Vertical transmission of Zika virus and its outcomes: a Bayesian synthesis of prospective studies

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Summary

Background Prospective studies of Zika virus in pregnancy have reported rates of congenital Zika syndrome and other adverse outcomes by trimester. However, Zika virus can infect and damage the fetus early in utero, but clear before delivery. The true vertical transmission rate is therefore unknown. We aimed to provide the first estimates of underlying vertical transmission rates and adverse outcomes due to congenital infection with Zika virus by trimester of exposure.

Methods This was a Bayesian latent class analysis of data from seven prospective studies of Zika virus in pregnancy. We estimated vertical transmission rates, rates of Zika-virus-related and non-Zika-virus-related adverse outcomes, and the diagnostic sensitivity of markers of congenital infection. We allowed for variation between studies in these parameters and used information from women in comparison groups with no PCR-confirmed infection, where available.

Findings The estimated mean risk of vertical transmission was 47% (95% credible interval 26 to 76) following maternal infection in the first trimester, 28% (15 to 46) in the second, and 25% (13 to 47) in the third. 9% (4 to 17) of deliveries following infections in the first trimester had symptoms consistent with congenital Zika syndrome, 3% (1 to 7) in the second, and 1% (0 to 3) in the third. We estimated that in infections during the first, second, and third trimester, respectively, 13% (2 to 27), 3% (–5 to 14), and 0% (–7 to 11) of pregnancies had adverse outcomes attributable to Zika virus infection. Diagnostic sensitivity of markers of congenital infection was lowest in the first trimester (42% [18 to 72]), but increased to 85% (51 to 99) in trimester two, and 80% (42 to 99) in trimester three. There was substantial between-study variation in the risks of vertical transmission and congenital Zika syndrome.

Interpretation This preliminary analysis recovers the causal effects of Zika virus from disparate study designs. Higher transmission in the first trimester is unusual with congenital infections but accords with laboratory evidence of decreasing susceptibility of placental cells to infection during pregnancy.

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Introduction

The Zika virus outbreaks in Central and South America in 2015–16 were accompanied by a high incidence of congenital microcephaly. The outbreaks caused widespread anxiety, with women in affected areas advised to avoid becoming pregnant, in addition to economic spread anxiety, with women in affected areas advised to avoid becoming pregnant, in addition to economic spread anxiety, with women in affected areas advised to avoid becoming pregnant, in addition to economic spread anxiety, with women in affected areas advised to avoid becoming pregnant, in addition to economic spread anxiety, with women in affected areas advised to avoid becoming pregnant, in addition to economic spread anxiety, with women in affected areas advised to avoid becoming pregnant, in addition to economic spread anxiety, with women in affected areas advised to avoid becoming pregnant, in addition to economic spread anxiety, with women in affected areas advised to avoid becoming pregnant, in addition to economic spread anxiety, with women in affected areas advised to avoid becoming pregnant, in addition to economic spread anxiety, with women in affected areas advised to avoid becoming pregnant. However, Zika virus can infect and damage the fetus early in utero, but clear before delivery. The true vertical transmission rate is therefore unknown. We aimed to provide the first estimates of underlying vertical transmission rates and adverse outcomes due to congenital infection with Zika virus by trimester of exposure.

Although the causal link between Zika virus and microcephaly is now established, the quantitative relation remains largely unknown. For other pathogens causing congenital infections, including cytomegalovirus, Toxoplasma, HIV, and hepatitis C virus, this knowledge has been gained through prospective cohort studies. These studies aim to estimate two target parameters: the vertical transmission rate, which is the probability of congenital infection following a maternal infection in pregnancy, and the rate of adverse outcomes due to congenital infection. Most vertical transmission studies include a paediatric control group of uninfected babies born to women infected in pregnancy. Comparisons between the congenitally infected and not congenitally infected groups can then establish the role of congenital infection in causing adverse outcomes while controlling for factors associated with maternal infection; this control group is essential to study less specific outcomes such as preterm delivery. Crucially, recruitment of women in such studies must be prospective and not the result of adverse findings on fetal or newborn examination. Otherwise the vertical transmission rate and the rate of sequelae are overestimated by the selective recruitment of pregnancies with adverse outcomes.

Zika virus presents considerable challenges because laboratory markers of congenital infection, although having reasonable analytical sensitivity, have poor diagnostic sensitivity. There is evidence that fetal infection can cause severe damage in utero, but that the infection then clears, leaving no immunological trace at delivery. The
true vertical transmission rate is therefore unobserved, and the rate of adverse outcomes following congenital infection cannot be estimated. In the absence of accurate diagnostic tests for congenital infection, inferences about the causal role of Zika virus in adverse pregnancy outcomes therefore require a second maternal control group of infants born to women who have not had a Zika virus infection during pregnancy.9

However, Zika virus poses further difficulties: although PCR testing in pregnancy is a highly specific indicator of infection, even the most intensive PCR testing protocol is likely to miss Zika virus infections because of the short duration of the PCR response, perhaps as short as 7 days.10 Seroconversion, IgG3, or IgM testing can identify infection in pregnancy, but lack specificity because of cross-reaction with other flaviviruses, and may only reflect an infection that cleared before delivery. As a result, the true vertical transmission rate in utero is unknown.

Adverse clinical outcomes have been observed in 7–45% of births to women with Zika virus infection in pregnancy. It is not clear how much of this variation is due to differences in diagnostic protocols or in criteria for adverse outcomes, or differences in vertical transmission rates between populations. In the absence of accurate diagnostics for maternal and congenital infection, conventional methods cannot estimate the causal effects of Zika virus in pregnancy.

Added value of this study
To our knowledge, this is the first reported synthesis of prospective studies of Zika virus in pregnancy, based on data from 1602 mother-child pairs. This Bayesian latent class analysis synthesises data from seven published studies with different designs, outcome definitions, and comparison groups, accounting for differences in diagnostic tests and protocols. Although highly preliminary, the findings are of scientific interest: average vertical transmission rates were estimated to be 47% in the first trimester, 28% in the second, and 25% in the third. The probability of outcomes consistent with congenital Zika syndrome was 9%, 3%, and 1% following maternal infection in the first, second, and third trimester, respectively. Diagnostic sensitivity of markers of congenital infection is lowest in the first trimester (42%), increasing to about 85% in the second trimester and 80% in the third.

To our knowledge, this study is the first to estimate vertical transmission rates and rates of adverse outcomes attributable to Zika virus infection, and to show how the key parameters of vertical transmission of Zika virus can be estimated.

Implications of all the available evidence
Latent class analysis can provide estimates of the vertical transmission rate and the risk of adverse outcomes following congenital infection, allowing for differences in study design and reporting. The finding of higher transmission rates in the first trimester is unusual with congenital infections, but it accords with laboratory studies showing that the susceptibility of specific placental cells to Zika virus decreases over the course of pregnancy.

Methods
Study identification and data extraction
We included prospective studies of women with Zika virus infection in pregnancy reporting adverse pregnancy and birth outcomes, markers of congenital infection, or both, based on a previous analysis and review of alternative designs for prospective studies of Zika virus in pregnancy.9 All the seven studies known to the authors...
as of May 31, 2019, were included in the analysis. To be as complete as possible, we considered studies in a published meta-analysis20 and did forward citation searches based on the seven studies and those in the meta-analysis. This process identified three further studies, all of which were excluded (appendix p 2). Among the seven studies, there were five prospective studies15–19 and two retrospectively reconstructed cohort studies based on registers.20,21

Table 1 shows the data in the form in which they were analysed from each study. Some studies provided data for both clinical outcomes and laboratory markers16,18,19 some for clinical outcomes alone.15,17,21 and one for laboratory searches based on the seven studies and those in the different criteria for adverse outcomes, which were not and ophthalmological outcomes, but source papers used adverse outcomes included other neurological, auditory, than those classified as congenital Zika syndrome. These all adverse outcomes reported in the source papers, other is examined in sensitivity analyses. OPZROs comprised information on the trimester distribution downs by trimester of maternal infection, but external material.15,16,18 Misclassification might have occurred, and classification was based on published supplementary studies not reporting congenital Zika syndrome, our regardless of the trimester of maternal infection. For fetal losses, stillbirths, and livebirth outcomes alike, PCR-confirmed, by trimester of infection

|                | PCR-confirmed, by trimester of infection | PCR-negative, by trimester of infection |
|----------------|----------------------------------------|----------------------------------------|
|                | 1           | 2           | 3  | Not reported | 1           | 2           | 3  | Not reported |
| OPZRO          |             |             |    |             |             |             |    |             |
| LMCI present   | 2           | 3           | 0  | …           | 0           | 0           | 0  | …           |
| LMCI absent    | 0           | 0           | 0  | …           | 0           | 0           | 0  | …           |
| OPZRO          |             |             |    |             |             |             |    |             |
| LMCI present   | 5           | 19          | 6  | …           | 0           | 0           | 0  | …           |
| LMCI absent    | 9           | 13          | 4  | …           | 0           | 0           | 0  | …           |
| No symptoms    |             |             |    |             |             |             |    |             |
| LMCI present   | 9           | 22          | 10 | …           | 0           | 0           | 0  | …           |
| LMCI absent    | 43          | 98          | 48 | …           | 0           | 0           | 0  | …           |
| Total mother–child pairs | 68          | 155         | 68 | …           | 0           | 0           | 0  | …           |
| Spanish cohort (PCR-negative patients reported as having a probable MIP)* |             |             |    |             |             |             |    |             |
| OPZRO          |             |             |    |             |             |             |    |             |
| LMCI present   | 1           | 2           | 5  | …           | 0           | 0           | 0  | …           |
| LMCI absent    | 0           | 2           | 4  | …           | 0           | 0           | 0  | …           |
| No symptoms    |             |             |    |             |             |             |    |             |
| LMCI present   | 1           | 6           | 3  | …           | 0           | 0           | 0  | …           |
| LMCI absent    | 2           | 16          | 12 | …           | 0           | 0           | 0  | …           |
| Total mother–child pairs | 4           | 26          | 24 | …           | 0           | 0           | 0  | …           |

Statistical model
We assumed that the observed data in the included studies were generated by the process shown

Articles
The number of mother–child pairs reported in each analysed study by clinical outcome, presence of congenital Zika syndrome (CZS) and maternal infection in pregnancy (MIP). The protocol was published previously.†

|                  | PCR-confirmed, by trimester of infection | PCR-negative, by trimester of infection |
|------------------|----------------------------------------|----------------------------------------|
|                  | 1 | 2 | 3 | Not reported | 1 | 2 | 3 | Not reported |
|                  |   |   |   |             |   |   |   |             |
| LMCI present     |   |   |   | 7           |   |   |   | 11           |
| LMCI absent      |   |   |   | 73          |   |   |   | 196          |
| Total mother–child pairs |   |   |   | 80          |   |   |   | 207          |
| Merriam et al (2020; PCR-negative patients reported as having a presumed MIP)+†† |   |   |   | 16          |   |   |   | 54           |

CZS=congenital Zika syndrome. MIP=maternal infection in pregnancy. LMCI=labatory markers of congenital Zika virus infection. OPZRO=other potentially Zika-virus-related outcome. *These unpublished data were provided by author Conners et al (2018; PCR-negative patients reported as having a suspected MIP)‡‡

‡‡For proportions of patients with confirmed or suspected infection in each trimester see the appendix (p 4).

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Table 1: The number of mother–child pairs reported in each analysed study by clinical outcome, presence of laboratory marker of congenital infection, and MIP status.

See Online for appendix

The number of mother–child pairs in each trimester of infection there is still a possibility of an OPZRO, but not CZS. In the absence of congenital infection, an OPZRO could either be Zika-virus-related or non-Zika-virus-related; both are included in probability $a$. The probability of a laboratory marker of congenital infection being present is $d$, the diagnostic sensitivity.

The group with a negative PCR test result is a mixture of women who have an infection in pregnancy (proportion $m$), and women without an infection in pregnancy (proportion $1−m$). In the absence of congenital infection there is still a possibility of an OPZRO, but now with probability $b$. The difference ($a−b$) therefore represents the proportion of OPZROs among those with congenital infection that is causally attributable to maternal Zika virus infection. Congenital Zika syndrome can only occur in the presence of congenital infection.

We assumed that the vertical transmission rate, the risks of congenital Zika syndrome and of other adverse outcomes following congenital infection, and diagnostic sensitivity (ie, the parameters $v$, $z$, $a$, and $d$) vary by trimester and also between centres. The between-study variation in these parameters was captured by a random effects meta-analytical model (appendix p 3). We assumed that diagnostic sensitivity is the same, regardless of whether the clinical outcome is congenital Zika syndrome, OPZRO, or asymptomatic (figure); this was tested in a sensitivity analyses. The parameters $b$ and $m$ were assumed to vary only by centre, not by trimester, because they were not related to Zika virus.

To see how the data inform the model parameters, consider the data relating to the first trimester from the study by Pomar and colleagues* (table 1). These six numbers estimate six probabilities as follows: the proportion with congenital Zika syndrome and positive laboratory markers (two of 68) is an estimate of the product $vzd$; the proportion with congenital Zika syndrome and negative laboratory markers (zero of 68) is an estimate of the product $v(1−d)$; the proportion with OPZROs and positive laboratory markers (five of 68) estimates $v(1−a)zd$; and the proportion with OPZROs and negative laboratory markers (nine of 68) could have Zika-virus-related or non-Zika-virus-related OPZROs and therefore estimates a sum of products $v(1−a)(1−d)+(1−v)b$. The same principle is followed for studies that do not report laboratory markers at all. For example, Hoen and colleagues‡ reported congenital Zika syndrome in 13 of 189 patients after confirmed infection in trimester one; this estimates the product $vz$.

Turning to outcomes in PCR-negative women, the Spanish cohort reported seven of 60 patients with OPZROs after an infection in trimester three (unpublished data provided by AS-A); this is an estimate of $m(v[1−z][1−a]d+[1−v][b]+(1−m)b)z$.

Although the relation between model parameters and data is complex, there are more items of data than model parameters, so they can all be estimated. The seven studies (table 1) each contribute directly or indirectly to every parameter; if any study is removed, all the estimates will change. Estimation was done by Bayesian Markov chain Monte Carlo methods. Details of the likelihood, model, prior distributions, model selection, convergence checks, and software are available in the appendix (pp 3-11) along with the program code.

**Sensitivity analyses**

Sensitivity analyses assessed the robustness of conclusions to: (1) the degree of between-study variation in parameters $v$, $z$, $a$, and $d$; (2) the exclusion of each data source in turn; (3) the classification of fetal loss with undetermined clinical outcomes as congenital Zika syndrome or asymptomatic, rather than OPZRO; (4) the definition of congenital Zika syndrome being less than 100% specific for congenital Zika virus infection; and (5) diagnostic sensitivity being greater for congenital Zika syndrome outcomes than for OPZRO and asymptomatic.

**Role of the funding source**

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study. The final decision to submit for publication was taken by CT as co-leader of the ZIKAction Vertical Transmission Work Package.
Results

We report fetal and newborn outcomes for 1602 mother–child pairs: 40 with congenital Zika syndrome, 181 with OPZROs, 1094 who were asymptomatic, and 287 who were unclassified. In our preferred model, selected on the basis of goodness-of-fit statistics and parsimony (appendix p 6), the estimated mean vertical transmission rate decreases from 47% (95% credible interval 26–76) for maternal infections in trimester one, to 28% (15–46) in trimester two, and 25% (13–47) in trimester three (table 2). The risk of congenital Zika syndrome conditional on congenital infection also decreased from 19% (8–37) in trimester one to 11% (5–25) in trimester two and 3% (0–12) in trimester three.

The absolute risk of congenital Zika syndrome in women infected in pregnancy (the product of these two parameters) was 9% (4–17), 3% (1–7), and 1% (0–3) in the first, second, and third trimesters, respectively.

Diagnostic sensitivity for congenital infection was estimated to be 42% (18–72) after infections in trimester one, but was nearly twice that in trimesters two and three (table 2).

The average rate of OPZROs in infants with congenital infection but without congenital Zika syndrome (parameter a in the figure) is approximately constant over the three trimesters (table 2). The overall rate of adverse outcomes, including congenital Zika syndrome, in those with a maternal infection in pregnancy was 24% (95% credible interval 14–37) in trimester one, 14% (8–23) in trimester two, and 11% (5–20) in trimester three (table 2). Estimated rates of adverse outcomes unrelated to congenital infection (b in the figure) range from 1% (0–4) to 17% (4–33; table 3), and average 11%. Subtracting this from the overall rate of adverse outcomes in congenital infection, we obtained approximate estimates of the risks of adverse outcomes other than congenital Zika syndrome that can be attributed to Zika virus in pregnancy. The results of this subtraction (13%, 3%, and 0% in trimesters one, two, and three, respectively) suggest that most adverse outcomes attributable to Zika virus are within the definition of symptoms consistent with congenital Zika syndrome.

The estimated proportion of women with maternal infection in pregnancy in the PCR-negative comparison groups was 12% in Brasil and colleagues’ study,20 2% in the Spanish cohort, in which this (IgG-positive, PRNT non-negative) group were described as having a probable infection;19 26% in Merriam and colleagues’ study21 (IgM-positive patients reported as having a presumed infection); and 52% in Conners and colleagues’ study20 (IgM-positive patients reported as having a suspected infection). These estimates have wide 95% credible intervals (table 3).

The sensitivity analyses establish that the general pattern of results in the base-case model is robust against a wide range of alternative assumptions (table 4). Estimated risks of congenital Zika syndrome are somewhat sensitive to the assumed level of between-study variation, with estimates from models that allow a little less (a factor of 1·5) or a little more (a factor of 3·0) variation by factors of 1·15 to 1·35 above and below the base-case estimates. Classification of fetal losses with undetermined clinical outcome as congenital Zika syndrome rather than OPZRO almost doubles from 1% (0–4) to 17% (4–33; table 3), and average 11%. Subtracting this from the overall rate of adverse outcomes in congenital infection, we obtained approximate estimates of the risks of adverse outcomes other than congenital Zika syndrome that can be attributed to Zika virus in pregnancy. The results of this subtraction (13%, 3%, and 0% in trimesters one, two, and three, respectively) suggest that most adverse outcomes attributable to Zika virus are within the definition of symptoms consistent with congenital Zika syndrome.

The estimated proportion of women with maternal infection in pregnancy in the PCR-negative comparison groups was 12% in Brasil and colleagues’ study,20 2% in the Spanish cohort, in which this (IgG-positive, PRNT non-negative) group were described as having a probable infection;19 26% in Merriam and colleagues’ study21 (IgM-positive patients reported as having a presumed infection); and 52% in Conners and colleagues’ study20 (IgM-positive patients reported as having a suspected infection). These estimates have wide 95% credible intervals (table 3).

The sensitivity analyses establish that the general pattern of results in the base-case model is robust against a wide range of alternative assumptions (table 4). Estimated risks of congenital Zika syndrome are somewhat sensitive to the assumed level of between-study variation, with estimates from models that allow a little less (a factor of 1·5) or a little more (a factor of 3·0) variation by factors of 1·15 to 1·35 above and below the base-case estimates. Classification of fetal losses with undetermined clinical outcome as congenital Zika syndrome rather than OPZRO almost doubles...
Target parameters: posterior summaries from the preferred model

Table 2: Data are median (95% credible interval). OPZRO=other potentially Zika-virus-related outcome.

| Parameter                                                                 | Maternal infection in trimester one | Maternal infection in trimester two | Maternal infection in trimester three |
|---------------------------------------------------------------------------|-------------------------------------|------------------------------------|--------------------------------------|
| Probability of vertical transmission given maternal infection in pregnancy | 47% (26 to 76)                      | 28% (15 to 46)                     | 25% (13 to 47)                       |
| Probability of congenital Zika syndrome given maternal infection in pregnancy | 19% (8 to 37)                      | 11% (5 to 25)                      | 3% (0 to 12)                         |
| Probability of congenital Zika syndrome given maternal infection in pregnancy | 9% (4 to 17)                       | 3% (1 to 7)                        | 1% (0 to 3)                          |
| Diagnostic sensitivity                                                   | 42% (18 to 72)                     | 85% (51 to 99)                     | 80% (42 to 99)                       |
| Probability of OPZRO given congenital infection                           | 42% (22 to 65)                     | 46% (27 to 64)                     | 43% (23 to 66)                       |
| Probability of any adverse outcome given maternal infection in pregnancy | 24% (14 to 37)                     | 14% (8 to 23)                      | 11% (5 to 20)                        |
| Probability of any adverse outcome attributable to congenital infection given maternal infection in pregnancy | 13% (2 to 27) | 3% (-5 to 14) | 0% (-7 to 11) |

Data are median (95% credible interval). OPZRO=other potentially Zika-virus-related outcome.

Table 3: Study-specific parameters: posterior summaries from the preferred model

| Study                        | Proportion of neonates with adverse outcomes in the absence of maternal Zika virus infection | Proportion of women in comparison group with Zika virus infection in pregnancy |
|------------------------------|-------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------|
| Pomar et al (2018)           | 9% (2–15)                                                                                        | -                                                                              |
| Nogueira et al (2018)        | 17% (4–33)                                                                                      | -                                                                              |
| Hoen et al (2018)            | 1% (0–4)                                                                                        | -                                                                              |
| Rodó et al (2019)            | 12% (9–19)                                                                                      | 2% (0–16)                                                                     |
| Brasil et al (2016)          | 13% (2–29)                                                                                      | 12% (1–49)                                                                    |
| Meriarn et al (2020)         | 11% (2–22)                                                                                      | 26% (1–91)                                                                    |
| Conners et al (2017)         | -                                                                                               | 52% (23–94)                                                                   |

Data are median (95% credible interval).

Discussion

Previous studies have reported rates of adverse outcomes of congenital Zika virus infection between 7% and 46%, and vertical transmission rates between 9% and 35%, but these rates are substantially underestimated because they take no account of the low diagnostic sensitivity of tests for fetal infection shown by our model (42% in the first trimester). Taking this into account, we found that vertical transmission rates declined with trimester of maternal infection, as did rates of congenital Zika syndrome. Following a Zika virus infection in pregnancy, the incidence of adverse outcomes, including congenital Zika syndrome, likely to be caused by maternal Zika virus infection was substantially higher in trimester one than in trimester two or three. Given the likelihood of error in the reported trimester of infection, it might be that all Zika-virus-related outcomes are due to infection in trimester one. From our results, we can deduce that, following an infection in pregnancy, about 35% of adverse outcomes are directly or indirectly attributable to maternal infection (or 55% after infections in trimester one), which is consistent with Pomar and colleagues’ estimate of 47% of adverse outcomes being due to Zika virus following diagnosed congenital infection.

Our findings, based on only seven studies done in diverse settings, many reporting incomplete data, are no more than preliminary. However, the findings are consistent with previous reports, and robust against challenge from a wide range of sensitivity analyses.

Latent class models could be extended to include a wider array of outcome categories, potential effect modifiers such as previous flavivirus infections, and extended to individual patient data. Information on the analytical sensitivity of diagnostic tests could also be incorporated. On the basis of our sensitivity analyses, tests on amniotic fluid and perhaps placental samples should be distinguished from tests on neonatal samples. With adequate data, the model we used (figure) can be modified to distinguish outcomes that are the result of congenital infection, such as congenital Zika syndrome, and outcomes such as fetal loss, stillbirth, or prematurity, which could be the result of congenital infection or of maternal Zika virus infection in the absence of congenital infection, as is seen with other infections in pregnancy. These additions to the model will require far more data.
### Table 4: Sensitivity analyses for each outcome by trimester of maternal infection

| Parameter | T1 | T2 | T3 | T1 | T2 | T3 | T1 | T2 | T3 | T1 | T2 | T3 | T1 | T2 | T3 | T1 | T2 | T3 | T1 | T2 | T3 |
|-----------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| Vertical transmission rate after maternal infection in pregnancy | 46% | 28% | 25% | 19% | 11% | 10% | 3% | 42% | 46% | 43% | 42% | 85% | 80% | 9% | 3% | 1% | 24% | 14% | 11% |
| CZS after congenital infection | 45% | 28% | 25% | 35% | 16% | 8% | 38% | 46% | 42% | 45% | 78% | 79% | 16% | 5% | 2% | 27% | 15% | 12% |
| OPZRO after congenital infection, given no CZS | 49% | 26% | 24% | 18% | 11% | 3% | 29% | 43% | 40% | 38% | 87% | 80% | 9% | 3% | 1% | 20% | 13% | 10% |
| Diagnostic sensitivity | 49% | 26% | 24% | 18% | 11% | 3% | 29% | 43% | 40% | 38% | 87% | 80% | 9% | 3% | 1% | 20% | 13% | 10% |
| CZS after maternal infection in pregnancy | 46% | 28% | 25% | 19% | 11% | 10% | 3% | 42% | 46% | 43% | 42% | 85% | 80% | 9% | 3% | 1% | 24% | 14% | 11% |
| CZS or OPZRO after maternal infection in pregnancy | 45% | 28% | 25% | 35% | 16% | 8% | 38% | 46% | 42% | 45% | 78% | 79% | 16% | 5% | 2% | 27% | 15% | 12% |

### Results

Results are awaited from several large, multicentre cohort studies of Zika virus in pregnancy (eg, NCT02856884, and ZIKAlliance, ZIKAction, and ZikaPLAN studies). Statistical plans for pooled data analysis have been, or are being, prepared by the Zika Virus Individual Participant Data Analysis Consortium, the European Commission consortia, and the Brazilian Ministry of Health. In the absence of accurate diagnostics for congenital infection, inference regarding the causal effects of Zika virus in pregnancy can only be made by comparing outcomes in women with and without infection in pregnancy, but—as we have seen—the only available control groups are mixtures of both infected and uninfected women. In one analysis plan, the intention is to regress the relative risk of pregnancy can only be made by comparing outcomes in women with and without infection in pregnancy. In one analysis plan, the intention is to regress the relative risk of vertical transmission, and diagnostic sensitivity varied randomly across studies. The estimated means are only averages over the studies included. We adopted informative priors for between-study variation, because there were insufficient data to estimate the extent of variation in all four parameters (v, z, a, and d), although sensitivity analyses suggest that results are robust to reasonable changes in previous assumptions. Analyses suggested a high level of between-study variation in the target parameters (appendix p 6), which is unexplained. The small quantity of data prevented many extensions and elaborations that would be required in a definitive analysis.

### Conclusions

Conclusions are limited to outcomes manifested by the end of the perinatal period and therefore do not account for more ZIKAlliance studies see https://zikalliance.tghn.org/
For more on ZIKAction studies see https://zikaction.org/
For more on ZikaPLAN studies see https://zikaplan.tghn.org/
for outcomes that resolve, and other outcomes that develop subsequently.19 The most important limitations arise from uncertainties about integrity of data collection and recruitment. Although all the studies included seemed to be consistent with prospective ascertainment, this can be difficult to implement, and there is a danger of selective recruitment of symptomatic cases, perhaps especially in retrospectively reconstructed or surveillance cohorts.19–21 This is more easily avoided in prospective studies in which every pregnant woman is recruited. Selective inclusion of congenital Zika syndrome detected on prenatal ultrasound might have occurred. In studies that include both a PCR-positive and PCR-negative comparison group,19 if recruitment was truly prospective, the distribution of cases across trimesters should be the same in both groups. The data from table 1 suggest that these studies might not have passed this test, and it is hard to rule out the possibility that neonates with more severe outcomes were tested more intensively. Our sensitivity analyses showed that higher diagnostic sensitivity with severe outcomes has little effect on estimates of vertical transmission or adverse outcome rates, but this does not address the potential for biases due to selective recruitment of patients with more severe outcomes.

The ZIKAlliance, ZIKAction, and ZikaPLAN studies are standard prospective designs offering recruitment to all eligible pregnant women. It is possible that these studies will generate superior data, especially on control groups in whom maternal infection can be ruled out with a high level of certainty. These studies will deliver better data on the risk of adverse outcomes in the absence of maternal infection and will help to distinguish the causal effects of congenital Zika virus infection from indirect effects of maternal Zika virus infection.

The high transmission rate in trimester one, an unusual finding with congenital infections, although not unique,7 is supported by experimental evidence. The haematogenic route for Zika virus vertical transmission relies on three possible entry sites to access fetal circulation: (1) the maternal decidual tissues and the juxtaposed fetal extravillous trophoblasts; (2) the syncytiotrophoblast layer covering the villous trophoblast; and (3) the amnionchorionic membrane surrounding the fetus. Each has been investigated in vitro to define when the placenta is most permissive to infection.

Villous and decidual explants of the first trimester are highly susceptible to Zika virus,12,13 as evidenced by a wide variety of virus-positive cells of both maternal and fetal origin, namely extravillous trophoblasts, proliferating trophoblasts, glandular cells, and decidual cells. Although decidual tissues maintain their susceptibility to Zika virus throughout pregnancy,13,14 chorionic villi gradually decrease their permissivity after the first trimester.15 Primary trophoblast cells and villous explants derived from the second and third trimesters are either resistant or poorly susceptible to Zika virus, because of the release of type III interferon, IFNγ, by the syncytiotrophoblast, acting both at a paracrine and autocrine level.13,16 Moreover, Sheridan and colleagues17 showed that trophoblasts derived from embryonic stem cells that are analogous to the primitive placental cells at the time of implantation support a quick and productive replication of the virus, whereas primary trophoblasts and syncytiotrophoblasts from term placentas are resistant to Zika virus infection. Regarding the amnionchorionic membrane, amniotic epithelial cells from midgestation generate higher infectious titres than the ones obtained from late-gestation placentas.18 These findings suggest a decreasing susceptibility of constituents of the placental barrier over the course of gestation, consistent with the vertical transmission risks we have described.

The causes of the between-study variation in vertical transmission rates and sequelae rates remain to be identified. Although WHO has produced standardised protocols for studies of Zika virus in pregnancy,19 studies have used a variety of clinical definitions, diagnostic tests, and testing schedules. Latent class analysis can remove the variation engendered by these incidental factors, allowing investigators to focus on the real causes of between-centre variation. An individual patient data analysis of datasets with harmonised definitions, protocols, and diagnostics is the ideal, but latent class analysis will still be required to handle diagnostic test inaccuracy. The objective now is to build a larger and more detailed evidence base for a more comprehensive analysis.

Contributors

AEA conceived the study, devised and carried out the statistical data analysis, and drafted the paper, with the help of all authors. AS-A and AA extracted the clinical classification of outcomes of pregnancy on the basis of material in the source publications, where this was not reported directly. All authors reviewed and approved the final draft. CT made the decision to submit for publication, as co-lead of the ZIKAction Vertical Transmission Work Package.

Declaration of interests

CT has received funding from AAbbVie and the Penta Foundation, outside of the submitted work. AEA, AS-A, FB, CT, and CSP are members of the ZIKAction consortium. CG is the principal investigator of the ZIKAction consortium. AA declares no competing interests.

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