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Title:

Simultaneous exposure to both Zika virus and household insecticides during pregnancy, and fetal growth and infant developmental behavior outcomes at 18 months, in Guadeloupe

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Authors' contributions

Study conception: GM, SC, BH, LMu, PQ, CC

Data collection, quality control: MK, LMi, BT

Data analysis: MK, CC

Writing – original draft: MK, GM, SC, LMu, PQ, CC

Writing – review editing: MK, GM, SC, LMi, BH, BT, LMu, PQ, CC

All authors have read and approved the final manuscript.

Abstract 300**Background**

Perinatal infection with Zika virus (ZIKV) could result in adverse growth, developmental and behavioral outcomes, while insecticides used to control mosquitoes are neurotoxic.

Objectives

We aim to study the role played by exposure during pregnancy to both ZIKV and household insecticides in newborn health, development and behavior at age of 18 months.

Methods

Maternal and cord blood samples from a cohort of pregnant women (created during Guadeloupe's Zika epidemic of 2016) were used to identify ZIKV infection during pregnancy. A self-administered questionnaire at birth documented prenatal household use of insecticides. Birth weight and head circumference were collected from maternity records (n=708). Infant development and behaviors were documented at 18 months of age through the Ages and Stages Questionnaire and the Quebec Longitudinal Study of Child Development (n=409). Logistic and linear regression models were performed, taking into account confounding factors.

Results

Use of household insecticides was associated with smaller head circumference and lower birth weight among newborns from mothers not exposed to ZIKV: -0.3 cm (95% CI: -0.6, 0) and -82 g (95% CI: -165, 0), respectively. Similar decreases were observed with ZIKV exposure among mothers not reporting household insecticides use, and with presence of both exposures. The combined presence of ZIKV exposure and insecticide use was associated with lower ASQ fine motor scores (-3.9; 95% CI: -7.3, -0.4), and higher hyperactivity scores (0.8; 95% CI: 0.0, 1.5), compared to no exposure to either. A higher opposition score was observed in association with ZIKV exposure among non-users of insecticide (0.6; 95% CI: 0.0, 1.2).

Conclusion

Adverse neurodevelopmental outcomes at 18 months of age were observed with prenatal ZIKV exposure, and with higher magnitude when mothers reported use of household insecticides. At birth, rates of adverse fetal growth were however similar for the combined presence of exposure and either of the exposures.

Key words: Zika, household insecticide exposure, fetal growth, infancy, development, pregnancy

Introduction

Zika virus (ZIKV) is a mosquito-borne flavivirus first identified in Uganda in 1952 (Dick et al. 1952). Three cases of human ZIKV infection were described in Nigeria in 1954 (Macnamara 1954), and the first ZIKV outbreaks occurred in the Pacific islands in 2007, then in French Polynesia in 2013 (Cao-Lormeau et al. 2014; Duffy et al. 2009; Musso 2015). The latest outbreak of the virus emerged in Brazil in 2016, before spreading to South America and the Caribbean. This epidemic raised concern because it was timely correlated to a rise in birth defects and thus became a growing public health concern (Heymann et al. 2016). The main mode of ZIKV transmission is through the *Aedes* mosquito, with the other modes being sexual or mother-child transmission, and blood transfusion (Brasil et al. 2016; D'Ortenzio et al. 2016; Driggers et al. 2016; Foy et al. 2011; McCarthy 2016; Musso 2015). The frequency of pauci or asymptomatic clinical forms following ZIKV infection is high, at around 70 to 80% (HCSP 2015). Therefore, about 20% of infected adults will develop either mild symptoms or more severe clinical forms (Guillain-Barré syndrome, meningo-encephalitis, myelitis, and ophthalmologic abnormalities) that are common with other arboviruses such as dengue or Chikungunya (Araujo et al. 2016; Cao-Lormeau et al. 2016; Petersen et al. 2016). ZIKV is however capable of crossing the placenta, and is the only arbovirus linked to congenital malformations in humans. An estimate of 4 to 9% of women having had a prenatal ZIKV infection result in a suspected congenital ZIKV syndrome (CZS) including brain and ocular malformations, microcephaly, congenital deafness, limb contractures, and arthrogryposis (Adams et al. 2016; Krauer et al. 2017; Melo et al. 2016; Oliveira Melo et al. 2016; Pomar et al. 2019; Rasmussen et al. 2016; Valentine et al. 2016; van der Linden et al. 2016). In the fetus, the principal targets are neural progenitor cells (NPC) and glial cells in the developing brain. The consequences are cell death and reduced NPC proliferation, which results in impaired brain development (Wen et al. 2017).

A ZIKV epidemic began in Guadeloupe (French West Indies, FWI) in April 2016, reaching its peak during the first week of June. In total, it lasted 22 weeks, with more than 30,500 clinically suggestive cases (SpF 2017) in a population of 390,253, including 639 cases biologically confirmed in pregnant women by Reverse Transcription Polymerase Chain Reaction (RT-PCR). Among these women, in January 2017, 8 microcephaly and 5 other fetal brain malformations had been detected on ultrasound (SpF 2017).

To reduce the risk of ZIKV exposure, the local vector control plan against arboviruses includes a social mobilization and community participation component in which people are recommended: (i) to get rid of any water tank, container or plant trays (ARS Guadeloupe 2018), (ii) to use repellents, especially on uncovered body parts; to sleep at night under an insecticide-treated mosquito net if possible; to wear light, full-cover clothing – and in the event of significant risk, clothing impregnated with insecticides (SpF 2019). Household insecticides (such as sprays, diffusers and spirals) are also regularly used in households, as suggested by a representative survey conducted in Guadeloupe in 2015 (February to July), which reported that 84% of households had used insecticides in the past 12

months (ANSES 2021). Most of these products contain insecticide molecules designed to target the insect's nervous system. The families of insecticides most widely used in household products throughout the world are pyrethroids, neonicotinoids, organophosphates, and carbamates. Most of them can act by modifying acetylcholinesterase, GABA receptors, sodium channels, glutamate-dependent chloride channels or the nicotinic acetylcholine receptor; most of these biological mechanisms are also present in mammals and humans (INSERM 2013). In humans, neurological effects are well-known for most insecticides following high or repeated exposure, generally in circumstances of acute intoxication (attempted suicide, poisoning) or professional use (Kreiger and Krieger 2001). Numerous epidemiological studies have also suggested that exposure to insecticides, whether via domestic products, those used in neighboring agricultural areas or food contamination (especially during critical periods such as pregnancy) could lead to reduced head circumference at birth as well as neurological impairment (Berkowitz et al. 2004; Dalsager et al. 2019; Eskenazi et al. 2008; INSERM 2013; Munoz-Quezada et al. 2013; Oulhote and Bouchard 2013; Viel et al. 2015).

There is yet no identified mechanism hypothesis supporting possible synergic effect between insecticides and ZIKV (Wen et al. 2017). Prenatal exposure to them may however constitute hazards of a similar nature for neurodevelopment. The study of the consequences on child's health of their co-exposure, which is particularly likely in an epidemic context, might thus help to identify whether any confounding or synergetic processes exist. This study's aim is to document the combined effect (*i.e.* interaction) of simultaneous ZIKV infection and household insecticide during pregnancy on fetal growth and subsequent development and behavior during infancy.

Materials and Methods

Population and data collection

The ZIKA-DFA-FE/BB studies were conducted during the 2016 Zika epidemic in the French Overseas Departments of America (which includes the islands of Guadeloupe, Martinique, Saint-Martin, and Saint-Barthelemy, as well as French Guiana in South America). These studies aimed to address the natural history of Zika infection during pregnancy (Hoen et al. 2018). A cohort was created of pregnant women and their infants (who were monitored at birth and up to the age of 2 years). Clinical and paraclinical information (including ultrasounds and biological results) was collected within the framework of usual care practices, and a biological collection was constituted to determine ZIKV infection during pregnancy. This study received ethical approval from the Comité de Protection des Personnes Sud-Ouest et Outre-Mer III (ZIKA-DEF-FE: DC-2016-2636, ZIKA-DFA-BB: 2016-A00684-47). All participants provided written informed consent. Both studies were implemented by the CIC Inserm 1424.

The present study is an ancillary of the ZIKA-DFA-FE cohort study. Eligible women were those who delivered live birth in the maternity unit of Pointe à Pitre / Abymes hospital (Guadeloupe) between 14 October 2016 (start date of the ancillary study) and the end of February 2017. A total of 740 women (of 837) were enrolled in the present study, with whom a maternal face-to-face interview was conducted either at the maternity unit after delivery (n=684), or when they left the hospital, prior to a phone interview (n=25) or home visit (n=31) by trained research staff, to collect socioeconomic and demographic characteristics as well as data on domestic use of household insecticides (against flying insects such as mosquitoes) during pregnancy. In addition to the blood sample collected within the context of the ZIKA-DFA-FE study, a maternal hair sample was collected just after birth. After the exclusion of 13 twins and 122 other infants (from whose mothers no biological samples had been taken), 605 infants were eligible for the 18-month follow-up. Of those, 3 died before reaching the age of 18 months, 32 families did not agree to participate, and 149 were not reachable at time of data collection. Of the 421 families participating in the 18-month follow up, we collected data on infant development and behavior, domestic use of household insecticides over the past 12 months, maternal lifestyle and socioeconomic characteristics via an interview conducted either by phone (n=300) or in person at the hospital (n=119) or at home (n=2). A posteriori exclusion criteria included maternal conditions during pregnancy as well as pregnancy characteristics highly likely to impact fetal growth and/or infant development, such as a history of human immunodeficiency virus infection (n=4) and antiepileptic treatment (n=1), twin pregnancies (n=23) and premature delivery (very (28-32 weeks of gestation) and extremely premature babies (<28 weeks of gestation) (n=4). Our study is based on 708 mother-child dyads at birth and 409 at the 18-month follow-up (**Figure 1**).

Definition of the ZIKV infection during pregnancy

Since Zika virus infection can be asymptomatic, and even when it results in clinical signs (which are non-specific), definition of a ZIKV(+) case requires biological confirmation. For molecular diagnostics, the ‘windows’ during which it is possible to reveal the ZIKV genome by Polymerase Chain Reaction in the blood or urine are narrow: respectively 7 and 10 days from the date of the first clinical signs (HAS 2016). Therefore, all women included in the ZIKA-DFA-FE cohort had benefited from an anti-ZIKV ELISA serological test (Euroimmun ©, Hully 2016), conducted on a blood sample taken at the time of delivery (maternal and cord blood), regardless of their status during pregnancy or childbirth with regard to ZIKV infection (asymptomatic, symptomatic, PCR negative or positive). Therefore, confirmed ZIKV(+) and ZIKV(-) cases were defined according to an algorithm used in the ZIKA-DFA-FE cohort taking into account clinical status, result of the PCR prescribed if necessary during pregnancy, ZIKV serology result (IgM and / or IgG detection) in maternal or cord blood, date of conception and the 2016 Guadeloupe Zika epidemic period (calendar weeks 17 to 39), April 25 to September 25 (Hoen et al. 2018). Where the algorithm was unable to classify women into confirmed ZIKV(+) or ZIKV(-) cases (approximately 40%), we defined “likely ZIKV-exposed” according to the date of conception, taking the epidemic curve into account. Since the epidemic peak was week 23, the end of week 27 (10 July 2016) was chosen as a ZIKV exposure limit during pregnancy. If the date of conception was before the start of calendar week 28, the woman was classified as a “probable ZIKV-exposed case”. If the date of conception was after calendar week 28 and the serological results were inconclusive, she was classified as a “probable ZIKV-non-exposed case”. Regarding Zika infection status, two variables were considered: one taking into account confirmed cases only (based on the ZIKA-DFA-FE algorithm in supplemental data): n=438 mother-child pairs at birth, and n=252 at the 18-month follow-up), and the other combining both confirmed and likely cases, with this last underpinning our main results.

Users of household insecticides during pregnancy

At birth, mothers were questioned about their domestic use of household insecticides during their pregnancy; where they had used ‘natural’ or ‘organic’ products, they were specifically asked to say so. Additional questions regarding frequency of use (less than once a month, one to three times a month, one to three times a week, daily or more often) and methods of use (aerosol or spray, electric or non-electric diffuser, spirals or coils, mosquito-repellent mats/stickers, anti-mosquito misting systems, among others). Where natural or organic products were used in a “lemongrass candle” or “smoke” format, women were classified as “non-users” since these products were unlikely to contain insecticides. In the end, we built two variables: a binary one (user/non-user), and one ordinal based on frequency of use (non-user, one to three times a month or less, between one and three times weekly, daily or more) to analyze a possible dose-response relationship with outcomes.

Outcomes

Neurodevelopment at 18 months of age was documented using the French 18-month-old version of the Ages and Stages Questionnaire (ASQ). This is a screening test aimed at identifying children at risk of developmental delay (Squires and Bricker 2009). Fine motor, gross motor, personal-social, problem-solving and communication subscales were answered by the mother; each subscale contains 6 items. The mothers had to answer each item using a 3-point Likert-scale from 10 “yes”, 5 “sometimes” or 0 “not yet”. The sum of response items provided total scores for each subscale, and the sum of the five subscale scores provided a total ASQ score. Higher continuous scores are indicators of more advanced development in each domain. Examination of the variable distributions revealed that the gross motor development subscale score lacked variability, as a ceiling effect was observed. We therefore dichotomized this subscale score at the median value of 60. Fifteen questions from the Quebec Longitudinal Study of Child Development (QLSCD) were used to document the following externalizing behaviors: hyperactivity (6 items), opposition (3 items), inattention (3 items) and physical aggression (3 items) (Orri et al. 2021). These questions were shown to be associated with factors relating to behavioral impairment, such as prenatal tobacco exposure, family type, maternal age and maternal depression (Ahun et al. 2018; Galera et al. 2011; Huijbregts et al. 2007, 2008). Mothers had to answer each question describing her child’s behavior using a 3-point Likert-scale from 1: “never”, 2: “sometimes” or 3: “often”. Higher scores denote more serious behavioral difficulties. Reduced head circumference has been related to lower scores in early life cognitive tests (Broekman et al. 2009; Gale et al. 2006; Heinonen et al. 2008) as well as, occupational prestige in later life (Dekhtyar et al. 2014). Fetal growth was characterized through weight (in g) and head circumference at birth (in cm) obtained from medical records.

Other data

Gestational age at delivery was expressed as weeks of gestation, based on the first trimester ultrasound scan (WG). Maternal socio-demographic and lifestyle characteristics were collected from the questionnaire after delivery: maternal date of birth, pre-pregnancy height (cm) and weight (kg), parity (primiparous vs. multiparous), place of birth (French West Indies for Martinique / Guadeloupe; other West Indies for Saint-Martin / Saint-Barthelemy / Santo Domingo / Cuba / Dominican Republic; metropolitan France, Europe and others), family situation (with a partner, alone with family members, alone), education level (≤ 12 years, > 12 years), and smoking status during pregnancy (no, yes). Maternal pre-pregnancy body mass index (BMI) was calculated as weight (kg)/height (m)². The number of days of pregnancy spent during the rainy season (June to November) were calculated using the date of conception and delivery date.

At the 18-month follow-up, the following information was obtained from maternal interviews: breastfeeding duration (number of months); maternal depression symptoms using the third quartile (> 12) as a threshold for recourse to the abbreviated (10-item) Center for Epidemiologic Studies

Depression Scale (CES-D-10; (Radloff 1977); quality of maternal stimulation provided to the child was estimated using 5 questions: “Do you sing along with your child?”, “Do you read stories or show pictures in a book to your child?”, “Do you play with games with your child at home?”, “Are you taking your child for a walk at the beach or the park?”, and “Do you play physical games (ball) with your child?”. Mothers were asked to specify how often they interact with their child in such ways: (3) almost every day, (2) several times a week, or (1) less than once a week or almost never. The sum of responses to these questions ranged from 5 to 15, where a higher score suggests greater opportunities for stimulation.

Statistical analysis

For all scores calculated, where less than 50% of items were missing, a correction method was applied: $\text{Score} = \sum \text{items of score} * (\text{N total of items} / \text{N of non-missing items})$.

Study population characteristics were described using means, standard deviation and percentages. The comparison of included vs excluded population and determinants of ZIKV infection and users of insecticide products were studied using the Student's t-test, Wilcoxon test, χ^2 test and Fisher test to describe differences between infected/exposed and non-infected/non-exposed mothers.

Using simple linear and logistic models, head circumference and birthweight were analyzed alongside development and behavior scores at 18 months in relation to use of household insecticides, ZIKV infection and the interaction of household insecticide use and ZIKV exposure. The analysis using frequency of household insecticide use is presented as secondary analysis in the supplemental material. Multivariable linear regression models for continuous outcomes and logistic models for dichotomous outcomes were used.

The list of covariates tested in the model, were chosen a priori as these were the main risk factors associated with both fetal growth and infant development and externalized behavior from the literature: maternal age at delivery, pre-pregnancy BMI, parity, educational level, family situation, prenatal tobacco use, gestation duration and sex of child for the birth outcomes. In analyses involving 18-month outcomes, we added breastfeeding duration, maternal depression and stimulation scores. Where the covariates were continuous, a linearity test was used to decide how to consider them, comparing embedded models with likelihood ratio test with the ordinal variable declared as continuous or as categorical. Where linearity was not accepted, these were categorized in quartiles. Covariates associated at a p value < 0.20 were initially included together in multiple regression models (to assess their confounding influence) and in a second step these were removed one by one, starting with the least significant to outcomes. The covariates were kept in the model where the p value < 0.20 .

The statistical interaction between prenatal ZIKV exposure and household use of insecticide products was tested assuming additive or multiplicative scale when using linear or logistic regression models, respectively. When the interaction term was statistically significant at p -value < 0.10 , adjusted means of the outcome were presented graphically for each stratum built by ZIKV exposure and

household use of insecticide products. To study the robustness of the associations observed, three series of additional sensitivity analyses were performed. (i) Exclusion of microcephaly, defined using the INTERGROWTH-21st Zscores for GA and sex ($<2SD$) (Villar et al. 2014), in order to verify that the associations observed were not exclusively driven by those cases. 43 newborns were classified as microcephalic, and by the time of the 18-month follow-up, 24 of these remained in the study. (ii) In addition to the main analyses of all cases ($n=692$), analyses using a missing data method (for demographic and socioeconomic characteristics) were performed. The missingness of each variable was less than 9% (8.5% for maternal depression score; 2.7% for maternal stimulation score; 1.4% for BMI; 0.7% for breastfeeding duration; 0.3% for maternal education level; 0.3% for parity; 0.1% for gestational age at delivery; 0.1% for family situation; and 0% for all other variables of interest). For covariates having missingness of less than 5%, the median or mode was imputed. For the maternal depression score, predicted values of a linear regression with selected covariates ($p<0.20$) were used. (iii) Where infants were excluded because of having those medical conditions most likely to impact infant development (Sickle cell disease ($n=1$), infants hospitalized for convulsion without fever ($n=6$)), the significance level was set at 0.10.

Results

The sociodemographic and medical characteristics of participating mothers and infants are presented in **Table 1**. The mean age at delivery was 30 years of age. Most of the women were born in French West Indies (FWI, 58%), and had completed high school (57%). 25% of participating mothers were obese before the pregnancy, 10% smoked cigarettes during pregnancy and 65% reported use of household insecticides during pregnancy. Comparable characteristics were observed between included and excluded populations is presented in **Supplementary Table 1**, and did not show differences in terms of socioeconomic characteristics. Women infected by ZIKV during pregnancy were more likely to be younger at delivery, to have been born in the FWI and to have used tobacco during pregnancy. They were also more likely to have had more days of their pregnancies fall during the rainy season (**Table 1**).

At the 18-month follow-up, participating women were comparable to the population at birth, except that they were more frequent with completed high school (63%) (**Supplementary Table 2**). The variables associated with ZIKV infection during pregnancy were fairly similar, and in addition, infected women were more likely to score higher for symptoms of depression. Breastfeeding duration was 7.5 months on average, and the mean age of infants at follow-up was 17.3 months (age corrected for gestational age) (**Supplementary Table 2**). Women who reported having used household insecticides during pregnancy were more likely to be overweight or obese, and more likely to be living alone or with family members (rather than with a partner). A higher proportion of women who used household insecticides during pregnancy were infected by ZIKV during pregnancy than those not declaring household insecticide use (44% vs. 34%) (**Table 1**). At the 18-month follow-up, the variables associated with use of household insecticides from delivery to interview were age at delivery and obesity status (**Supplementary Table 2**). **Table 2** presents descriptive statistics for the outcomes under study. Bivariate associations between household use of insecticide products and ZIKV infection during pregnancy and outcomes of interest are presented in **Supplementary Table 3**.

Table 3a shows that ZIKV exposure during pregnancy was associated with reduced head circumference (in cm; -0.4; 95% CI: -0.9, 0.0) and lower birth weight (in g; -130; 95% CI: -242, -19) among non-users of household insecticides. Prenatal use of household insecticides was also associated with reduced head circumference (in cm; -0.3; 95% CI: -0.7, 0.0) and lower birth weight (in g; -82; 95% CI: -165, 0) among mothers not exposed to ZIKV. However, there was no clear dose-response trend with frequency of household insecticide use (**Supplementary Tables 4 and 5a**). On average, the smaller head circumference and/or lower birth weight in the babies of mothers exposed during pregnancy to both ZIKV infection and household insecticide use were similar to those exposed to just one of these factors. The interaction term between both factors was 0.04 for birth weight, which suggests that the changes in birth weight associated with the combined presence of ZIKV exposure and insecticide use

differ from the sum of changes observed for each. This is shown in **Figure 2**, which presents adjusted means for birthweight according to the four strata, by ZIKV infection and household insecticide use.

Table 3b shows that the combined presence of ZIKV exposure and household use of insecticides during pregnancy was associated with decreased ASQ Fine Motor subscale scores, in comparison with no exposure to either. These results are shown in **Figure 3** (-3.9 ; 95% CI: $-7.3, -0.4$); p -interaction= 0.09 with additive scale). A lower score with the combined presence of both factors was observed for ASQ Personal-Social and Total scores, though without reaching statistical significance. A decrease of ASQ Fine Motor and Total scores was also observed (though at a lower magnitude) in association with ZIKV infection among those mothers not reporting household insecticide use. No evidence of association was observed with household insecticide use alone (**Table 3b, Supplementary Table 4 and Table 5b**).

In terms of externalized behavior, ZIKV exposure during pregnancy was related to a higher opposition score ($\beta=0.6$; 95% CI: $0.0, 1.2$ among non-users, **Table 3c**), with no effect-heterogeneity observed in line with use of household insecticides (p -interaction >0.10). Neither ZIKV nor insecticide use (regardless of frequency) was associated with hyperactivity, inattention or physical aggression scores. Combined exposure to both factors was however associated with a greater number of behavioral problems (close to statistical significance), especially for the hyperactivity phenotype ($\beta=0.8$; 95% CI: $-0.0, 1.5$) (**Table 3c, Supplementary Tables 4 and 5c, and Figure 1**), and reaching statistical significance level for oppositional behaviors ($\beta=1.0$; 95% CI: $0.5, 1.5$).

When excluding infants with likely ZIKV exposure from our analysis, the results were fairly similar, albeit at a higher magnitude for some results: among non-users of household insecticides, ZIKV infection during pregnancy was associated with reduced head circumference of 0.7 cm (95% CI: $-1.2, -1.7$), and reduced birthweight of 240 g (95% CI: $-381, -97$). Combined exposure to ZIKV and household insecticides during pregnancy was significantly associated with higher opposition scores $\beta=0.9$ (95% CI: $0.3, 1.6$), and among non-users of household insecticides, ZIKV infection was associated with higher opposition scores $\beta=1.0$ (95% CI: $0.3, 1.7$) (**Supplementary Tables 6a, 6b and 6c**). Results were very similar after excluding from the analysis those newborns with microcephaly (6% of newborns) (**Supplementary Tables 7a, 7b and 7c**). Lastly, using imputed data for the few covariables having missing data (**Supplementary Tables 8a, 8b and 8c**) or excluding infants having medical conditions that could lead to impaired development (**Supplementary Tables 9a, 9b and 9c**) did not affect the results.

Discussion

The aim of this study was to document whether the combined effect (*i.e.* the interaction) of simultaneous ZIKV infection and use of household insecticides during pregnancy was related to a decrease in fetal growth and subsequent non-optimal development and behavior at the age of 18 months.

In the present study, joint exposure to ZIKV infection and household insecticides during pregnancy was associated with both reduced head circumference at birth and reduced birthweight. Decreases of a similar (or slightly higher) magnitude were observed after insecticide use among the ZIKV-unexposed mothers, and after ZIKV infection among non-users of household insecticides. To our knowledge, no previous studies have examined the combined effect of ZIKV infection and use of household insecticides during pregnancy on child's health. Our results on birth outcomes did not show an adverse combined effect indeed, they suggest both that the use of household insecticides does not modify the risk of adverse health outcomes at birth associated with prenatal ZIKV infection, or the reverse (*i.e.* ZIKV infection neither increases nor decreases the risks related to exposure to insecticides). Though use of household insecticides during pregnancy is usually recommended in vector control (especially during a ZIKV epidemic), our results could also call into question the role of household insecticides, which appear to report an adverse impact on newborn health at birth comparable to that of ZIKV infection.

Most studies published to date on the effect of ZIKV exposure have focused on microcephaly (usually defined as head circumference less than 2 SD below the mean for sex and gestational age) (De Carvalho et al. 2016; Hoen et al. 2018). No study has yet investigated the possible effect on head circumference on average. Some epidemiological studies and reviews have suggested that exposure related to insecticides or the domestic use of pesticides, in particular during such critical periods as pregnancy, could lead to reduced head circumference at birth (Berkowitz et al. 2004; INSERM 2013; Munoz-Quezada et al. 2013), which is consistent with our present study. Moreover, when newborns with microcephaly were excluded, the significant reduction in fetal growth both remained significant, and was even more marked. Our findings of reduced birth weight are also in line with the association shown in a Brazilian cross-sectional study of 393 infants with CZS (Carvalho-Sauer et al. 2019). However, in the Japan Environment and Children's Study of 71,116 mother-child pairs, frequency of use of household pesticides was not associated with reduced birth weight (Matsuki et al. 2020).

Furthermore, the association of very low birthweight (VLBW) and very preterm birth (VPTB) with lower cognitive development is well documented. In recent meta-analysis of 30 studies, which included a total of 10,293 VLBW or VPTB infants, the pooled prevalence of cognitive and motor delays, evaluated using developmental tests, was estimated at 16.9% (95% CI 10.4–26.3) and 20.6% (95% CI 13.9–29.4%) respectively. Mild delays were more frequent than moderate-to-severe delays (Pascal et

al. 2018). In order to clearly differentiate between the combined effect of ZIKV infection and use of household insecticides on cognitive development, and a possible mediating effect of VLBW and VPTB, we excluded from our analysis those deliveries likely to represent the lowest birthweight, i.e. very and extremely premature infants.

In terms of infant development at 18 months of age, combined exposure during pregnancy to ZIKV and household insecticides was associated with a significant decrease in the fine motor domain, and this was marginally statistically significant with the ASQ Personal-Social subscale score and Total score. A decrease of lower magnitude in ASQ Fine Motor and Total scores was also observed with ZIKV exposure among mothers not reporting use of insecticide products during pregnancy, though this did not reach statistical significance. With regard to the behavioral characteristics documented, our study showed higher frequency of opposition and hyperactivity behavioral disorders in 18-month-old children when women were exposed during pregnancy to both ZIKV infection and the household insecticides. The present study also showed a higher frequency of opposition disorders in the 18-month-old children of women exposed during pregnancy to ZIKV. There was no evidence of any association between prenatal insecticide use and any developmental and behavioral outcomes documented at 18 months.

An earlier study of the same ZIKA-DFA-FE/BB cohort, extended to Martinique, French Guiana and Guadeloupe, reported comparable cognitive performances (using the 24-month ASQ) among children at 24 months, whether or not they had been exposed to ZIKV infection during pregnancy. In this earlier study, only normocephalic infants (using INTERGROWTH charts) were included (Grant et al. 2021). When we excluded the microcephalic infants from the present analysis, the reduction in the ASQ Fine Motor score at 18 months of age persisted. We cannot rule out an attenuation effect with child age. Another study that included 47 Brazilian infants with confirmed CZS, found a decreased Fine Motor score at 16 months of age (Wheeler et al. 2018). Three other studies have linked exposure to ZIKV with infant development, two of which showed increased risk of developmental delays, while the other showed none (Blackmon et al. 2022; Key et al. 2021; Mulkey et al. 2020). In the Key et al. cohort study, the Baley Score of Infant and Toddler Development Screening Test (cognitive development, communication skills and motor development) was used for 194 normocephalic Brazilian infants aged 3 months to 24 months. For the communication domain, between 5.7% and 26.3% of infants were classified as “emerging” or “at risk” (Key et al. 2021). In the Mulkey et al. cohort study, both the Warner Initial Developmental Evaluation of Adaptive and Functional Skills (WIDEA total score, self-care, mobility, communication and social cognition domain) and the Alberta Infant Motor Scale (AIMS) were used at 1 or 2 time points between 4 to 18 months for 77 normocephalic Colombian infants. The WIDEA total score and self-care domain showed unadjusted curvilinear association with age. Other domains and the AIMS scores showed unadjusted negative linear association with age (Mulkey et al. 2020). In the Blackmon et al. nested case control study, the INTER-DNA assessment (communication, language and motor items) was used between the ages of 22 to 30 months for 68 exposed and 63 unexposed

Grenadian infants. There were no group differences in standardized INTER-DNA scores between infants exposed or not exposed to ZIKV (Blackmon et al. 2022).

To our knowledge, no previous study has linked externalizing trait behavior (or more specifically ADHD spectrum) with prenatal ZIKV infection.

Insecticides and household uses of pesticides have, in several epidemiological studies, been associated with a reduction in sensory, motor and cognitive scores and behavioral scores in children (INSERM 2013; Oulhote and Bouchard 2013; Viel et al. 2015). In the present study, we did not observe evidence of adverse neurodevelopmental outcomes after use of household insecticides during pregnancy, other than for externalizing trait behavior among ZIKV-exposed mothers.

According to a representative French survey (PestiHome DROM), most of the active substances found in the household insecticides products stored and used by Guadeloupean families, were pyrethroids, cypermethrin being the most frequently used molecule of this family (57.3%), followed by d-phenothrin (53.1%) (ANSES 2021). This finding is consistent with the correlation between reported use of household insecticides during pregnancy and urinary pyrethroid metabolite levels observed in the French ELFE cohort study (n=1077) (Dereumeaux et al. 2018). The next two most-commonly used molecules are the synergizer Piperonyl butoxide (PBO, 20.3%), and the repellent DEET (17.7%), (ANSES 2021). PBO has been associated with a Bayley Scale of Infant Development decrease at 36 months of age in a prospective cohort of Dominican mothers (N=348) (Horton et al. 2011). DEET has yet to be linked to adverse neurotoxic effects (Swale and Bloomquist 2019). The majority of the women in our study (77%) declared use of insect repellents during pregnancy, as observed in the PestiHome DROM survey. Most (67%) were also using household insecticides. Pyrethroids were described as having diverse toxic actions and pharmacological effects on voltage-sensitive sodium channels, calcium and chloride channels, while peripheral-type benzodiazepines were involved in the neurotoxicity of pyrethroids (Soderlund et al. 2002). But, to our knowledge, there is no identified mechanism hypothesis supporting possible synergic effect between pyrethroid or repellent and ZIKV (Wen et al. 2017).

In the specific setting of ZIKV infection vector control, the use of household insecticides gives rise to many questions as to both their actual effectiveness and their possible consequences for environment and human health. In 2016, use of insect repellents to prevent ZIKV infection was recommended. This recommendation may have been due to the (as yet unforeseen) possible combined effect of exposure during pregnancy to both household insecticides and ZIKV (Wylie et al. 2016). Studies analyzing the determinants of ZIKV infection prevention measures for pregnant women have shown that behaviors were dependent on socioeconomic status, with repellent use being more common in those of higher socioeconomic status, and mosquito nets used mainly by those of lower socio economic status (Dantas Melo et al. 2019; Quintana-Domeque et al. 2018). In the present study,

the higher frequency of ZIKV(+) in women declaring use of household insecticides may be linked to the fact that they were probably more exposed to mosquito bites than non-users, so that ultimately, their risk of being infected was higher among users (44.3% ZIKV(+)) than non-users (33.6%). Pregnant women's knowledge of ZIKV infection still needs to be improved (Pooransingh et al. 2018). Targeted messages should address the prevention of both use of household insecticides, and ZIKV infection.

Our study has several limitations that should be mentioned. Despite the fact that biological samples were available for all study participants, ZIKV infection status could not be determined for some of them, so that as many as 40% of participants (N=270) were not classified as confirmed ZIKV(+) or ZIKV(-) cases. The present findings were similar, however, when using the stricter definition of confirmed ZIKV infection. Data on exposure to household insecticides was collected via self-reporting, which allows the possibility of recall bias. Since in our study we were focusing on assessing exposure during pregnancy to insecticides, using a questionnaire after delivery (during the hospital stay of 1-3 days), we can hypothesize that recall bias was minimized. In addition, data were not collected on certain confounding factors (such as surrounding exposure to other insecticide uses). Nevertheless the spread of insecticides in the neighborhood reported in the questionnaire was not associated with any particular household's use. We did not collect the ZIKV infection status during the early postnatal life. The 2016 ZIKV outbreak in Guadeloupe lasting approximately 5 months, only minor confounding by postnatal ZIKV exposure might have been possible. Among the strengths of our study design are its cohort size and longitudinal design (which enables measurement of exposure upstream of outcome evaluation). We were then able, during the prenatal and postnatal periods, to collect data on environmental exposures from multiple sources as well as on potential prenatal and postnatal confounding variables in the socioeconomic, maternal and family domains. Several sensitivity analyses were performed, allowing the robustness of the results to be tested.

Conclusion

Both ZIKV exposure and use of household insecticides during pregnancy have been associated with adverse birth outcomes, without any exacerbating effect for their combined exposure. Fine motor development in particular, as well as externalizing behavior at 18 months of age, were adversely associated with ZIKV exposure during pregnancy, with a higher magnitude of associations among mothers who also used household insecticides during pregnancy. While no mechanistic hypothesis has yet supported a possible synergic effect between insecticide molecules and ZIKV on neurodevelopment, insecticide-free ways of preventing mosquito bites should be encouraged.

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Figure 1: Flow Chart

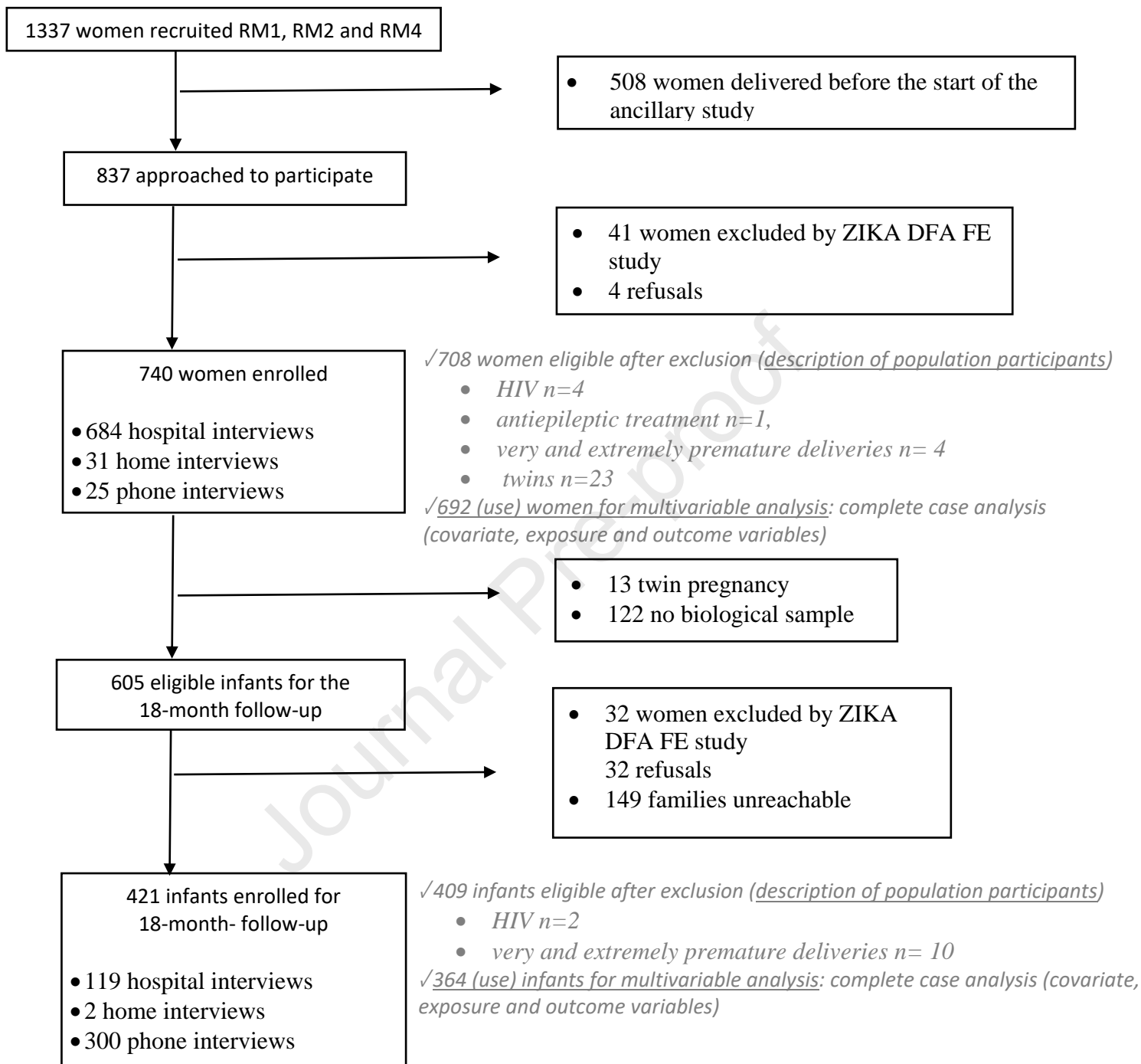


Table 1: Population characteristics (N=708) and determinants of ZIKV exposure and household insecticide during pregnancy

		Birth N=708	Domestic use of household insecticides			ZIKV exposure		
			No use (n=247)	Use (n=461)		No (n=421)	Yes (n=287)	
	MV	% (N) or mean (±SD)	% (N) or mean (±SD)	% (N) or mean (±SD)	p*	% (N) or mean (±SD)	% (N) or mean (±SD)	p*
Maternal and pregnancy characteristics								
Age at delivery (years)	0	30.2 (±6.4)	29.6 (±6.2)	30.5 (±6.6)	0.09	30.8 (±6.4)	29.3 (±6.4)	0.00
BMI (kg/m²)	10	26.45 (±6.4)	25.8 (±6.5)	26.7 (±6.3)	0.07	26.2 (±6.2)	26.7 (±6.7)	0.25
Obesity	10							
Underweight		4.2% (29)	7.0% (17)	2.6% (12)	0.01	4.8% (20)	3.2% (9)	0.65
Normal		45.1% (315)	48.8% (119)	43.2% (196)		44.3% (184)	46.3% (131)	
Overweight		26.2% (183)	21.3% (52)	28.9% (131)		27% (112)	25.1% (71)	
Obese		24.5% (171)	23.0% (56)	25.3% (115)		23.9% (99)	25.4% (72)	
Maternal weight gain during pregnancy (kg)	11	9.4 (±7.2)	9.2 (±7.6)	9.5 (±7.0)	0.58	9.3 (±7.2)	9.64 (±7.3)	0.51
Parity	2							
Primiparous		37.7% (266)	38.2% (94)	37.4% (172)	0.83	36.7% (154)	39.2% (112)	0.50
Multiparous		62.3% (440)	61.8% (152)	62.6% (288)		63.3% (266)	60.8% (174)	
Education level	2							
≤12 years		43.3% (306)	41.1% (101)	44.6% (205)	0.37	43.2% (181)	43.6% (125)	0.93
>12 years		56.7% (400)	58.9% (145)	55.4% (255)		56.8% (238)	56.4% (162)	
Place of birth	0							
French West Indies		58.1% (411)	53.0% (131)	60.7% (280)	0.11	56.1% (236)	61.0% (175)	0.03
Other West Indies		26.4% (187)	28.3% (70)	25.4% (117)		25.4% (107)	27.9% (80)	
Metropolitan France, Europe and other		15.5% (110)	18.6% (46)	13.9% (64)		18.5% (78)	11.1% (32)	
Tobacco use during pregnancy	0							
No		89.8 (636)	89.5% (221)	90.0% (415)	0.82	92.2% (388)	86.4% (248)	0.01
Yes		10.2 (72)	10.5% (26)	10.0% (46)		7.8% (33)	13.6% (39)	
Length of pregnancy in wet season (days)	1	119.8 (±39.7)	118.3 (±40.6)	120.6 (±39.2)	0.51	101.9 (±41.8)	145.9 (±13.2)	<.0001
Family situation	1							
With a partner		51.8% (366)	58.3% (144)	48.3% (222)	0.02	55.1% (232)	46.8% (134)	0.06
Single, with family members		18.4% (130)	14.2% (35)	20.7% (95)		16.2% (68)	21.7% (62)	

Single		29.4% (211)	27.5% (68)	31.0% (143)		28.7% (121)	31.5% (90)	
ZIKV exposure during pregnancy	0							
No		59.5% (421)	66.4% (164)	55.7% (257)	0.01			
Yes		40.5% (287)	33.6% (83)	44.3% (204)				
Infants characteristics								
Gestational age at delivery (weeks)	2	38.7 (± 1.7)	38.6 (± 1.7)	38.7 (± 1.6)	0.21	38.6 (± 1.7)	38.8 (± 1.6)	0.06
Sex	0							
Male		53.4 (378)	50.6% (125)	54.9% (253)	0.28	53.0% (224)	53.7% (154)	0.91
Female		46.6 (330)	49.4% (122)	45.1% (208)		47.0% (197)	46.3% (133)	

MV: Missing Values; ZIKV: Zika virus

* p-values obtained from Student's t, Wilcoxon, χ^2 or Fisher tests

Table 2 Descriptives for birth and 18-month outcomes

	Birth (N=708)			18-month follow-up (N=409)		
	MV	mean (\pm SD)	range	MV	mean (\pm SD)	range
Birth outcomes						
Gestational age at delivery (weeks)	2	38.7 (\pm 1.7)	33-41	0	38.7 (\pm 1.7)	33-41
Head circumference (cm)	0	33.8 (\pm 1.9)	27-39.5	0	33.8 (\pm 1.8)	27-38
Birth weight (g)	0	3109 (\pm 518)	1270-4680	0	3120 (\pm 517)	1270-4680
Development and behavior at 18-month						
ASQ Total score				0	224.6 (\pm 38.7)	0-300
ASQ communication				0	41.1 (\pm 149)	0-60
ASQ Gross motor				0	56.8 (\pm 7.7)	0-60
ASQ Fine motor				0	46.2 (\pm 12.7)	0-60
ASQ Personal-social				0	44.2 (\pm 9.7)	0-60
ASQ Problem solving				0	36.2 (\pm 13.9)	0-60
Hyperactivity				9	12.6 (\pm 2.7)	6-18
Inattention				9	5.2 (\pm 1.5)	3-9
Opposition				9	8.1 (\pm 1.78)	4-12
Physical aggression				9	5.2 (\pm 1.8)	3-9

MV: missing values, ASQ: Ages and Stages Questionnaire

Table 3a: Multivariable analysis between household insecticide use and Zika infection during pregnancy, and birth outcomes (n=692; Guadeloupe, France)

	Head circumference (cm)	Birth weight (g)
	β^a [95% CI]	β^b [95% CI]
Non-users and ZIKV(-)	ref	ref
Users and ZIKV(-)*	-0.3 [-0.6; 0.0]	-82 [-165; -0.3]
Non-users and ZIKV(+)**	-0.4 [-0.9; 0.0]	-130 [-242; -19]
Combined exposure: User and ZIKV(+)**	-0.3 [-0.7; 0.0]	-72 [-159; 13]

ZIKV: Zika virus, β coefficient and 95% CI obtained from linear regression

^a adjusted for gestational duration (from date of last menses), maternal pre-pregnancy body mass index, parity and education level, and fetal sex

^b additionally adjusted for gestational duration squared

* Average change estimate of users vs. non-users among ZIKV(-) mothers

** Average change estimate of ZIKV(+) vs. ZIKV(-), among non-users of household insecticides

*** Estimate of average change due to the combined presence of ZIKV infection and household insecticide use (vs. ZIKV(-) and non-users)

Figure 2: Birth weight adjusted means according to ZIKV exposure and household insecticide use during pregnancy (n=692; Guadeloupe, France); p-value of interaction (additive scale) = 0.04

Legends: Yellow dots: ZIKV(-), Gray dots ZIKV(+)

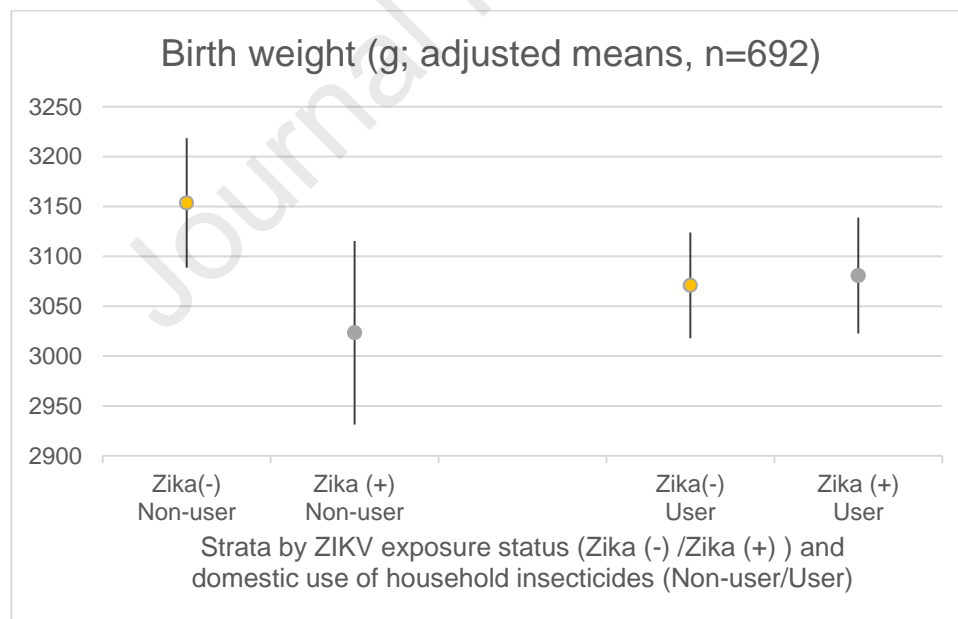


Table 3b: Multivariable analysis between household insecticide use and ZIKV infection during pregnancy, and ASQ scores at 18 months of age (n=364; Guadeloupe, France)

	Total score	Gross Motor	Fine Motor	Personal-social	Problem solving	Communication
	$\beta^{a, b, e}$ [95% CI]	OR ^{a, c, d, e} [95% CI]	$\beta^{a, b, c, e}$ [95% CI]	β^a [95% CI]	β^a [95% CI]	$\beta^{a, b, d, e}$ [95% CI]
Non-users and ZIKV(-)	ref	ref	ref	ref	ref	ref
Users and ZIKV(-)*	1.7 [-7.8; 11.2]	1.3 [0.7; 2.5]	3.1 [-0.1; 6.2]	-1.4 [-4.0; 1.2]	-0.6 [-4.1; 3.1]	1.1 [-2.8; 4.9]
Non-users and ZIKV(+)**	-5.7 [-18.2; 6.7]	1.1 [0.5; 2.4]	-2.4 [-6.6; 1.7]	-0.0 [-3.4; 3.4]	-0.7 [-5.5; 4.0]	-1.5 [-6.5; 3.6]
Combined exposure: User and ZIKV(+)**	-9.1 [-19.4; 1.1]	0.8 [0.4; 1.5]	-3.9 [-7.3; -0.4]	-2.7 [-5.5; 0.1]	-1.2 [-5.1; 2.7]	-1.2 [-5.4; 2.9]

ZIKV: Zika virus, ASQ: Ages and stages questionnaire, β coefficient and 95%CI obtained from linear regression, OR: Odds Ratio

Gross motor subscale dichotomized score at the median value of 60

^aadjusted for maternal body mass index before pregnancy and maternal stimulation score

^b additionally adjusted for breastfeeding duration (months), maternal education level and depression score

^c additionally adjusted for family situation (single, with a partner, single with family members)

^d additionally adjusted for maternal tobacco use during pregnancy (yes/no)

^e additionally adjusted for infant sex

* Average change estimate of users vs. non-users among the ZIKV(-) mothers

** Average change estimate of ZIKV(+) vs. ZIKV(-), among non-users of household insecticides

*** Estimate of average change due to the combined presence of ZIKV infection and household insecticide use (vs. ZIKV(-) and non-users)

Figure 3: Fine motor ASQ-score adjusted means according to ZIKV infection and household insecticide use during pregnancy (n=364; Guadeloupe, France); p-value of interaction (additive scale) = 0.09

Legends: Yellow dots: ZIKV(-), Gray dots ZIKV(+)

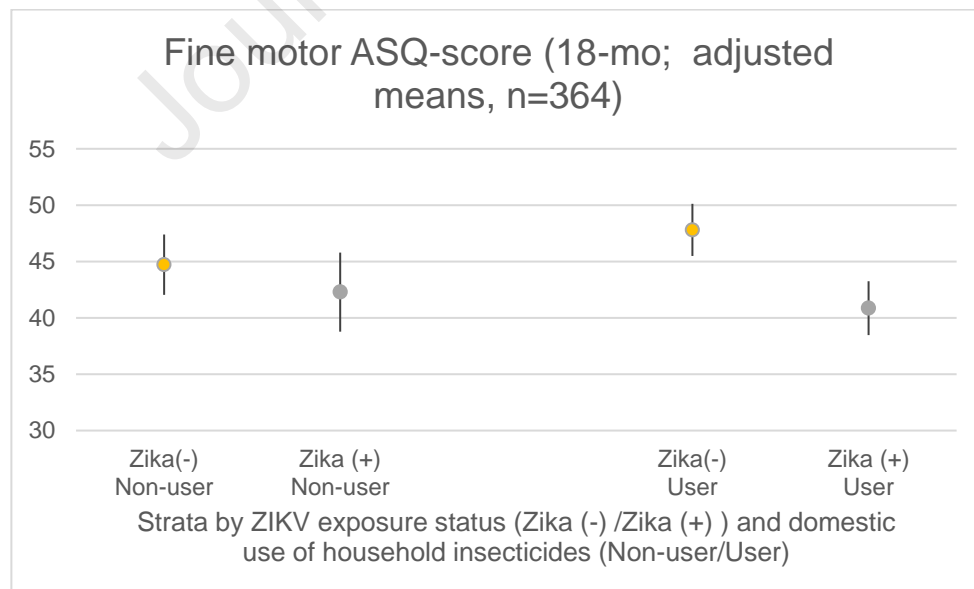


Table 3c: Multivariate analysis between frequency of household insecticide use and ZIKV infection during pregnancy, and externalizing behaviors at 18 months (N=364; Guadeloupe, France)

	Hyperactivity	Opposition	Inattention	Physical aggression
	β^a [95% CI]	β^b [95% CI]	β^c [95% CI]	β^d [95% CI]
Non-users and ZIKV(-)	ref	ref	ref	ref
Users and ZIKV(-)*	0.2 [-0.5; 1.0]	0.2 [-0.3; 0.6]	0.1 [-0.3; 0.5]	-0.1 [-0.6; 0.6]
Non-users and ZIKV(+)**	0.4 [-0.6; 1.3]	0.6 [0.0; 1.2]	-0.0 [-0.5; 0.5]	0.1 [-0.6; 0.7]
Combined exposure: User and ZIKV(+) ***	0.8 [-0.0; 1.5]	1.0 [0.5; 1.5]	0.4 [-0.1; 0.8]	0.4 [-0.1; 0.9]

ZIKV: Zika virus, QLSCD : Quebec Longitudinal Study of Child Development, β , coefficient and 95% CI obtained from linear regression

^a adjusted for maternal age at delivery, tobacco use during pregnancy, depression score and fetal sex

^b adjusted for gestational age at delivery, parity, maternal education level, family situation, tobacco use during pregnancy, depression and stimulation scores, and infant sex

^c adjusted for parity, maternal education level, tobacco use during pregnancy and stimulation score

^d adjusted for maternal age at delivery, parity, depression and stimulation scores, and infant sex

* Average change estimate of users vs. non-users among ZIKV- mothers

** Average change estimate of ZIKV+ vs. ZIKV-, among non-users of household insecticides

*** Estimate of average change due to the combined presence of ZIKV infection and household insecticide use (vs. ZIKV(-) and non-users)

Highlights: Simultaneous exposure to both Zika virus and household insecticides during pregnancy, and fetal growth and infant developmental behavior outcomes at 18 months, in Guadeloupe

- Zika virus could result in adverse growth, developmental and behavioral outcomes
- Insecticides used to control mosquitoes are neurotoxic
- Combined neurodevelopmental effect (interaction) between both should be tested
- Altered neurodevelopmental at 18 months of age were observed with Zika exposure
- Higher magnitude observed when mothers reported use of household insecticides

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: