Researching Zika in pregnancy: lessons for global preparedness

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Abstract

Our understanding of congenital infections is based on prospective studies of women infected in pregnancy. The EU has funded three consortia to study Zika virus (ZIKV), each including a prospective study of pregnant women. Another has been funded by National Institutes of Health. This personal view sets out the study designs required to research ZIKV, and questions whether funding academics in the EU and US to work with collaborators in outbreak areas is an effective strategy. Three years after the 2015-2016 outbreaks, these collaborations have taught us little about ZIKV vertical transmission. In the time taken to approve funding, agree contracts, secure ethics approval, and equip laboratories, Zika had largely disappeared. By contrast, prospective studies based on local surveillance and standard of care (SOC) protocols have already provided valuable data. Threats to fetal and child health pose new challenges for global preparedness requiring support for the design and implementation of locally appropriate protocols. These can answer the key questions earlier and at lower cost. Local protocols can also provide a framework for recruitment of unexposed controls, required to study less specific outcomes. Other priorities include accelerated development of non-invasive tests, and longer-term storage of neonatal and antenatal samples to facilitate retrospective reconstruction of cohort studies.

Introduction

Past epidemics have triggered global initiatives to strengthen preparedness against emerging infectious disease threats, focusing on surveillance, detection and outbreak containment.[1–4] It has been recognized that the higher risks faced by pregnant women and their infants during epidemics have often been overlooked within global preparedness frameworks,[5,6] but emerging infections with teratogenic effects pose an entirely new set of challenges, particularly around research preparedness.

The Zika virus (ZIKV) epidemic in 2015/2016, following the re-emergence of ZIKV in Asia and the Pacific in 2013[7], was unexpected.[4] During the outbreak in Brazil from 2015[8,9], detection of babies with microcephaly and other abnormalities[10,11] led to the identification of ZIKV as a teratogen, as seen for other congenital infections such as rubella or cytomegalovirus (CMV), but not previously observed for arboviruses. The Pan-American Health Organization issued an Epidemiological Alert in May 2015[12] and WHO declared a Public Health Emergency of International Concern in February 2016.[13] The need for a coordinated research response was recognized quickly. The Global Research Collaboration for Infectious Disease Preparedness was engaged (www.glopid-r.org), and the European Commission (EC) issued a call for research and preparedness activities. The three EC consortia (Zika-PLAN (zikaplan.tghn.org/),[14] ZIKAlliance (zikalliance.tghn.org), and ZIKAction (zikaction.org/)) are multi-disciplinary programmes, each including prospective vertical transmission cohort studies. In the US, the National Institutes for Health launched the ZIP (Zika in Pregnancy) Study (clinicaltrials.gov/ct2/show/NCT02856984). These projects have multiple field sites in the Caribbean, and Central and South America.

Over three years since the Zika alerts were issued several important prospective vertical transmission studies have been published,[15–18] but none from these externally-funded programmes. ZIKAlliance has pointed to "familiar barriers", citing delays in the funding process and ethical approvals, and diagnostic challenges.[19] They have argued for a "permanent … research capacity" with structured funding for a rapid response infrastructure.

In this paper, we explain why prospective studies to investigate Zika VT require particular design features, and consider other ways in which such studies could be originated and set in motion. Should it be the traditional pattern whereby Western academics are granted funds to work with research collaborators in affected areas, or can prospective studies be built onto appropriately designed and implemented standard of care (SOC) protocols?

Role of prospective cohort studies in understanding vertical transmission

Understanding of congenital infections, such as rubella, CMV, toxoplasmosis, hepatitis C virus (HCV) and HIV, has been based largely on prospective cohort studies of pregnant women and their children.[20–24] Pregnancies at risk are identified by screening women for markers of the relevant pathogen, and then followed forward with a more intensive care protocol, the outcome of pregnancy is recorded, and the newborn tested for infection acquired *in utero* or intrapartum.

The protocol for identifying pregnancies at risk depends on the pathogen. With chronic infections, such as HIV and HCV, IgG antibody testing identifies pregnancies at risk.[24,25] With toxoplasmosis the fetus is at risk only following primary infection in pregnancy, with at-risk pregnancies identified by repeat serological testing of susceptible (IgG-negative) women. This protocol would be less effective in detecting pregnancies at risk of congenital CMV, where non-primary infections also pose a risk.[26]

The key property of the prospective cohort design is that it is women who are recruited, as early as possible before delivery, not the babies. Otherwise there is a risk of selectively recruiting pregnancies with adverse outcomes. Ascertainment of infection in pregnancy must therefore pre-date, or at least be independent of, any examination of the fetus (e.g. ultrasound scan) or newborn.

Prospective studies aim to estimate the vertical transmission rate - the probability that the fetus/newborn is infected, given an infected mother. In classic studies of HIV,[27,28] CMV[29] and toxoplasmosis,[22,30,31] the second key parameter is the rate of adverse outcomes in vertically-infected infants (Table 1). With less specific outcomes, uninfected infants of women infected during pregnancy constitute a control group (Control group 1 in Table 1).[32,33] Infants of unexposed women, who have not been infected in pregnancy, can form a second control group (Table 1)[34]; this is not routinely included in many prospective studies, but essential to study outcomes resulting directly from maternal infection, without the infection crossing to the fetus. Examples are prematurity, low birthweight and miscarriage, which have been reported with dengue,[35] malaria[36] and other infections.[37]

The prospective study design as applied to Zika

Applying the prospective design to ZIKV is not straightforward. Firstly, only a few of the components of Congenital Zika Syndrome (CZS) are specific to ZIKV and some require intensive investigation to detect.[38] A control group is therefore essential. Second, PCR+ or IgM+ findings in newborn samples confirm congenital infection, but these markers are absent in a high proportion of CZS cases,[39,40] despite their relatively high analytic sensitivity. Clearance of virus from amniotic fluid,[16,41] and

transient viremia in fetal blood, accompanied by *post mortem* isolation of ZIKV from brain tissue,[42] suggest that the virus can infect the fetus, causing serious damage but then clearing without leaving an immunological trace. As presence or absence of congenital infection cannot be reliably established, a control Group 1 cannot be identified, and unexposed women and their offspring (Control Group 2) are required (Table 1).

Identifying maternal infection in pregnancy (MIP) is also difficult with ZIKV. While MIP can be confirmed by PCR RNA, even the most intense PCR-based screening programme is likely to miss cases of MIP because most ZIKV infections are asymptomatic, and the PCR response may last no more than 7 days.[43] Thus, failure to detect ZIKV RNA does not demonstrate "No MIP". An IgG negative test in a woman at delivery is suggestive of No MIP, but little is known about ZIKV serology dynamics. In any case, this marker would only be useful in Zika-naïve populations exposed over a short period. Seroconversion is a reliable indicator of MIP if cross-reactions to other flavivirus antibody can be excluded.[44] Tests of recent infection such as IgM, IgG3, or avidity assays have poor specificity, and even if cross-reactions are ruled out, may only reflect an infection that cleared before pregnancy.[45] Probably, the best classification of maternal infection that can be achieved is likely to be along the lines of: confirmed, suspected, and no evidence of MIP (although tested).

Standard of care protocols for women exposed to Zika in pregnancy

Recruitment into a formal prospective study requires informed consent to investigations that would not ordinarily be carried out under a SOC protocol. The question for study designers is therefore: what additional investigations are required, and in which patients, that are not already specified for SOC?

Most countries in Latin America and the Caribbean made ZIKV a notifiable disease, creating a *de facto* surveillance system in which (symptomatic) pregnant women could be referred into a protocol for care in pregnancy. Early protocols in affected countries were therefore restricted to women with symptoms in pregnancy (Table 2). Later, in better-resourced settings, testing was extended to all pregnant women exposed to the outbreak, for example based on repeat serology.[46] The current CDC protocol specifies 3 PCR tests through pregnancy. However, the key issue is whether the SOC provides for testing for congenital infection and follow-up of *all* newborns delivered to women with a confirmed infection in pregnancy, or only if there are congenital abnormalities (Table 2). Guidance from the Brazilian Ministry of Health has followed the latter course, in common with May 2016 guidelines issued by WHO.[47] The four prospective studies funded through international collaborations (Table 3) offer a similar mix of protocols.

The response of WHO to ZIKV has been confused. Like the earlier CDC guidance,[48] WHO recommended testing of women reporting symptoms with additional ultrasound to identify fetal malformations. WHO argued against testing asymptomatic women, even in areas of high ZIKV incidence, as it failed on three of the Wilson & Junger screening criteria:[49]   poorly understood natural history , lack of effective treatment, and low specificity of diagnostic tests. But it is unclear why these criteria would not also rule out testing symptomatic women. On the other hand, the design of SOC and prospective study protocols alike must be sensitive to the cultural, religious, and medico-legal context: in some countries a confirmed diagnosis of ZIKV in pregnancy may be of little benefit to the mother, and could even lead to harm.[50] In such circumstances protocols that go beyond routine ultrasound may not be feasible.

More critically, the WHO protocol[47] made no provision for laboratory testing and follow-up of infants of women with confirmed or suspected ZIKV in pregnancy, unless congenital anomalies were present. Remarkably, positive ZIKV results in pregnancy are described as "false positives" if not resulting in microcephaly. WHO also recommended TORCH testing of neonates born to women with suspected ZIKV in pregnancy, but no tests for congenital ZIKV[51] WHO guidance may have discouraged some Latin American countries from adopting SOCs that would have provided appropriate care to families at risk, and which - as we show below - would concurrently have generated prospective study data on a very large scale.

Prospective studies based on surveillance and SOC for Zika in pregnancy.

During a ZIKV outbreak, pregnant women can be referred into a SOC protocol in two ways: either through a ZIKV surveillance system, if they have symptoms; or through repeat serological or virological testing offered by a maternity care provider. Once an SOC is operational, it inevitably generates cohorts of women with confirmed or suspected ZIKV in pregnancy. A "prospective study" is therefore generated simply by carrying out SOC investigations and recording results. All the prospective studies published so far (Table 3) have been generated in this way.

Equally, the same SOC protocols also generate unexposed controls groups (Control Group 2), at least to the extent that this is possible for ZIKV. Thus, some studies have reported outcomes in women who were PCR negative in pregnancy, interpretable as "No Evidence of infection in pregnancy" (Table 3). However, detailed imaging, testing and follow-up of apparently healthy newborns born to women without evidence of MIP is explicitly ruled out in CDC guidelines,[52] and is likely to require informed consent in any jurisdiction. It is at this point that a formal prospective study can go further than an SOC protocol, by obtaining consent for additional investigations on women and especially

their newborns. However, the identification of these infants, and ethical approval and logistics for follow-up, will be facilitated if their mothers are already part of a SOC protocol for ZIKV-exposed pregnant women. Follow-up of healthy children with no evidence of MIP raises some specific problems, which we return to below.

Table 4 illustrates that prospective studies based on local SOCs are a viable alternative to collaborative projects funded through US or EC partners (Table 3), even in the absence of funding.[16]

In the US, pregnancy outcomes in women potentially ZIKV-exposed and under the CDC protocol are recorded in the US Zika Pregnancy Register (USZPR). This has documented serious brain abnormalities with or without microcephaly in 6% to 15% of pregnancies with confirmed infection, with higher rates following first trimester infection.[53–55] The authors acknowledge that these are likely to be over-estimates, as USZPR includes pregnancies in which Zika involvement was recognized retrospectively, following detection of anomalies on routine imaging, or at delivery.

Such registers represent a third, and massively under-exploited way of generating prospective data on ZIKV in pregnancy. They can either recruit directly into prospective studies,[56,57] or be converted into prospective cohort studies retrospectively, by checking the dates of maternal tests relative to delivery, and removing mother-child pairs in which infection in pregnancy was retrospectively ascertained. Just such a retrospective reconstruction of a prospective cohort based on the USZPR was conducted in New York City,[58] reporting markers of congenital infection in 11% and 7% of infants of mothers with "confirmed" and "probable" infection in pregnancy respectively (Table 4).

Other opportunities for retrospective reconstruction of prospective studies

SOC protocols that provide for infant testing and follow-up cannot be established immediately in the context of outbreaks that emerge rapidly and without warning. There will therefore always be a need for retrospective reconstructions. One option is to reconstruct prospective studies from surveillance records of pregnant women who reported symptoms and were tested.

With hindsight, it now seems remarkable that in many countries these pregnancies were not referred to ZIKV-related care protocols in maternity hospitals for additional investigations and infant follow-up. This should remind us that whatever protocols are in place, there may be insufficient resources for laboratory infrastructure and follow-up. However, this process can be carried out retrospectively as a review of maternity and delivery records. Consent could also be sought for clinical follow-up of the infants.

An interesting addition to retrospective review would be to use residual samples routinely collected for newborn sickle cell and/or metabolic screening, as dried blood spots. Many countries in Latin America and the Caribbean have routine newborn screening in national, regional and hospital laboratories.[59] Newborn dried blood spots have been tested in retrospectively reconstructed cohort studies of congenital infections[60] but also in irreversibly anonymized studies retaining demographic information.[61,62] Stored residual newborn dried blood spots could be tested for ZIKV RNA and IgM, retaining information on confirmed or suspected maternal ZIKV infection, and trimester in which symptoms occurred. Laboratory markers cannot be found in a proportion of congenital infection,[41,42] but studies would set a lower limit on the vertical transmission rate. A sample of newborns of women with no evidence of Zika could also be included, as well as randomly selected controls whose mothers were not notified to surveillance.

A second potential approach to reconstruct prospective studies post-epidemic is the use of stored samples collected during routine antenatal care whilst the outbreak was ongoing. These could be tested for markers of infection; confirmed and suspected cases, plus a sample of those with "No Evidence of MIP", could again be entered into a record review of pregnancy and neonatal outcomes as above, with the option of obtaining consent for pediatric follow-up, and testing of stored residual newborn screening samples. Joint testing of linked anonymized antenatal and neonatal samples creates many further opportunities for large-scale reconstruction of incidence in pregnant women and vertical transmission, although is logistically more complex.

These designs could be applied now to study the 2015-16 Zika outbreak, but they have a wider significance. There will always be a role for retrospectively reconstructed cohort studies in future outbreaks of pathogens that affect fetal health, especially when no clear diagnostic pathway, or even the pathogen, is identified until later. Such studies may be especially valuable in countries where medical, religious, legal or cultural constraints limit the benefit of a prospective diagnosis of ZIKV in pregnancy. There are logistic requirements: if conducted prospectively, studies based on surveillance reports require only coordination between the surveillance centre, the relevant laboratory and the maternity hospital.[15,17,18] However, in a retrospective design, these three sets of patient records have to be linked. This can be done using names, addresses, and dates of birth, but record linkage can be greatly facilitated by having unique common identifier, such as the mother's national security number, on all records, including those of the infant.

Retrospective designs require neonatal and antenatal samples to be stored for longer, which has a cost. Resources could be conserved by focusing on a small number of maternity hospitals serving representative populations, or by initiating sample storage only when an outbreak is detected.

Retrospective studies including "control" mother-child pairs may also require consent, which could perhaps be obtained on a universal basis during an outbreak.

Role of international agencies and funders in global preparedness

Only prospective studies can fully answer the key scientific and public health questions around ZIKV infection in pregnancy and its consequences, ideally including an unexposed control group. We have argued that SOC protocols for pregnant women provide a feasible approach, allowing autonomy of local institutional review boards, and ensuring access to the relevant diagnostics and to locally trained research and nursing staff integrated within the health care system. This resolves the delays and barriers[19] that confront prospective studies established by externally-funded researchers (ethics approvals, creation of data collection systems and laboratory infrastructure). Furthermore, externally-originated research protocols may do little to foster global preparedness if the infrastructure is unsupported once the project ends.

One of the difficulties in studying outcomes of ZIKV in pregnancy is testing and follow-up of healthy children, especially when maternal infection is not apparent. It is recognized that global preparedness demands much faster development of diagnostics,[63] but the particular need for non-invasive tests may not have been appreciated. Capillary blood samples collected by heel prick are well accepted, widely used with rapid diagnostic tests (RDTs) and can be dried and stored long-term. Oral fluid samples contain the same antibody pattern as serum and are used for measles and rubella case-based surveillance,[64,65] for HIV RDTs,[66] and extensively for virus detection by PCR. Throat swabs are used for diagnosis of congenital CMV.[67]

We believe that global preparedness must focus more on the care of pregnant women and their children, with the primary task being the development of culturally sensitive SOC protocols that are appropriate to settings and resources available, backed up by assistance in implementing them, including approaches to data capture, recording and linkage. These SOCs represent *de facto* prospective studies that will answer the key research questions more rapidly than internationally-led research studies. Furthermore, prospective studies can be reconstructed on a very large scale, with appropriate filtering out of retrospectively-ascertained maternal infection, from registers recording outcomes under SOC. For more subtle outcomes, informed consent would be needed to follow-up unexposed "control" infants, but this can be built onto SOC protocols.

Strengthened surveillance is a key component of preparedness:[6] a focus on pregnancy would prioritize surveillance for stillbirths, neonatal mortality, congenital malformations, auditory and ophthalmological problems. Meanwhile, between epidemics, and before the next new pathogen

emerges with potential for congenital infection, international agencies should devise mechanisms for accelerated development of non-invasive diagnostics, and promote facilities for longer-term storage of routinely collected antenatal and neonatal samples, which can be linked to maternity and pediatric records. No matter how rapid the response to an emerging pathogen, retrospective studies based on stored samples will always be needed.

Contributors

The paper arose from discussions at the November 2017 ZIKAction consortium meeting at University of Florida, Gainesville, FL. AEA drafted the paper with input from all co-authors. The final decision to submit was taken by CDCC and CT, who co-lead the ZIKAction vertical transmission work package. CG is the ZIKAction principal investigator. All co-authors read, contributed to and approved the final version.

Declaration of interests

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TABLES

Table 1. Design of a prospective vertical transmission study. The table shows cell frequency counts *a,b…f* of birth outcomes broken down by congenital infection status, presence or absence of adverse outcomes, and maternal infection status. The Vertical Transmission rate is estimated by *(a+b)/(a+b+c+d)*. The rate of adverse outcomes conditional on congenital infection is *a/(a+b)*. For less specific adverse outcomes this can be compared with the rate of outcomes in newborns with no congenital infection, *c/(c+d)*, whose mothers were infected in pregnancy (Control Group 1). An overall adverse event rate can be estimated, *(a+c)/(a+b+c+d)*, pooling congenital infection and No congenital infection. Follow-up of births where there has been no maternal infection in pregnancy (MIP) represent a second control group (Control Group 2), with a rate of adverse outcomes, *e/(e+f)*. In the absence of clear diagnostic criteria for congenital infection, as with ZIKV, this second control group is needed.

Some cells have zeros as there can be no congenital infection without maternal infection in pregnancy.

<table>
<tr><td rowspan="2" colspan="3"></td><td colspan="2">Maternal Infection in Pregnancy (MIP) Status</td></tr>
<tr><td>MIP</td><td>No MIP</td></tr>
<tr><td rowspan="4">Congenital Infection (CI) Status</td><td rowspan="2">CI</td><td>Adverse outcomes</td><td>*a*</td><td>*0*</td></tr>
<tr><td>No Adverse outcomes</td><td>*b*</td><td>*0*</td></tr>
<tr><td rowspan="2">No CI</td><td>Adverse outcomes</td><td>*c*</td><td>*E*</td></tr>
<tr><td>No Adverse outcomes</td><td>*d*</td><td>*F*</td></tr>
</table>

Table 2. Standard protocols of care for women exposed to Zika outbreaks

State	Date	Provision for screening women potentially exposed during pregnancy	Care of newborn mother with confirmed ZIKV in pregnancy
USA	January 2016[48]	PCR, IgM if symptomatic, additional U/S if asymptomatic	Laboratory testing for congenital ZIKV, clinical follow-up
Brazil	January 2016[68]	Testing and U/S in symptomatic	Follow-up if microcephaly
France	January 2016 [69]	PCR, IgM additional U/S if symptomatic	Laboratory testing for congenital ZIKV, clinical follow-up
France	April 2016[70]	PCR, IgM testing regardless of symptoms	Laboratory testing, clinical follow-up
WHO	May 2016[47]	Testing and U/S if symptomatic	Follow-up if microcephaly
WHO	August 2016[51]	Not applicable	TORCH screen, but no tests for Congenital ZIKV
Brazil	November 2016[71]	Testing and U/S if symptomatic	Follow-up if symptoms of ZIKA in first 3 years
USA	July 2017[72]	3 PCR tests, additional U/S, regardless of symptoms	Laboratory testing for congenital ZIKV, clinical follow-up
Spain	April 2017[73]	PCR, IgM and IgG regardless of symptoms, alongside U/S; if IgG(+) then PRNT-ZIKV	Laboratory testing for congenital ZIKV and clinical follow-up for all born to ZIKV-infected mothers (probable and confirmed)

Table 3. Protocols of four international collaborative prospective studies, funded through US and EU partners

Study	Funder	Provision for screening women potentially exposed during pregnancy	Care of newborn of mother with confirmed ZIKV in pregnancy
ZIKAlliance	European Commission	Repeat serological and PCR tests	Laboratory testing for congenital ZIKV, clinical follow-up
ZIKAction	European Commission	Repeat serological tests	Laboratory testing for congenital ZIKV, clinical follow-up
Zika PLAN	European Commission	ZIKV Symptoms in pregnancy	Clinical follow-up from week 6.
Zika In Pregnancy (ZIP)	National Institutes of Health	Repeat PCR tests	Laboratory testing for congenital ZIKV, clinical follow-up

Table 4. Prospective studies published before April 2019: method of recruitment, cohorts followed, prevalence of adverse outcomes and of markers of congenital infection (CI).

Region	Recruitment	Cohorts reported	Adverse Outcomes, %	Markers of CI, %
Brazil[18]	Rash in pregnancy, dengue surveillance programme	1. Confirmed MIP (n=134) 2. No Evidence of MIP (n=73)	46% "Adverse outcomes" 11.5%	NR NR
Texas, USA[56]	Prospective USZPR	1. Confirmed or probable MIP (n=28) 2. No Evidence of MIP (n=306)	4% CZS NR	7% NR
French Guiana[46]	Three PCR tests on all pregnant women	1. Confirmed MIP (n=301) 2. No Evidence of MIP (n=399)	9% "CNS involvement" 4%	NR NR
French Guiana [74]	Three PCR tests on all pregnant women	1. Confirmed MIP (n=291)	4% "Severe complications"	35%
Martinique & Guadeloupe [15]	ZIKV Surveillance, symptomatic women	1. Confirmed MIP (n=555)	10% "Neurological and ocular defects"	NR
Colombia[75]	National surveillance	1. ZIKV Symptoms in pregnancy, 3rd trimester (n=c.570)	0% "microcephaly <3SD or brain abnormalities	NR
New York City, USA [58]	Retrospective reconstruction USZPR,	1. Confirmed (n=80) 2. Suspected (n=207)	NR NR	11% 7%
Brazil [17]	Symptoms of ZIKV in pregnancy	1. Confirmed MIP (n=57)	28% "Adverse outcomes"	26%
Barcelona[57]	ZIKV surveillance travellers	1. Confirmed MIP (n=9) 2. Probable MIP (n=62)	33% "Adverse outcomes" 0%	NR NR

NR: Not Reported; MIP: Maternal Infection in Pregnancy; USZPR United States Zika in Pregnancy Register

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