

### c Microcephaly and neurological complications post-Zika virus infection: evaluating the evidence for causality

The WHO Strategic Response Framework and Joint Operations Plan includes research into 'the reported increase in incidence of microcephaly and neurological syndromes including their possible association with Zika virus infection'. The determination of a causal association of a micro-organism with a specific disease syndrome has been deliberated since the discovery of micro-organisms (1676, Van Leeuwenhoek) and the proposal of the 'germ-theory of disease' (in the 1700s). Robert Koch, who discovered the causative organisms of tuberculosis, anthrax and cholera, proposed criteria for causality, now known as 'Koch's postulates' in 1880 while he was a government advisor with the Imperial Department of Health in Prussia. Koch's postulates require that the implicated microorganisms should be present in every case of disease, should be grown in pure culture from an infected person, and when re-inoculated into a susceptible laboratory animal, the organisms should cause the same disease, and be isolated in pure culture from the same laboratory animal. While these criteria are sound, Koch's postulates are limited by the requirement for infection and isolation from susceptible laboratory animals: many, if not most human pathogens do not cause disease in animals.

In the 1950s, Austin Bradford Hill proposed a set of criteria by which to evaluate an apparently causal relationship between exposure and disease. These criteria (Table 2) are helpful today to examine the evidence for the hypothesized causal association of Zika virus with microcephaly or neurological complications such as Guillian-Barré syndrome. Evidence for the strength and consistency of the association between Zika and microcephaly is mounting. A Slovakian woman who had worked in northern Brazil and experienced a Zika-like illness in the week 13 of pregnancy, returned to Europe at week 28 and had a termination of pregnancy on account of severe fetal abnormalities including microcephaly. Zika virus was detected by RT-PCR, and flavivirus-like particles were visualized in brain tissue using electron microscopy. No other recognized cause of fetal abnormalities were identified (Mlakar *et al*, NEJM February 10 2016). In a prospective study in Brazil amongst 88 pregnant women with clinical symptoms compatible with Zika, 72 had laboratory-confirmed Zika virus infection; amongst the 42 who had a fetal ultrasound, 12 (29%) had abnormalities detected ranging from growth retardation to cerebral calcification and microcephaly. Of the 6 births that had occurred by the time of publication, all had

abnormalities (2 x stillbirth, 1 x microcephaly, and 3 x small for gestational age with ocular abnormalities) (Brasil *et al*, NEJM, March 4, 2016). In a retrospective study of the Zika outbreak in French Polynesia that occurred from October to April 2014, 8 of 4 100 infants born during the outbreak had microcephaly. The authors modeled the epidemic based on population serosurveys, and established that the incidence of microcephaly increased from 2 cases/10 000 women infected in the first trimester, to 95 cases/10 000 women infected in the first trimester, representing a risk ratio of approximately 50%.

The temporal association between the increase in cases of microcephaly and the Zika epidemic has been shown in a number of studies, but is nicely illustrated in Figure 3, showing the peak of the epidemic curve of microcephaly cases roughly 9 months after the peak of the outbreak in a province in Brazil (de Oliveira, MMWR, March 11, 2016). It is plausible, and biologically coherent that Zika virus may be teratogenic: other viral infections, notably rubella lead to similar clinical presentations, and there is evidence for neurotropism and interference by Zika with neural progenitor cells. By way of analogy, other flaviviruses may cause neurological sequelae in animals (Wesselsbron and Japanese encephalitis virus).

Certain of the Bradford-Hill criteria remain unfulfilled: studies of the association between maternal Zika viral load and risk of microcephaly will be difficult to establish (biological gradient). The neurological syndrome of microcephaly has multiple aetiologies, leaving the specificity of Zika virus infection for microcephaly difficult to establish. It may not be possible to demonstrate the association by experiment, as an animal model for Zika virus infection does not exist at the present moment. In conclusion, it appears increasingly likely that Zikavirus infection in pregnant women does cause fetal abnormalities.

#### Recommended reading:

- Maestre AM, Caplivski D, Fernandez-Sesma A. Zika virus: More questions than answers. EBioMedicine. 2016 Mar 11;5:2-3.  
Rasmussen SA, Jamieson DJ, Honein MA, Petersen LR. Zika Virus and birth defects - Reviewing the evidence for causality. N Engl J Med. 2016 Apr 13  
Frank C, Faber M, Stark K. Causal or not: applying the Bradford Hill aspects of evidence to the association between Zika virus and microcephaly. EMBO Mol Med. 2016 Mar 14.

**Source:** Division of Public Health, Surveillance and Response, NICD-NHLS, (outbreak@nicd.ac.za)

Strength of the association
Consistency
Specificity
Temporality
Biological gradient
Plausibility
Coherence
Experiment
Analogy

**Table 2 (left).** Bradford Hill criteria for assessment of a relationship between an exposure and disease

**Figure 3 (below).** An epidemic curve showing the number of cases of microcephaly by epidemiological week relative to the week when authorities confirmed the transmission of Zika virus in Pernambuco province, Brazil through laboratory testing. (de Oliveira, MMWR, March 11, 2016)

