

ZIKA Virus and Neuroscience: the Need for a Translational Collaboration

Lavínia Schuler-Faccini¹ · Paulo Roehé² · Eduardo R. Zimmer^{3,4} ·
André Quincozes-Santos³ · Adriano M. de Assis³ · Elizabeth Obino Cirne Lima⁵ ·
Jorge Almeida Guimarães⁶ · Cesar Victora⁷ · Vivaldo Moura Neto⁸ · Diogo O. Souza³

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Abstract Zika virus (ZIKV) has become a major challenge for scientists and health agencies. ZIKV's involvement with human fetal microcephaly and Guillain-Barré syndrome and its transmission through *Aedes africanus* and *Aedes aegypti* mosquitos highlighted the epidemiological and neurological risks associated to ZIKV infection. In 2013, ZIKV arrives in Brazil but the first outbreak in the country was reported in 2015. Here, we used the *Web of Science* as a search tool for comparing the evolution of world and Brazilian scientific research on dengue virus (DENV)—also present in mosquito—,

ZIKV and microcephaly. The association between ZIKV and microcephaly was only evidenced in 2015. Interestingly, Brazil and the USA are the responsible for most of these reports. Furthermore, the level of double-counted articles indicates a high degree of international collaborative effort in studying ZIKV and microcephaly. The ZIKV research clearly requires multidisciplinary expertise including epidemiologic, clinical, virological, and neurochemical backgrounds. This letter intends to emphasize the need of multidisciplinary studies and put forward some as yet unanswered questions in attempting to contribute to the understanding of this multifaceted health problem. In line with this, we recently constituted a collaborative and multidisciplinary taskforce encompassing eight Brazilian scientific institutions of excellence, *The ZIKV translational* research taskforce. This taskforce comprises a vast international network of collaborators and welcomes additional collaborators. We intend to advance fast in terms of mechanisms, which can potentially contribute to treat or halt ZIKV spreading around the world.

✉ Diogo O. Souza
diogo@ufrgs.br

¹ Department of Genetics, Institute of Biosciences, Federal University of Rio Grande do Sul, Porto Alegre, Brazil

² Microbiology, Immunobiology and Parasitology, Laboratory of Virology, Federal University of Rio Grande do Sul, Porto Alegre, Brazil

³ Department of Biochemistry, ICBS, Federal University of Rio Grande do Sul, 2600 Ramiro Barcelos St, Santana, Porto Alegre, RS 90035003, Brazil

⁴ Brain Institute (Bralns) of Rio Grande do Sul, Pontifical Catholic University of Rio Grande do Sul (PUCRS), Porto Alegre, Brazil

⁵ Embryology and Cell Differentiation Laboratory, Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil

⁶ Hospital de Clínicas de Porto Alegre and Laboratory of Biochemical Pharmacology, Centre of Biotechnology, UFRGS, Porto Alegre, Brazil

⁷ International Center for Equity in Health, Post-Graduation Program in Epidemiology, Federal University of Pelotas, Rua Marechal Deodoro, Pelotas, Brazil

⁸ Instituto Estadual do Cérebro Paulo Niemeyer, Secretaria Estadual de Saúde do Rio de Janeiro, Rio de Janeiro, Brazil

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Dear Editor,

Zika virus (ZIKV) has become a major challenge for scientists and health agencies, particularly since the identification of its potential involvement with microcephaly and Guillain-Barré syndrome in 2015 [1, 2]. After its accidental identification in Uganda in 1947 as a virus infecting captive *Rhesus* monkeys and its subsequent (1948) identification in the mosquito *Aedes africanus*, it sooner proved to be also infective to humans in Nigeria in 1954. During the following 50 years, it spread to other areas of Africa. In 1969, ZIKV was detected in *Aedes aegypti* mosquitos in Malaysia. In Oceania, the virus

would cause the first large outbreak in the Island of Yap in 2007 and subsequently in the French Polynesia in 2014. However, ZIKV was initially underestimated due to its mild clinical manifestations in infected people. Usually, the infection would undergo a subclinical course; eventually, low-grade fever, and often, cutaneous rash, and arthralgia were the main signs of a self-limited infection [3].

In 2013, ZIKV arrives in Brazil but its first outbreak in the country was reported during the first semester of 2015 [4, 5]. In the second semester, its history dramatically changes, as an important rise in the birth of babies with microcephaly is reported in the state of Pernambuco, northeastern Brazil. As a consequence, a burst of publications about ZIKV and microcephaly came out rather way in Brazil and abroad. As shown in Table 1, while several articles about dengue were published since 1945, in the case of ZIKV the publications started much later, reaching 230 articles in 2016. Moreover, the first report on microcephaly only came out in 2015 (one article) and mostly in the first semester of 2016 (110 articles). Interestingly, Brazilian and North American researchers are the most responsible for these reports as depicted in Table 2. Although recent subject of research, the level of double-counted articles indicate a high proportion of international collaborative effort in studying ZIKV and microcephaly.

The increase in the number of babies born with microcephaly was confirmed and further detected in other Brazilian states. Although initially contested, a geographic and temporal link between ZIKV infections and microcephaly was rapidly observed. Moreover, maternal history compatible with ZIKV infection mostly during the first half of pregnancy and brain images suggestive of

congenital infections (periventricular and cortical calcifications, lissencephaly) were reported in the great majority of cases. Recently, the association between prenatal exposure to ZIKV and brain disruption was recognized [6]. However, the fast spread of ZIKV to other countries in the Americas, including the USA, prompted a worldly concern and mobilization of scientists and health agencies, since effective vaccines or treatments for ZIKV are still unavailable. In addition, the battle against the major vector—*Aedes* species mosquito—remains barely ineffective and other forms of transmission, including sexual transmission, are raising concerns over the spread of the infection around the world.

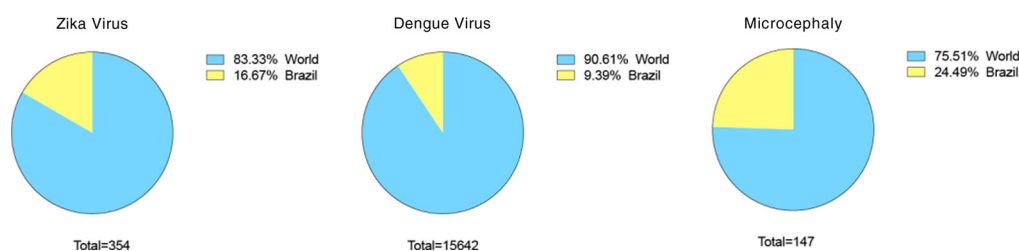
Although the cause-consequence relationship between ZIKV prenatal infection and brain damage currently seems unquestionable, the number of unanswered questions and concerns has multiplied. One of the major issues is related to the precise pathogenic mechanisms of ZIKV, which seems to affect neural progenitor cells pointing out to the high susceptibility of the developing central nervous system (CNS) [7, 8]. Thus, experimental studies—both in vitro and in vivo—are being very helpful to elucidate and to propose mechanisms by which the virus infects progenitor neural cells.

Currently, ZIKV infection is a very relevant health public problem, which clearly requires multidisciplinary expertise—including epidemiologic, clinical, virological, and neurochemical backgrounds—to advance in the understanding of this severe health problem, which will potentially affect all countries in the world. In the following paragraphs, we attempt to shed light on until now major

Table 1 Evolution of scientific publication on Zika virus, dengue, and microcephaly

World			Brazil				
Years	Zika virus	Dengue	Microcephaly	Years	Zika virus	Dengue	Microcephaly
1945-1985	10	641	0	1945-1985	0	0	0
1986-1995	2	711	0	1986-1995	0	32	0
1996-2005	3	2,173	0	1996-2005	0	188	0
2006-2011	9	4,190	0	2006-2011	0	518	0
2012-2015	41	5,501	1	2012-2015	3	610	0
2016	230	957	110	2016	56	121	36
Total	295	14,173	111	Total	59	1,469	36

*Source: Web of Science – Thomson Reuters, Access August 22, 2016



unanswered questions on ZIKV (for specific key questions see Box 1).

Box 1. Key Questions

- How do distinct neural cells react to ZIKV infection?
- Is the Central Nervous System (CNS) the only affected organ by ZIKV?
- What are the long-term effects of ZIKV?
- Does ZIKV infection affects adult human healthy humans?
- Are there any potential interactions between ZIKV infection and the so-called neurodegenerative disorders?
- What is the risk of a newborn presenting with microcephaly or other types of brain damage if the pregnant woman is infected, according to gestational age?
- May brain damage occur post-natally in infants who are infected through mosquito bites?

Clinical and Epidemiological Outstanding Issues

There are now strong evidences that not only infections in the first trimester of pregnancy lead to brain damage but also infections in the third trimester may cause adverse neurological outcomes even in babies born with head circumference within the normal range [1]. Arthrogryposis is frequently observed in these babies [9]. In fact, one could propose that arthrogryposis can be a consequence of CNS injury or even

represent a direct damage in the peripheral nervous system. On the other side it is also important to know whether other factors can collaborate in the outcome of infected babies, for example genetic variations in genes involved in CNS development or in the inflammatory response pathways. Other environmental risks or protective factors might play additional roles such as nutritional state, previous *flavivirus* infections or vaccinations, use of anti-inflammatory drugs, among others.

Virological Outstanding Issues

Investigations on ZIKV must include virus-related factors, which may affect the outcome of the infection. Strain-related differences in pathogenicity may also account for some of the effects that are currently being observed. Virus-related differences in pathogenicity in experimentally infected animals have been recognized since the 1950s [10] and now being reevaluated in view of the catastrophic impact of ZIKV infection in pregnancy. Serological cross-reactions between flaviviruses have been recognized for decades; the phenomenon called “antibody dependent enhancement” (ADE) of infection may lead to exacerbation of signs in infected hosts with partial immunity to a cross-reacting virus, or a different variant of a particular virus; likewise, dengue virus (DENV)

Table 2 Top 20 countries that published article on Zika virus, microcephaly, and dengue

Zika virus (1952–2016)			Microcephaly (2015–2016)			Dengue (1945–2016)		
Ranking	Country	Article (n)	Ranking	Country	Article (n)	Ranking	Country	Articles (n)
1	USA	108	1	USA	42	1	USA	4670
2	Brazil	59	2	Brazil	36	2	Brazil	1469
3	France	30	3	England	12	3	India	1148
4	England	21	4	China	7	4	France	1020
5	FR Polynesia	19	5	FR Polynesia	5	5	Thailand	976
6	Germany	19	6	France	5	6	England	974
7	Italy	19	7	Italy	4	7	Australia	801
8	Singapore	13	8	Canada	3	8	Singapore	704
9	Canada	11	9	Japan	3	9	China	601
10	China	11	10	Netherlands	3	10	Japan	552
11	Thailand	8	11	Pakistan	3	11	Taiwan	541
12	Australia	7	12	Singapore	3	12	Malaysia	500
13	Senegal	7	13	Australia	2	13	Germany	460
14	Spain	7	14	Germany	2	14	Mexico	141
15	Japan	6	15	Saudi Arabia	2	15	Netherlands	282
16	Saudi Arabia	6	16	Scotland	2	16	Canada	275
17	New Caledonia	5	17	South Korea	2	17	Vietnam	271
18	Reunion	5	18	Spain	2	18	Italy	269
19	Switzerland	5	19	Taiwan	2	19	Switzerland	242
20	India	4	20	Turkey	2	20	Cuba	228
Total					370			16397
World without double-counting					295			14173
World with double-counting					448			20741
% World double-counting					51.9%			46.6%

Source: Web of Science–Thomson Reuters, Access August 22, 2016.

Double-counting of articles resulted from co-authorship publications made by authors from two or more countries

superinfection in association with a distinct virus type may severely aggravate the outcome of DENV infection and then progresses to what is known as the “dengue shock syndrome”. Recently, sera from DENV-infected patients were shown to enhance ZIKV’s infection on Fc gamma receptor (FcγR)-bearing cells in vitro [11]. Moreover, Dejnirattasai and colleagues have shown that antibodies to the immunodominant epitope on DENV were able to bind ZIKV but were unable to neutralize the virus [12, 13]. Additionally, this promoted ADE suggests that DENV infection might lead to increased ZIKV replication. Even more, the structure of such cross-reactivity has recently been studied by crystallography. In fact, it is suggested that a same antibody could neutralize epitopes on both DENV’s and ZIKV’s envelope protein [14]. It is worth highlighting here another arboviruses serological cross-reactivity: in view of the apparent initial concentration of cases of ZIKA-associated microcephaly outside the area of vaccination to yellow fever (YFV) in Brazil, it has been suggested that YFV vaccine may induce some protective effect on ZIKV infections [15]. If such observation is confirmed and cross-protective immunity might indeed confer protection to microcephaly in pregnant women, then health authorities would already be able to at least minimize the damages caused by ZIKV by increasing the area of coverage of YFV vaccination. However, additional experiments must be carried out in order to confirm such an attractive hypothesis. Regardless of the outcome, it should not be surprising to identify a role for infections with other arboviruses in the outcome of ZIKV-associated disease.

The comments above are intended to provide a quick glance at the complexity of the subject, involving the classical triad “host, agent, and environment”, and whose implications on the pathogenesis and outcome of ZIKV infection still remain to be more deeply investigated.

Neurochemical Outstanding Issues

Classically, the CNS is target of several pathogens, particularly virus infection that affects the brain function [16]. In this sense, ZIKV strongly alters the cytoarchitecture and functionality of neural cells and morphological studies from newborns with microcephaly showed neuron-shaped calcifications, diffuse astrogliosis, activated microglial cells, and presence of macrophages in brain tissue [17]. Additionally, many reports have been linked changes in signaling pathways associated to neuronal apoptosis that might impair neurogenesis inducing neuropathological dysfunction [7, 8]. Although, all events in microcephaly have been implicated in neuronal cell fate, other cell types in the CNS are now gaining attention.

In line with this, ZIKV infection seems closely associated to glial cells namely astrocytes, oligodendrocytes, and

microglia: (I) astrocytes express the AXL receptor tyrosine kinase that is necessary to ZIKV to infect the human brain [17, 18]; (II) microglial cells are crucial component in immune response [19]; and (III) dysfunctional oligodendrocytes are potentially involved in the link between ZIKV infection and the development of Guillain-Barré syndrome [20]. However, it remains unclear if ZIKV induces disturbance in glial cell development, distribution, or functionality.

For studying ZIKV effects on the brain development, the role of neural crest (NC) in early stages of brain maturation has attracting considerable attention. The NC cells colonize several tissues [21] and its ablation affects brain and craniofacial development [22]. Based on that, it is very likely that, in vivo and in vitro experimental models will advance in the understanding of mechanisms involved in ZIKV-induced microcephaly.

Concluding Remarks

In this context, this letter intends to emphasize the need of multidisciplinary studies to rapidly advance in the understanding of this multifaceted health problem. In keeping with this, we recently constituted a collaborative and multidisciplinary taskforce encompassing eight Brazilian scientific institutions of excellence, *The ZIKV translational* research taskforce, which was recently supported by the Brazilian Agency, CNPq/MCTIC. This taskforce has a wide international network of collaborators and it is willing to support other researchers and incorporate additional collaborators. Our main goal is to advance fast in terms of mechanisms, which can potentially contribute to treat or halt ZIKV spreading around the world.

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