

Zika and Other Potential Causes of Microcephaly in Brazil: Status March 9, 2016

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Abstract

We review the current information about causes of microcephaly in Brazil in 2015-6, including speculative ones, in order to provide the best possible clarity about the information that is available as of the publication date. We review the leading candidate, Zika virus infections, the second candidate, the pesticide pyriproxifen, as well as DPT immunizations, and GM mosquitoes. Each of these candidates has been considered because of an increase at very roughly the time of the increase in microcephaly cases. The strongest evidence is in favor of Zika, through the observation of Zika virus in neural tissue, though a key piece of evidence is missing in the expected rise of cases in other locations, specifically Colombia. Evaluation of the potential role of pyriproxifen is difficult due to the limited number and nature of available studies, which should be revisited as they include some evidence for neurodevelopmental toxicity. The possibility of DPT immunizations of pregnant women as a factor is largely ruled out by an increase in immunization in countries in which microcephaly cases are not being reported. There is no direct evidence for GM mosquitoes as a cause. If there is a dramatic increase in cases of microcephaly in Columbia in the next three months, the case for Zika will be dramatically strengthened, and the case for pyriproxifen and GM mosquitoes will be essentially ruled out. On the other hand if the cases do not materialize, Zika will essentially be ruled out and pyriproxifen would become the strongest case with GM mosquitoes a speculative alternative along with other environmental toxins.

I. INTRODUCTION:

A dramatic increase in the incidence of newborn microcephaly cases has been observed in Brazil, as well as potentially associated still-births and other developmental problems. Zika virus is currently suspected to be the cause, but medical authorities state this connection is suggestive rather than conclusive [1]. Connections between viruses and developmental problems, including microcephaly, have been made, but Zika has not yet been established conclusively as the cause. There remains a possibility that other causes are responsible. Alternatives that have been suggested include the use of a pesticide that has developmental effects in insects and therefore perhaps in human beings, genetically modified mosquitoes due to the suggestion that transposable elements might transfer and cause genetic modifications in human beings, and immunizations due to ongoing public concerns about immunization involvement in cognitive developmental problems.

Public policy actions and public concerns make causal understanding important, including making sure that alternative hypotheses are evaluated, as the public may take steps that are inconsistent with scientific understanding if concerns are ignored and/or if they are misled. Many people are sick, afraid, and may be deciding not to have children or to abort them based upon this information. Thus, there is an immediate need to find out if individuals are right to be concerned. Here we present the current evidence and counter evidence for potential causes of microcephaly.

The World Health Organization (WHO) divides the most common causes of microcephaly into four categories [2]:

- Infections in the womb: toxoplasmosis (caused by a parasite found in undercooked meat), rubella, herpes, syphilis, cytomegalovirus and HIV;
- Exposure to toxic chemicals: maternal exposure to heavy metals like arsenic and mercury, alcohol, radiation, and smoking;
- Genetic abnormalities such as Down syndrome;
- Severe malnutrition during fetal life.

The association of causes of the increase of microcephaly with potential causes relies first on an increase in the cause with the increase in number of cases. However, the precise dates

of the increase are believed to be uncertain because of problems with reporting. According to a CDC report [1], before the medical alert in Brazil related to the manifest increase in number of cases, descriptions of congenital anomalies were reported but infant head circumference was not routinely recorded. Cases of microcephaly may not have been identified. Surveillance and reporting of suspected cases have increased since the medical alert, the increased levels of reliable reporting (and perhaps over reporting) makes determination of the precise timing of the outbreak more difficult. Without accurate timing and even with accurate timing, it is difficult to identify a specific cause for microcephaly.

We review the evidence and counter-evidence for four potential causes that have been cited in the professional and public discourse.

II. ZIKA VIRUS INFECTIONS:

An outbreak of Zika virus began in early 2015. Increasing cases of microcephaly were reported beginning in August, 2015 [1]. The roughly 9 months between outbreak times provides the first evidence for the role of Zika in microcephaly and other developmental problems. Additional evidence has been compiled through observation of the Zika virus in tissues of a few specific cases, observation of the infection of neural stem cells, and a review that identifies the incidence of Zika in cases of microcephaly. Possible counter evidence includes the reporting of cases before Zika in Brazil, and the absence of a correspondingly large number of cases in Colombia. Additional support is found in reports of a few cases associated with an outbreak in French Polynesia. More specifically:

- The presence of Zika virus in an aborted fetus with microcephaly after the mother had symptoms of infection in the 13th week of gestation. The virus was specifically found in neurological tissue [3].
- The observation of Zika virus infecting neural stem cells and affecting their growth [4].
- Zika virus found in the amniotic fluid of two pregnant Brazilian women, after both showed possible signs of infection, including fever and a rash [5].
- In a public release on February 12, 2016, Brazil's health ministry reported 462 confirmed cases of microcephaly or other alterations to the central nervous system, after

investigation of 1,227 of 5,079 suspected cases of microcephaly recorded from October 22, 2015 until February 6, 2016. 3,852 remain under investigation. Brazil has confirmed 41 of these cases of microcephaly are combined with “evidence of Zika infection...either in the baby or in the mother.” It is unclear from the report what is the Zika infection status of the microcephaly cases for which evidence of Zika infection is not reported [6, 7], i.e. whether they were investigated and had no evidence of infection or whether they were not investigated.

- In a release reported on November 14, 2015, the French Polynesian health authorities reported 17 (possibly 18) cases of central nervous system malformations, including 9 cases of microcephaly [8, 9]. A direct comparison of case counts in proportion to infections is difficult as the infection is not uniform nor complete in Brazil but the numbers are at the same order of magnitude with 17 in the Polynesian population of 270×10^3 giving a proportion of 6×10^{-5} . Assuming the same proportion of confirmed to reported cases as those that have been investigated and about 10% of Brazil having been affected (20 million) we obtain a proportion of 1×10^{-4} . However, given the small number of cases and given the uncertainty in the number of Brazilians that have been exposed this comparison is highly uncertain.
- Zika is known to cause neurological damage in adults, typically leading to transitory paralysis, i.e. Guillian-Barre syndrome [10, 11].

Possible counter-evidence:

- A recent report by Dr. Sandra Mattos found that, of 100,000 newborns in a database born in or after 2012, “at least 1,600 babies born in the last years had microcephaly or smaller-than-normal heads” [12].
- Absence of cases in Colombia. Only a single microcephaly case has been associated with the Zika epidemic in Columbia [13]. The Colombia outbreak of Zika grew during the late summer early fall, and particularly in October [14]. If more cases of microcephaly are not reported by June/July of 2016 then this would be evidence against the causal link of Zika to microcephaly [15].

III. PYRIPROXYFEN:

A group of physicians in Argentina and Brazil have suggested that the widespread use of pesticides to reduce mosquito populations may be the cause of microcephaly [16]. In particular, the pesticide pyriproxyfen has been used in the epicenter of Zika to reduce mosquito populations, including in the water supply. Its impact on insect development raises concerns that it might affect development in human fetuses. Exposure to toxic chemicals is one of the potential causes of microcephaly. The insecticide is used around the world, but not commonly in the water supply. A recent article on the inadequacy of neurodevelopmental toxicity testing states [17]: “The developing human brain is uniquely vulnerable to toxic chemical exposures, and major windows of developmental vulnerability occur in utero and during infancy and early childhood. During these sensitive life stages, chemicals can cause permanent brain injury at low levels of exposure that would have little or no adverse effect in an adult.”

We obtained detailed information about the tests performed that prompted regulatory approval [18], by request from the California regulatory authority. These tests were performed by its producer Sumitomo Chemical. Testing is limited and inconclusive, but includes some evidence for neurodevelopmental toxicity (see below). Such tests should be made public so that they can be critiqued by the scientific community, not just by regulatory authorities whose public summaries are not adequate for evaluation [19, 20]. This is a quite general problem, as the inadequacy of neurodevelopmental toxicity testing has been identified as a global risk [17].

Evidence of pyriproxyfen toxicity:

- Pyriproxyfen, used to curb the population of mosquitoes believed to be the carriers of the Zika virus, has been applied to the water supply of Brazil since 2014 [21].
- As a developmental toxin, Pyriproxyfen directly affects the development of mosquitoes, suggesting that it may also interfere in human development [16].
- Some (but limited) evidence from developmental toxicology studies indicates neurodevelopmental toxicity:

Developmental toxicology studies: Pyriproxyfen has been tested on rats, rabbits and other species of animals with low levels of toxicity for adult animals. Reproductive

and developmental toxicity has been tested in rats and rabbits. One rat study considered brain and behavioral effects. The experimental group of 36 pregnant rats in each of four test groups were fed dosage levels of 0, 100, 300 and 1000 mg/kg/day, during days 7 to 17 of gestation, which lasts 21 days. The pups were checked for physiological deformations and organs weighed.

From each dosage level litters of pups were obtained that were investigated for developmental problems. For 99 pups in the 100 mg/kg and 78 pups of the 1000 mg/kg dosage groups no relevant developmental disorders were found. Of the 99 pups in the 300 mg/kg dosage 1 (1%) had Arhinencephaly and 1 (1%) had Thyroid hypoplasia, The former would be consistent with concerns about neurodevelopmental disorders of the type of microcephaly.

Of the resulting offspring, 2 male and 2 female per dosage were kept alive for emotional/mental testing at 4 and 6 weeks of age and their brains were subsequently weighed at 8 weeks. One of the groups, the males of the 300 mg/km group had lower brain weight at 8 weeks, implying that at least one of the only 12 pups tested in this way had substantially reduced brain weight. (Note: The study description makes it possible that only half of this number, 1 male and 1 female per dosage, was tested).

Four problems suggest that this study is too limited to conclusively exclude the responsibility of pyriproxyfen for microcephaly:

- The period of administration does not include the very beginning of development, i.e. the first 7 out of 21 days in rats (It is noted in the study that 5 animals were exposed starting in day 6 rather than day 7 and their results were excluded from the study without further explanation).
- The number of animals that were exposed is small so that a low incidence condition would not be observed, and even so at least one of the 12 whose brains was studied was found to be low in mass.
- It is not clear that the more widely performed visual observations would identify microcephaly in rats.
- As with all animal experiments, the transferability of results to human beings is uncertain.

There are enough questions raised by this study to warrant further research.

IV. DPT IMMUNIZATION:

DPT vaccines, a combination vaccine for diphtheria, pertussis, and tetanus, has been suggested by some as the cause of the birth defects. The global incidence and deaths of children from pertussis [22] has led to recent increases in use of DPT vaccinations in pregnant women. The increase in use of the DPT vaccination in pregnant women began in Brazil in 2014, leading to claims of its relevance to the cases of microcephaly [23]. However, the DPT vaccine has been widely administered in the UK to pregnant women [24] without reports of increases in cases of microcephaly. Additional reasons to consider this association are not currently apparent.

V. GM MOSQUITOES

In order to control the mosquito populations that spread Dengue (and now Zika) the Brazilian authorities released GM mosquitoes in 2015 and previously. The male mosquitoes released have a baseline of 95% sterility and therefore should mostly die without offspring, inhibiting mosquito reproduction [25, 26]. While claims that the release was successful as a mosquito control effort are discounted by the subsequent Zika epidemic, it has been asked whether the release might instead be the cause of microcephaly cases. A highly speculative scenario has been presented [27], where a combination of (1) the override of the death programming because of the presence of its antidote tetracycline in agriculture, (2) the Zika virus adopting GM genetic materials including transposable elements, (3) transfer of the DNA from the mosquito to human beings, (4) transposable elements in that DNA acting as genetic modifiers of human genes together lead to developmental disruption in fetuses [28]. Evidence has been provided against the step of this scenario where Zika virus DNA receives DNA from the GM mosquito (see [27]). Overall, the scenario raises some speculative questions about previously unknown mechanisms, but there is no evidence for this scenario at this time. We note, however, that the precautionary principle implies that GM mosquitoes should not be used for vector control without adequate testing of both health and environmental impacts, standards for which have yet to be established [29].

VI. CONCLUSIONS:

We summarize the currently available evidence for causes of microcephaly. **Zika virus infection:** The predominant evidence is that microcephaly is caused by Zika infections of mothers in the first trimester due to direct infection of the neural brain tissue. **Pyriproxyfen:** The potential of a developmental insecticide to cause human neuro-developmental problems is credible but the limited testing currently available means that no conclusions pro or con can be reached. In the single available study of neurodevelopment in rats a reported case of Arhinencephaly and another of low brain mass in a pup provides sufficient motivation to warrant additional studies. **DPT immunizations:** There is direct evidence against any role of DPT immunizations in microcephaly due to its widespread use in the UK. **GM mosquitoes:** There is no evidence for a role of GM mosquitoes in microcephaly. Standards for testing of GM impacts have not been established, so the use of GM mosquitoes for vector control should be avoided due to the precautionary principle.

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- [1] L. Schuler-Faccini, E.M. Ribeiro, I.M.L. Feitosa, D.D.G. Horovitz, D.P. Cavalcanti, A. Pessoa, M.J.R. Doriqui, J.I. Neri, J.M. de Pina Neto, H.Y.C. Wanderley, M. Cernach, A.S. El-Husny, M.V.S. Pone, C.L.C. Serao, M.T.V. Sanseverino, Brazilian Medical Genetics Society - Zika Embryopathy Task Force, Possible association between Zika virus infection and microcephaly - Brazil, 2015, Morbidity and Mortality Weekly Report 65(3), 59-62 (2016), <http://www.cdc.gov/mmwr/volumes/65/wr/mm6503e2.htm>.
 - [2] Microcephaly: Fact sheet, World Health Organization (March 2, 2016), <http://www.who.int/mediacentre/factsheets/microcephaly/en/>.
 - [3] J. Mlakar, M. Korva, N. Tul, M. Popović, M. Poljšak-Prijatelj, J. Mraz, M. Kolenc, K.R. Rus, T.V. Vipotnik, V.F. Vodusek, A. Vizjak, J. Pizem, M. Petrovec, T.A. Županc, Zika virus associated with microcephaly, The New England Journal of Medicine doi: 10.1056/NEJMoa1600651 (2016), <http://www.nejm.org/doi/full/10.1056/>

[NEJMoa1600651#t=articleTop](#).

- [4] H. Tang, C. Hammack, S.C. Ogden, Z. Wen, X. Qian, Y. Li, B. Yao, J. Shin, F. Zhang, E.M. Lee, K.M. Christian, R.A. Didier, P. Jin, H. Song, G. Ming, Zika virus infects human cortical neural progenitors and attenuates their growth, *Cell Stem Cell* doi: 10.1016/j.stem.2016.02.016 (2016), <http://dx.doi.org/10.1016/j.stem.2016.02.016>.
- [5] G. Calvet, R.S. Aguiar, A.S.O. Melo, S.A. Sampaio, I. de Filippis, A. Fabri, E.S.M. Araujo, P.C. de Sequeira, M.C.L. de Mendonça, L. de Oliveira, D.A. Tschoeke, C.G. Schrago, F.L. Thompson, P. Brasil, F.B. dos Santos, R.M.R. Nogueira, A. Tanuri, A.M.B. de Filippis, Detection and sequencing of Zika virus from amniotic fluid of fetuses with microcephaly in Brazil: A case study, *Lancet Infectious Diseases* (February 17, 2016), [http://www.thelancet.com/pdfs/journals/laninf/PIIS1473-3099\(16\)00095-5.pdf](http://www.thelancet.com/pdfs/journals/laninf/PIIS1473-3099(16)00095-5.pdf).
- [6] S. Nebehay, B. Hirschler, Zika link to birth defects could be proven within weeks: WHO, Reuters (February 16, 2016) <http://www.reuters.com/article/us-health-zika-idUSKCN0VLOTQ>.
- [7] Ministério da Saúde investiga 3.852 casos suspeitos de microcefalia no país, Agência Saúde (February 12, 2016), <http://portalsaude.saude.gov.br/index.php/cidadao/principal/agencia-saude/22145-ministerio-da-saude-investiga-3-852-casos-suspeitos-de-microcefalia-no-pais>.
- [8] Zika virus microcephaly and Guillain-Barré syndrome, Situation report, World Health Organization (February 26, 2016), <http://www.who.int/emergencies/zika-virus/situation-report-26-02-2016.pdf>.
- [9] Rapid risk assessment: Microcephaly in Brazil potentially linked to the Zika virus epidemic, European Centre for Disease Prevention and Control (November 24, 2015), <http://ecdc.europa.eu/en/publications/Publications/zika-microcephaly-Brazil-rapid-risk-assessment-Nov-2015.pdf>.
- [10] V. Cao-Lormeau, A. Blake, S. Mons, S. Lastre, C. Roche, J. Vanhomwegen, T. Dub, L. Baudouin, A. Teissier, P. Larre, A. Vial, C. Decam, V. Choumet, S.K. Halstead, H.J. Willison, L. Musset, J. Manuguerra, P. Despres, E. Fournier, H. Mallet, D. Musso, A. Fontanet, J. Neil, F. Ghawch, Guillain-Barr Syndrome outbreak associated with Zika virus infection in French Polynesia: a case-control study, *The Lancet* (2016) [http://www.thelancet.com/journals/lancet/article/PIIS0140-6736\(16\)00562-6/abstract](http://www.thelancet.com/journals/lancet/article/PIIS0140-6736(16)00562-6/abstract).

- [11] G. Vogel, K. McLaughlin, Why does Zika leave some patients paralyzed?, Science (2016) <http://www.sciencemag.org/news/2016/03/why-does-zika-leave-some-patients-paralyzed>.
- [12] M. Chastain, Brazil: Medical study finds more than a thousand microcephaly cases before Zika, Breitbart (February 11, 2016), <http://www.breitbart.com/national-security/2016/02/11/doctors-find-microcephaly-predates-zika-virus-in-brazil/>.
- [13] D. Butler, First Zika-linked birth defects detected in Colombia, Nature News (March 4, 2016) <http://www.nature.com/news/first-zika-linked-birth-defects-detected-in-colombia-1.19502>.
- [14] Zika virus infection, Brazil and Colombia, Disease outbreak news, World Health Organization (October 21, 2015), <http://www.who.int/csr/don/21-october-2015-zika/en/>.
- [15] J.S. Cobb, L.J. Acosta, Colombia's forecast on Zika-linked birth defect may be too high: Minister, Reuters (February 17, 2016), <http://news.yahoo.com/colombia-global-relevance-zika-microcephaly-fight-minister-180421923.html>.
- [16] Report from physicians in the crop-sprayed villages regarding Dengue-Zika, microcephaly, and mass-spraying with chemical poisons, Red Universitaria de Ambiente y Salud (February 3, 2016), http://www.reduas.com.ar/wp-content/uploads/downloads/2016/02/Informe-Zika-de-Reduas_TRAD.pdf.
- [17] P. Grandjean, P.J. Landrigan, Neurobehavioural effects of developmental toxicity, Lancet Neurology 13(3), 330-338 (2014), <http://www.thelancet.com/journals/laneur/article/PIIS1474-4422%2813%2970278-3/fulltext>.
- [18] WHO Specifications And Evaluations for Public Health Pesticides: Pyriproxyfen, WHO (2006) http://www.who.int/whopes/quality/en/pyriproxyfen_eval_specs_WHO_jul2006.pdf.
- [19] Pyriproxyfen, Toxnet (2010) <http://toxnet.nlm.nih.gov/cgi-bin/sis/search/a?dbs+hsdb:@term+@DOCNO+7053>.
- [20] Pyriproxyfen in drinking-water: Background document for development of WHO guidelines for drinking-water quality, World Health Organization (2004), http://www.who.int/water_sanitation_health/dwq/chemicals/pyriproxyfen.pdf.
- [21] D. Bowater, Zika virus: Brazil dismisses link between larvicide and microcephaly, The Telegraph (February 15, 2016), <http://www.telegraph.co.uk/news/worldnews/zika/12157747/>

- [Zika-virus-Brazil-dismisses-link-between-larvicide-and-microcephaly.html](#).
- [22] Pertussis, World Health Organization (June 21, 2011), <http://www.who.int/immunization/topics/pertussis/en/>.
- [23] Brazilian Shrunk Head Babies: Zika or TDAP?, <https://brazilianshrunkenheadbabies.wordpress.com/about/>.
- [24] Whooping cough vaccination in pregnancy, National Health Service (September 24, 2014), <http://www.nhs.uk/conditions/pregnancy-and-baby/pages/whooping-cough-vaccination-pregnant.aspx>.
- [25] J.L. Reynolds, Same area where GM mosquitoes were released in 2015 is the epicenter for the Zika outbreak, Natural News (February 2, 2016), http://www.naturalnews.com/052836_Zika_virus_GM_mosquitoes_Brazil.html.
- [26] E. Griffiths, Was Zika outbreak caused by release of genetically modified mosquitoes in Brazil?, Mirror (January 31, 2016), <http://www.mirror.co.uk/news/world-news/zika-outbreak-caused-release-genetically-7281671>.
- [27] N. Berning, Genetically modified mosquitoes' survival rate concealed, Friends of the Earth (January 12, 2012), <http://www.foe.org/news/news-releases/2012-01-genetically-modified-mosquitoes-survival-rate>.
- [28] O. Tickell, Pandora's box: How GM mosquitos could have caused Brazil's microcephaly disaster, Ecologist (February 1, 2016), http://www.theecologist.org/News/news_analysis/2987024/pandoras_box_how_gm_mosquitos_could_have_caused_brazils_microcephaly_disaster.html.
- [29] N.N. Taleb, R. Read, R. Douady, J. Norman, Y. Bar-Yam, The precautionary principle (with application to the genetic modification of organisms), arXiv:1410.5787 (2014).