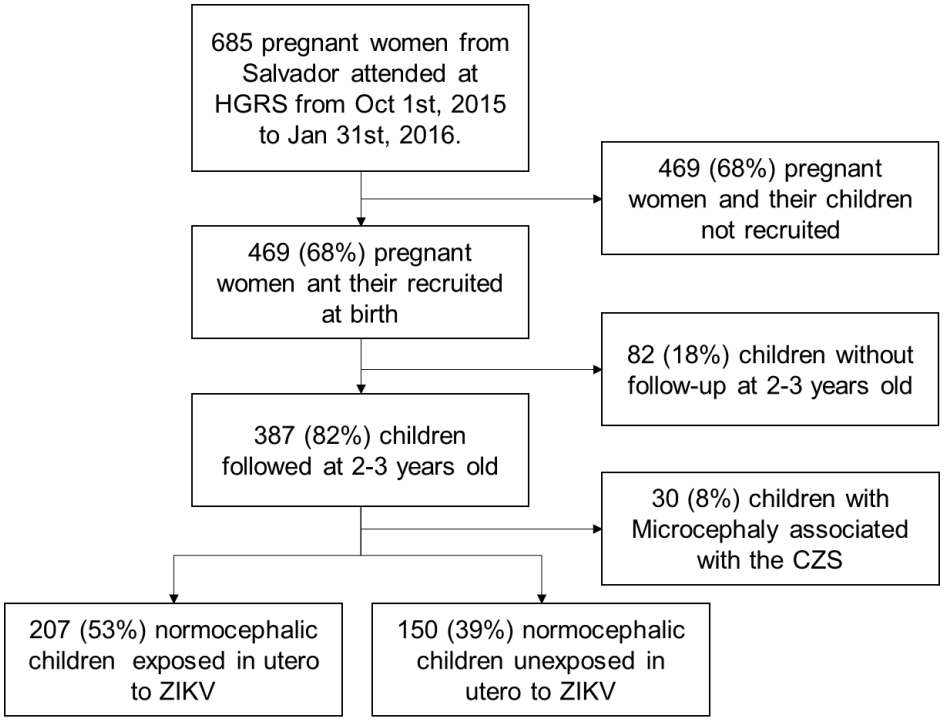

Fig 1. Study flow chart, pregnant women and their children recruited at the HGRS, Salvador, Brazil

Table 1. Comparison of the maternal characteristics between those who tested positive for ZIKV and those who tested negative.

Characteristic	Mother ZIKV negative (N = 150)	Mother ZIKV positive (N = 207)	p value
Maternal sociodemographic			
Age (years)	27 (7)	26 (7)	**0.042**
Ethnicity			>0.99
White	6 (4.1)	8 (3.9)	
Negro	75 (51)	105 (51)	
Brown	63 (43)	89 (44)	
Others	2 (1.4)	2 (1.0)	
Education			**0.03**
Up to 9th year	118 (79)	139 (68)	
More than 9th year	32 (21)	66 (32)	
Work before pregnancy	77 (51)	92 (45)	0.24
Worked with a formal contract	44 (35)	41 (27)	0.15
Dissatisfaction with family income	79 (53)	118 (58)	0.33
Income			>0.99
Up to 1 salary	11 (11)	15 (12)	
More than 1 salary	85 (89)	114 (88)	
Food insecurity in the Screening	80 (53)	128 (63)	0.081
Concern about lack of food	78 (52)	126 (61)	0.083
Reduction or food restriction for lack of money	51 (34)	95 (46)	**0.029**
Receives government financial assistance	17 (53)	36 (60)	0.66
Clinical history			
Poor self-perceived health	5 (3.4)	12 (5.9)	0.32
Previous pregnancies	1.56 (1.44)	1.49 (1.29)	0.65
Folic acid	117 (78)	165 (80)	0.69
Ferrous sulfate	130 (87)	178 (86)	0.88
Other vitamins	65 (43)	97 (47)	0.52
Pre-existing diseases	21 (15)	54 (27)	**0.011**

Prior sexually transmitted disease	8 (5.8)	15 (7.5)	0.66
Habits during the pregnancy			
Alcohol consumption	31 (21)	62 (30)	0.066
Cigarettes	6 (4.1)	16 (7.8)	0.18
Use of illicit drugs	3 (2.1)	4 (2.0)	>0.99
Sytomps during the preganacy			
Rash	3 (2.0)	34 (16)	**<0.001**
Fever	5 (3.3)	23 (11)	**0.009**
Cough	3 (2.0)	8 (3.9)	0.37
Headache	5 (3.3)	24 (12)	**0.005**
Arthralgia	4 (2.7)	26 (13)	**<0.001**
Muscle ache	4 (2.7)	27 (13)	**<0.001**
Conjunctivitis	0 (0)	4 (1.9)	0.14

1 Table 2. Clinical and developmental outcomes associated with in utero ZIKV exposure.

Characteristic	**N**	**Total** (N = 357)[1]	**Mother ZIKV negative and child without CZS** (N = 150)[1]	**Mother ZIKV positive and child without CZS** (N = 207)[1]	**p-value**[2]
Child's age in months on evaluation	357	26.0 (24.0, 30.0)	26.0 (23.2, 30.0)	26.0 (24.0, 30.0)	0.55
Anthropometric assessment					
Cephalic perimeter score Z	350	0.60 (-0.13, 1.20)	0.65 (0.00, 1.28)	0.55 (-0.16, 1.11)	0.090
Length score Z	349	-0.02 (-0.73, 0.79)	0.06 (-0.71, 0.85)	-0.05 (-0.77, 0.75)	0.83
Weight score Z	349	0.31 (-0.41, 1.28)	0.44 (-0.28, 1.40)	0.21 (-0.44, 1.15)	0.15
Neurological examination					
Median HINE total score	338	78.00 (78.00, 78.00)	78.00 (78.00, 78.00)	78.00 (78.00, 78.00)	0,34
HINE total score >74	338				>0.99
Abnormal		15 (4.4%)	6 (4.1%)	9 (4.7%)	
Normal		323 (95.6%)	140 (95.9%)	183 (95.3%)	
Auditory evaluation					
Absence cochlear-palpebral reflex, n (%)	329	327 (99.4%)	138 (100.0%)	189 (99.0%)	0,51
Auditory behavior test	335	13 (3.9%)	6 (4.3%)	7 (3.6%)	0,78
Abnormal Auditory development, n (%)	344	319 (92.7%)	137 (95.1%)	182 (91.0%)	0,21

Auditory response, n (%)	338				>0.99
No response		0 (0.0%)	0 (0.0%)	0 (0.0%)	
Doubtful reaction to stimuli or asymmetry of response		15 (4.4%)	6 (4.1%)	9 (4.7%)	
Reacts to stimuli from both sides		323 (95.6%)	140 (95.9%)	183 (95.3%)	
Ophthalmologic evaluation					
Ophthalmologic alteration	281	18 (6.4%)	5 (4.2%)	13 (8.0%)	0,23
Retina alteration, n (%)	287	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Strabismus	294	10 (3.4%)	6 (4.8%)	4 (2.4%)	0,33
Nystagmus	293	1 (0.3%)	0 (0.0%)	1 (0.6%)	>0.99
Neurodevelopmental function					
Bayley-III screening evaluation	357				0,066
At risk		29 (8.1%)	7 (4.7%)	22 (10.6%)	
Emergent		82 (23.0%)	40 (26.7%)	42 (20.3%)	
Normal		246 (68.9%)	103 (68.7%)	143 (69.1%)	
Bayley-III complete evaluation	357				0,067
Abnormal		34 (9.5%)	9 (6.0%)	25 (12.1%)	
Normal		323 (90.5%)	141 (94.0%)	182 (87.9%)	
<-1SD	357	29 (8.1%)	8 (5.3%)	21 (10.1%)	0,12
<-2SD	357	5 (1.4%)	1 (0.7%)	4 (1.9%)	0.40
Cognitive evaluation	356	9 (2.5%)	2 (1.3%)	7 (3.4%)	0,31

<-1SD	356	5 (1.4%)	1 (0.7%)	4 (1.9%)	0,4
<-2SD	356	4 (1.1%)	1 (0.7%)	3 (1.5%)	0,64
Language evaluation	353	17 (4.8%)	7 (4.7%)	10 (4.9%)	>0.99
<-1SD	353	14 (4.0%)	6 (4.0%)	8 (3.9%)	>0.99
<-2SD	353	3 (0.8%)	1 (0.7%)	2 (1.0%)	>0.99
Motor evaluation	348	7 (2.0%)	2 (1.4%)	5 (2.5%)	0,7
<-1SD	348	6 (1.7%)	2 (1.4%)	4 (2.0%)	>0.99
<-2SD	348	1 (0.3%)	0 (0.0%)	1 (0.5%)	>0.99

2

Table 3. Regression model including risk factors associated with neurodevelopmental impairments.

Characteristic	Neurodevelopmental test			Bivariate analyses		Multivariate analyses	
	N	Total (N = 357)	Abnormal (N = 34)	OR	95% CI	OR	95% CI
Mother's age (years)	357	26 (21, 32)	31 (24, 35)	1.06	1.01, 1.12	1.08	1.02, 1.14
Education	355						
Up to 9th year		257 (72.4%)	23 (8.9%)	—	—		
More than 9th year		98 (27.6%)	11 (11.2%)	1.29	0.58, 2.69		
Sex	357						
Male		206 (57.7%)	23 (11.2%)	—	—		
Female		151 (42.3%)	11 (7.3%)	0.63	0.28, 1.30		
Pre Term	330						
Yes		52 (15.8%)	4 (7.7%)	0.81	0.23, 2.19		
No		278 (84.2%)	26 (9.4%)	—	—		
Child's age in months on evaluation	357	26.0 (24.0, 30.0)	24.0 (22.2, 26.8)	0.87	0.78, 0.97		
Cephalic perimeter score Z	350	0.60 (-0.13, 1.20)	0.55 (-0.02, 1.26)	1.05	0.77, 1.42		
Neurological examination	338						
Abnormal		15 (4.4%)	7 (46.7%)	10.4	3.41, 31.5	9.89	3.05, 31.8
Normal		323 (95.6%)	25 (7.7%)	—	—	—	—
Auditory response, n (%)	338						
Doubtful reaction to stimuli or asymmetry of response		15 (4.4%)	11 (73.3%)	39.5	12.4, 153		
Reacts to stimuli from both sides		323 (95.6%)	21 (6.5%)	—	—		
ZIKV exposed	357						
Zika positive		207 (58.0%)	25 (12.1%)	2.15	1.01, 5.01	2.36	1.05, 5.80
Zika negative		150 (42.0%)	9 (6.0%)	—	—	—	—

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PLOS ONE

RESEARCH ARTICLE

Heterogeneous development of children with Congenital Zika Syndrome-associated microcephaly

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Abstract

Objective

To describe the neurological and neurodevelopmental outcomes of children with Congenital Zika Syndrome (CZS) associated microcephaly beyond 2 years of age.

Method

We followed children with CZS-associated microcephaly in an outpatient clinic in Salvador, Brazil. Neurological and neurodevelopmental assessments were performed using the Hammersmith Infant Neurological Examination (HINE) and Bayley Scales of Infant and Toddler Neurodevelopment (Bayley-III) respectively.

Results

Of the 42 children included, 19 were male (45.2%); median (interquartile range) age at neurological evaluation was 28 (25–32) months, and 36 (85.7%) had severe microcephaly. HINE and Bayley-III results were completed for 35/42 (83.3%) and 33/42 (78.5%) children respectively. Bayley-III identified a severe developmental delay in 32/33 (97.0%) children while 1/33 (3.0%) had only a mild delay. In the multivariable analysis, we found that Bayley-

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III and HINE scores were correlated. Better HINE scores were associated with higher Bayley-III cognitive raw scores (β = 0.29; CI 95% = 0.02–0.57) and motor raw scores (β = 0.43; CI 95% = 0.04–0.82) after adjusting for head circumference, prematurity, and age at neurodevelopmental evaluation. Furthermore, we found that greater head circumference at follow up was associated with higher cognitive (β = 1.27; CI 95% = 0.01–2.53) and motor raw scores (β = 2.03; CI 95% = 0.25–3.81).

Conclusion

Children with CZS-associated microcephaly demonstrate severe neurodevelopmental delays and slower growth rates than their peers over time. Still, they have remarkably heterogeneous neurodevelopmental profiles according to neurological exam scores which correlate with their long-term outcomes. We found that HINE scores effectively captured the heterogeneity of neurological capabilities among these children and could be predictive of cognitive and motor development progress.

Introduction

Congenital Zika Virus (ZIKV) infection is associated with severe neurological abnormalities such as microcephaly and central nervous system malformation [1]. Congenital Zika syndrome (CZS) refers to the presence of microcephaly or other congenital anomalies and alterations such as brain disruption sequence, ocular lesions, congenital contractures and neurodevelopmental impairments which have also been associated with congenital ZIKV infection [2, 3].

The clinical evolution and prognosis of children with CZS-associated microcephaly may vary significantly and has not yet been fully delineated after these children reach two years of age. Previous studies have found major neurodevelopmental delays of around 20 months in children less than 2 years of age with CZS-associated microcephaly, [4] although cases have also been identified in which children with CZS-associated microcephaly underwent approximately normal neurodevelopment [5–8], including one case report which describes a child showing normal neurodevelopment despite CZS-associated microcephaly (head circumference Z-score −2.4 SD) [9]. These heterogeneous findings suggest significant variation in developmental outcomes for children exposed to ZIKV *in utero*. Given that neurodevelopment is a dynamic process, it is important to track these children past two years of age in order to learn whether these differences persist over time. This heterogeneity has not been well described, which may, in part, be due to difficulties in effectively assessing developmental differences among children with severe disabilities [10]. Furthermore, there is little information about factors associated with this heterogeneity. This study aims to characterize the neurodevelopmental outcomes of children with CZS-associated microcephaly between 24–40 months of age.

Materials and methods

Study site and participants

We performed a prospective study of children from the Microcephaly Outpatient Clinic at the *Hospital Geral Roberto Santos* (HGRS) in Salvador, Bahia, Brazil. We enrolled children with a head circumference more than two standard deviations (<-2 SD) below average, according to the standards set by the International Fetal and Newborn Growth Consortium for the 21st

century (INTERGROWTH-21st), who were born during the peak of the ZIKV epidemic in Salvador between October 1st, 2015 and January 31th, 2016 at HGRS or nearby health centers and who received follow-up attention at the HGRS Microcephaly Outpatient Clinic.

A multidisciplinary team composed of physiotherapists, speech therapists, nurses, and physicians, including an ophthalmologist and a neurologist, collected information on mothers and their children based on interviews, medical records, and clinical evaluations. Sociodemographic statistics were obtained during the interviews at birth and at follow-up. All data were recorded and managed in REDCap (Research Electronic Data Capture).

Intrauterine ZIKV exposure

Congenital ZIKV exposure was defined using a Plaque Reduction Neutralization Test (PRNT50) for maternal serum samples. These serum samples were collected either at the child's birth or during the follow up evaluation. Serum samples were considered positive when anti-ZIKV neutralizing antibody titer was $\geq$ 1: 20 for ZIKV.

Clinical and developmental outcomes

INTERGROWTH-21st Fetal Growth Standards were used to evaluate children's head circumferences (HC) at birth and to adjust for gestational age and sex [11]. This was used to define microcephaly (<-2 SD) as inclusion criteria for this study. World Health Organization (WHO) parameters were used to evaluate the child's anthropometric growth during the follow up in the second year of life. The child's length and weight were also measured and adjusted by age and sex [12].

We performed neurological evaluations using the Hammersmith Infant Neurological Examination (HINE) [12]. HINE is a scorable clinical neurological exam comprised of three sections. The first section is the main section, and is a neurological assessment which includes 26 items (score range between 0–78) to evaluate cranial nerve function, posture, movements, tone, and reflexes. The second section is comprised of 8 items (score range between 0–26) and evaluates motor function by age relative to standard milestones in childhood development. The third section is composed of three items and evaluates child behavior with regard to consciousness or alertness, emotional state, and social orientation, (score range between 3–15). In children over 18 months of age, an optimal HINE score for the first section is $\geq$74 [12]. In children with neurodevelopmental impairments, a score <40 was associated with severe motor impairment while scores between 41 and 60 were associated with less severe motor impairment [13]. Previous studies in the literature focus on the first section, and so although we performed all three components, our subsequent analysis focused on the first section while the others were only used in our initial characterizations of the children.

We also used the Bayley Scales of Infant and Toddler Development, 3rd edition (Bayley-III) to evaluate cognitive, language (expressive and receptive), and motor (fine and gross) development of children in our cohort[14]. Bayley-III is a neurodevelopmental tool for children between 16 days and 42 months of age. For each scale, raw scores, age-corrected scores, percentile scores, and composite scores were obtained. Children were classified based on their composite scores as having severely delayed (composite score $\leq$70 [$\leq$-2 SD]), mildly delayed (71 to 85 [-2 SD to -1 SD]), or normal development (>85) [14–16].

Ophthalmologic evaluations were performed using standard techniques to identify and evaluate CZS-associated abnormalities. These included external ocular examination, functional ocular examinations, ocular biomicroscopy, and indirect ophthalmoscopy with pupillary dilation. The visual function assessment included the following items: visual acuity, sensitivity to light, ocular fixation, stability, object tracking, visual contact, social smile

response, facial responses to visual stimuli, and facial expression imitation. They were evaluated by an ophthalmologist and a determination of inadequate visual function was defined as children with one or more abnormal responses during the assessment. Auditory evaluations included behavioral hearing screening, development of auditory skills and cochleo-palpebral reflex which were performed using an adapted conditioned play audiometry test (Simonek hearing kit), designed to screen children up to 48 months of age [16]. This test evaluates reflexes, attention, location, and orientation in front of different auditory stimuli using objects with different frequency levels (from 38.2 to 95.1 decibels). Furthermore, brainstem evoked response audiometry (BERA) and transient evoked otoacoustic emissions (TEOAE) tests were performed. Cranial computed tomography scan (CT) was obtained during the first year of life at the HGRS.

Statistical analysis

Data analysis was performed using R Studio v3.6.1. Data were summarized using descriptive statistics. To evaluate the differences between head circumference Z-scores at birth and during the follow up, we used the paired samples Wilcoxon test. We used Spearman's rank correlation to evaluate the relationships between Bayley scales, age at follow up, and HINE scores. In the multivariable analysis, we excluded children with incomplete neurological and neurodevelopmental evaluations. Stepwise linear regression analyses were performed to calculate the association between each independent variable and neurodevelopment (Bayley scales). Results were considered statistically significant at $p < 0.05$.

Ethical aspects

The study was approved by the Institutional Review Boards of Yale University (1006006956) and the ethics committee of the Hospital Geral Roberto Santos–Bahia (1.866.918). Written informed consent was obtained from the parents of all studied patients.

Results

We included 42 children in our study, 29 of whom were born at HGRS and 13 which were born at another health center but followed at the HGRS Microcephaly Outpatient Clinic. Among them, 19/42 (45.2%) were male and 4/42 (9.5%) were born prematurely (<37 weeks of gestation), 2/42 (4.5%) born with 33 and 34 weeks (moderate preterm) and 2/42 (4.5%) born with 36 weeks (late preterm) and 19/28 (67.9%) presented with postneonatal epilepsy (Table 1). Sociodemographic characteristics of the mothers are summarized in the S1 Table. Median maternal age at delivery was 25 years (range 15–42), 20/37 (54.1%) self-declared as black, and 11/36 (30.6%) reported having less than nine years of schooling. ZIKV exposure was positive in 40/40 (100%) cases with available positive PRNT result (Table 1). We were not able to collect blood sample of two children, so laboratory results were not available. However, those two children were included in the study and followed because they had clinical indications of CZS (microcephaly and severe neurodevelopmental delays), neuroimaging findings characteristic of CZS, and no serologic evidence of other congenital infections (TORCHS). CT scans were available for 39/42 (92.6%) participants. Among them, the most frequent findings were ventriculomegaly 34/39 (87.2%), parenchymal 33/39 (84.6%) and subcortical 30/39 (76.9%) calcification, and simplified gyral pattern 31/39 (79.5%) (S2 Table).

The median age at anthropometric follow up evaluation was 28.2 months (IQR 23.9–32.9). At follow up, two children with a previous history of hydrocephalus had a HC Z-score between 0 and -2. Nineteen (45.2%) had a length Z-score less than -2, 18 (42.9%) had a weight Z-score less than -2, and 36 (85.7%) had a HC Z-score less than -3 (considered severe microcephaly)

Table 1. Demographic characteristics and clinical, neurodevelopmental, and laboratory outcomes of children with CZS-associated microcephaly.

Characteristics	Children with CZS- associated microcephaly	
	No of participants	n (%) or median (IQR)
Male, n (%)	42	19 (45.2)
Preterm, n (%)	42	4 (9.5)
CT scan abnormalities, n (%)	39	39 (100.0)
Postneonatal epilepsy, n (%)	28	19 (67.9)
Anthropometric growth		
Age in months, median (IQR)	42	28.2 (23.9–32.9)
Length score Z, median (IQR)	42	-1.5 (-3.3 –-0.5)
Weight score Z, median (IQR)	42	-1.3 (-3.1–0.2)
HC score Z, median (IQR)[a]	42	-6.4 (-7.5 –-3.9)
Neurological evaluation		
Age in months, median (IQR))	35	27.8 (25.1–31.6)
HINE neurologic scale, median (IQR)	35	25.0 (20.5–33.0)
Range score 0–78		
HINE motor scale, median (IQR)	35	2.0 (1.0–4.0)
Range score 0–26		
HINE behavior scale, median (IQR)	35	13.0 (11.3–15.0)
Range score 3–15		
Neurodevelopmental Evaluation		
Bayley-III summary[b]		
Age in months, median (IQR)	33	31.7 (29.4–37.5)
Cognitive scale percentile, median (IQR)	33	0.1 (0.1–0.1)
Language scale percentile, median (IQR)	33	0.05 (0.05–0.05)
Motor scale percentile, median (IQR)	33	0.05 (0.05–0.05)
Corrected age according Bayley raw scores		
Cognitive corrected age in months + days, median (IQR)	33	2.3 (1.3–4.0)
Receptive language corrected age in months + days, median (IQR)	33	4.3 (2.3–7.0)
Expressive language corrected age in months + days, median (IQR)	33	2.6 (2.0–5.0)
Fine motor corrected age in months + days, median (IQR)	33	0.6 (0.5–1.6)
Gross motor corrected age in months + days, median (IQR)	33	1.6 (0.5–3.0)
Ophthalmological evaluation		
Age in months, median (IQR)	28	29.2 (28.1–32.3)
Inadequate visual function, n (%)	28	25 (89.3)
Abnormalities at funduscopic eye exam, n (%)	26	15 (57.7)
Auditory evaluation		
Age in months, median (IQR)	34	27.9 (25.7–30.5)
Abnormal BERA	22	4(18.2)
Abnormal OEA	18	3(16.7)
Auditory response, n (%)		
No response	32	3 (9.4)
Doubtful reaction to stimuli or asymmetry of response	32	8 (25.0)
Reacts to stimuli from both sides	32	21 (65.6)
Absence of cochlear-palpebral reflex, n (%)	34	4 (11.8)
Abnormalities at Auditory behavior tests, n (%)	34	25 (73.5)
Abnormal Auditory development, n (%)	34	29 (85.3)
ZIKV laboratory result		
PRNT positive at birth or during the follow up	42	37 (88.1)

(*Continued*)

Table 1. (Continued)

Characteristics	Children with CZS- associated microcephaly	
	No of participants	n (%) or median (IQR)
PRNT positive result during pregnancy obtained at an outside laboratory not through the study	42	3 (7.4)
Unavailable	42	2 (4.5)

CT: computerized tomography; ZIKV: Zika virus; PRNT: Plaque reduction neutralization test; HINE: Hammersmith Infant Neurological Examination; Bayley-III: Bayley Scales of Infant and Toddler 3rd edition; TEOEA; transient evoked otoacoustic emissions BERA: brainstem electric response audiometry.

[a] Two children had a HC > -2 SD.

[b] Only one child had reached composite scores corresponding to mild delay (71 to 85 [-2 SD to -1 SD]) in the all-Bailey's Bayley's scales.

The number of participants reflects the availability of specific information.

Conversion factors: 1 month = 30.417 days and 0.1 months = 3.0417 days.

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(Table 1 and Fig 1). When we compared HC at birth [Z score of -3.8(IQR -4.5 –-2.7)] with HC at the follow-up [Z score of -6.4(IQR -7.4 –-3.9)], we found a significant decrease ($p < 0.001$) in HC (Fig 1).

All children had cerebral palsy, bilateral spasticity that compromised their movement, poor head control and hyperreflexia. This required all children to be transported in a manual wheelchair in all settings. All children were categorized by a neuropediatrician as Level V according to the Gross Motor Function Classification System. Among 35/42 (83.3%) children with HINE results, median age was 27.8 months (IQR 25.1–31.6). All children had low scores in the HINE neurological section and significant delays in achievement of motor milestones. The median HINE neurological section score was 25.0 (20.5–33.0) and median motor score was 2.0 (IQR 1.0–4.0). The mean score for the HINE behavioral section was much better at 13.0 (IQR 11.3–15.0) (Table 1 and Fig 2).

For the 33/42 (78.5%) children with a complete Bayley-III, median age was 31.7 months (IQR 29.4–37.5). In our evaluation of child development using Bayley scales, 32/33 (97.0%) had a severe developmental delay in all Bayley-III scales while 1/32 (3%) had only a mild delay in all Bayley-III scale evaluated (Table 1). Composite and raw scores did not show any trend when compared with the age at follow up (Fig 3).

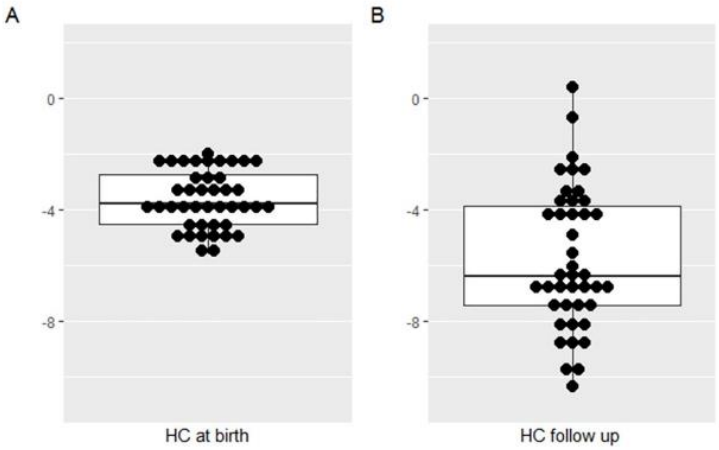

Fig 1. Head circumference of children with Congenital Zika Syndrome-associated microcephaly. Comparison of A) Z-score at birth (INTERGROWTH-21 parameters and B) Z-score at follow up (WHO parameters). The two children with HC Z-score >-2 SD at follow up both had a history of hydrocephalus.

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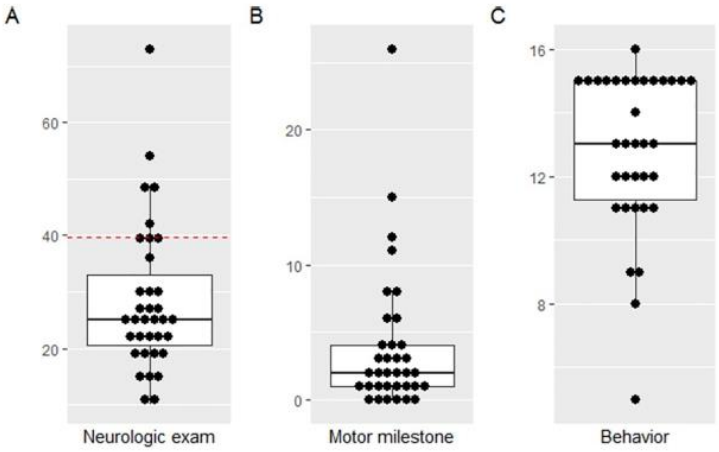

Fig 2. Hammersmith Infant Neurological Examination scores for children with Congenital Zika Syndrome associated microcephaly. A) The main section–neurological exam; B) The second section–motor milestone; and C) The third section—child behavior.

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When we compared developmental outcomes based on Bayley scales with the neurological manifestations summarized in the HINE neurological section, HINE scores were positively correlated with the Bayley cognitive ($R^2 = 0.14$; p = 0.032) and motor ($R^2 = 0.12$; p = 0.047)

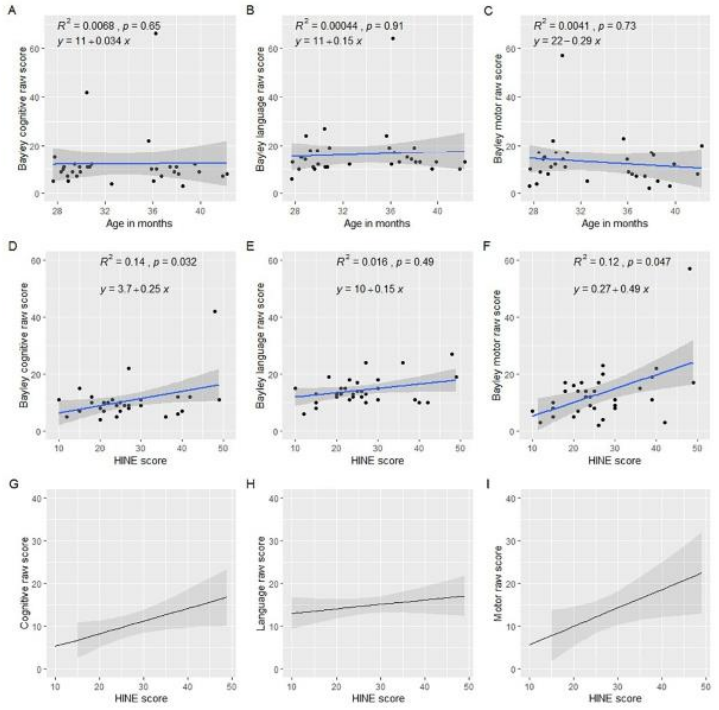

Fig 3. Neurodevelopment of children with Congenital Zika Syndrome-associated microcephaly. Raw Bayley scores over time (A, B, and C). Correlation of raw Bayley scores with the HINE neurological assessment (first section) score (D, E, and F). Linear regression model of the association between HINE score and Bayley raws scores adjusted by prematurity, child age and head circumference (G, H, and I).

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Table 2. Linear regression, factors associated with Bayley-III raw scores in children with microcephaly associated with the Congenital Zika Syndrome.

Factors	Bayley cognitive scale raw score			Bayley language scale raw score			Bayley motor scale raw score		
	β	CI	p	β	CI	P	β	CI	p
HINE neurological score	0.29	0.02 – 0.57	**0.035**	0.10	-0.10 – 0.31	0.311	0.43	0.04 – 0.82	**0.030**
Age at time of Bayley exam	-0.11	-0.68 – 0.45	0.681	-0.13	-0.56 – 0.29	0.526	-0.31	-1.11 – 0.49	0.427
Preterm	2.04	-6.05 – 10.14	0.607	0.79	-5.33 – 6.90	0.792	2.80	-8.65 – 14.25	0.618
HC Z-score	1.27	0.01 – 2.53	**0.048**	0.46	-0.49 – 1.41	0.326	2.03	0.25 – 3.81	**0.027**

β: standardized (regression) coefficients; HINE: Hammersmith Infant Neurological Examination.

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domains (Fig 3). Furthermore, head circumference Z-scores also were correlated with motor domain ($R^2 = 0.30$; p = 0.005) (S1 Fig). In total, the linear regression included 28/42 (66.7%) children. In this regression, we found that better neurological HINE scores were associated with better cognitive raw scores (β = 0.29; CI 95% = 0.02–0.57) and with improved motor raw scores (β = 0.43; CI 95% = 0.04–0.82) after adjusting for head circumference, prematurity and age at neurodevelopmental evaluation. Furthermore, we found that greater head circumference at follow up was associated with higher cognitive and motor raw scores (β = 1.27; CI 95% = 0.01–2.53) and (β = 2.03; CI 95% = 0.25–3.81) (Table 2 and Fig 3). Ophthalmological and auditory findings are summarized in Table 1 and CT scan findings are summarized in S2 Table.

Discussion

This is one of the few studies following children with CZS-associated microcephaly beyond two years of age. As expected, these children demonstrate severe language, cognitive, and motor delays as measured by the Bayley-III neurological exam, as well as severe neurological symptoms including bilateral spasticity that compromises their movement, posture, and balance. Among these children, however, there are important differences in their neurological and neurodevelopmental profiles that need to be understood.

Although widely used tools like the composite scores from the Bayley III exam are effective ways to characterize most children [14], we found that they have difficulties differentiating between children with CZS-associated microcephaly, due to the severity of developmental delays within this population [5]. Furthermore, as these children grow, and their gap relative to the standard reference groups for their age used by these tests increases, this effect may worsen. Thus, in order to effectively characterize differences among these children, alternative approaches may be needed.

To that end, we found that HINE scores and Bayley-III raw scores were able to capture the heterogeneity of neurological capabilities among children with CZS-associated microcephaly. Furthermore, we observed that HINE scores could be predictive of cognitive and motor developmental progress at the time of follow-up. A few studies have previously used HINE to evaluate infants with CZS-associated microcephaly, however ours is the first to associate HINE scores with the cognitive and motor development of these children [5, 7]. This is important because HINE is an early neurological examination tool, which is short and easy to perform, and which unlike Bayley does not require specific materials. Furthermore, HINE scores are already used to evaluate developmental and functional prognoses for other developmentally impaired children, such as those with cerebral palsy [13, 17]. While we found that composite scores from Bayley scales were useful in evaluating children without microcephaly and helped to understand the trends of their neurodevelopment [15, 16, 18], children with CZS-associated

microcephaly often had severe neurological alterations, including neurosensory impairment which complicated performing an evaluation as active as Bayley-III [2, 3, 7, 19–21].

In a 2011 paper, Jary et al. discuss the importance of using alternatives to the Bayley-II exam composite scores when evaluating children with severe impairments [10]. In particular, they highlight the ability of Bayley raw scores to demonstrate the heterogeneous development of severely developmentally delayed children. This effect held true in our cohort when using the more recent edition of the Bayley exam, Bayley III. In our study, all children were around the 0.05th percentile, and raw score analysis was necessary to observe the differences between children that was present in their HINE scores.

Head circumference is another easy measurement that we found was associated with neurodevelopment. In a study in Salvador of children with CZS-associated microcephaly, it was described that between follow ups at one and two years after birth, there was a positive correlation between HC and both cognitive and motor performance [5, 6]. In our study, when focusing only on children with HC more than 2SD below average and excluding one child with only mild global delay, we found that HC and HINE scores were positively correlated with cognitive and motor development. This underscores the value of following a simple protocol which includes both these measurements during evaluations of children born with potentially CZS-associated neurological impairments.

It is important to note that while reduced HC is a useful marker of potential neurological alterations due to CZS, children born with a normal head circumference should still undergo further evaluation [3]. Notably, in our study, two children with both a diagnosis of microcephaly and hydrocephaly had a normal head circumference at the time of follow up, and were not included in the head circumference analysis. This is consistent with previous work by Pereira H. et al., which describes nineteen children with a normal head circumference at birth and neuroimaging findings typical for CZS patients, of which fifteen developed postnatal microcephaly and only four maintained normal head circumference through the second year of life [3]. Pereira H et al. concluded that this phenomenon could be due to the total or partial resolution of ventriculomegaly [3]. It is likely that the two children with normal head circumference at follow up in our study will continue to develop slowly.

Our study has some relevant limitations. First, the number of participants in our cohort was small, which limited the study's statistical power. That said, this study represents a unique opportunity to understand a rare event in the form of microcephaly, which is a severe consequence of congenital Zika virus infection. Second, despite multiple attempts, it was difficult to complete all clinical assessments for all children, which may have affected the rate of defects identified in our study. This is primarily because the extensive number of assessments involved deterred some mothers and their children from persisting with evaluations. In order to combat this, the research team often provided families with transportation or performed home visits for these evaluations, however some assessments could not be performed outside of the hospital.

Another limitation of our study is the use of HINE exams in children older than 24 months. Previous studies had identified neurodevelopmental delays of approximately 20 months in children with CZS-associated microcephaly by age two, indicating that the HINE exam might still be an appropriate tool to use when evaluating them. This is consistent with our findings from the Bayley-III evaluations in which all children scored below the 0.05th percentile, corresponding to an adjusted relative age between 20 days to 4 months. A similar age correction was used by Lind A. et al. for preterm children (gestational age between 23–35 weeks) [22]. They performed HINE exams based on a corrected age of two years, equivalent to a time of 26 to 29 months after birth [22]. Furthermore, a study by Nielsen-Saines K. et al. performed HINE exams on children with previous intrauterine ZIKV exposure including children with

CSZ-associated microcephaly aged 7 to 32-months, indicating that it can still be used for older children [7]. Another reason to use the HINE exam is that, unlike the Bayley III exam, it does not require extensive tools or materials to perform, making it practical in resource-poor settings, as in our study site.

We were not able to relate the link between HINE and Bayley results to imaging results because CT scans were only available for the first year of life. Additionally, it would have been ideal to also perform MRIs in order to better identify migrational defects. Finally, we were limited to use of a cross-sectional study design which makes it difficult to establish a firmly causal association between the factors examined and the outcomes we explore in our study. Thus our findings are correlational instead, and further prospective studies that follow children with CZS-associated microcephaly during the growth are necessary.

Conclusions

We found that children with CZS-associated microcephaly experience major neurodevelopmental delays and severe neurological outcomes including spasticity which compromises their movement, posture, and balance. Still, there is significant variation among these children and they demonstrate heterogeneous development patterns. In order to better understand the differences between these children and to identify early interventions which will reduce the disease burden, it is first necessary to develop either new evaluative tools or standardized adjustments to existing ones, which reflect this heterogeneity and can be used to follow and evaluate the progression of the disease. Health professionals need easy, practical, and reliable tests which can predict the longer term outcomes of their patients, and can help them to design plans for their therapy and treatment.

Supporting information

S1 Data. Excel spreadsheet containing, in separate spreadsheets, the data used in tables and figures.
(XLSX)

S1 Table. Mother characteristics.
(DOCX)

S2 Table. Cranial computed tomography scans (CT) of children with Congenital Zika Syndrome associated microcephaly.
(DOCX)

S1 Fig. Head circumference of children with CZS associated microcephaly associated with neurodevelopmental and neurological outcomes. A) Cognitive Bayley III scale raw score, B) Language Bayley III scale raw score C) Motor Bayley III raw score and D) Hine neurological section score.
(TIF)

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11. CONSIDERAÇÕES FINAIS

Este estudo investigou a transmissão do vírus Zika em comunidades vulneráveis e os impactos clínicos e de desenvolvimento em crianças expostas durante a epidemia de 2015 em Salvador, Brasil. Os resultados destacam a importância de abordar os determinantes sociais da saúde para reduzir a ameaça da transmissão do ZIKV. Foi identificado que a falta de emprego formal e insegurança alimentar são fatores associados com o aumento da probabilidade da infecção. Tais desfechos podem auxiliar na identificação de áreas e indivíduos com maior risco de infecção em áreas urbanas e em comunidades informais urbanas. Ademais, essas informações podem ser combinadas com o conhecimento pré-existente sobre fatores ambientais e relacionados com o controle de vetores, que são importantes na transmissão de arbovírus, como ZIKV e DENV. Dessa forma, é possível criar intervenções de saúde pública que visem a redução das desigualdades sociais, além de promover o controle de vetores e outras intervenções ambientais.

O presente estudo também revelou que comportamentos sexuais de risco aumentam a probabilidade de infecção pelo ZIKV, especialmente entre homens que não usam preservativos, têm múltiplos parceiros sexuais ou se envolvem em relações sexuais casuais. Embora a transmissão sexual por si só não seja suficiente para sustentar a disseminação do vírus devido à curta duração da capacidade de infectar, é fundamental que clínicos e formuladores de políticas desenvolvam e promovam medidas para aconselhar os parceiros sexuais sobre como reduzir o risco de transmissão sexual do ZIKV. Entre estas atividades, é de extrema importância a promoção do uso de preservativos e outras estratégias preventivas para residentes e viajantes em áreas com transmissão contínua de ZIKV.

Em relação aos efeitos clínicos e de desenvolvimento em crianças sem microcefalia ou outra malformação congênita aparente associada ao ZIKV, o estudo demonstrou que a exposição intrauterina ao vírus está associada a um atraso leve no neurodesenvolvimento, especialmente na linguagem. Embora as sequelas mais leves no neurodesenvolvimento sejam difíceis de diagnosticar nos primeiros dias de vida, estas podem se tornar mais perceptíveis com o acompanhamento a longo prazo. No entanto, quanto mais cedo for feito o diagnóstico, mais oportuna pode ser a intervenção para limitar ou minimizar as sequelas relacionadas à infecção congênita do ZIKV. Consequentemente é fundamental realizar o acompanhamento das crianças expostas com e sem microcefalia durante as fases pré-escolar e escolar para identificar atrasos no neurodesenvolvimento em um estágio inicial e

implementar intervenções para alcançar resultados ideais, ou limitar a carga das sequelas. Em contraste, crianças com microcefalia associada à síndrome congênita do Zika apresentaram atrasos graves em todas as dimensões avaliadas, incluindo linguagem, cognição e motricidade, que estavam associadas à idade materna mais avançada e alterações neurológicas detectadas no exame físico precoce. Também é necessário que o sistema de saúde seja adapto e o pessoal de saúde capacitado para realizar avaliações com maior cuidado nas crianças com SCZ, com uma maior ênfase na avaliação neurológica e do neurodesenvolvimento. À medida que as alterações sejam detectadas e diagnosticadas, podem ser geradas estratégias para reabilitação e estimulação precoce, atividades necessárias para diminuir os efeitos das alterações na infância, que é o período importante para o desenvolvimento.

Finalmente, a coorte de Pau da Lima tem aproximadamente duas décadas de seguimento até o momento da escrita deste trabalho e vem elucidando diversas questões associadas aos fatores sociais e ambientais de doenças infecciosas emergentes. A coorte do HGRS é um dos poucos estudos que acompanharam crianças nascidas de mulheres expostas à infecção sintomática e assintomática pelo ZIKV. Embora limitados pelo contexto do inesperado surto do ZIKV e outras características intrínsecas ao método empregado, discutido nos artigos, ambas as coortes são alguns dos poucos estudos que conseguiram realizar o seguimento análise da transmissão do ZIKV e das sequelas associadas com a exposição intrauterina.

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Apêndice A. Autobiografia e recorrido durante a pós-graduação

Meu nome é Juan Pablo Aguilar Ticona. Sou médico boliviano e esta tese é o resultado da minha etapa como estudante de doutorado em saúde pública no Instituto de Saúde Coletiva da Universidade Federal da Bahia (ISC-UFBA) em Salvador, Brasil.

Concluí a faculdade de medicina em 2014 na Universidad Mayor de San Andrés em La Paz, Bolívia. Durante essa fase inicial da minha carreira, participei de projetos de saúde pública e tive um interesse particular em questões relacionadas à promoção da saúde e prevenção de doenças. Em seguida, de 2014 a 2015, trabalhei como coordenador clínico em um projeto de telemedicina (RAFT-Altiplano) para melhorar o acesso à saúde em comunidades isoladas na Bolívia. Em 2015, a Organização dos Estados Americanos (OEA) e a Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) me concederam uma bolsa de estudos para um mestrado em epidemiologia clínica na UFBA. No entanto, minha preferência me levou a buscar um segundo mestrado em Saúde Coletiva e nos últimos anos, de 2017 a 2023, cursei o doutorado em Saúde Pública no ISC-UFBA que tem como resultado esta tese. Durante esse período, tive a oportunidade de avaliar o impacto e a prevenção de doenças infecciosas ao fazer parte do grupo de pesquisa do Prof. Federico Costa. Além disso, durante o último semestre do doutorado, recebi uma bolsa para realizar uma parte do meu doutorado na Escola de Saúde Pública da Universidade de Yale, sob a orientação do professor Albert Ko, graças à bolsa de doutorado sanduíche da CAPES..

Como estudante de pós-graduação, meu objetivo foi entender a transmissão e o controle de doenças infecciosas, incluindo vírus emergentes como o Zika e, na última parte do doutorado, o SARS-CoV-2, em comunidades urbanas economicamente desfavorecidas. Tive a oportunidade de me capacitar no grupo de pesquisa composto por pesquisadores do ISC, Fiocruz e Yale. Junto a eles, adquiri habilidades epidemiológicas, bioestatísticas e sociológicas, e trabalhei principalmente com coleta de dados primários para estudos observacionais. Participei da avaliação das crianças nascidas durante a epidemia de Zika no Brasil, avaliando atrasos no desenvolvimento e sua associação com a exposição intrauterina ao Zika, o que foi descrito nesta tese. Também participei da avaliação dos soroinquéritos durante a pandemia de COVID-19 e planejei e coordenei o trabalho de campo de busca ativa de casos durante a onda Ômicron.

Sem um tratamento eficaz e antes da aprovação da vacina contra a COVID-19, a higiene das mãos e respiratória eram as principais estratégias para controlar a epidemia. No entanto, fatores de risco estruturais, como pobreza e superlotação, dificultaram sua implementação nesses ambientes e, como resultado, nossa equipe identificou taxas significativas de infecção nessas comunidades economicamente desfavorecidas. Portanto, após concluir meu treinamento de doutorado, gostaria de colaborar e conduzir atividades de intervenção para promover a higiene e, consequentemente, reduzir as taxas de doenças infecciosas, inicialmente como objetivo de curto e mediano prazo.

Apêndice B. Produção cientifica durante o doutorado

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