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Supplementary appendix 2

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Max R, Toval-Ruiz C, Becker-Dreps S, et al. Neurodevelopment in preschool children exposed and unexposed to Zika virus in utero in Nicaragua: a prospective cohort study. *Lancet Glob Health* 2024; **12:** e1129–38.

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Additional Methodological Detail on Enrollment, Exposure, and Outcome Measures

Participant enrollment and follow-up

In Leon, mothers were initially approached by telephone to explain the study aims and asked to receive a household visit by field staff to provide further information and obtain informed consent. If study staff were unable to contact them by phone, a household visit was performed. If the mother stated she no longer desired to be in the study, no further phone calls or visits were attempted. If the mother was not present during the household visit for MSL and ASQ assessment, at least 2 more attempts were made. Participants in Managua were recruited during visits to the health center by a physician collaborating in with this study and all visits occurred at the health center.

In Leon, phone contact was performed by the field coordinator and the visits were performed by the field staff that included the psychologist and nurse. Participants in Managua were recruited during the visits to the health center by a physician collaborating in with this study.

In Leon, informed consent was obtained during the household visit and it was obtained by the field staff (Psychologist or Nurse). In Managua, informed consent was obtained by the physician, nurses, or psychologists collaborating in this study.

MSEL Scores

Scores for individual domains and composite MSEL scores, as well as t- and z-scores for individual participants, were calculated based on the standard methodology for this instrument.

The MSEL instrument is proprietary and we are unfortunately not able to include the questionnaire itself with our study materials although its contents can be accessed at https://www.pearsonassessments.com/store/usassessments/en/Store/Professional-Assessments/Developmental-Early-Childhood/Mullen-Scales-of-Early-Learning/p/100000306.html?tab=product-details

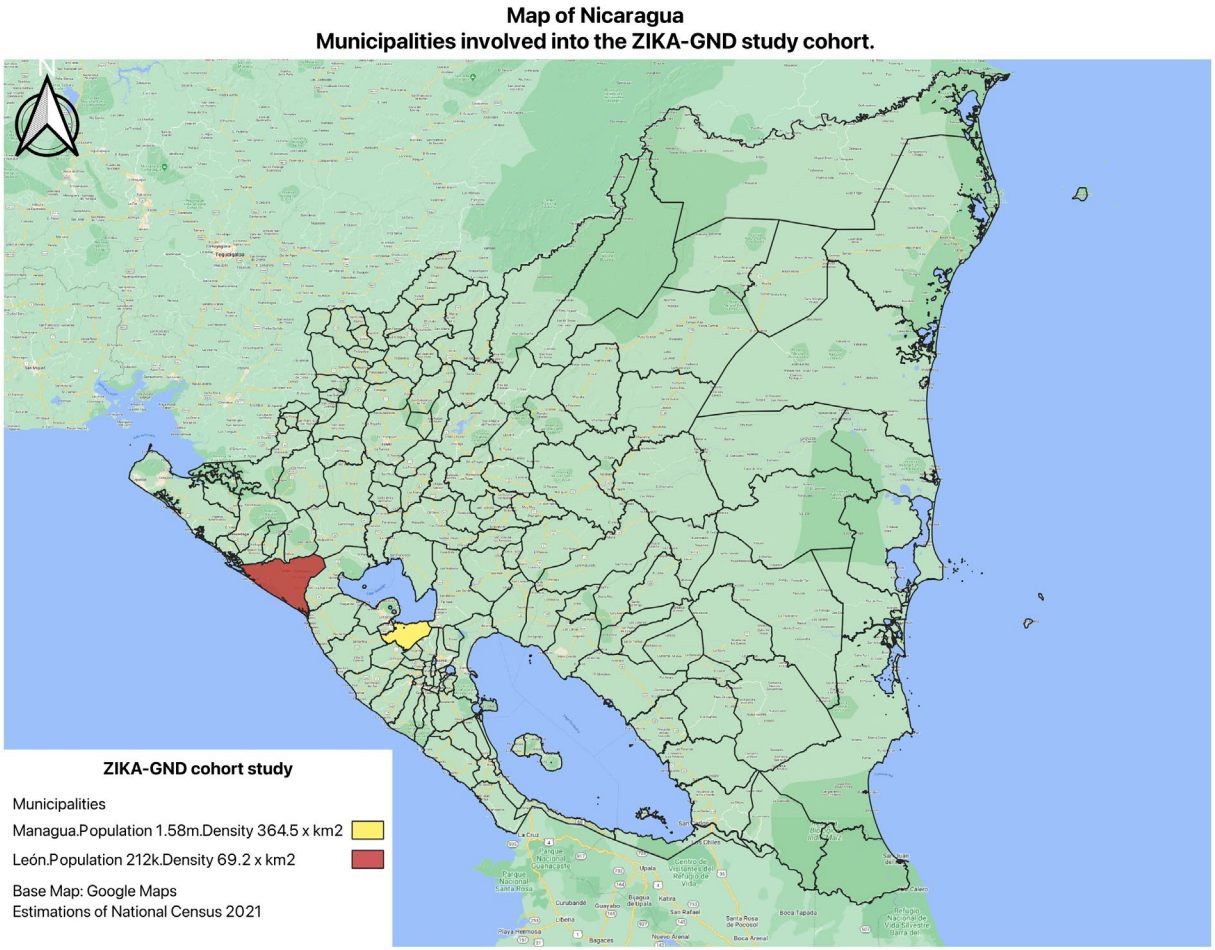
Poverty Index

The methods for calculating the CIDS are available in the referenced article (Pena, 2008)

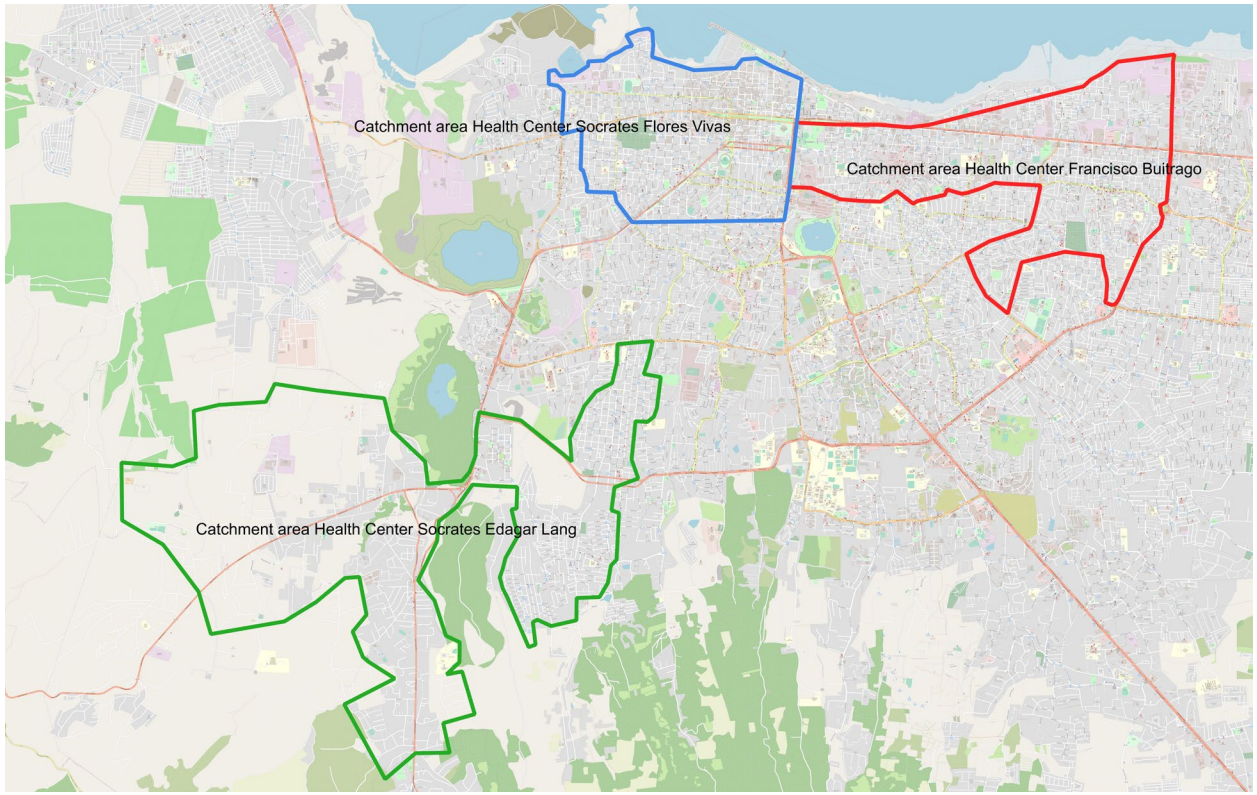
Table S1. Proportion of children with "very low" or "below average" scores by *in utero* ZIKV exposure status at 30, 36, and 48 months of age

	León Cohort				Managua Cohort			
	In utero ZIKV exposure (n=33)	No *in utero* ZIKV exposure (n=270)	OR (95% CI)	p-value	*In utero* ZIKV exposure (n=219)	No *in utero* ZIKV exposure (n=100)	OR (95% CI)	p-value
Early learning composite								
28.5- 31.5 months, (%)	6/29 (20.7%)	75/241 (31.1%)	0.29 (0.18-1.54)	0.29	-------	-------	-------	-------
34.5 – 37.5 months	9/33 (27.3%)	59/238 (24.8%)	1.14 (0.44-2.71)	0.83	35/204 (17.2%)	21/90 (23.3%)	0.68 (0.36-1.32)	0.28
46.5 – 49.5 months	12/30 (40.0%)	27/86 (31.4%)	1.45 (0.55-3.72)	0.50	65/219 (29.7%)	34/97 (35.1%)	0.78 (0.46-1.35)	0.41
Gross motor								
28.5- 31.5 months, (%)	2/29 (6.9%)	22/240 (9.2%)	0.73 (0.08-3.28)	>0.99	-------	-------	-------	-------
34.5 – 37.5 months	-------	-------	-------	-------	-------	-------	-------	-------
46.5 – 49.5 months	-------	-------	-------	-------	-------	-------	-------	-------
Fine motor								
28.5- 31.5 months, (%)	3/29 (10.3%)	50/241 (20.7%)	0.44 (0.08-1.53)	0.22	-------	-------	-------	-------
34.5 – 37.5 months	7/33 (21.2%)	35/238 (14.7%)	1.55 (0.53-4.06)	0.31	58/207 (28.0%)	33/91 (36.3%)	0.69 (0.39-1.20)	0.20
46.5 – 49.5 months	7/30 (23.3%)	13/86 (15.1%)	1.70 (0.51-5.27)	0.40	57/221 (25.8%)	29/99 (29.3%)	0.84 (0.48-1.48)	0.61
Visual reception								
28.5- 31.5 months, (%)	4/29 (13.8%)	33/241 (13.7%)	1.01 (0.24-3.20)	>0.99	-------	-------	-------	-------
34.5 – 37.5 months	11/33 (33.3%)	79/238 (33.2%)	1.01 (0.42-2.29)	>0.99	38/207 (18.3%)	24/90 (26.7%)	0.62 (0.33-1.17)	0.14
46.5 – 49.5 months	17/30 (56.7%)	34/86 (39.5%)	1.99 (0.79-5.08)	0.16	39/221 (17.6%)	16/99 (16.2%)	1.11 (0.57-2.26)	0.87
Expressive language								
28.5- 31.5 months, (%)	10/29 (34.5%)	76/241 (31.5%)	1.14 (0.45-2.73)	0.91	-------	-------	-------	-------
34.5 – 37.5 months	5/33 (15.2%)	25/238 (10.5%)	1.52 (0.42-4.50)	0.38	20/207 (9.7%)	6/91 (6.6%)	1.51 (0.56-4.77)	0.52
46.5 – 49.5 months	10/30 (33.3%)	25/86 (29.1%)	1.22 (0.44-3.20)	0.65	54/220 (24.5%)	23/98 (23.4%)	1.06 (0.59-1.95)	0.95
Receptive language								
28.5- 31.5 months, (%)	2/29 (6.9%)	47/241 (19.5%)	0.31 (0.03-1.30)	0.13	-------	-------	-------	-------
34.5 – 37.5 months	9/33 (27.3%)	52/238 (21.8%)	1.34 (0.52-3.21)	0.51	31/206 (15.1%)	16/91 (17.6%)	0.83 (0.41-1.73)	0.72
46.5 – 49.5 months	6/30 (20.0%)	17/86 (19.8%)	1.01 (0.29-3.11)	>0.99	65/219 (29.7%)	36/98 (36.7%)	0.73 (0.43-1.25)	0.26

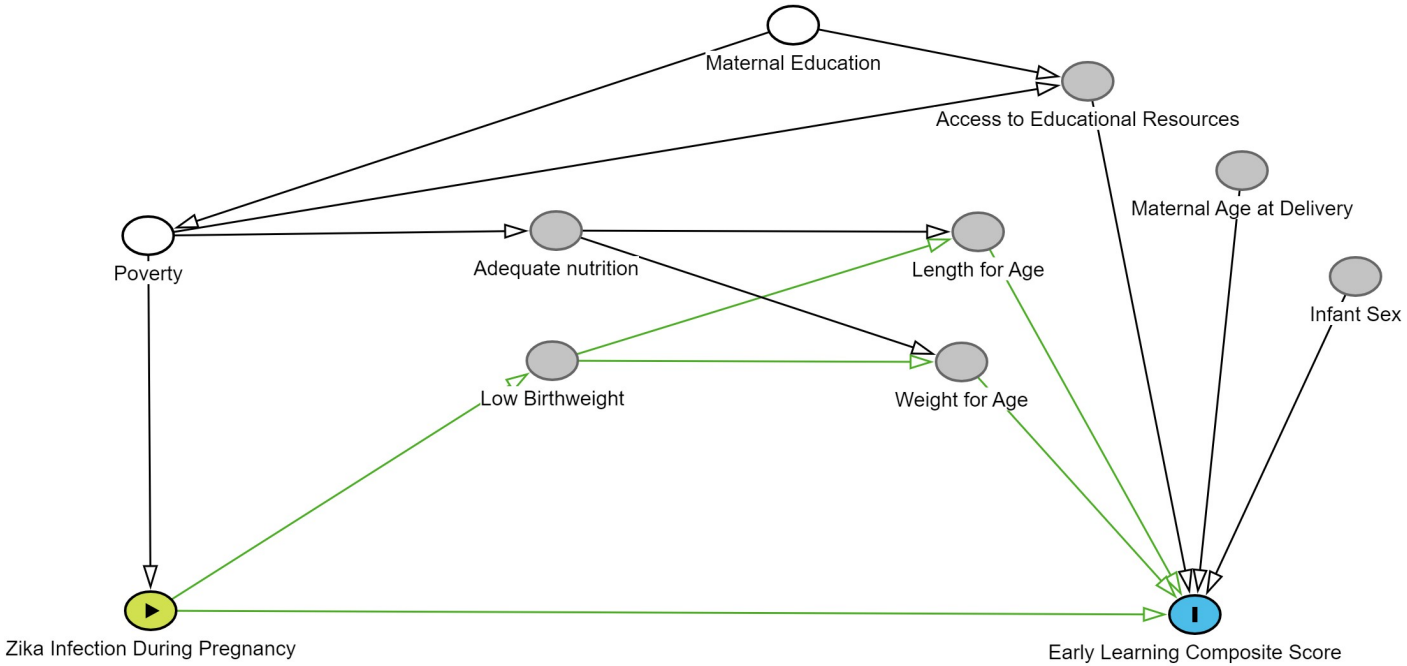
Leon and Managua Study Sites Map with Population Density



Managua Study Site Health Center Catchment Area



Directed Acyclic Graph (DAG) of Proposed Causal Diagram for Zika Exposure and Neurodevelopmental Scores



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SIGNATURE PAGE

The signature below documents the review and approval of this protocol and provides the necessary assurances that this study will be conducted according to the protocol, including all statements regarding confidentiality, and according to national, regional, and local legal and regulatory requirements.

_____

Site Principal Investigator Name (Print)

Signature

Date

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Protocol Summary

Protocol Title	**Understanding Maternal-Fetal Zika Virus Transmission and its Complications in Nicaragua**
Study Design	This study which will be performed in Leon, Nicaragua to understand the effect of in utero ZIKV exposure and infection on infant growth and neurodevelopment. We propose to follow a cohort of three groups of newborns: ZIKV infected, ZIKV exposed-uninfected and ZIKV unexposed.
Study Population	Infants born to pregnant women who participated in ongoing city-wide surveillance of antenatal and delivery blood specimens at one clinic in Leon and who were enrolled in a separate cohort study in Managua, Nicaragua

Number of subjects	Up to 730 infants born to women who participated in ongoing city-wide surveillance of antenatal and delivery blood specimens in Leon and another cohort in Managua
Number of sites	2; Leon and Managua, Nicaragua
Clinical Samples	*Blood samples*
Estimated Start of Enrollment	___ 2018___
Estimated Time to Completion	Five years
Protocol Duration	Infants will be followed from 18-24 months to 84 months of age

1.0 OBJECTIVE

It took less than a year for Zika virus (ZIKV) to sweep across large portions of Latin America and the Caribbean. In León, Nicaragua, where UNC has a long-standing research collaboration, there have been approximately 150 cases of local transmission, and the virus is expected to become epidemic over the next 12-24 months. Although ZIKV is not usually morbid among non-pregnant adults, it is now established that the virus can be transmitted *in utero* from mother to fetus and that the consequences of congenital infection can be severe. While the developmental deficits of vertically-acquired ZIKV are increasingly apparent, it is currently not known what risk (if any) attends infants who are exposed to the virus *in utero*, but are uninfected at birth. The rate of incident ZIKV infection in early childhood among infants unexposed during pregnancy is also unknown.

This investigation aims to **enroll a prospective cohort of infants in León, Nicaragua for a longitudinal study to evaluate the effect on infant growth and neurodevelopment of *in utero* ZIKV exposure, *in utero* ZIKV infection, and incident ZIKV infection and follow children for up to 84 months of age.** The cohort will be comprised of 3 groups of newborns: ZIKV infected, ZIKV exposed-uninfected, and ZIKV unexposed. They will be followed for up to 84 months and assessed with serial neurologic exams, development assessments (Mullen scales of early learning), hearing and eye exams, and parental interviews. We hypothesize that ZIKV-exposed infants who do not meet definitive criteria for vertical ZIKV infection will have higher rates of developmental delay than unexposed infants.

2.0 INTRODUCTION

2.1 Background and Significance

Since its initial detection in Brazil in early 2015, Zika virus (ZIKV) has spread rapidly throughout Latin America and the Caribbean, with nearly all countries in the region and several US states now reporting local viral transmission.[1] Evidence is now definitive[1-6] that ZIKV can be transmitted in utero from mother to fetus, resulting in fetal loss, intrauterine growth restriction, amniotic fluid disturbances, and/or various neurological anomalies –a constellation of findings now known as congenital Zika virus syndrome.[7-9] Microcephaly is the most dramatic and morbid birth defect attributed to ZIKV, prompting intense concern among health officials and the public because of its association with profound and irreversible developmental and cognitive deficits.[4,8,10-13] New data also show that affected infants may have sensorineural hearing loss, seizures, and other brain abnormalities, including subtle deficits that may not be recognized until months after birth.[14-17]

The rapidity with which Dengue (DENV) and Chikungunya Virus (CHIKV) have blanketed Central and South America has raised widespread concern that ZIKV will follow the same explosive pattern, resulting in a commensurate rise in microcephaly and possibly other birth defects. In response to evolving data, the US Centers for Disease Control and Prevention (CDC) and other public health agencies worldwide have taken the dramatic measure of recommending that pregnant women avoid travel to areas where ZIKV is circulating.[18] Officials in several affected countries have advocated that women defer pregnancy altogether.[19] CDC has been prompted to make such sweeping recommendations because so little is understood about ZIKV and the consequences can be so dire. More than a year after the first cases of microcephaly were reported in the Americas,

travel advisories remain in place for pregnant women and those planning to become pregnant.

Despite recent emerging data on perinatal transmission of ZIKV and some limited follow up of ZIKV-infected infants,[20] many knowledge gaps remain.[7,16] Our group has published key research priorities for ZIKV,[21] which include the need to: 1) reliably diagnose ZIKV infection in pregnancy, both in individual mother-infant pairs, and at the population level; 2) estimate the frequency of adverse outcomes associated with fetal ZIKV infection; 3) better understand the role that timing of infection with respect to gestational age plays in maternal-fetal ZIKV transmission and pathogenesis; and 4) identify risk factors for ZIKV transmission during pregnancy and risk factors for adverse outcomes when vertical transmission does occur. Our study will provide much needed data on exposed and unexposed infants.

In January 2018 the CDC published their first report of population-based birth defect surveillance of birth defects potentially associated with Zika virus infection[31] which compared birth outcomes in the first half of 2016 and the second half of 2016 in in 15 states and US territories with reported ZIKV transmission. They found a 21% increase in brain abnormalities, microcephaly, eye abnormalities and consequences of central nervous system dysfunction in areas with local ZIKV transmission in the second half of 2016 compared to the first half. There were no differences in neural tube defects.

CDC has also updated their interim guidance for US health care providers taking care of infants with possible congenital Zika virus infection. CDC decided to stratify infants born to mothers with possible Zika virus infection in pregnancy into three categories. 1) Those with clinical evidence of CZS 2) infants without clinical findings consistent with congenital Zika syndrome who were born to mothers with possible Zika virus infection 3) infants without clinical findings consistent with CZS born to mothers without evidence of Zika infection. CDC has broad recommendations for infants who may have been exposed to Zika in utero that mostly include evaluations of hearing and vision within the first three to six months of life as well as head ultrasound and ongoing assessments for growth and neurodevelopment if any disabilities are noted with appropriate referrals.[32] Specifically, CDC recommends that all neonates and infants with suspected ZIKV exposure in utero have their visual function assessed during routine well child examinations and if external eye abnormalities or abnormal visual function tests are detected that referral for further eye exams be conducted. [32] CDC also recommends that all infants who are suspected to be ZIKV exposure have auditory assessments with age-appropriate hearing screening which includes either auditory brainstem response (ABR) or otoacoustic emissions test. There is no evidence to support delayed onset hearing loss and so CDC recommends a baseline hearing test only if the baseline is normal.

Our understanding of the long-term effects of Zika exposure in pregnancy on newborns is in the primary stages because many exposed infants are only now reaching two years of age.

Available neurodevelopmental data can be extrapolated from infants born with CMV and microcephaly. Literature on the long-term outcomes of children with symptomatic congenital CMV infection is concerning. In one prospective cohort of 76 children followed up

to 13 years of age, 43% had some hearing loss, 27% had vision impairment and 43% had some level of intellectual disability. Microcephaly was significantly associated with all three of poor these outcomes.[33]

While cognitive outcomes are clearly affected in infants with microcephaly stemming from a variety of cause, outcomes range from mild to severe developmental delays depending on the degree of brain anomalies. Much less is known on the impact of ZIKV exposure on children without overt signs of CZS. Even minor hearing and vision deficits can further complicate child developmental outcomes and affect a child's ability to meet developmental milestones. Motor and cognitive impairments can also likely have long term impacts on social and behavioral outcomes of infants.

Our study seeks to better understand the impact of ZIKV exposure on neurodevelopmental outcomes with the goal of identifying the most high-risk children so that interventions can begin early.

2.2 Rationale

The World Health Organization recommends that infants who are diagnosed with congenital Zika virus syndrome be followed quarterly for 2 years.[32] The WHO is silent on clinical follow up for infants who were exposed to ZIKV in utero but do not show signs at birth. The diagnosis of ZIVK exposure and ZIKV infection in areas where DENV is co-circulating is not a simple matter; it is entirely plausible that some exposed infants are in fact infected but escape detection. The purpose of this study is to examine the effects of both exposure and infection on child outcomes. The clinic system in Leon is well suited for close follow up of infants delivered to mothers in our study, owing to a robust tracking system that is already in place, supported by the government. Each clinic keeps detailed registers of infants born in the community to track immunizations and well baby visits, and field workers are used to follow-up missed visits. We will follow infants participating in the cord blood surveillance exercise for up to 84 months of age.

3.0 STUDY DESIGN:

Enroll a prospective cohort of infants in León, Nicaragua and another cohort of infants identified retrospectively in Managua to evaluate the effect on infant growth and neurodevelopment of *in utero* ZIKV exposure, *in utero* ZIKV infection, and incident ZIKV infection among infants unexposed during pregnancy

Rationale for our approach

Epidemiological reports are now supported by clear biology, showing the neurotropic properties of ZIKV, leading the CDC and others to conclude that ZIKV causes fetal microcephaly, arthrogryposis, and other birth defects. New data also show that affected infants can have sensorineural hearing loss, seizures, and other brain abnormalities[15-17] and it is suspected that more subtle deficits including neurologic, ophthalmic and hearing problems may be identified in children without overt microcephaly or visible deficits at birth

during the first year of life.[51-54] Based on increasing evidence that ZIKV is neurotropic,[8] there is an urgent need to understand the long term effects of ZIKV on the infants through at least 12 months of follow up after birth.

Cohort procedures

In the ongoing surveillance, all infants have a physical exam at birth that includes basic anthropometry (head circumference) and makes note of any obvious morphologic abnormalities. The documentation of this care – like that of antenatal and delivery care – is already quite complete at HEODRA, but we have budgeted additional personnel to ensure completeness. Key aspects of pregnancy and delivery are also already being collected. These include characteristics of labor, mode of delivery, chorioamnionitis, gestational age at birth, birth weight, Apgar scores. All infants are assessed prior to discharge and no more than 72 hours after birth, if possible.

In the current proposal, after discharge from the hospital, infants will be brought to the health posts where their mother received antenatal care. Every week a list of enrolled mothers and newly delivered infants will be created and categorized according to where they live and which health post the mother attended for care. The visit schedule for all infants is listed below. (Table 1) At three months of age, all infants will have a neurodevelopmental evaluation with a Mullen developmental assessment. The Mullen assessment will be completed on each infant again every 3 months until 24 months. After 24 months, the Mullen will be performed every 12 months up to 68 months of age. An Ages and Stages assessment will be performed every three months up to five years of age. After 68 months of age, children will be evaluated with the Kaufman Assessment Battery (KABC-II) and the test of variables of attention (TOVA) yearly until the end of the study. All infants in the cohort will have blood spots collected for ZIKV PCR at every 3 months between delivery and 24 months; the PCR tests will be used to identify incident infections. After 24 months of age, the children will have blood collected every 6 months until 48 months of life and then yearly. A hearing assessment using the otoacoustic emissions technique at 6, 12, 18, and 24 months of age and a vision assessment will be conducted with a standard eye exam to evaluate tracking, nystagmus, or roving eye movements at 6 and 18 months on all infants. All infants will have a retinal examination by an ophthalmologist at 12 and 24 months of age. Mothers will also have their blood collected every year for ZIKV antibody decay and for full blood count.

In addition, GPS coordinates will be collected from in front of participants' homes using a Garmin unit. This will be done without added face to face contact with participants in order to avoid additional risks to the health of participants or study staff.

Referral for Infants with adverse outcomes

All ZIKV exposed infants with any neurologic, developmental, or physical signs of being affected by ZIKV (see definition below) will

also be screened for Cytomegalovirus, Toxoplasmosis, Rubella, Syphilis, and Herpes Virus and have a complete blood count, complete metabolic panel, and liver function tests performed in addition to the serial neurologic assessments and developmental assessments at the time the diagnosis is made. These infants will be referred to the pediatric group at HEODRA and continue to be followed in the cohort study. This procedure will also be followed for any infant or child wo is noted to have developmental delay for the duration of the study.

Training for neurodevelopment assessments

ASQ-3 and the MSEL will be performed by certified, trained psychologists or other appropriate health care providers. Psychologists in León have already been trained in administering the MSEL exam and will undergo a refresher course as part of a broader training course for health care providers from Managua. They will be trained on reliability in the examination procedures and in administering the Mullen evaluation in a 4-day, hands-on workshop for practitioners from both Managua and León. Examiner certification at sites will be obtained by the successful completion of two videotaped demonstrations of accurate performance and scoring of a 24-month-old infant. The trainers, including Dr. Boivin, will review videotapes throughout the study as needed for quality assurance and provide feedback/retraining as necessary to ensure high quality data collection. Additional training will occur later in the study for the KABC-II and TOVA

Statistical considerations

WHO Child Growth Standards (WCGS) for attained head circumference will be used to interpret the head circumference measurement for full term infants. For preterm neonates, Intergrowth-21 preterm postnatal growth standards[55] for attained head circumference will be used to interpret postnatal changes of head circumference until 64 weeks postmenstrual age. After this, WCGS for attained head circumference will be used to interpret the head circumference measurement. Microcephaly will be diagnosed if the neonatal head circumference is 3 or more standard deviations below the gestational-age-adjusted mean; we will make our calculations based on WHO nomograms.[56]

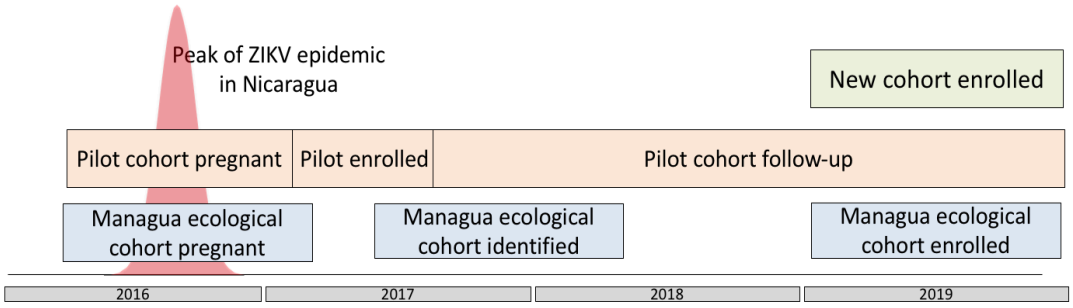
The **primary outcome** of this study will be the Early Learning Composite calculated from the average of 4 Mullen sub-domains at 12 months of age. This normalized score has a mean of 100 points and standard deviation (SD) of 15. We will also define a dichotomous outcome of neurodevelopmental impairment among children who meet any of the following criteria: (1) Hearing loss or deficit requiring amplification, (2) Motor delay on the Mullen scale defined as T-score more than two standard deviations below the mean, or (3) Cognitive delay based on T scores more than two standard deviations below the mean

We expect to enroll up to 730 exposed and unexposed infants at both sites. We also expect to enroll at least 15 of whom will be infected.[15]

4.0 SELECTION AND ENROLLMENT OF PARTICIPANTS

4.1 Inclusion Criteria

All infants born to women in the cord blood surveillance are eligible for the cohort. As stated above we will follow all infants from the cord blood surveillance as well as infants from another cohort who were identified in Managua. In Managua, categorization of ZIKV incident infections in pregnancy was based on the timing of the ZIKV epidemic. There is documentation from a large cohort study in Managua that the epidemic occurred only between June 15-September 30, 2016. Therefore, the Managua pregnancy cohort includes pregnant women who had an LMP prior to June 15, 2016, and delivered prior to March, 2017. NSI-BOB testing was performed in October, 2017 on all cohort members. An incident ZIKV infection was defined as those cohort women who had a positive NSI-BOB assay at the time of testing.



4.2 Study Enrollment Procedures

Infants will be enrolled in the cohort study after delivery before they are discharged from the hospital through a process by which the infant's mother will be consented.

Infants who are currently being followed in the existing cohorts will be enrolled into the long term follow up study.

All infants will be assessed every six months in the home or clinic for up to five years from enrollment. The baseline assessment will include a household survey, a maternal and infant survey, and the Family Care Indicators (FCI) survey which assesses home

stimulation. Assessments every six months will include infant height and weight, any updates on socioeconomic status and health status including seizure activity or dysphagia, and an Ages and Stages Questionnaire®-3 (ASQ-3) evaluation. [FIGURE 1]. A Mullen exam will be performed every six to twelve months through 60 months of age as described below. All infants will be assessed by a pediatrician or a pediatric health care provider yearly at which time an updated medical history and physical exam will occur. Physical exam findings of CZS include arthrogryposis, clubfeet with brain anomalies, hip dysplasia, and microcephaly will be noted.

All cohort members will undergo a hearing assessment using the otoacoustic emissions screening test between 24 to 30 months of age, a vision assessment with a standard eye exam to evaluate tracking, nystagmus, or roving eye movements during the same time period, and a retinal examination by an ophthalmologist. Eye exams will evaluate for coloboma, cataract, intraocular calcification, atrophy or scarring, optic nerve atrophy. If the hearing exam and eye exam are normal at the time of the first exam, no further exams will be performed as part of the study. If there are deficits noted at the initial exam, the infant will be followed every six to twelve months with repeated exams and referred to the appropriate specialist. Baseline hearing screen will be performed through otoacoustic emission testing.

Neurodevelopmental assessments will occur every six to twelve months using the AGES and STAGES developmental assessment (which is available in Spanish and currently in use for ongoing ZIKV infant follow-up studies in Managua and León) and the MSEL for neurodevelopment assessment.[34] We have chosen the MSEL because each assessment only takes 15 to 60 minutes depending on the child's age, can be used from birth to 68 months of age (for the cognitive scales), and can be administered by well-trained lay personnel. After 68 months of age, children will be evaluated with the Kaufman Assessment Battery (KABC-II) only.. Further, co-investigator Dr. Michael Boivin and colleagues have extensive experience administering these evaluations in low-income settings, including in non-English speaking populations. The sub-scales of the MSEL consist of: Gross-Motor (GM), Visual Reception (VR), Fine-Motor (FM), Receptive Language (RL), Expressive Language (EL), and Early Learning Composite (comprised of the sum of VR+FM+ RL+ EL). *T scores*, percentile ranks, and age equivalents can be computed for the five scales separately. Additional detailed socioeconomic status information, including maternal and paternal education and occupation, marital status, income level, and a detailed interim medical history will be updated at each visit.

Maternal follow up

The mothers of all infants will have updated medical history recorded once a year throughout the course of the study including repeat pregnancies. A depression screen will be performed at 24 months using the Johns Hopkins depression scale because maternal depression can affect infant outcomes. The Johns Hopkins depression scale has been validated in Latin and Central American countries.

5.0 CLINICAL AND LABORATORY EVALUATIONS

5.1Schedule of Events

Infant assessment	3mo	6mo	9mo	12mo	18mo	24mo	30mo	36mo	42mo	48mo	54mo	60mo	66mo	72mo	78mo	84mo
Consent	X															
Maternal health questionnaire	X			X	X	X	X	X	X	X	X	X	X	X	X	X
Infant health questionnaire	X			X	X	X	X	X	X	X	X	X	X	X	X	X
Infant/child exam						X		X		X		X		X		X
Household SES questionnaire	X					X				X						
Maternal depression screen						X										
Family Care Indicators (FCIs)	X					X				X						
MSEL	X	X	X	X	X	X	X	X		X		X				
ASQ-3	X	X	X	X	X	X	X	X	X	X	X	X				
KABC-II														X		X
TOVA														X		X
Baseline hearing assessment						X										
Baseline vision assessment						X										
Maternal blood draw						X		X		X		X		X		X
Infant blood draw						X	X	X	X	X		X		X		X
Blood storage						X	X	X	X	X		X		X		X

We will not be performing the TOVA but rather only the KABC-II

5.2 Entry Evaluations

Infants will be assessed per the visit schedule in section 5.1.

5.3 Documentation of ZIKV

Unexposed infants: Infants whose maternal blood that does not contain antibodies to ZIKV will be chacterized as unexposed.

We have defined maternal ZIKV infection status in León in the following ways:

Incident ZIKV infection: 1) Women who are ZIKV IgG positive and have high titers (FRNT>3000) at the time of birth were designated as infected in pregnancy. 2) The same designation was assigned if the FRNT50 value increased 4-fold between the early and late sample. 3) Any woman with a positive IgM in pregnancy was diagnosed with an incident infection

ZIKV Pre-immune: FRNT<3000 that remained stable over the course of the pregnancy were designated ZIKV immune, meaning the ZIKV infection likely happened prior to pregnancy.

ZIKV Naïve: 1) ZIKV IgG negative. 2) ZIKV IgG positive, but eFRNT <200. 3) ZIKV IgG positive, eFRNT ≥200, but FRNT50 <40 (negative).

ZIKV unknown timing: A portion of ZIKV-immune subjects did not readily fit readily into either category, mostly due to inadequate sample availability from early time points in pregnancy.

Infected infants: Infants whose cord blood was positive for ZIKV PCR or peripheral blood at 3 or 6 months of age was positive for ZIKV PCR and whose maternal blood contained antibodies to ZIKV.

Incident infections: Unexposed infants at delivery (ie, maternal blood does not contain antibodies to ZIKV) who develop ZIKV infection during study follow up (peripheral blood ≥3 months is positive for ZIKV PCR or ZIKV antibodies)

5.3.1 Clinical Assessments

At birth, each infant will have a complete physical exam performed. At each additional visit, the infant will have a developmental assessment and a neurologic assessment as outline in the visit schedule.

5.3.2 Laboratory Evaluations

All infants will undergo ZIKV testing every 3 months between 3 and 24 months and then blood drawn every six months until 48 months of age and then every year until the end of the study. Maternal blood will also be tested for the presence of ZIKV IgG and leftover blood will be stored for future studies.

Genetic polymorphisms can affect differences in metabolism of choline and folate. How pregnant women and infants metabolize choline and folate has been shown to have an important impact on child development. We would like to understand if this is a factor affects child development among the infants we already enrolled in our study. We will seek separate permission to run GWAS studies on individuals—mothers and children—enrolled in our study.

5.3.3 Procedures *(as required)*

For studies that require procedures, include them in the protocol where appropriate in the Schedule of Events.

All infants will have a hearing assessment, a vision assessment at 24 months. At one time during the study, the participants will have an ophthalmic examination by a trained ophthalmologist to look for eye changes that may be due to Zika infections. This will include the use of standard diagnostic eye drops.

6.0 ADVERSE EVENT REPORTING

6.1 Definitions

An adverse event (AE) is any untoward medical occurrence in a patient or clinical investigation participant administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

An unexpected (unlisted) adverse event is an adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g., Investigator's Brochure for an unapproved investigational product or package insert/summary of product characteristics for an approved product).

A serious adverse event (SAE) is any untoward medical occurrence that at any dose:

- results in death;
- is life-threatening;
- requires inpatient hospitalization or prolongation of existing hospitalization;
- results in a persistent or significant disability/incapacity;

or

- is a congenital anomaly/birth defect

Important medical events including adverse events and social harms as assessed by medical and scientific judgment may also be considered SAEs by the investigator and should be reported in an expedited fashion.

Unanticipated problems as defined by the UNC IRB:

Unanticipated problems involving risks to participants or others" or "Unanticipated Problem" (UP) refers to any incident, experience, or outcome that:

- is unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- is related or possibly related to a participant's participation in the research; and
- suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) related to the research than was previously known or recognized.

Unanticipated Problems that are serious adverse events should be reported to the UNC IRB within one (1) week of the investigator becoming aware of the event. Any other Unanticipated Problem should be reported to the UNC IRB within two (2) weeks of the investigator becoming aware of the problem. If the Unanticipated Problem Report cannot be completed in its entirety within the required time period, a preliminary report should be submitted. The Unanticipated Problem Report should be amended once the event is resolved and/or more information becomes available.

All individual AE Safety Reports shall be maintained by the Principal Investigator. For those events that are not reportable to the UNC IRB, a summary (i.e., not individual reports) of all adverse events that have occurred within the last approval period should be submitted to the UNC IRB at the time of continuing review.

Reports from a DSMB (Data and Safety Monitoring Board)/DMC (Data Monitoring Committee) or other independent safety monitoring group should be provided to the UNC IRB on a regular basis, generally at least as often as the study undergoes continuing review. Reports should include findings from adverse event reports and recommendations derived from data and safety monitoring.

7.0 STATISTICAL CONSIDERATIONS

WHO Child Growth Standards (WCGS) for attained head circumference will be used to interpret the head circumference measurement for full term infants. For preterm neonates, Intergrowth-21 preterm postnatal growth standards[55] for attained head circumference will be used to interpret postnatal changes of head circumference until 64 weeks postmenstrual age. After this, WCGS for attained head circumference will be used to interpret the head circumference measurement. Microcephaly will be diagnosed if the neonatal head circumference is 3 or more standard deviations below the gestational-age-adjusted mean; we will make our calculations based on WHO nomograms.[56]

The **primary outcome** of this study will be the Early Learning Composite calculated from the average of 4 Mullen sub-domains 84 months of age. This normalized score has a mean of 100 points and standard deviation (SD) of 15. We will also define a dichotomous outcome of neurodevelopmental impairment among children who meet any of the following criteria: (1) Hearing loss or deficit requiring amplification, (2) Motor delay on the Mullen scale defined as T-score more than two standard deviations below the mean, or (3) Cognitive delay based on T scores more than two standard deviations below the mean.

7.1 Endpoints

The **primary outcome** of this study will be the Early Learning Composite calculated from the average of 4 Mullen sub-domains at 84 months of age. This normalized score has a mean of 100 points and standard deviation (SD) of 15. We will also define a dichotomous outcome of neurodevelopmental impairment among children who meet any of the following criteria: (1) Hearing loss or deficit requiring amplification, (2) Motor delay on the Mullen scale defined as T-score more than two standard deviations below the mean, or (3) Cognitive delay based on T scores more than two standard deviations below the mean.

7.2 Randomization and Stratification

Patients will be enrolled in one of 3 groups based on their ZIKV status: ZIKV infected, ZIKV exposed-uninfected and ZIKV unexposed.

7.3 Sample Size and Accrual

We expect to follow up to 730 ZIKA exposed and unexposed infants.

7.5 Analyses

The **primary outcome** of this study will be the Early Learning Composite (a measure of cognitive function) calculated from the sum of the 4 MSEL sub-domains (Visual Reception, Fine Motor, Receptive Language and Expressive Language). This normalized score has a mean of 100 points and standard deviation (SD) of 15. We will compare the mean Mullen score between the group of exposed vs unexposed infants. We will also define a dichotomous outcome of neurodevelopmental impairment among children who meet any of the following criteria: (1) Hearing loss or deficit requiring amplification, (2) Motor delay on the MSEL Gross Motor scale defined as *T-score* more than two standard deviations below the mean.

Sample size calculation

As outlined above, we expect to have identified 210 infants with *in utero* ZIKV exposure.

Under these assumptions, our sample is large enough to detect relatively small differences in MSEL scores between groups (TABLE 2).

Table 2 Minimum detectable difference in Composite Mullen scores between

Comparison	n	Minimum detectable difference in scores
Exposed vs unexposed	210 vs 520	3.6

We will use a t-test for the comparison between ZIKV exposed and unexposed infants. In addition, we will describe baseline characteristics of mother-infant pairs in each of the exposure categories with medians and interquartile ranges for continuous variables and percentages for categorical variables. Baseline characteristics and those covariates that have been found in prior research to be associated with neurocognitive delay (e.g., birth asphyxia, chorioamnionitis, preterm birth) will be evaluated with univariate linear regression. Associations that meet a threshold of $p < 0.01$ will be included in a multivariable regression model with our 2-level nominal exposure variable (ZIKV unexposed, ZIKV exposed-uninfected).

8.0 STUDY MANAGEMENT

8.1 Institutional Review Board (IRB) Review and Informed Consent

This protocol and the informed consent document and any subsequent modifications (amendments) will be reviewed and approved by the IRB or ethics committee responsible for oversight of the study including UNC IRB and the local IRB.

It is expected that the IRB will have the proper representation and function in accordance with federally mandated regulations. The IRB should approve the consent form(s) and protocol.

In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s), and should adhere to Good Clinical Practice (GCP) and to ethical principles that have their origin in the Declaration of Helsinki.

Before recruitment and enrollment onto this study, the participant will be given a full explanation of the study and will be given the opportunity to review the consent form(s). Each consent form must include all the relevant elements currently required by the FDA Regulations and local or state regulations, which include elements such as the purpose of the study, the procedures to be followed, and the risks and benefits of participation. Once this essential information has been provided to the participant and the investigator is assured that the participant understands the implications of participating in the study, the participant will be asked to give consent to participate in the study by signing an IRB-approved consent form.

Prior to a participant's participation in the trial, the written informed consent form(s) should be signed and personally dated by the participant or the participant's legally authorized representative (legal guardian or person with power of attorney for participants who cannot consent for themselves), and by the person who conducted the informed consent discussion.

A copy of the consent form will be given to the participant or legal guardian, and this fact will be documented in the participant's record.

8.2 Adherence to the Protocol

Except for an emergency situation in which proper care for the protection, safety, and well-being of the study participant requires alternative treatment, the study shall be conducted exactly as described in the approved protocol. Any deviation from the protocol must have prior approval by the Principal Investigator and IRB, and must be recorded and explained.

8.3 Amendments to the Protocol

Should amendments to the protocol be required, the amendments will be originated and documented by the Principal Investigator at UNC. The written amendment will be sent to investigators and must be submitted to the IRB at the investigator's site for approval. It should also be noted that when an amendment to the protocol substantially alters the study design or the potential risk to the participant, a revised consent form might be required.

8.4 Record Retention

Study documentation includes all Case Report Forms, data correction forms or queries, source documents, Sponsor-Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed participant consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

Government agency regulations and directives require that all study documentation pertaining to the conduct of a clinical trial must be retained by the study investigator. In the case of a study with a drug seeking regulatory approval and marketing, these documents shall

be retained for at least two years after the last approval of marketing application in an International Conference on Harmonization (ICH) region. In all other cases, study documents should be kept on file until three years after the completion and final study report of this investigational study.

8.5 Obligations of Investigators

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki. The Principal Investigator is responsible for personally overseeing the treatment of all study participants. The Principal Investigator must assure that all study site personnel, including co-investigators and other study staff members, adhere to the study protocol and all FDA/GCP regulations and guidelines regarding clinical trials both during and after study completion.

The Principal Investigator at each institution or site will be responsible for assuring that all the required data will be collected and entered onto the Case Report Forms (CRFs). Periodically, monitoring visits will be conducted and the Principal Investigator will provide access to his/her original records to permit verification of proper entry of data. Site monitors will visit participating sites to review the individual participant records, including consent forms, CRFs, supporting data, laboratory specimen records, and medical records (physicians' progress notes, nurses' notes, individuals' hospital charts), to ensure protection of study participants, compliance with the protocol, and accuracy and completeness of records. The monitors also will inspect sites' regulatory files to ensure that regulatory requirements are being followed and sites' pharmacies to review product storage and management. At the completion of the study, all case report forms will be reviewed by the Principal Investigator and will require his/her final signature to verify the accuracy of the data.

The site investigator will make study documents (e.g., consent forms, drug distribution forms, CRFs) and pertinent hospital or clinic records readily available for inspection by the local IRB, the site monitors, the FDA, the NIAID, the OHRP, and the pharmaceutical supporter or designee for confirmation of the study data.

8.6 Participant Confidentiality

All laboratory specimens, evaluation forms, reports, and other records that leave the site will be identified by coded number only to maintain participant confidentiality. All records will be kept locked. All computer entry and networking programs will be done with coded numbers only. Clinical information will not be released without written permission of the participant, except as necessary for monitoring by the ACTU, IRB, the FDA, the NIAID, the OHRP, or the pharmaceutical supporters or designee.

8.7 Study Discontinuation

The study may be discontinued at any time by the ACTU, IRB, the NIAID, the pharmaceutical supporters, the FDA, the OHRP, or other government agencies as part of their duties to ensure that research participants are protected.

9.0 BIOHAZARD CONTAINMENT

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood and shipping and handling of all specimens for this study, as currently recommended by the Centers for Disease Control and Prevention and the NIH.

All dangerous goods materials, including diagnostic specimens and infectious substances, must be transported using packaging mandated by CFR 42 Part 72. Please refer to instructions detailed in the International Air Transport Association (IATA) Dangerous Goods Regulations.

10.0 ETHICS AND REGULATORY CONSIDERATIONS

Ethical Standards

The procedures set forth in this study protocol are designed to ensure that the site investigators abide by International Council on Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Good Clinical Practice (GCP) guidelines, and the US FDA regulations as specified in 21 CFR parts 50, 56, and 312 in the conduct, evaluation, and documentation of this study

This study will be carried out in accordance with the principles set forth in The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research of the US National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR Part 46 and/or the ICH E6; 62 Federal Regulations 25691 (1997) and US 21 Code of Federal Regulations dealing with clinical studies (including parts 50 and 56 concerning informed consent and IRB regulations).

Site Investigators agree, when signing the protocol, to adhere to the instructions and procedures described in it and thereby to adhere to the principles of GCP that it conforms to.
The investigator will ensure that the study will be conducted in accordance with all applicable national, regional, and local regulations.

This study will request a waiver of informed consent, consistent with CFR Title 45 part 46.116(d). The study does not involve direct interaction with human subjects. The medical records of patients admitted to the hospital will be screened and data collected from those records according this protocol. The patients will not be approached to obtain information, no intervention is being tested or specimens are being collected. The sample

of CRE isolates will be collected. Therefore, the study should be considered no more than minimal risk, and the waiver will not adversely affect the rights and welfare of the patients observed.

10.1 Data Confidentiality

Each participating site will maintain appropriate medical and research records for this study, in compliance with ICH E6, Section 4.9 and regulatory and institutional requirements for the protection of confidentiality of participants. The site investigator will prepare and maintain complete and accurate study documentation in compliance with GCP standards and applicable federal, state, and local laws, rules and regulations; and, for each participant participating in the study, promptly complete all eCRFs and such other reports as required by this protocol following completion or termination of the clinical study or as otherwise required.

By signing the protocol, the site investigator acknowledges that, within legal and regulatory restrictions and institutional and ethical considerations, study documentation will be promptly and fully disclosed to the sponsor or their representative by the site investigator upon request and also shall be made available at the site investigator's site upon request for inspection, copying, review and audit at reasonable times by representatives of the sponsor or by responsible government agencies as required by law

PROTOCOLO

Versión 2.0

Investigadores Principales:

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1. **Título:** Impacto De La Transmisión Materno-Fetal Del Virus Zika Y Sus Complicaciones En Nicaragua

2. **Proyectos Relacionados.**
   - Estudio de Efectos del Virus Zika en el Embarazo (ZPO, por sus siglas in inglés)

3. **Naturaleza y Propósito**

   Antecedentes.

Desde su detección inicial en Brasil a inicios del 2015, el ZIKV se ha diseminado rápidamente a través de Latinoamérica y el Caribe, con casi todos los países en la región y varios estados de los EEUU reportando ahora transmisión viral local. La evidencia es ahora definitiva que el ZIKV puede ser transmitido in útero, resultando en pérdida fetal, restricción del crecimiento intrauterino, disturbios en el líquido amniótico, y/o varias anomalías neurológicas- una constelación de hallazgos conocido como síndrome congénito del virus Zika. La microcefalia es el defecto más dramático y mórbido del nacimiento atribuido al ZIKV, provocando intensa preocupación entre autoridades de salud y la población debido a su asociación con déficits cognitivos profundos e irreversibles. Nuevos datos también muestran que los niños afectados podrían tener pérdida auditiva sensorineural, convulsiones y otras anormalidades del cerebro, incluidos déficits sutiles que no pueden ser reconocidos hasta meses después del nacimiento.

La rapidez con que el virus dengue (DENV) y virus Chikungunya (CHIKV) ha cubierto Centro y Sudamérica ha incrementado la preocupación que el ZIKV siga el mismo patrón explosivo, resultando en un conmensurado incremento en Microcefalia y otros defectos del nacimiento. En respuesta a cómo evolucionan los datos el Centro para la Prevención y Control de Enfermedades (CDC) y otras agencias de salud pública en el mundo entero han tomado la medida dramática de recomendar que las mujeres embarazadas eviten viajar a áreas donde el ZIKV está circulando. Autoridades de Salud Pública en varios países afectados han abogado que las mujeres posterguen sus embarazos.

El CDC ha estado solicitando hacer esas dramáticas recomendaciones porque se conoce poco a cerca del ZIKV y las consecuencias pueden ser muy graves. Después de un año después que los primeros casos de Microcefalia fueron reportados en las Américas, los avisos de viaje permanecen en su lugar para las mujeres embarazadas y aquellas planeando a quedar embarazadas. (me parece repetitivo estos dos párrafos)

A pesar de datos recientes emergentes sobre la transmisión perinatal del ZIKV y algún seguimiento limitado de niños infectados con el ZIKV, existen vacíos en el conocimiento. Nuestro equipo ha identificado prioridades de investigación para el ZIKV, que incluye la necesidad de: 1) Diagnosticar de manera confiable la infección por ZIKV en el embarazo en el binomio madre-hijo y al nivel poblacional; 2) Estimar la frecuencia de eventos adversos asociados con la infección fetal al ZIKV; 3) Entender mejor el rol del tiempo al momento de la infección con respecto a la edad gestacional en la transmisión materno-fetal del ZIKV y en la patogénesis y; 4) Identificar factores de riesgo para la transmisión del ZIKV durante el embarazo y para eventos adversos cuando ocurre la transmisión vertical. El estudio proveerá información necesaria en infantes expuesto y no expuestos.

En enero del 2018 el CDC publicó su primer reporte de vigilancia de defectos del nacimiento potencialmente asociados a infección por ZIKV basados en la población que comparó efectos en el nacimiento en la primera mitad del 2016 y a segunda mitad de 2016 en 15 estados y territorios de EEUU con transmisión al ZIKV reportada. Ellos encontraron un incremento del 21% en anormalidades del cerebro, microcefalia, anormalidades oculares consecuencias en la disfunción en el sistema nervioso central en áreas con transmisión local al ZIKV en la segunda mitad del 2016 comparado a la primera mitad. No hubo diferencias en defectos del tubo neural.

El CDC ha actualizado su guía interina para proveedores del sistema de salud de EEUU que tienen a su cuido infantes con posible infección congénita al ZIKV. El CDC decidió estratificar a los infantes nacidos de madres con posible infección al virus en el embarazo en tres categorías: 1) Aquellos con evidencia de Síndrome Congénito por Zika (SCZ) 2) Infantes sin hallazgos clínicos consistentes con síndrome congénito por Zika que nacieron de madres con posible infección al virus Zika, 3) Infantes sin hallazgos clínicos consistentes con SCZ nacidos de madres sin evidencia de infección por Zika. El CDC tiene amplias recomendaciones para los infantes que han sido expuestos al Zika in útero que mayormente incluyen evaluaciones auditivas o visuales en los primeros tres a seis meses de vida, así como ultrasonido cefálico y evaluaciones de seguimiento para el crecimiento y neurodesarrollo si alguna discapacidad es observada que incluye referencias apropiadas. Específicamente, CDC recomienda que todos los neonatos e infantes con exposición sospechosa al ZIKV in útero tengan su función visual evaluada durante examinaciones pediátricas de rutina y si hay anormalidades oculares externas y exámenes de función visual anormales se realice referencia para más exámenes visuales. El CDC también recomienda que todos los infantes que son sospechosos de exposición al ZIKV tengan evaluaciones auditivas con tamizajes auditivos apropiados para la edad que incluye: Respuesta Auditiva del Tronco Cerebral (ABR, por sus siglas en inglés) o prueba de emisiones otoacústicas. No hay evidencia que apoye la pérdida de audición de inicio tardío y por lo tanto el CDC recomienda una prueba de audición como línea base.

Nuestro entendimiento de los efectos a largo plazo de la exposición del virus Zika en el embarazo en los recién nacidos está en los estadíos primarios, ya que, muchos infantes expuestos están alcanzando hasta ahora los dos años de edad.

Los datos de neurodesarrollo disponibles pueden ser extrapolados de infantes nacidos con Citomegalovirus (CMV) y microcefalia. La literatura disponible de los efectos a largo plazo de niños con infección congénita por CMV sintomático es de referencia. En una cohorte prospectiva de 76 niños seguidos hasta los 13 años de edad, 43% presentaron algo de pérdida auditiva, 27% discapacidad visual y 43% algún nivel de discapacidad intelectual. La microcefalia estaba significativamente asociada con estos 3 efectos.

Mientras que los efectos cognitivos están claramente evidentes en infantes con microcefalia proveniente de una variedad de causas, los efectos de retraso en el desarrollo varían de leves a severo dependiendo en el grado de anormalidad cerebral. Mucho menos es conocido en el impacto de exposición al ZIKV sobre los niños sin signos evidentes de SCZ.

Inclusive déficits auditivos y visuales menores pueden complicar más los efectos en el desarrollo del niño y afectar la habilidad del infante de alcanzar los hitos del desarrollo. Las deficiencias motoras y cognitivas pueden tener probablemente impacto en el largo plazo en los resultados sociales y de conducta de los niños.

Nuestro estudio busca entender mejor el impacto de la exposición al ZIKV en los efectos del neurodesarrollo con el objetivo de identificar los niños en mayor riesgo de tal modo que las intervenciones puedan iniciar temprano.

Nicaragua detectó su primer caso en enero de 2016 y habia una epidemia fuerte entre junio y septiembre 2016. Es enteramente desconocido si los sitios afectados por Zika experimentarán una segunda ola Zika, y dada la incidencia y el tamaño de la epidemia en Nicaragua, esta es una gran incertidumbre y muchos en el sector de la salud no esperan tener otra ola en el futuro. Con el fin

de aprovechar esta oportunidad única de aprender más sobre la infección por el virus Zika en mujeres embarazadas en una población de Zika-naïve, buscamos a hacer un seguimiento de largo plazo a los niños de mujeres embarazadas durante la epidemia.

Objetivo del estudio:

Evaluar los efectos en el crecimiento infantil y neurodesarrollo de la exposición in útero al virus Zika (ZIKV) en una cohorte de niños en Managua, Nicaragua a través de un estudio longitudinal (damos seguimiento a los niños hasta que tengan 7 años). Los niños serán evaluados con exámenes neurológicos, evaluaciones de desarrollo (Escala Mullen de aprendizaje temprano), exámenes auditivos y oftalmológicos básicos y entrevistas a los padres. La hipótesis es que los niños expuestos al ZIKV tendrán tasas más altas de retraso en el desarrollo que infantes no expuestos.

Instituciones Colaboradoras:

Ministerio de Salud de Nicaragua (MINSA)

El Ministerio de Salud de Nicaragua, a través del SILAIS Managua, será un colaborador clave para asegurar el éxito del estudio. Las entidades del MINSA involucradas en este estudio son:

Centro de Salud Sócrates Flores Vivas, Centro de Salud Edgard Lang y Centro de Salud Francisco Buitrago. Los tres centros de salud han colaborado con el ICS en otros estudios de Zika. El ingreso y las visitas del estudio tendrán lugar en estos Centros de Salud.

El Laboratorio Nacional de Virología, Centro Nacional de Diagnóstico y Referencia, Ministerio de Salud, Managua, si sea necesaria, realizará todas las pruebas serológicas para el diagnóstico de Zika, procesará las muestras colectadas y se encargará de su almacenamiento como parte del estudio propuesto.

El Hospital Infantil Manuel de Jesús Rivera (HIMJR), Managua, es el hospital pediátrico de referencia nacional. Es la institución de mayor resolución en el país. Los casos que ameriten valoración neurológica serán referidos al HIMJR

Instituto de Ciencias Sostenibles (ICS)

Es un organismo sin fines de lucro en Nicaragua que ha estado trabajando en colaboración con la Universidad de California en Berkeley (UCB) y con Dra. Eva Harris desde su incorporación en 2004. El ICS será responsable de proveer el personal del estudio y

supervisar la recolección de datos y procedimientos del estudio en Nicaragua mediante visitas a los Centros de Salud. EL ICS en convenio con el MINSA realizará este estudio.

Universidad de Carolina de Norte (UNC – por sus siglas en ingles)

Es una universidad publica en los estados unidos con una historia largo de investigaciones de salud. La investigador pricipal, Dra. Elizabeth Stringer esta basada en UNC en el departamento de Obstetricia y Ginecología como obstetra/ginocologa y profesora. Dra. Stringer formó su socio con ICS en 2018 para colaborar en este estudio.

4. Sujetos

Población del Estudio

Todos los participantes serán residentes de Nicaragua. No tendrá lugar discriminación en términos de raza o etnia.

Participarán las madres y sus niños que tengan 2-3 años de edad al momento de inscribirse en el estudio. Todos estaban participantes en el Estudio del efecto de Zika en embarazo en Nicaragua (codigo # NIC-MINSA/CNDR CIRE-13/11/17-091.ver1.) o en el Estudio de Mujeres Embarazadas Positivas al Virus Zika. Todas las mujeres eran embarazadas durante la epidemia de Zika en 2016. Como parte del estudio anterior, realizamos serología para conocer cuales eran expuesto al virus de Zika.

Algunos participantes y sus familias pueden pertenecer a tres categorías de poblaciones vulnerables: los iletrados, los pobres y los que tienen baja escolaridad.

El número máximo de participantes será de 1200 (hasta 600 binomio madre/infante).

5. Reclutamiento

Se está finalizando un estudio denominado "Estudios de Efectos del Zika en el embarazo (ZPO), siguiendo binomio madre/niños y el Estudio de Mujeres Embarazdas Positivas al Virus Zika (Estudio NZP) finalizó en 2019. La población del estudio será extraída de la población del estudio ZPO y del Estudio NZP. Habrá 2 opciones para el reclutamiento del estudio: 1) Cuando la madre/niño acuda o sea contactado para su última visita del estudio ZPO se le preguntará si le gustaría participar en este nuevo estudio 2) Personal de reclutamiento visitará al participante en su casa y le preguntará si le gustaría conocer más sobre este nuevo estudio.

Materiales para el reclutamiento

Debido a que la población del estudio será extraída de la población de un estudio previo el reclutamiento será hecho verbalmente (en persona o vía telefónica) por personal calificado del estudio. Se usará una guía de reclutamiento para contactar a las participantes.

6. Tamizaje

Los participantes serán extraídos de dos estudios previos. Los criterios de inclusión para este estudio son los siguientes: 1) Ser participante del Estudio de Efectos del Virus Zika en el Embarazo (ZPO, por sus siglas en inglés o haber participado en el Estudio NZP) 2) Estar de acuerdo en acudir a todas las visitas del estudio planificadas durante 5 años y 3) Participante no tiene planes de mudarse fuera del área de estudio por los próximos 5 años.

7. Procedimientos del estudio

Se realizará un total de 7 visitas del estudio (una visita para inscribirse y 6 visitas anuales). Habrá 5 llamadas telefónicas del estudio adicionales entre cada visita del estudio. En el caso que una participante responda su teléfono para las llamadas telefónicas, se puede hacer una visita al domicilio y la visita de llamada puede ser completada en persona. En algunos casos la visita de inscripción al estudio y la primera visita anual pueden ser combinadas en la misma visita. Personal médico y de enfermería realizará estas visitas.

Ingreso

Completar las preguntas de tamizaje del estudio, formulario de consentimiento e instrumento de recolección de datos personales. El personal del estudio capacitado, completará la información de consentimiento e ingreso en aplicaciones en tabletas o celulares en la unidad de salud.

Visitas Anuales del Estudio (Meses 24, 36, 48, 60, 72, 84)

Se realizarán los siguientes procedimientos a los participantes.

Ver apéndice A para detalles del horario.

Batería de Evaluación de Kauffman II, es una medida de la habilidad cognitiva y los conocimientos académicos para sujetos entre 2 ½ y 12 ½ años. Consta de dos grandes escalas: una de procesamiento mental, que incluye las escalas de procesamiento secuencial y simultáneo, y otra de conocimientos académicos.

El Examen de Variables de Atención (TOVA, por sus siglas en inglés) es una prueba sistematizada que sirve para apoyar el diagnóstico y evaluar la respuesta al tratamiento del Trastorno por Déficit de Atención e Hiperactividad (TDAH). También se puede valorar la hiperactividad e impulsividad, así como lesiones cerebrales por traumatismos y otros trastornos de la conducta.

La Batería de Evaluación de Kauffman II y el Examen de Variables de Atención serán realizados por psicólogo entrenado.

La Escala Mullen de Aprendizaje Temprano sirve con el propósito de evaluar la habilidad motora y cognitiva. Las cinco escalas - motora gruesa, recepción visual, motora fina, lenguaje expresivo y lenguaje receptivo- son usadas para identificar debilidades y fortalezas en niños. El examen de Mullen es usado para evaluar desarrollo intelectual y preparación para la escuela. Esta prueba va a ser realizado por personal de enfermería entrenado.

El Examen oftalmológico básico/Oftalmoscopía indirecta (será realizada solamente en la primera o segunda visita del estudio si el niño no tuvo un examen oftalmológico realizado en la visita final del estudio ZPO (CPHS-2017-05-9943).

El Tamizaje auditivo de Emisiones Otoacústicas (será realizado solamente en la primera o segunda visita del estudio si el niño no tiene un tamizaje realizado durante la visita final del estudio ZPO (CPHS 2017-05-9943) La prueba de emisiones otoacústicas será realizado por personal de enfermería entrenado.

Llamadas telefónicas del estudio (horario laboral)

Ver apéndice A para detalles del horario.

El Cuestionario de Tamizaje de Edades y Etapas-3® (ASQ-3) contiene preguntas sobre las siguientes áreas del desarrollo: 1) comunicación, mide las habilidades verbales del niño 2) motora gruesa, explora como el niño utiliza los brazos, piernas y otros músculos para sentarse, caminar o correr 3) motora-fina, mide la coordinación ojos-manos y manipulación de objetos pequeños 4) resolución de problemas, explora como el niño soluciona problemas y juega 5) personal-social, explora la capacidad del niño para ayudarse a sí mismo y de interacción con las demás personas. Llamadas telefónicas serán realizadas por personal de enfermería o personal encuestador entrenado para ello.

El período de ventana para completar las visitas anuales del estudio y las llamadas telefónicas será un mes antes o después de la edad indicada del niño para la visita (Edad para visita del estudio +/- 1 mes). En casos donde la visita o la llamada no pueda ser completada dentro del período de ventana, será aceptado hacer la visita o la llamada hasta 4 meses antes o después de la edad indicada para la visita (Edad de la visita del estudio +/- meses).

Todas las visitas del estudio, incluyendo el ingreso del estudio tendrán lugar en el Centro de Salud o en el hogar del niño. El lugar de preferencia para la visita del estudio es el Centro de Salud, pero si la madre/niño no puede atender una visita en el centro de salud se realizará un intento para completar la visita en el hogar del participante, realizándose por personal del estudio.

Las llamadas telefónicas del estudio pueden ser reemplazadas por una visita en persona al hogar si el participante no se logra localizar vía telefónica.

El ingreso al estudio tomará aproximadamente 20-30 minutos.

Todas las otras visitas del estudio durarán entre 1.5-4 horas

Llamadas del estudio durarán alrededor de una hora

Apéndice A: Tabla de Procedimientos del Estudio

	Enrolamiento	Ingreso	Llamada 1	Visita 2	Llamada 2	Visita 3	Llamada 3	Visita 4	Llamada 4	Visita 5	Llamada 5	Visita 6
Evaluación		**24 Mes**	**30 Mes**	**36 Mes**	**42 Mes**	**48 Mes**	**54 Mes**	**60 Mes**	**66 Mes**	**72 Mes**	**78 Mes**	**84 Mes**
Firma Carta Consentimiento	X											
Formulario Datos del Participante	X											
Cuestionario Demográfico		X										
Cuestionario Salud Materna		X	X	X	X	X	X	X	X	X	X	X

Cuestionario Salud Infantil		X	X	X	X	X	X	X	X	X	X	X
Examen físico Infante/Niño		X		X		X		X		X		X
Cuestionario ESE del Hogar		X	X	X	X	X	X	X	X	X	X	X
Tamizaje de Depresión Materna		X		X		X		X		X		X
Indicadores del Cuidado de la Familia (FCIs)		X		X		X		X		X		X
Escala Mullen de Aprendizaje Temprano (MSEL)		X		X		X		X				
Tamizaje Edades y Etapas 3 (ASQ-3)			X		X		X		X			
Batería de Evaluación de Kaufman para niños (KABC-II)										X		X
Examen de Variables de Atención (TOVA)										X		X
Evaluación auditiva de base		X		X*								
Evaluación visual de base		X		X*								
Muestra de sangre materna		X		X		X		X		X		X
Muestra de sangre Infante		X		X		X		X		X		X

*Solo si no estaba completado a la visita de 24 meses

8. Beneficios

Los niños en el estudio podrían beneficiarse de examinaciones adicionales que podrían identificar retraso en el desarrollo más temprano que si no estuvieran participando en el estudio. Las madres podrían beneficiarse en el caso que tuvieran depresión identificada y poder ser referidas a médicos locales o psicólogos. Un examen de BHC se realizará anualmente para revisar el estado general del niño.

9. Riesgos y Malestar

Riesgos/Malestar psicológico: algunas de las preguntas de las entrevistas pueden causar malestar ya que ellas abordan temas sensibles del estado socioeconómico, depresión materna, etc. Se permite a los participantes negarse a contestar cualquier pregunta que los haga sentir inconfortable.

Muestreo de sangre: hay un riesgo de hematoma, sangrado y/o infección en el sitio donde si introduce la aguja. Técnicos y enfermeras entrenadas, utilizando técnicas con asepsia extraerán la muestra de sangre para minimizar los riesgos.

Hay un pequeño riesgo de incomodidad con los exámenes/pruebas de audición y oftalmológica. Personal entrenado realizará estos procedimientos para minimizar los riesgos.

Violación de la confidencialidad: hay siempre riesgo que la información pudiera verse comprometida.

10. Confidencialidad

Todos los datos del participante serán etiquetados con un código del estudio y la información clínica será mantenida en bases de datos sin identificadores personales. La base de datos separada protegida por contraseña vinculando el código del estudio con identificadores personales será accesible solamente a coordinadores del estudio y personal clave.

Los médicos, enfermeras, otro personal del Centro de Salud y personal de laboratorio que trabaja en el estudio tendrán acceso a identificación personal identificable en expedientes clínicos tan necesario para proveer cuidado médico y seguimiento en los centros de salud. Aparte de este personal clínico, solamente los administradores de datos y personal de entrada de datos, Coordinadores de Sitio y de Estudio y el Investigador Principal tendrán acceso a información identificable. Mientras que el nombre de los participantes aparecerá en los formularios clínicos,

que son los documentos fuente y parte de los registros médicos de los participantes, nombres y otra información identificable no estará presente en las bases de datos del estudio donde la información clínica, de laboratorio y demográfica colectada de los sujetos será almacenada para propósitos de investigación.

Los datos que son requeridos para ser compartidos para condiciones de salud notificables puede ser compartido con el Ministerio de Salud local, pero toda la otra información del paciente será mantenida confidencialmente como sea posible por el investigador y el personal del estudio.

Registros en gabinetes en llevados y los resultados de los exámenes serán codificados para prevenir asociación con los nombres de los participantes. Datos digitados en los archivos computarizados serán accesibles por personal autorizado directamente envuelto con el estudio (protegido por contraseña) y serán codificadas.

Registros del estudio (Consentimiento y copias físicas de los formularios de recolección de datos) serán retenidos en un lugar seguro para revisión o consultas en el centro de salud o en la oficina central. Registros no incluidos como parte del expediente médico del paciente serán destruidos por trituración 7 años después de concluido el estudio para todos aquellos que no dieron permiso para uso futuro.

Una vez que el consentimiento informado es obtenido, al participante le será otorgado un único número que está codificado. La llave para este código y el número único del participante será mantenida en una base de datos del estudio que está encriptado y protegida por contraseña. La llave identificadora por si misma será por si misma protegida por contraseña y encriptado antes que sea almacenada en una base de datos del estudio. Solamente personal clave del estudio y administradores de datos tendrán acceso a la base de datos donde ambos el número único del participante y los datos identificables son almacenados juntos. Cuando sea posible, datos codificados serán compartidos en lugar de datos identificables.

La llave para los datos codificados será destruida 7 años después de terminado es estudio para todos aquellos que no concedieron permiso para uso futuro. Los datos serán almacenados indefinidamente para aquellos que dieron permiso para uso futuro.

Los datos identificables pueden ser transmitidos vía email o internet. En estos casos datos serán encriptados previo a cargarlos o transferirlos. Todas las contraseñas encriptadas cumplen la (CPHS, por sus siglas en inglés) definición de una contraseña segura. Ninguna información identificable es almacenada en la nube. Cuando se transporte las muestras biológicas tendrán solamente código del participante en lugar de datos identificables.

Información específica del participante podría ser proveída a otro personal médico apropiado solamente con el permiso del participante del estudio. Todos los exámenes físicos tendrán lugar en cuartos de examen privados. Entrevistas/cuestionarios serán conducidos en cuartos de examen privados cuando sea posible, o en el lugar más privado como sea posible, en contacto cercano con el entrevistador para asegurar privacidad.

11. Consentimiento Informado

A los posibles participantes, les será leída una carta de consentimiento informado escrita en español por personal entrenado en este proceso. El entrevistador solicitará una firma en la carta de consentimiento al posible participante después de asegurarse que él/ella entiende la naturaleza del estudio. A las participantes maternas que son iletrados, se les solicitará de marcar su firma con la huella digital del pulgar, y la carta será firmado por un testigo, tal y como es requerido por la ley nicaragüense y las normas de buena práctica clínica para las personas iletradas. En caso que la posible participante materna sea menor de 18 años, el padre/tutor legal deberá estar en el momento de solicitud del consentimiento informado y firmar la carta de consentimiento si está de acuerdo con la participación en el estudio. El entrevistador también firmará la carta de consentimiento como un representante del estudio. Una copia firmada de la carta de consentimiento servirá como copia de la información describiendo los procedimientos del estudio, riesgos, beneficios e información de contacto y será entregada al participante del estudio.

12. Compensación y costos

Un pequeño regalo (valuado en aproximadamente US $6-$10) será proveído como una muestra de agradecimiento por su tiempo por participar al momento del ingreso. Un pequeño regalo adicional (valuado en aproximadamente US $6-$10) será proveído en las visitas anuales.

Este regalo muestra nuestro aprecio por su participación sin ser coercitivos. En Nicaragua el salario mínimo varía de $125-$275 dependiendo en el área de trabajo. Como tal el valor del regalo (valuado en $6-$10) es equivale aproximadamente a 1-1.5 días de salario mínimo.

El proyecto de investigación será responsable de todos los costos que se originen de la participación en el estudio exceptuando el cuidado médico de rutina. No habrá costos a los sujetos por los procedimientos médicos que sean parte del estudio. Los sujetos serán responsables por los costos de transporte de/hacia el Centro de Salud. Muchos participantes caminan o usan un vehículo personal. El costo del pasaje de bus (transporte público) cuesta aproximadamente US $0.10 centavos - $0.20 centavos. Si una familia presenta dificultades financieras extremas para presentarse a las visitas, un vehículo del estudio puede ser usado para transportar a los participantes o un pequeño estipendio de transporte (~2$) para pagar por un taxi para que se presenten a la visita.

13. Eventos Adversos

Todos los participantes recibirán atención médica adecuada en Nicaragua. Si un evento adverso no serio (alguna infección como resultado de toma de muestra de sangre) ocurre como resultado

de cualquier procedimiento del estudio, el participante será tratado en el Centro de Salud del estudio sin ningún costo a él. Cualquier compensación por el evento adverso seguirá la política de UCB. Si un evento adverso serio (que resulte en hospitalización) ocurre, el sujeto será transferido por personal del estudio a un Hospital del Ministerio de Salud en Managua, donde la atención es proveída sin costo alguno. Cualquier compensación por el evento adverso serio seguirá la política de la UCB.

En el caso de cualquier evento adverso relacionado a la participación en el estudio, el personal del estudio notificará al Comité Protección de Sujetos Humanos (CPHS, por sus siglas en Inglés). Un reporte inicial será hecho por teléfono o correo al Director de CPHS tan pronto como sea posible, pero no más de 1 semana (7 días calendario) de que el Investigador Principal tenga conocimiento del incidente. El reporte inicial será seguido por un reporte formal escrito dentro de no más de dos semanas (14 días calendario) de que el Investigador Principal tenga conocimiento del Incidente. Igualmente será notificado el Comité Institucional de Revisión Ética del CNDR.

Firma:

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