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Seroconversion and seroprevalence of TORCH infections in a pregnant women cohort study, Mombasa, Kenya, 2017-2019

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Summary

Women infected during pregnancy with TORCH (Toxoplasmosis, Other, Rubella, Cytomegalovirus, and Herpes simplex viruses) pathogens have a higher risk of adverse birth outcomes including stillbirth and miscarriage because of mother-to-child transmission. To investigate these risks in pregnant women in Kenya, we analyzed serum specimens from a pregnancy cohort study at three healthcare facilities. A sample of 481 participants were selected for TORCH pathogen antibody testing to determine seroprevalence. A random selection of 285 from the 481 participants were selected to measure seroconversion. These sera were tested using an IgG enzyme linked immunosorbent assay against 10 TORCH pathogens. We found that the seroprevalence of antibodies against all but three of the 10 TORCH pathogens at enrollment was >30%, except for *Bordetella pertussis* (3.8%), *Treponema pallidum* (11.4%) and varicella zoster virus (0.5%). Conversely, very few participants seroconverted during their pregnancy and of those were herpes simplex virus type 2 (n=24, 11.2%), parvovirus B19 (n=14, 6.2%), and rubella (n=12, 5.1%). For birth outcomes, 88% of the participant had live births and 12% had stillbirths or miscarriage. Cytomegalovirus positivity at enrollment had a statistically significant positive association with a live birth outcome (p=0.0394). Of the 10 TORCH pathogens tested, none had an association with adverse pregnancy outcome.

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Introduction

Pathogens that cause congenital infections worldwide are referred to as TORCH pathogens. Initially, TORCH pathogens have primarily included Toxoplasmosis gondii, Other (syphilis, varicella zoster virus, parvovirus B19) rubella, cytomegalovirus (CMV), and herpes simplex virus type 1 and 2 (HSV-1 and HSV-2) when detected during the early stages of pregnancy. Over the years, this list of pathogens has grown significantly to include, most recently, Zika virus (ZIKV), *Chlamydia trachomatis*, and human immunodeficiency virus (HIV). Depending on when the expecting mother is exposed to any of these pathogens, a myriad of different adverse outcomes may arise. Some of the notable adverse outcomes upon exposure during the first trimester of pregnancy include congenital malformations, intrauterine growth restriction or fetal death, whereas exposure during late pregnancy (i.e., third trimester) may result in adverse outcomes that present later after delivery.

In this study, we aimed to detect pre-existing antibodies against TORCH pathogens in pregnant women to understand previous exposure to these pathogens, susceptibility, and risk factors associated with infection of any of these pathogens during pregnancy. This information could tailor control and prevention programs by public health authorities towards these specific high-risk pathogens in Kenya. However, some of these TORCH pathogens can reinfect the host or reactivate in regions where they lie dormant within the host, such as several of the *Herpesviridae* family of viruses (i.e., HSV-1, HSV-2, CMV, and VZV). In sub-Sahara African countries such as Kenya, there is limited information on the burden and epidemiology of these pathogens in pregnant women. A recent study in the Kenya coastal region identified a 1.9% microcephaly birth outcome rate that was not associated with the ZIKV in newborns (Barsosio et al. 2019). For these microcephaly cases, the causative etiology was not determined; however, the authors state that most of these microcephaly cases were also in infants determined to be small for gestational age (SGA). Of the limited information available from many African countries, epidemiological studies have shown that the burden of disease is greatest, where TORCH infections significantly contribute to prenatal and infant morbidity and mortality.

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We conducted a study on ZIKV amongst pregnant women in Mombasa, Kenya. This ZIKV pregnancy cohort study was designed to detect active transmission of ZIKV and determine if infection during pregnancy with the African strain of ZIKV caused adverse birth outcomes (Osoro et al. 2022). Using this pregnancy cohort study, we conducted a secondary analysis to determine the presence of other TORCH pathogens (excluding ZIKV) and their association with adverse birth outcomes. Thus, we determined the seroprevalence of 10 different TORCH pathogens within this pregnancy cohort and their association with adverse birth outcomes. In addition, we measured the seroconversion rate for these pathogens during the participant's pregnancy and matched this information to observed adverse birth outcomes. A secondary objective was to examine the seroprevalence by maternal age and HIV status as predictors of infection with TORCH pathogens.

METHODS

Pregnancy cohort

From October 2017 - March 2019, we consented and enrolled pregnant women at <28 weeks estimated gestational age visit and ≥ 15 years of age that presented at antenatal clinics in 3 hospitals: Bomu, Coast General, and Port Ritz hospital (1 private and 2 public, respectively) in Mombasa, Kenya. Each enrolled participant was administered a questionnaire, was evaluated clinically, an obstetric ultrasound was conducted, and was asked to provide a blood sample of 5mL at enrollment and then monthly throughout their pregnancy until delivery. During the first antenatal care (ANC) visit, we collected a urine specimen as well as clinical, obstetric history, demographic, socioeconomic status and exposure to mosquito bites from a total of 2312 participants. Monthly follow-up visits were performed where the participants completed a questionnaire on risk factors and submitted a 5ml blood specimen. During these follow-up visits, participants were asked of any symptoms of fever or rash within the last 7 days as part of the ZIKV monitoring. After delivery, we collected birth outcome data, including stillbirth and miscarriage, and postnatal parameters (such as head circumference, length, and birth weight) within 24 hours of birth. We did not measure neurological outcomes such as hearing loss often

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associated with CMV infections. We randomly selected 481 women from the pregnancy cohort study who were ZIKV negative with an enrollment specimen (Figure 1). A subset of women of the initial random selection (n=285), were selected based on the availability of both enrollment and delivery specimens. From this subset, whose enrollment specimen for each TORCH pathogen was seronegative, were selected to assess for seroconversion of TORCH pathogens.

Laboratory diagnostic testing

Commercial enzyme linked immunoassays (ELISAs) were used to detect IgG antibodies against 10 different TORCH pathogens (Euroimmun, Germany). The 10 TORCH pathogens included: *Toxoplasmosis gondii* (*T. gondii*), cytomegalovirus (CMV), *Bordetella pertussis* (*B. pertussis*), parvovirus B19, herpes simplex viruses 1 and 2 (HSV-1, 2), rubella, *Treponema pallidum*, varicella zoster virus (VZV) and *Chlamydia trachomatis*. Both enrollment and delivery blood specimens from the participants were tested for the presence of IgG for all 10 TORCH pathogens. Sera collected from 5 mL of venous blood were separated by centrifugation, and 5ul of serum from each participant was tested by ELISA in duplicates according to the manufacturer's recommendations. See appendix for manufacturer's validation studies for each TORCH test.

Statistical analysis

The study cohort enrolled 2312 participants, among whom 1967 (85.1%) formed the sampling frame for the current study because they had complete outcome data (birth weight, miscarriage, stillbirth, live birth). Previous studies have reported TORCH seroprevalence estimates ranging from 2-98% in Kenya (Nisbet et al. 2018; Otieno-Nyunya et al. 2011). Using this information, we assumed a seroprevalence of at least 6% for the TORCH pathogens tested, an absolute precision level of 2%, and applied finite population correction for a minimum sample size of 425.

We determined seroprevalence, defined as the proportion of participants who tested positive for any of the TORCH pathogens at enrollment. Furthermore, seroconversion was defined as a participant

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who tested negative for any of the 10 TORCH pathogens at enrollment, followed by a positive test at delivery.

We described the study variables (demographic variables and pathogen status) using frequencies and percentages (95% confidence interval (CI)), and graphically using bar charts. Seroprevalence and seroconversion were calculated by maternal age category, HIV status, and SGA status. SGA was defined as a birth weight z-score of ≤ 1.28 at birth (equivalent to the 10th percentile) and extreme SGA as a birth weight z-score of ≤ 1.88 (equivalent to the 3rd percentile) based on INTERGROWTH-21st standards, and the corresponding p-values were estimated using either Pearson Chi-Square or Fisher's Exact test (Villar et al. 2014). For participants who had birth outcome data, we investigated the association of each pathogen with adverse birth outcomes (stillbirth or miscarriage) using logistic regression and quantified the association with the odds ratio (OR). We calculated the 95% CI for the OR estimate. A multivariable regression analysis was not performed on this data set because no independent variables reached a $p<0.1$ cut-off value. The analyses were conducted using SAS software version 9.4 (SAS Institute Inc, Cary, NC). The level of significance was defined as $\alpha \leq 0.05$.

RESULTS

The study

A total of 481 participants who were ZIKV negative and had an enrollment specimen were selected using simple random selection from the pregnancy cohort and tested for 10 TORCH pathogens. A second subset of 285 participants who had both an enrollment specimen and a delivery specimen available were tested for the 10 TORCH pathogens to determine seroconversion rates. ZIKV positive specimens were excluded from this study analysis and previously published (Osoro et al. 2022). A total of 62 (13.4%) of the women were ≤20 years old, 333 (71.9%) were between the ages of 21-35 years old, and 68 (14.7%) were >35 years old. There were 130 (27%) enrolled during their first trimester, 296 (61.5%) that were enrolled within the second trimester [14-26 weeks], and 55 (11.4%) enrolled during their third trimester (Table 1).

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Participants' demographics at enrollment

Fewer than half the women enrolled in this study had a secondary-school level of education, and almost equal numbers of women had either a primary or below (27.5%) or tertiary education (29.3%) (Table 1). Similar proportion of pregnant women were employed (42.6%), unemployed (41.5%), and 15.9% were self-employed. There was also a similar distribution of participants from the three study sites in Mombasa (Bomu 34%, Coast General 29.,9% and Port Reitz 36.1%). The majority were HIV negative (79.3%), between 21-35 years old (71.9%,) and enrolled within the 2nd trimester of their pregnancy (61.5%).

Seroprevalence: Past exposure to 10 TORCH pathogens

Of the 481 pregnant women tested at enrollment, all had detectable IgG to at least one of the 10 TORCH pathogens tested (HSV-1 (97.3%), CMV (94%), rubella (86.5%), parvovirus B19 (55.4%), HSV-2 (48.9%), *Chlamydia trachomatis* (39.9%), *B. pertussis* (3.8%), *T. pallidum* (11.4%) and VZV (0.5%)) (Figure 2). All participants were simultaneously seropositive to at least two TORCH pathogens during either enrollment or delivery, driven primarily by HSV-1 and CMV (Table 2). Also, 78% of enrollment samples and 75% of delivery samples were positive for 4 to 6 TORCH pathogens.

When categorizing the cohort by age, women ≤20 years old, representing the age group for adverse birth outcomes (Cavazos-Rehg et al. 2015), had lower seroprevalence to CMV (88.1% vs. 94%), *Chlamydia trachomatis* (35.9% vs. 39.9%) *T. gondii* (28.6% vs. 33.1%), VZV (0% vs. 0.5%), HSV-2 (39.3% vs. 48.9%) and rubella (83.9% vs. 86.5%) compared to the overall rates for all age groups. The seroprevalence of those within the age group (21-35 years old) had similar or higher seroprevalence compared to the overall rates of the entire cohort for most of the 10 TORCH pathogens except for parvovirus B19 (52.9% vs. 55.4%), and *T. gondii* (31.8% vs. 33.1%). The age category (>35 years old) had higher seroprevalence than the overall rate of the entire cohort for parvovirus B19 (71.2% vs. 55.4%), *T. gondii* (46.9% vs. 33.1%), VZV (1.7% vs. 0.5%), HSV-2 (63.3% vs. 48.9%) and rubella (92.9% vs. 86.5%).

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Seroconversion to TORCH pathogens during pregnancy

Of the 285 women that were tested at enrollment and delivery, we observed seroconversion to TORCH pathogens for HSV-2 (11.2%), parvovirus B19 (6.2%), rubella (5.1%), *T. pallidum* (3.2%), *Chlamydia trachomatis* (2.1%), CMV (1.6%), HSV-1(1.6%), and VZV (0.4%) (Figure 3). When we analyzed seroconversion results by age groups, we observed that none of the low-risk age groups of ≤20 years had seroconversion to any of the 10 TORCH pathogens. The medium-risk group (21-35 years old) had similar seroconversion results as the overall cohort, and the high-risk age group >35 years old had higher seroconversion for 7 of the 10 TORCH pathogens than the overall cohort: CMV (5.3% vs. 1.6%), *Chlamydia trachomatis* (7.1% vs. 2.1%), parvovirus B19 (12.1% vs. 6.2%), *T. gondii* (5.6% vs. 2.7%), *T. pallidum* (9.1% vs. 3.2%), HSV-1 (2.5% vs. 1.6%), and HSV-2 (16.7% vs. 11.2%).

Birth outcomes related to seroprevalence of 10 TORCH pathogens

Of the 481 study participants selected for TORCH testing at enrollment, 362 (75.2%) had live births, 52 (10.8%) had miscarriages, 21 (4.4%) had stillbirths, and 46 (9.5%) did not have an outcome recorded (Table 3). CMV positivity at enrollment was the only serostatus group that had statistically significant decreased odds of non-live birth (OR=0.378 (0.145; 0.985) and p-value=0.0394).

When analyzing the seroprevalence of TORCH pathogens in women with the birth outcome of SGA, we found that, among live births, 225 (62.1%) deliveries had normal birth weight, 18 (5.0%) had low birth weight (<25,00g) and 119 (32.8%) did not have birth weight recorded (Table 4). For pathogen seroprevalence by SGA, none of the TORCH pathogens were associated with a significant difference in the birth outcome of SGA.

The association of HIV status and seroconversion and seroprevalence of 10 TORCH pathogens

We also determined the seroprevalence by HIV status and found that none of the *B. pertussis* and VZV positive cases were HIV-positive (Table 5). However, *T. gondii* (45.7% p=0.046) and HSV-2 (73.2% p<.001) were significantly higher in HIV-positive women, whereas *B. pertussis* (0%, p=0.046) was

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significantly higher in HIV-negative women. When comparing HIV status and seroconversion, only *T. gondii* was significantly higher among HIV-positive women (p=0.03) (Table 6). Seroconversion for all other pathogens did not differ by HIV status.

DISCUSSION

Our objective was to study the seroprevalence of TORCH pathogens using specimens collected from a cohort of pregnant women enrolled in a ZIKV study in Mombasa, Kenya. Our study showed detectable IgG antibodies in all women for at least one of the 10 TORCH pathogens. We found that there was relatively low seroconversion in this cohort for most of the TORCH pathogens suggesting that few acquired an infection during their pregnancy, thus, risk factors associated with acute infections could not be calculated. Among those that had detectable seroconversion, the majority were women that fell within the two ages categories (21-25 and >35 years old) compared to the ≤20 years old. Overall, *B. pertussis* had very low seroprevalence (3.8%) and no seroconversion, whereas HSV-1 (97.3%), CMV (94%), and rubella (86.5%) had the highest seroprevalence, and HSV-2 (11.2%), parvovirus B19 (6.2%) and rubella (5.1%) had the highest seroconversion within this cohort. Neither CMV and rubella seroprevalence were associated with SGA or HIV status; however, CMV was significantly associated with live birth outcome, suggesting that prior exposure was protective.

There were two viruses, of the four tested in this study, belonging to the *Herpesviridae* family (HSV-1 and CMV) had the highest percentage of seropositives within this cohort. This family of viruses are well adapted to their human host and thus highly infectious even within the general population. HSV-1 antibodies were detected in 97.3% of all the women selected for testing and had the highest seroprevalence of all the pathogens tested. Although HSV-1 had the highest seroprevalence within this cohort, it had a very low seroconversion rate of 1.6%, perhaps suggesting that few of the study participants acquired HSV-1 during their pregnancy, and almost all had prior immunity upon entering the study. When comparing miscarriage or stillbirth with live birth outcomes for HSV-1, the majority of live births were seropositive for HSV-1 (96.6%) and within the category of stillbirths and miscarriages

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most were also seropositive for HSV-1 (98.6%) which may have falsely suggest that this adverse birth outcomes could have been associated with HSV-1. However, when we compared the live birth to non-live birth overall odds ratio, there was no significant difference between that observed with HSV-1. Interestingly HSV-2, another virus belonging to the *Herpesviridae* family, had a much lower seroprevalence (48.6%) compared to HSV-1 but had the highest seroconversion rate (11.2%) of all the TORCH pathogens tested and the majority of the detectable seroconversion were within the 21–35-year-old group (75%).

CMV from the *Herpesviridae* virus family had the second highest seroprevalence (94%) compared to the other TORCH pathogens tested in our cohort. CMV is a very common infection with an overall seroprevalence among adults in the United States of America of 50% (Boeckh and Geballe 2011) and has been found to be even higher in LMIC (Shet 2011). The greatest risk to the fetus is found with exposure to CMV within the first trimester of pregnancy compared to the second or third trimesters (Gandhi and Khanna 2004). Primary CMV infection can cause a range of clinical symptoms, including permanent hearing and vision loss, neurological impairments, and even death by miscarriage (Cannon and Davis 2005). CMV is the leading cause of long-term disabilities in children and the leading cause of hearing loss worldwide. In pregnant women, the most common source of transmission of CMV is either sexual transmission or contact with urine or saliva from young children (Pass et al. 1986). Previous studies in Africa indicated that there is an overall high prevalence of CMV in pregnant women, demonstrated in multiple African countries, including Kenya (Hamdan et al. 2011; Kamel et al. 2014; Maingi and Nyamache 2014; Yeshwondm et al. 2016). A study in Thika, Kenya, showed 77% prevalence with 8% IgM positivity suggesting recent infection (Maingi and Nyamache 2014). Although the prevalence of CMV in our study was much higher (94%) than previously shown in Kenya, we only had 4 women (1.6%) who seroconverted in this cohort, suggesting that few of the expecting mothers were either infected early in their pregnancy prior to our sampling or perhaps had prior immunity to CMV. We suspect the latter since high seroprevalence was associated with lower odds of non-live birth (stillbirth or miscarriage) that was found to be significant (OR= 0.378 p-value 0.0394) compared to the overall birth outcomes within this cohort. Unfortunately, we were unable to measure infant outcomes

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such as hearing loss or other neurological impairments following delivery. The high seroprevalence of CMV may be associated with household size related to transmission within this setting (Hoehl et al. 2020).

Rubella had the third highest seroprevalence (86.5%) and the third highest seroconversion rate (5.1%) in our cohort. Rubella vaccination was incorporated into Kenya's national childhood immunization schedule in 2016 [1]. Therefore, Rubella exposure or seroconversion among study participants was likely due to infection rather than vaccination.

Women who have no immunity to rubella are at very high risk for congenital rubella infection and potentially adverse outcomes (congenital rubella syndrome (CRS)). CRS can only be detected many months after the baby is born; we were unable to determine if any of the infants in our cohort had CRS since we did not follow the women and infants after delivery. Although the exact number of CRS cases is unknown due to the lack of CRS surveillance, prior studies indicated that 6% of measles/rubella cases were women of reproductive age in Kenya (Njeru et al. 2015). Most participants were enrolled during the second trimester when the risk of CRS following rubella infection is low. The rubella vaccine program for Kenya has had various interruptions since 2002. In 2016, Kenya introduced the combined measles and rubella (MR) vaccine in an attempt to improve vaccine coverage, but the coverage in pregnant women is not well documented, including in our study cohort participants (Manakongtreecheep and Davis 2017). Since our study was conducted between 2017-2019, the participants were not eligible to receive the rubella vaccine through the Kenya routine vaccination (https://www.afro.who.int/news/kenya-rolls-out-massive-measles-rubella-and-tetanus-campaign). The results from this study may provide sufficient information to estimate the seroprevalence of rubella and support policy for strengthening vaccination efforts in Kenya.

In our study, *T. pallidum* had a relatively high seroprevalence of 11.4% compared to that observed in North America (0.67%) (Neu et al. 2015). A recent study by Warnecke et al. in 2020, demonstrated that the average seroprevalence of *T. pallidum* was between 1-3% in 6 different countries (Mexico, China, Poland, Germany, Brazil, and Turkey), demonstrating that this cohort in

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Kenya had between 3-10-fold higher levels in comparison to all 6 countries (Warnecke et al. 2020). Overall countries in Africa tend to have much higher prevalence of syphilis than the global average ranging from an average of 4.6% in Eastern African countries to 6.5% in Southern African countries (Joseph Davey et al. 2016). Although our results are ~2-fold higher than the average reported prevalence for the East African region, the ELISA used in our study was validated by the manufacturer with 139 clinical specimens using the gold standard TPHA test and the performance of their test indicated 100% sensitivity and specificity. One of the most surprising findings from this study was the very low seroprevalence (0.5%) and seroconversion (0.4%) of VZV amongst pregnant women in Kenya. Again, in Warnecke *et al.*, the authors reported that all 6 countries tested had very high VZV seroprevalence with a mean of 95.9% and a range of 92.3-99.4% (Warnecke et al. 2020). There are very few studies of prevalence of VZV in Kenya however a study by Hussey et al., 2017 showed a VZV prevalence of 23% which is also significantly lower than detected globally (Hussey et al. 2017; Korenromp et al. 2019). Our findings are significantly lower than even the Hussey et al., study and this could be due to the ELISA test parameters used to measure anti-VZV IgG antibodies. The manufacturer may have used a higher titer antibody cut-off to detect acute cases. Although the test application is to confirm a VZV suspected infection and/or reactivation, the manufacturer also claims that the test can determine immune status in early pregnancy. Nevertheless, our results are much lower than expected and must be taken with caution and requires further verification of this finding which we were unable to perform.

There were study limitations that should be considered. First, most of the cases were enrolled in the second trimester, thus missing the window of identifying potential seroconversions that may have happened earlier in the pregnancy. Also, we relied on seroconversion as a surrogate marker for acute infection leading to vertical transmission to the fetus. Second, most of the cohort fell into a single age category of 21-35 years old (71.9%), where age-related adverse outcomes are not commonly observed. Third, the vaccine status for some of the vaccine-preventable diseases was not known. Fourth, a full description of poor infant outcomes, such as hearing, or vision loss was outside of the scope of our study, thus limiting our analysis. Our analysis of co-infection used seroprevalence results

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due to the limited seroconversions observed; however, co-infections with any of these TORCH pathogens often culminate into a higher risk of adverse birth outcomes especially spontaneous abortions (Rasti et al. 2016). The causes of stillbirth and miscarriage could not be further determined and only 66 stillbirths were examined at delivery with no gross anomalies noted. Finally, the results may need to be interpreted with caution since multivariable models could not be used due to because no independent variables reached a $p<0.1$ cut-off value.

In summary, this is the first study to measure 10 TORCH pathogens in a large pregnancy cohort in Kenya. The seroprevalence of many of the TORCH pathogens in this cohort resembled those findings in Brazil except for *T. pallidum* and VZV. We found that only CMV antibodies at enrollment was negatively associated with adverse birth outcomes; however, prior exposure to other TORCH pathogens was not associated with adverse pregnancy outcomes. These findings support the inclusion of routine screening for those TORCH pathogens in national guidelines, especially those with high seroprevalence, where there are vaccines or prophylactic treatments available.

Ethical Approval and consent to participants:

This study was approved by the University of Nairobi/ Kenyatta National Hospital ethics and research committee (P71/02/2017). Concurrence from the Kenya Medical Research Institute's Scientific Ethics and Research Unit (SERU), Washington State University Institutional Review Board (IRB). It was also reviewed by CDC and was conducted consistent with applicable federal law and CDC policy[§]. [§See 45 C.F.R. part 46; 21 C.F.R. part 56]. All participants enrolled in this study consented to participate. Ethical approval was obtained through SERU and CDC ethical committee.

Availability of data and materials: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

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REFERENCES:

Barsosio HC, Gitonga JN, Karanja HK, Nyamwaya DK, Omuoyo DO, Kamau E, Hamaluba MM, Nyiro JU, Kitsao BS, Nyaguara A, Mwakio S, Newton CR, Sang R, Wright D, Sanders EJ, Seale AC, Agoti CN, Berkley JA, Bejon P and Warimwe GM (2019) Congenital microcephaly unrelated to flavivirus exposure in coastal Kenya. *Wellcome Open Research* **4,** 179. https://doi.org/10.12688/wellcomeopenres.15568.1.

Boeckh M and Geballe AP (2011) Cytomegalovirus: pathogen, paradigm, and puzzle. *Journal of Clinical Investigation* **121**(5)**,** 1673-1680. https://doi.org/10.1172/JCI45449.

Cannon MJ and Davis KF (2005) Washing our hands of the congenital cytomegalovirus disease epidemic. *BMC Public Health* **5,** 70. https://doi.org/10.1186/1471-2458-5-70.

Cavazos-Rehg PA, Krauss MJ, Spitznagel EL, Bommarito K, Madden T, Olsen MA, Subramaniam H, Peipert JF and Bierut LJ (2015) Maternal age and risk of labor and delivery complications. *Maternal Child Health Journal* **19**(6)**,** 1202-1211. https://doi.org/10.1007/s10995-014-1624-7.

Gandhi MK and Khanna R (2004) Human cytomegalovirus: clinical aspects, immune regulation, and emerging treatments. *Lancet Infectious Diseases* **4**(12)**,** 725-738. https://doi.org/10.1016/S1473-3099(04)01202-2.

Hamdan HZ, Abdelbagi IE, Nasser NM and Adam I (2011) Seroprevalence of cytomegalovirus and rubella among pregnant women in western Sudan. *Virolology Journal* **8,** 217. https://doi.org/10.1186/1743-422X-8-217.

Hoehl S, Berger A, Ciesek S and Rabenau HF (2020) Thirty years of CMV seroprevalence-a longitudinal analysis in a German university hospital. *European Journal of Clinical Microbiology and Infectious Diseases* **39**(6)**,** 1095-1102. https://doi.org/10.1007/s10096-020-03814-x.

Hussey H, Abdullahi L, Collins J, Muloiwa R, Hussey G and Kagina B (2017) Varicella zoster virus-associated morbidity and mortality in Africa - a systematic review. *BMC Infectious Diseases* **17**(1)**,** 717. https://doi.org/10.1186/s12879-017-2815-9.

Joseph Davey DL, Shull HI, Billings JD, Wang D, Adachi K and Klausner JD (2016) Prevalence of Curable Sexually Transmitted Infections in Pregnant Women in Low- and Middle-Income Countries From 2010 to 2015: A Systematic Review. *Sexually Transmitted Diseases* **43**(7)**,** 450-458. https://doi.org/10.1097/OLQ.0000000000000460.

Kamel N, Metwally L, Gomaa N, Sayed Ahmed WA, Lotfi M and Younis S (2014) Primary cytomegalovirus infection in pregnant Egyptian women confirmed by cytomegalovirus IgG avidity testing. *Medical Principles and Practices* **23**(1)**,** 29-33. https://doi.org/10.1159/000354758.

Korenromp EL, Rowley J, Alonso M, Mello MB, Wijesooriya NS, Mahiane SG, Ishikawa N, Le LV, Newman-Owiredu M, Nagelkerke N, Newman L, Kamb M, Broutet N and Taylor MM (2019) Global burden of maternal and congenital syphilis and associated adverse birth outcomes-Estimates for 2016 and progress since 2012. *PLoS ONE* **14**(2)**,** e0211720. https://doi.org/10.1371/journal.pone.0211720.

Maingi Z and Nyamache AK (2014) Seroprevalence of Cytomegalo Virus (CMV) among pregnant women in Thika, Kenya. *BMC Research Notes* **7,** 794. https://doi.org/10.1186/1756-0500-7-794.

Manakongtreecheep K and Davis R (2017) A review of measles control in Kenya, with focus on recent innovations. *Pan African Medicine Journal* **27**(Suppl 3)**,** 15. https://doi.org/10.11604/pamj.supp.2017.27.3.12118.

https://doi.org/10.1017/S0950268824000165 Published online by Cambridge University Press

Neu N, Duchon J and Zachariah P (2015) TORCH infections. *Clinical Perinatology* **42**(1)**,** 77-103, viii. https://doi.org/10.1016/j.clp.2014.11.001.

Nisbet AI, Omuse G, Revathi G and Adam RD (2018) Seroprevalence data at a private teaching hospital in Kenya: An examination of Toxoplasma gondii, cytomegalovirus, rubella, hepatitis A, and Entamoeba histolytica. *PLoS ONE* **13**(10)**,** e0204867. https://doi.org/10.1371/journal.pone.0204867.

Njeru I, Onyango D, Ajack Y and Kiptoo E (2015) Rubella outbreak in a Rural Kenyan District, 2014: documenting the need for routine rubella immunization in Kenya. *BMC Infectious Diseases* **15,** 245. https://doi.org/10.1186/s12879-015-0989-6.

Osoro E, Inwani I, Mugo C, Hunsperger E, Verani JR, Omballa V, Wamalwa D, Rhee C, Nduati R, Kinuthia J, Jin H, Okutoyi L, Mwaengo D, Maugo B, Otieno NA, Mirieri H, Shabibi M, Munyua P, Njenga MK and Widdowson MA (2022) Prevalence of microcephaly and Zika virus infection in a pregnancy cohort in Kenya, 2017-2019. *BMC Medicine* **20**(1)**,** 291. https://doi.org/10.1186/s12916-022-02498-8.

Otieno-Nyunya B, Bennett E, Bunnell R, Dadabhai S, Gichangi AA, Mugo N, Wanyungu J, Baya I, Kaiser R and Kenya AISST (2011) Epidemiology of syphilis in Kenya: results from a nationally representative serological survey. *Sexually Transmissible Infections* **87**(6)**,** 521-525. https://doi.org/10.1136/sextrans-2011-050026.

Pass RF, Hutto C, Ricks R and Cloud GA (1986) Increased rate of cytomegalovirus infection among parents of children attending day-care centers. *New England Journal of Medicine* **314**(22)**,** 1414-1418. https://doi.org/10.1056/NEJM198605293142204.

Rasti S, Ghasemi FS, Abdoli A, Piroozmand A, Mousavi SG and Fakhrie-Kashan Z (2016) ToRCH "co-infections" are associated with increased risk of abortion in pregnant women. *Congenital Anomalies (Kyoto)* **56**(2)**,** 73-78. https://doi.org/10.1111/cga.12138.

Shet A (2011) Congenital and perinatal infections: throwing new light with an old TORCH. *Indian Journal of Pediatrics* **78**(1)**,** 88-95. https://doi.org/10.1007/s12098-010-0254-3.

Villar J, Cheikh Ismail L, Victora CG, Ohuma EO, Bertino E, Altman DG, Lambert A, Papageorghiou AT, Carvalho M, Jaffer YA, Gravett MG, Purwar M, Frederick IO, Noble AJ, Pang R, Barros FC, Chumlea C, Bhutta ZA, Kennedy SH, International F and Newborn Growth Consortium for the 21st C (2014) International standards for newborn weight, length, and head circumference by gestational age and sex: the Newborn Cross-Sectional Study of the INTERGROWTH-21st Project. *Lancet* **384**(9946)**,** 857-868. https://doi.org/10.1016/S0140-6736(14)60932-6.

Warnecke JM, Pollmann M, Borchardt-Loholter V, Moreira-Soto A, Kaya S, Sener AG, Gomez-Guzman E, Figueroa-Hernandez L, Li W, Li F, Buska K, Zakaszewska K, Ziolkowska K, Janz J, Ott A, Scheper T and Meyer W (2020) Seroprevalences of antibodies against ToRCH infectious pathogens in women of childbearing age residing in Brazil, Mexico, Germany, Poland, Turkey and China. *Epidemiology and Infections* **148,** e271. https://doi.org/10.1017/S0950268820002629.

Yeshwondm M, Balkachew N, Delayehu B and Mekonen G (2016) Seroepidemiology Study of Cytomegalovirus and Rubella among Pregnant Women at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia. *Ethiopian Journal of Health Sciences* **26**(5)**,** 427-438. https://doi.org/10.4314/ejhs.v26i5.4.

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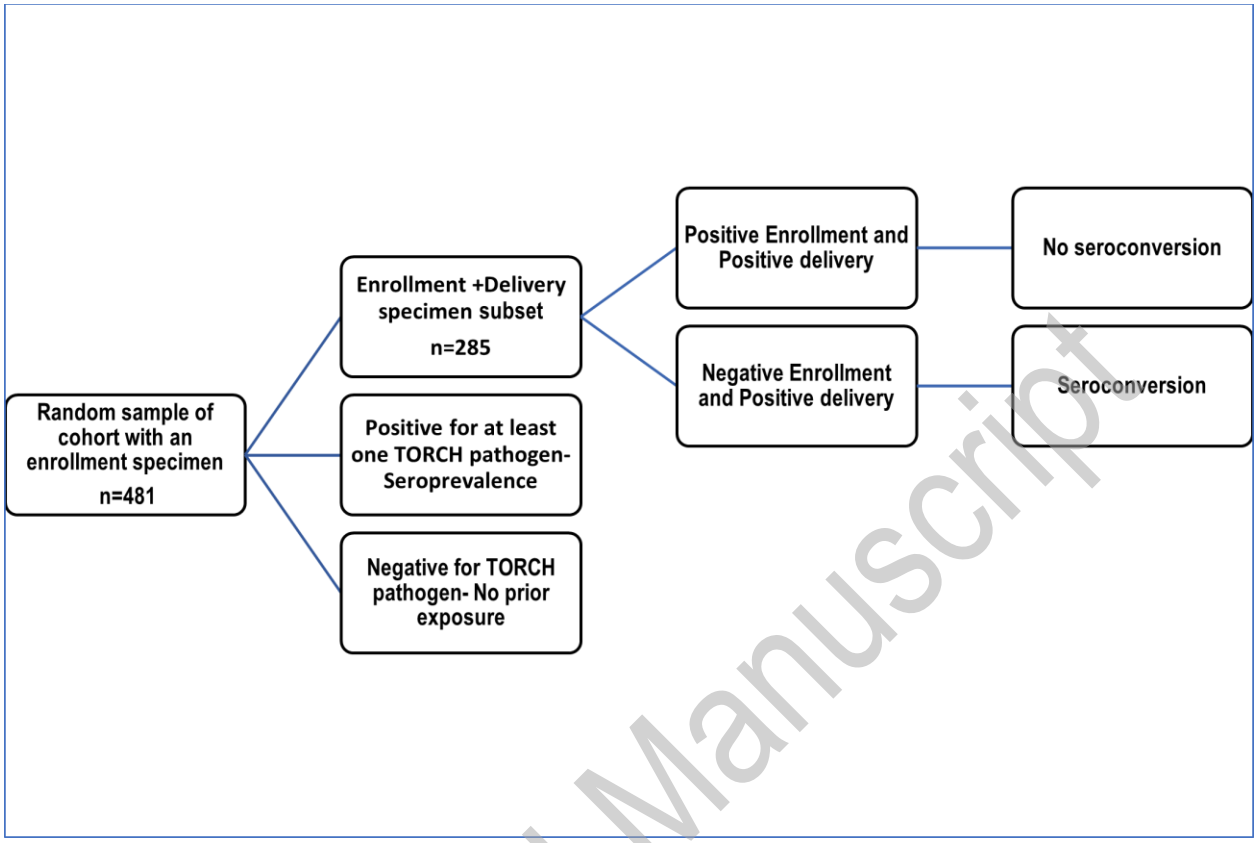
412 **Figure 1.** Participant selection criteria, testing and outcome schematic.



413

414

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Figure 2. Seroprevalence of TORCH pathogens by age group. CMV=cytomegalovirus; HSV-1=herpes simplex virus type 1; HSV-2=herpes simplex virus type 2; VZV=varicella zoster virus.

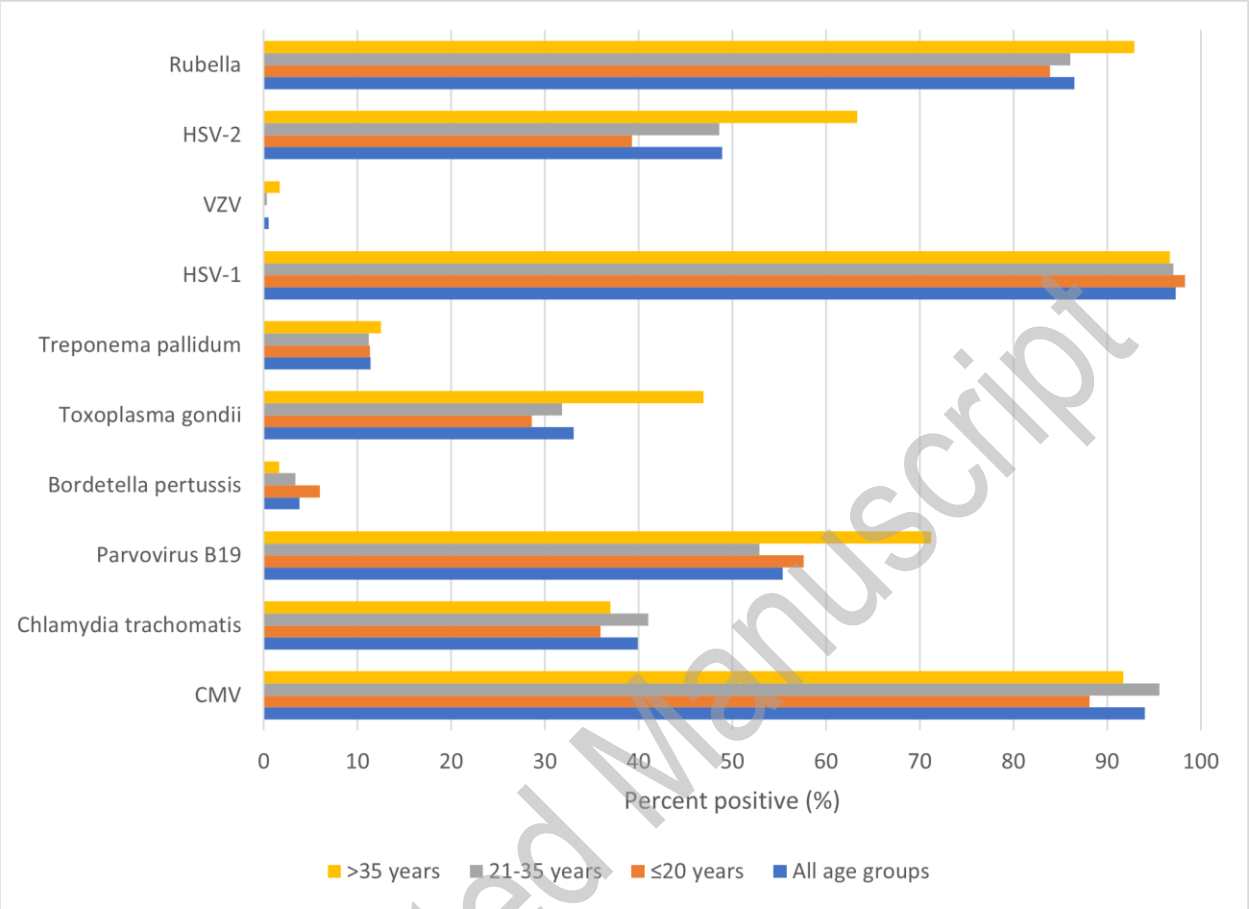

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Figure 3. Seroconversion of TORCH pathogens by age group. Note that there were no seroconversions observed in age group <20 years old. CMV=cytomegalovirus; HSV-1=herpes simplex virus type 1; HSV-2=herpes simplex virus type 2; VZV=varicella zoster virus.

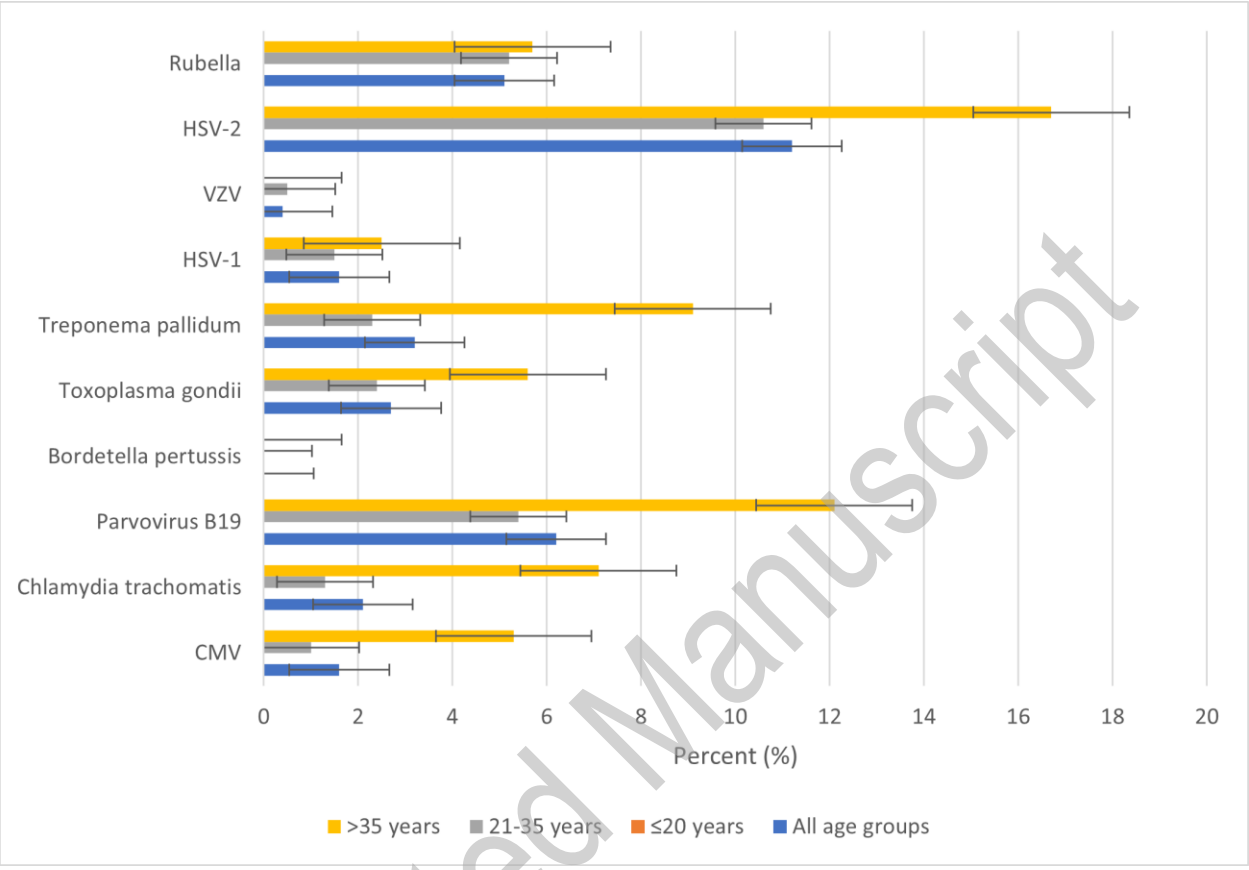

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Table 1. Participants demographic characteristics from single specimen taken at enrollment (N=481) and participants with two-specimens obtained at enrollment and delivery (N=285)

	Enrolment (N= 481)		With enrolment and delivery (N=285)	
Demographic characteristics	n	Percent (95% CI)	n	Percent (95% CI)
Education				
· Primary and below	119	27.5 (23.3; 31.7)	79	28.3 (23.1; 34.0)
· Secondary	187	43.2 (38.5; 48.0)	118	42.3 (36.4; 48.3)
· Tertiary	127	29.3 (25.1; 33.9)	82	29.4 (24.1; 35.1)
Occupation				
· Employed	188	42.6 (38.0; 47.4)	118	41.4 (35.6; 47.4)
· Self employed	70	15.9 (12.6; 19.6)	52	18.3 (13.9; 23.2)
· Unemployed	183	41.5 (36.9; 46.3)	115	40.4 (34.6; 46.3)
Site				
· Bomu	150	34.0 (29.6; 38.6)	97	34.0 (28.6; 39.9)
· Coast General	132	29.9 (25.7; 34.4)	90	31.6 (26.2; 37.3)
· Port Reitz	159	36.1 (31.6; 40.7)	98	34.4 (28.9; 40.2)
HIV status				
· Yes	85	20.7 (16.9; 25.0)	62	23.2 (18.3; 28.8)
· No	325	79.3 (75.0; 83.1)	205	76.8 (71.2; 81.7)
Maternal age (years)				
· ≤ 20	62	12.9 (10.0; 16.2)	10	3.5 (1.7; 6.4)
· 21-35	351	73.0 (68.8; 76.9)	229	80.4 (75.3; 84.8)
· > 35	68	14.1 (11.2; 17.6)	46	16.1(12.1; 20.9)
Gestational age at enrollment (weeks)				
· 0-13 (1st trimester)	130	27.0 (23.1; 31.2)	60	21.1 (16.5; 26.3)
· 14-26 (2nd trimester)	296	61.5 (57.0; 65.9)	192	67.4 (61.6; 72.8)
· 27 + (3rd trimester)	55	11.4 (8.7; 14.6)	33	11.6 (8.1; 15.9)

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Table 2. Seroprevalence of TORCH pathogens and the number of pathogens exposures from enrollment and delivery. CMV=cytomegalovirus; HSV-1=herpes simplex virus type 1; HSV-2=herpes simplex virus type 2; VZV=varicella zoster virus.

TORCH target	Enrollment, N = 159[1]	Delivery N = 159[1]
B. pertussis	6 (3.8%)	4 (2.5%)
HSV-1	153 (96%)	157 (99%)
chlamydia	61 (38%)	55 (35%)
CMV	155 (97%)	154 (97%)
HSV-2	78 (49%)	76 (48%)
rubella	124 (78%)	130 (82%)
VZV	0 (0%)	0 (0%)
T. gondii	52 (33%)	50 (31%)
Parvo B19	80 (50%)	79 (50%)
Treponema pallidum	19 (12%)	14 (8.8%)
No. of TORCH pathogens that were seropositive		
2	8 (5.0%)	9 (5.7%)
3	18 (11%)	23 (14%)
4	50 (31%)	48 (30%)
5	49 (31%)	43 (27%)
6	26 (16%)	28 (18%)
7	7 (4.4%)	7 (4.4%)
8	1 (0.6%)	1 (0.6%)

[1]n (%)

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Table 3. Distribution of pathogen status at enrollment by newborn outcomes non-live birth outcome status, the odds ratio quantifies the association of presence of an antibody to a pathogen at enrollment with the odds of a non-live birth outcome. CMV=cytomegalovirus; HSV-1=herpes simplex virus type 1; HSV-2=herpes simplex virus type 2; VZV=varicella zoster virus.

PATHOGEN AT ENROLLMENT		OUTCOMES		ODDS OF NON-LIVEBIRTH	
	Status	Non-livebirth (miscarriage/ stillbirth)	Live birth	OR (95% CI)	P-value
OVERALL*		73	362		
CMV	Pos	62	305	0.378 (0.145; 0.985)	0.0394
	Neg	7	13		
CHLAMYDIA TRACHOMATIS	Pos	26	119	0.954 (0.554; 1.642)	0.8648
	Neg	41	179		
PARVOVIRUS B19	Pos	42	171	1.514 (0.876; 2.619)	0.1357
	Neg	24	148		
BORTEDELLA PERTUSSIS	Pos	2	11	0.872 (0.189; 4.030)	0.8608
	Neg	64	307		
TOXOPLASMA GONDII	Pos	12	66	0.857 (0.410; 1.793)	0.6821
	Neg	28	132		
TREPONEMA PALLIDUM	Pos	8	34	1.128 (0.496; 2.562)	0.7738
	Neg	58	278		
HSV-1	Pos	70	316	2.436 (0.309; 19.179)	0.3824
	Neg	1	11		
VZV	Pos	0	2	----[a]	0.5240
	Neg	64	315		
HSV-2	Pos	37	145	1.373 (0.803; 2.346)	0.2458
	Neg	29	156		
RUBELLA	Pos	58	270	1.350 (0.579; 3.147)	0.4855
	Neg	7	44		

[a]-OR could not be calculated due to 0 positive cases

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Table 4. Pathogen seroprevalence for birth outcome of small for gestational age (SGA) among live births (n=362)

PATHOGEN	SGA		P-VALUE[A]
	No	Yes	
CMV	182/189 (96.3)	22/22 (100)	1.000
CHLAMYDIA TRACHOMATIS	70/173 (40.5)	8/24 (33.3)	0.503
PARVOVIRUS B19	99/188 (52.7)	12/23 (52.2)	0.965
BORDETELLA PERTUSSIS	5/195 (2.6)	1/23 (4.4)	0.492
TOXOPLASMA GONDII	35/112 (31.3)	4/11 (36.4)	0.742
TREPONEMA PALLIDUM	21/186 (11.3)	4/21 (19.1)	0.294
HSV-1	191/195 (98.0)	24/24 (100)	1.000
VZV	0/188 (0.0)	0/25 (0.0)	1.000
HSV-2	88/180 (48.9)	7/21 (33.3)	0.177
RUBELLA	155/186 (83.3)	21/24 (87.5)	0.773

[A] P-value calculated using either Pearson Chi-Square or Fisher's Exact Test

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Table 5. Pathogen seroprevalence by human immunodeficiency virus (HIV) status

PATHOGEN	HIV STATUS		P-VALUE[A]
	Yes	No	
CMV	73/75 (97.3)	273/290 (94.1)	0.386
CHLAMYDIA TRACHOMATIS	23/72 (31.9)	109/269 (40.5)	0.185
PARVOVIRUS B19	40/73 (54.8)	164/290 (56.6)	0.787
BORDETELLA PERTUSSIS	0/80 (0.0)	14/284 (4.9)	**0.046**
TOXOPLASMA GONDII	21/46 (45.7)	52/180 (28.9)	**0.030**
TREPONEMA PALLIDUM	9/75 (12.0)	32/281 (11.4)	0.883
HSV-1	73/75 (97.3)	293/300 (97.7)	1.000
VZV	0/74 (0.0)	2/285 (0.7)	1.000
HSV-2	52/71 (73.2)	122/276 (44.2)	**< 0.001**
RUBELLA	66/74 (89.2)	239/282 (84.8)	0.332

[A] P-value calculated using either Pearson Chi-Square or Fisher's Exact Test

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Table 6. Pathogen seroconversion by human immunodeficiency virus (HIV) status.

PATHOGEN	HIV STATUS		P-VALUE[A]
	Yes	No	
CMV	0/52 (0.0)	3 /176 (1.7)	1.000
CHLAMYDIA TRACHOMATIS	1/41 (2.4)	3/137 (2.2)	1.000
PARVOVIRUS B19	5/44 (11.4)	8/165 (4.9)	0.153
BORDETELLA PERTUSSIS	0/53 (0.0)	0/163 (0.0)	--[b]
TOXOPLASMA GONDII	3/30 (10.0)	1/111 (0.9)	**0.030**
TREPONEMA PALLIDUM	0/50 (0.0)	6/153 (3.9)	0.340
HSV-1	1/51 (2.0)	2/180 (1.1)	0.529
VZV	0/48 (0.0)	1/167 (0.6)	1.000
HSV-2	6/43 (14.0)	18/160 (11.3)	0.601
RUBELLA	5/52 (9.6)	7/169 (4.1)	0.159

[A] P-value calculated using either Pearson Chi-Square or Fisher's Exact Test; [b] P-value cannot be calculated due to zero counts

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