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

A E Ades, Antoni Soriano-Arandes, Ana Alarcon, Francesco Bonfante, Claire Thorne, Catherine S Peckham, Carlo Giaquinto

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.

Funding European Union Horizon 2020 programme.

Introduction

The Zika virus outbreaks in Central and South America in 2015–16 were accompanied by a high incidence of congenital microcephaly.[1] The outbreaks caused widespread anxiety, with women in affected areas advised to avoid becoming pregnant, in addition to economic damage from adverse travel advisories. Although Zika virus incidence has subsided,[2] quantifying the risks for the fetus, neonate, and child following Zika virus infection in pregnancy remains a priority to inform the public health response to future outbreaks.

Although the causal link between Zika virus and microcephaly is now established,[1,3] the quantitative relationship 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.[4–7] 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.[8] 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.[9] The

Lancet Infect Dis 2020

Published Online
October 14, 2020
https://doi.org/10.1016/S1473-3099(20)30432-1

See Online/Comment
https://doi.org/10.1016/S1473-3099(20)30454-0

Department of Population Health Science, University of Bristol Medical School, Bristol, UK (Prof A E Ades PhD); **Pediatric Infectious Diseases and Immunodeficiencies Unit, Hospital Universitari Vall d'Hebron, Vall d'Hebron Research Institute, Barcelona, Spain** (A Soriano-Arandes PhD); **Department of Neonatology, Hospital Universitari Sant Joan de Déu, Sant Joan de Déu Research Institute, Barcelona, Spain** (A Alarcon PhD); **Laboratory of Experimental Animal Models, Division of Comparative Biomedical Sciences, Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy** (F Bonfante DVM); **Population Policy and Practice Programme, University College London Great Ormond Street Institute of Child Health, London, UK** (Prof C Thorne PhD, Prof C S Peckham MD); **and Dipartimento di Salute della Donna e del Bambino, Università degli Studi di Padova, Padua, Italy** (Prof C Giaquinto MD)

Correspondence to:
Prof A E Ades, Department of Population Health Science, University of Bristol Medical School, Bristol BS8 2PS, UK
t.ades@bristol.ac.uk

Research in context

Evidence before this study

Although the causal link between Zika virus in pregnancy and microcephaly has been established, the quantitative risks to the fetus and neonate can only be established by prospective follow-up of women with Zika virus infection in pregnancy. We identified seven studies published before May 31, 2019, for inclusion in our analysis using previously published criteria and forward citation searches. These studies had varied designs, recruiting women in different ways, some including a comparison group of PCR-negative women, others not; some have reported on markers of congenital infection, some on adverse outcomes, and others on both.

Reported vertical transmission rates following PCR-confirmed Zika virus infection in pregnancy range from 9% to 35%, but the standard laboratory markers of congenital infection, IgM and PCR, have low diagnostic sensitivity: it seems that the virus can infect the fetus, sometimes causing profound damage, but clear 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.

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.[10]

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.[11] 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 pregnancy.[12] Therefore, control groups consisting of women with a negative PCR test result for Zika virus, with or without serological evidence of infection, will comprise infected and uninfected women in a proportion determined by the diagnostic schedule, the sensitivity and specificity of the diagnostic tests, and the relative incidence of Zika virus and other flaviviruses.

This paper presents a Bayesian latent class analysis of the seven prospective studies available so far. Latent class analysis is often used when observations fall into unobserved (latent) categories in unknown proportions, for example when estimating the accuracy of diagnostic tests with no gold standard.[13] Our results, although preliminary and based on imperfect data, are of scientific interest in their own right, and, to our knowledge, provide the first estimates of underlying vertical transmission rates and adverse outcomes due to congenital infection, by trimester of exposure.

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.[10] 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-analysis[14] 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 studies[15–19] 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 markers,[16,18,19] some for clinical outcomes alone,[15,17,21] and one for laboratory markers alone.[20] Two studies[20,21] did not provide breakdowns by trimester of maternal infection, but external information on the trimester distribution was available and used in the analysis (see appendix p 4).

All included studies reported fetal and neonatal outcomes in women with confirmed (PCR-positive) infection in pregnancy. Four of these studies[15,19–21] also included a group of women who tested PCR-negative for Zika virus; each of these groups comprised an unknown mixture of infected and uninfected women. In three of these four comparison groups, women had serological markers suggestive of possible infection in pregnancy, based on IgM testing[20,21] or IgG with non-negative plaque reduction neutralisation testing (PRNT).[19]

Clinical outcomes were categorised as symptoms consistent with congenital Zika syndrome, other potentially Zika-virus-related outcomes (OPZROs), and no symptoms. Regarding congenital Zika syndrome, a paediatric infectious disease consultant (AS-A) and a neonatologist (AA) applied Hoen's criteria[17] based on earlier work[23] to fetal losses, stillbirths, and livebirth outcomes alike, regardless of the trimester of maternal infection. For studies not reporting congenital Zika syndrome, our classification was based on published supplementary material.[15,16,18] Misclassification might have occurred, and is examined in sensitivity analyses. OPZROs comprised all adverse outcomes reported in the source papers, other than those classified as congenital Zika syndrome. These adverse outcomes included other neurological, auditory, and ophthalmological outcomes, but source papers used different criteria for adverse outcomes, which were not detailed in full. Note that OPZROs include both Zika-virus-related and non-Zika-virus-related outcomes. Cases of fetal loss or stillbirth for which the clinical classification was undetermined or unreported were classified as OPZROs, but sensitivity analyses were run in which they were all classified as congenital Zika syndrome or all classified as asymptomatic. Full details of the data sources and data from individual cases, where possible, are shown in the appendix (pp 1, 2).

Statistical model

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

	PCR-confirmed, by trimester of infection				PCR-negative, by trimester of infection			
	1	2	3	Not reported	1	2	3	Not reported
Pomar et al (2018)[16]								
CZS								
LMCI present	2	3	0	..	..	..	..	..
LMCI absent	0	0	0	..	..	..	..	..
OPZRO								
LMCI present	5	19	6	..	..	..	..	..
LMCI absent	9	13	4	..	..	..	..	..
No symptoms								
LMCI present	9	22	10	..	..	..	..	..
LMCI absent	43	98	48	..	..	..	..	..
Total mother–child pairs	68	155	68	..	..	..	..	..
Nogueira et al (2018)[18]								
CZS								
LMCI present	0	0	0	..	..	..	..	..
LMCI absent	0	0	0	..	..	..	..	..
OPZRO								
LMCI present	1	2	5	..	..	..	..	..
LMCI absent	0	2	4	..	..	..	..	..
No symptoms								
LMCI present	1	6	3	..	..	..	..	..
LMCI absent	2	16	12	..	..	..	..	..
Total mother–child pairs	4	26	24	..	..	..	..	..
Spanish cohort (PCR-negative patients reported as having a probable MIP)*								
CZS								
LMCI present	0	1	0	..	0	0	0	..
LMCI absent	2	0	0	..	0	0	0	..
OPZRO								
LMCI present	1†	0	0	..	0	0	0	..
LMCI absent	0	1†	0	..	6+1†	6	7	..
No symptoms								
LMCI present	0	0	0	..	0	0	0	..
LMCI absent	2	6	1	..	28	48	53	..
Total mother–child pairs	5	8	1	..	35	54	60	..
Hoen et al (2018)[17]								
CZS	13	3	1	..	..	..	..	..
OPZRO	11	6	5	..	..	..	..	..
No symptoms	165	243	108	..	..	..	..	..
Total mother–child pairs	189	252	114	..	..	..	..	..
Brasil et al (2016; PCR negative patients showed no evidence of MIP)[15]								
CZS	2	5	0	..	0	0	0	..
OPZRO	4+5†	29+3†	9+1†	..	3	2	1+1†	..
No symptoms	9	34	24	..	1	33	20	..
Total mother–child pairs	20	71	34	..	4	35	22	..

(Table 1 continues on next page)

	PCR-confirmed, by trimester of infection				PCR-negative, by trimester of infection			
	1	2	3	Not reported	1	2	3	Not reported
(Continued from previous page)								
Conners et al (2018; PCR-negative patients reported as having a suspected MIP)[20]‡								
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)[21]‡								
CZS	..	..	..	1	..	..	..	0
OPZRO	..	..	..	1+1†	..	..	..	6+1†
No symptoms	..	..	..	13	..	..	..	47
Total mother–child pairs	..	..	..	16	..	..	..	54

CZS=congenital Zika syndrome. MIP=maternal infection in pregnancy. LMCI=laboratory markers of congenital infection. OPZRO=other potentially Zika-virus-related outcome. *These unpublished data were provided by author AS-A. The protocol was published previously.[19,22] †Fetal losses with undetermined or unreported clinical categorisation. ‡For proportions of patients with confirmed or suspected infection in each trimester see the appendix (p 4).

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

schematically in the figure. Women with PCR-confirmed infection in pregnancy transmit Zika virus to their fetus with probability v, and this can lead either to congenital Zika syndrome with probability z, or not. If there is congenital infection but no congenital Zika syndrome, the outcome might be OPZRO with probability a, or no symptoms with probability $1–a$. Note that in fetuses with 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[16] (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 $vz(1–d)$; the proportion with OPZROs and positive laboratory markers (five of 68) estimates $v(1–z)ad$; 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 $v(1–z)a(1–d)+(1–v)b$. The same principle is followed for studies that do not report laboratory markers at all. For example, Hoen and colleagues[17] 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 $m(v[1–z]a[1–d]+[1–v]b)+(1–m)b$.

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.

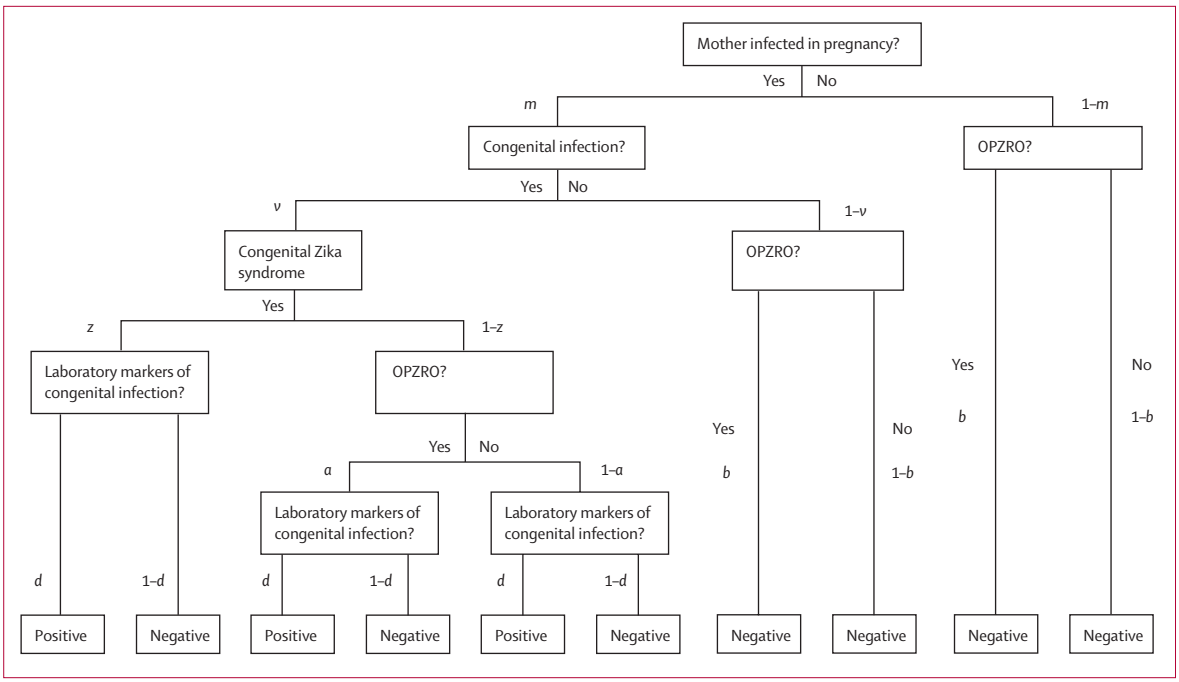

Figure: **Data generation process, showing the links between the observed data and the unobserved model parameters**
OPZRO=other potentially Zika-virus-related outcome.

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 is 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;[15] 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' study[21] (IgM-positive patients reported as having a presumed infection); and 52% in Conners and colleagues' study[20] (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

	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 congenital infection	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 2: **Target parameters: posterior summaries from the preferred model**

	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)[16]	9% (2–15)	..
Nogueira et al (2018)[18]	17% (4–33)	..
Hoen et al (2018)[17]	1% (0–4)	..
Rodó et al (2019)[19]	13% (9–19)	2% (0–16)
Brasil et al (2016)[15]	13% (2–29)	12% (1–49)
Merriam et al (2020)[21]	11% (2–22)	26% (1–91)
Conners et al (2017)[20]	..	52% (23–94)

Data are median (95% credible interval).

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

the congenital Zika syndrome rate in trimester one. Removal of each study in turn generated a series of estimates of the congenital Zika syndrome rate in trimester one that varied from 7% to 11% (base case 9%) but did not affect the overall pattern of results. However, removal of the study by Pomar and colleagues[16] substantially reduced diagnostic sensitivity in trimesters one and two; in this study, a high proportion of congenital infection was diagnosed by PCR testing of placental samples.

If the diagnostic sensitivity is lower for OPZROs and asymptomatic outcomes than for congenital Zika syndrome, even by a log odds ratio of 2, the predicted vertical transmission rate is increased by a factor of no more than 1·1, and the rate of congenital Zika syndrome conditional on vertical transmission is decreased by the same amount, so the overall effect on the predicted rate of congenital Zika syndrome is negligible. The results were more sensitive to the assumption that our definition of congenital Zika syndrome is 100% specific for congenital Zika virus: lowering the positive predictive value to 80% or 60% had the effect of decreasing both the estimated congenital Zika syndrome rate conditional on vertical transmission and the absolute congenital Zika syndrome rate by the same proportion. Decreasing the positive predictive value also results in an increase in the estimated vertical transmission rate, especially in trimester one, whereas estimated diagnostic sensitivity is decreased.

Discussion

Previous studies[15–21] 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.[16]

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,[24,25] 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.[26–28] These additions to the model will require far more data.

	Vertical transmission rate after maternal infection in pregnancy			CZS after congenital infection			OPZRO after congenital infection, given no CZS			Diagnostic sensitivity			CZS after maternal infection in pregnancy			CZS or OPZRO after maternal infection in pregnancy		
	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3
Base case	46%	28%	25%	19%	11%	3%	42%	46%	43%	42%	85%	80%	9%	3%	1%	24%	14%	11%
Between-trial variation: base case assumes that 95% of study-specific outcomes are within a factor of 2 above or below the median																		
Factor of 1·0: fixed effect	28%	23%	22%	22%	10%	3%	33%	31%	33%	62%	96%	89%	6%	2%	1%	14%	9%	8%
Factor of 1·5: random effect	48%	26%	26%	22%	15%	4%	40%	44%	42%	45%	88%	82%	8%	3%	1%	22%	13%	10%
Factor of 3·0: random effect	48%	29%	26%	20%	12%	4%	43%	47%	44%	39%	82%	78%	9%	3%	1%	26%	15%	12%
Factor of 1200: random effect	46%	31%	26%	36%	24%	8%	45%	49%	46%	35%	78%	75%	16%	7%	2%	30%	19%	13%
Classification of fetal loss outcomes with undetermined status as OPZRO, CZS, or asymptomatic: base case classifies them as OPZRO																		
CZS	45%	28%	25%	35%	16%	8%	38%	46%	42%	45%	78%	79%	16%	5%	2%	27%	15%	12%
Asymptomatic	49%	26%	24%	18%	11%	3%	29%	43%	40%	38%	87%	80%	9%	3%	1%	20%	13%	10%
Removal of data, one study at a time, and removal of all data																		
Pomar et al (2018)[16]	48%	28%	21%	24%	12%	5%	46%	39%	56%	27%	64%	73%	11%	3%	1%	27%	13%	12%
Nogueira et al (2018)[18]	43%	24%	22%	22%	14%	4%	45%	51%	39%	40%	83%	74%	9%	3%	1%	24%	14%	9%
Hoen et al (2018)[17]	47%	37%	30%	14%	10%	2%	47%	52%	46%	42%	75%	75%	7%	4%	1%	25%	21%	14%
Rodó et al (2019)[19]	44%	31%	27%	16%	8%	0%	41%	42%	40%	49%	84%	80%	7%	2%	1%	22%	14%	11%
Brasil et al (2016)[15]	39%	21%	21%	21%	11%	5%	34%	36%	41%	43%	90%	81%	8%	2%	1%	18%	9%	9%
Merriam et al (2020)[21]	48%	29%	26%	20%	11%	3%	43%	47%	43%	41%	84%	79%	9%	3%	1%	26%	15%	12%
Conners et al (2017)[20]	51%	33%	29%	17%	9%	3%	40%	42%	42%	46%	85%	82%	8%	3%	1%	25%	16%	13%
All data removed	50%	50%	50%	50%	50%	50%	50%	50%	50%	50%	50%	50%	18%	18%	18%	34%	34%	34%
Lower diagnostic sensitivity with OPZRO and asymptomatic outcomes relative to CZS: base case has equal diagnostic sensitivity																		
Log odds ratio 0·5	51%	30%	26%	17%	10%	3%	39%	44%	42%	49%	85%	84%	8%	3%	1%	25%	15%	12%
Definition of CZS not 100% specific for congenital Zika virus: base-case positive predictive value is 100%																		
Positive predictive value 80%	51%	28%	25%	14%	8%	2%	42%	46%	44%	36%	82%	78%	7%	2%	1%	25%	14%	11%
Positive predictive value 60%	64%	32%	27%	10%	6%	2%	36%	45%	44%	24%	73%	74%	6%	2%	0%	27%	15%	12%

Data are posterior median percent. CZS=congenital Zika syndrome. OPZRO=other potentially Zika-virus-related outcome. T1=trimester 1. T2=trimester 2. T3=trimester 3.

Table 4: **Sensitivity analyses for each outcome by trimester of maternal infection**

Results are awaited from several large, multicentre cohort studies of Zika virus in pregnancy (eg, NCT02856984, 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 Meta-analysis Consortium,[29] 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,[29] the intention is to regress the relative risk of adverse outcomes in PCR-positive women relative to PCR-negative women against estimates of test sensitivity and specificity. However, it is not clear that meta-regression would adjust for test inaccuracy: lack of either sensitivity or specificity will bias relative risks towards a null effect. Furthermore, the proportion of true and false positive diagnoses of maternal infection, which is our study-specific parameter m, is far more sensitive to the absolute and relative incidence of Zika virus and cross-reacting flaviviruses, than to sensitivity and specificity.

By contrast, Bayesian latent class analysis estimates the study-specific mixing proportions and delivers estimates of parameters that are crucial for scientific understanding and causal inference: namely the vertical transmission rate and rates of adverse outcomes attributable to Zika virus.

The models we have used have several limitations. We assumed that vertical transmission rates, congenital Zika syndrome and OPZRO rates conditional on 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 are limited to outcomes manifested by the end of the perinatal period and therefore do not account

For more on **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.[30] 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 ultrasonography might have occurred. In studies that include both a PCR-positive and PCR-negative comparison group,[15,19] 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 amniochorionic 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,[31,32] 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,[33,34] chorionic villi gradually decrease their permissivity after the first trimester.[33] 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, IFNλ1, by the syncytiotrophoblast, acting both at a paracrine and autocrine level.[35,36] Moreover, Sheridan and colleagues[37] 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 amniochorionic membrane, amniotic epithelial cells from mid-gestation generate higher infectious titres than the ones obtained from late-gestation placentas.[32] 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,[38] 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 AbbVie 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.

Acknowledgments

This project has received funding from the European Union Horizon 2020 programme under grant agreement number 734857. We thank Tom Byrne for assistance in preparing the manuscript for publication. This work was partly supported by the National Institute of Health Research Great Ormond Street Hospital Biomedical Research Centre.

References

1 Rasmussen SA, Jamieson DJ, Honein MA, Petersen LR. Zika virus and birth defects—reviewing the evidence for causality. *N Engl J Med* 2016; **374:** 1981–87.

2 Pan American Health Organization, WHO. Cases of Zika virus disease by country or territory: cumulative cases. http://www.paho.org/data/index.php/en/?option=com_content&view=article&id=524:zika-weekly-en&Itemid=352 (accessed Feb 5, 2020).

3 Krauer F, Riesen M, Reveiz L, et al. Zika virus infection as a cause of congenital brain abnormalities and Guillain-Barré syndrome: systematic review. *PLoS Med* 2017; **14:** e1002203.

4 Mandelbrot L, Mayaux MJ, Bongain A, et al. Obstetric factors and mother-to-child transmission of human immunodeficiency virus type 1: the French perinatal cohorts. *Am J Obstet Gynecol* 1996; **175:** 661–67.

5 Thiébaut R, Leproust S, Chêne G, Gilbert R. Effectiveness of prenatal treatment for congenital toxoplasmosis: a meta-analysis of individual patients' data. *Lancet* 2007; **369:** 115–22.

6 Stagno S, Pass RF, Dworsky ME, et al. Congenital cytomegalovirus infection: the relative importance of primary and recurrent maternal infection. *N Engl J Med* 1982; **306:** 945–49.

7 Miller E, Cradock-Watson JE, Pollock TM. Consequences of confirmed maternal rubella at successive stages of pregnancy. *Lancet* 1982; **2:** 781–84.

8 Preece PM, Blount JM, Glover J, Fletcher GM, Peckham CS, Griffiths PD. The consequences of primary cytomegalovirus infection in pregnancy. *Arch Dis Child* 1983; **58:** 970–75.

9 Schaub B, Vouga M, Najioullah F, et al. Analysis of blood from Zika virus-infected fetuses: a prospective case series. *Lancet Infect Dis* 2017; **17:** 520–27.

10 Ades AE, Thorne C, Soriano-Arandes A, et al. Researching Zika in pregnancy: lessons for global preparedness. *Lancet Infect Dis* 2020; **20:** e61–68.

11 Paz-Bailey G, Rosenberg ES, Doyle K, et al. Persistence of Zika virus in body fluids—preliminary report. *N Engl J Med* 2018; **379:** 1234–43.

12 Oduyebo T, Polen KD, Walke HT, et al. Update: interim guidance for health care providers caring for pregnant women with possible Zika virus exposure—United States (including U.S. territories), July 2017. *MMWR Morb Mortal Wkly Rep* 2017; **66:** 781–93.

13 Qu Y, Tan M, Kutner MH. Random effects models in latent class analysis for evaluating accuracy of diagnostic tests. *Biometrics* 1996; **52:** 797–810.

14 Campos Coelho AV, Crovella S. Microcephaly prevalence in infants born to zika virus-infectedwomen: a systematic review and meta-analysis. *Int J Mol Sci* 2017; **18:** 1–10.

15 Brasil P, Pereira JP Jr, Moreira ME, et al. Zika Virus infection in pregnant women in Rio de Janeiro. *N Engl J Med* 2016; **375:** 2321–34.

16 Pomar L, Vouga M, Lambert V, et al. Maternal-fetal transmission and adverse perinatal outcomes in pregnant women infected with Zika virus: prospective cohort study in French Guiana. *BMJ* 2018; **363:** k4431.

17 Hoen B, Schaub B, Funk AL, et al. Pregnancy outcomes after ZIKV infection in French territories in the Americas. *N Engl J Med* 2018; **378:** 985–94.

18 Nogueira ML, Nery Júnior NRR, Estofolete CF, et al. Adverse birth outcomes associated with Zika virus exposure during pregnancy in São José do Rio Preto, Brazil. *Clin Microbiol Infect* 2018; **24:** 646–52.

19 Rodó C, Suy A, Sulleiro E, et al. Pregnancy outcomes after maternal Zika virus infection in a non-endemic region: prospective cohort study. *Clin Microbiol Infect* 2019; **25:** 633.e5–9.

20 Conners EE, Lee EH, Thompson CN, et al. Zika virus infection among pregnant women and their neonates in New York City, January 2016–June 2017. *Obstet Gynecol* 2018; **132:** 487–95.

21 Merriam AA, Nhan-Chang C-L, Huerta-Bogdan BI, Wapner R, Gyamfi-Bannerman C. A single-center experience with a pregnant immigrant population and Zika virus serologic screening in New York City. *Am J Perinatol* 2020; **37:** 731–37.

22 Sulleiro E, Rando A, Alejo I, et al. Screening for Zika virus infection in 1057 potentially exposed pregnant women, Catalonia (northeastern Spain). *Travel Med Infect Dis* 2019; **29:** 69–71.

23 Moore CA, Staples JE, Dobyns WB, et al. Characterizing the pattern of anomalies in congenital zika syndrome for pediatric clinicians. *JAMA Pediatr* 2017; **171:** 288–95.

24 Turner LH, Kinder JM, Wilburn A, et al. Preconceptual Zika virus asymptomatic infection protects against secondary prenatal infection. *PLoS Pathog* 2017; **13:** e1006684.

25 Andrade DV, Harris E. Recent advances in understanding the adaptive immune response to Zika virus and the effect of previous flavivirus exposure. *Virus Res* 2018; **254:** 27–33.

26 Paixão ES, Costa MDCN, Teixeira MG, et al. Symptomatic dengue infection during pregnancy and the risk of stillbirth in Brazil, 2006–12: a matched case-control study. *Lancet Infect Dis* 2017; **17:** 957–64.

27 Rijken MJ, McGready R, Boel ME, et al. Malaria in pregnancy in the Asia-Pacific region. *Lancet Infect Dis* 2012; **12:** 75–88.

28 Cardenas I, Means RE, Aldo P, et al. Viral infection of the placenta leads to fetal inflammation and sensitization to bacterial products predisposing to preterm labor. *J Immunol* 2010; **185:** 1248–57.

29 Wilder-Smith A, Wei Y, Araújo TVB, et al. Understanding the relation between Zika virus infection during pregnancy and adverse fetal, infant and child outcomes: a protocol for a systematic review and individual participant data meta-analysis of longitudinal studies of pregnant women and their infants and children. *BMJ Open* 2019; **9:** e026092.

30 Nielsen-Saines K, Brasil P, Kerin T, et al. Delayed childhood neurodevelopment and neurosensory alterations in the second year of life in a prospective cohort of ZIKV-exposed children. *Nat Med* 2019; **25:** 1213–17.

31 Tabata T, Petitt M, Puerta-Guardo H, Michlmayr D, Harris E, Pereira L. Zika virus replicates in proliferating cells in explants from first-trimester human placentas, potential sites for dissemination of infection. *J Infect Dis* 2018; **217:** 1202–13.

32 Tabata T, Petitt M, Puerta-Guardo H, et al. Zika virus targets different primary human placental cells, suggesting two routes for vertical transmission. *Cell Host Microbe* 2016; **20:** 155–66.

33 Weisblum Y, Oiknine-Djian E, Vorontsov OM, et al. Zika virus infects early- and midgestation human maternal decidual tissues, inducing distinct innate tissue responses in the maternal-fetal interface. *J Virol* 2017; **91:** 1–13.

34 Hermanns K, Göhner C, Kopp A, et al. Zika virus infection in human placental tissue explants is enhanced in the presence of dengue virus antibodies in-vitro. *Emerg Microbes Infect* 2018; **7:** 198.

35 Corry J, Arora N, Good CA, Sadovsky Y, Coyne CB. Organotypic models of type III interferon-mediated protection from Zika virus infections at the maternal-fetal interface. *Proc Natl Acad Sci USA* 2017; **114:** 9433–38.

36 Bayer A, Lennemann NJ, Ouyang Y, et al. Type III interferons produced by human placental trophoblasts confer protection against Zika virus infection. *Cell Host Microbe* 2016; **19:** 705–12.

37 Sheridan MA, Yunusov D, Balaraman V, et al. Vulnerability of primitive human placental trophoblast to Zika virus. *Proc Natl Acad Sci USA* 2017; **114:** e1587–96.

38 Van Kerkhove MD, Reveiz L, Souza JP, Jaenisch T, Carson G, Broutet N. Harmonisation of Zika virus research protocols to address key public health concerns. *Lancet Glob Health* 2016; **4:** e911–12.