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Swedish University of Agricultural Sciences

**Fakulteten för veterinärmedicin
och husdjursvetenskap**
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Transmission and Expansion of the Zika Virus

Jacob Henrikson

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Jacob Henrikson

Supervisor: Mikael Berg, Department of Biomedical Sciences and Veterinary Public Health

Examiner: Eva Tydén, Department of Biomedical Sciences and Veterinary Public Health

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ABBREVIATIONS

ZIKV	Zika Virus
DENV	Dengue Virus
CHIKV	Chikungunya Virus
GBS	Guillain-Barré Syndrome

SUMMARY

There is a vast selection of vector-borne diseases circulating in the world today. The latest of these to have a globally recognised impact is the Zika Virus (ZIKV). The suggested link between ZIKV and the recent spike in microcephaly in Brazil has put the spotlight on this previously obscure virus. The potential for this virus to spread worldwide means that any suggested correlation between ZIKV and negative health effects should be extensively researched in case of a future global epidemic. This literature-review aims to assess how ZIKV is transmitted, through which vectors, its capacity for non-vector borne transmission, as well as highlighting the potential risks it poses to human health.

ZIKV is named after the Zika forest in Uganda where it was discovered in 1947, first isolated in a Rhesus monkey whilst studying yellow fever virus. The virus would for the next few decades go relatively undetected, with minor activity being reported in Africa and Asia, until 2007 when an outbreak occurred in Yap, Micronesia. Since then, epidemics have been observed in French Polynesia in 2013 and Brazil in 2016, with the virus being linked to both microcephaly and Guillain-Barré syndrome (GBS).

The Zika virus is of the *Flaviviridae* family, and so is related to Dengue virus, yellow fever virus and West Nile virus. It produces very similar febrile symptoms to some more commonly diagnosed arboviral infections such as chikungunya (CHIKV) and dengue virus (DENV), which has been suggested as a delaying factor in diagnosis. The low number of ZIKV cases previously recorded may be due to the occurrence of subclinical forms of ZIKV, as well as misdiagnosis as DENV or CHIKV.

Transmission of ZIKV is mostly vectorial through mosquitoes of the *Aedes* genus. The virus reproduces in the host vector with no effect on the host, and remains in the host until it dies. The *Aedes aegypti* and *Aedes albopictus* are the species most competent of transmission and pose a great public health concern, due to them being widely spread throughout tropical, sub-tropical (*Ae. aegypti*) and temperate (*Ae. albopictus*) regions. Non-vector borne transmission has also been described, with sexual, perinatal and blood transfusion-transmitted ZIKV all reported, although much less common than vector-borne transmission.

As mentioned previously, ZIKV has been linked to microcephaly and Guillain-Barré syndrome. The current ZIKV outbreak in Brazil has coincided with a spike in the number of babies born with microcephaly. Although not yet a proven link, pregnant women in the regions are being urged to take preventative measures against infection. Guillain-Barré syndrome has however already been shown to be a possible result of ZIKV infection. As ZIKV is rapidly spreading across

the Americas, these countries' healthcare systems must be prepared to accommodate patients with the potential complications that infection with the virus may lead to.

INTRODUCTION

Many of the most devastating diseases in less-developed countries are known to be vector-borne. The widening distribution of many of the vector organisms that transmit these diseases have led to an emerging threat of vector-borne diseases in the developed world. Mosquitoes are one of the most common and effective vectors for transmitting pathogens. Following the spread of Chikungunya and West Nile virus to the Americas, it seemed inevitable that additional mosquito-borne diseases would in time also spread to new geographic areas. To predict with certainty the next emerging threat would have been near impossible, considering the vastly expanding trade and travel networks that span the globe today, as well as the constantly changing climate. We now know that it would be the ZIKV. On 1 February 2016, the World Health Organization declared the growing ZIKV outbreak in the Americas, a Public Health Emergency of International Concern.

This literature-review aims to assess the existing knowledge and research that exists regarding the ZIKV, the different routes of transmission this virus can take and the potential health-complications it is feared that this virus may be the source of.

MATERIALS AND METHODS

The databases used to obtain literature were Pubmed, Web of Science and Primo. I used the search words (Zika Virus) AND (vector OR vectors) AND (transmission OR spread). I have chosen to primarily focus on articles relating to the transmission and distribution of the virus.

I chose to search for articles spanning 'All years', as I deemed all previous research on the subject to be of potential interest. The initial search for literature was carried out on February 12th 2016, and was then regularly repeated until the paper's time of completion. In addition to this I carried out hand searches on articles obtained from the databases, to find further literature I deemed relevant to my topic.

LITERATURE-REVIEW

HISTORY AND EXPANSION

The Zika Virus was discovered in 1947, in the Zika forest, Uganda. A rhesus monkey, Rhesus 766, was caged on a sentinel platform in the forest canopy as part of an ongoing study into sylvatic YFV transmission. A few days later febrile symptoms were observed and ZIKV was isolated from the monkey's serum (Dick et al., 1952). The same research group would later isolate the virus in the mosquito vector *Aedes Africanus*.

Transmission of ZIKV via artificially infected *Ae. aegypti* mosquitoes to mice and a monkey was observed in 1956 (Boorman et al, 1956).

Based on more recent phylogenetic analysis of the virus, it is thought to have emerged near this same location around the 1920s, before splitting into the 3 lineages we know to exist today (Haddow et al., 2012 & Faye et al., 2014). It is worth mentioning that the Asian lineage is thought to be responsible for the recent outbreak in Brazil (Zanluca et al.,2015).

In 1952, the first human cases of ZIKV in Uganda were identified (Dick et al., 1953). Cases would later be found in Nigeria in 1953, and by the late 70s ZIKV had spread to large parts of the African continent, including Sierra Leone, Senegal, Gabon, Egypt Ivory Coast and other Central African countries (Robin et al., 1975, Fagbami et al., 1977, Jan et al., 1978 , Renaudet et al., 1981, Saluzzo et al., 1981).

Up until this point there was no evidence of ZIKV circulation outside of Africa. However in Indonesia, between 1977 and 1978 (Olson et al., 1981), 30 patients submitted with acute febrile symptoms were found to have ZIKV antibodies in their sera. This marked the first migration of the virus outside the African continent, and should have been an indication of the virus' future epidemiological potential.

For the rest of the 20th century it would appear that ZIKV remained only within the African and Asian continents. In 2007 however, an outbreak of illness on Yap Island, Micronesia, characterised by rash, conjunctivitis and arthralgia, was reported by physicians. Subsequent testing led to detection of ZIKV RNA in serum samples from the patients (Duffy et al., 2009). The spread of ZIKV to Yap island was suspected to be through either a viremic traveller or via importation of infected mosquitoes (Duffy et al., 2009 & Musso et al., 2015). Following this, the disease spread rapidly through the pacific islands, with a large outbreak being detected in French Polynesia in 2013 (Cao-Lormeau et al., 2013). This outbreak saw an increase in cases of Guillain-Barré syndrome, suggesting a link between GBS and ZIKV. A 2016 study provided the first evidence for ZIKV causing GBS (Cao-Lormeau et al., 2016), highlighting the need for adequate preparation of

the healthcare systems in at-risk countries. A few cases of foetal microcephaly were also observed during this outbreak, however further investigations are needed to establish any causal link.

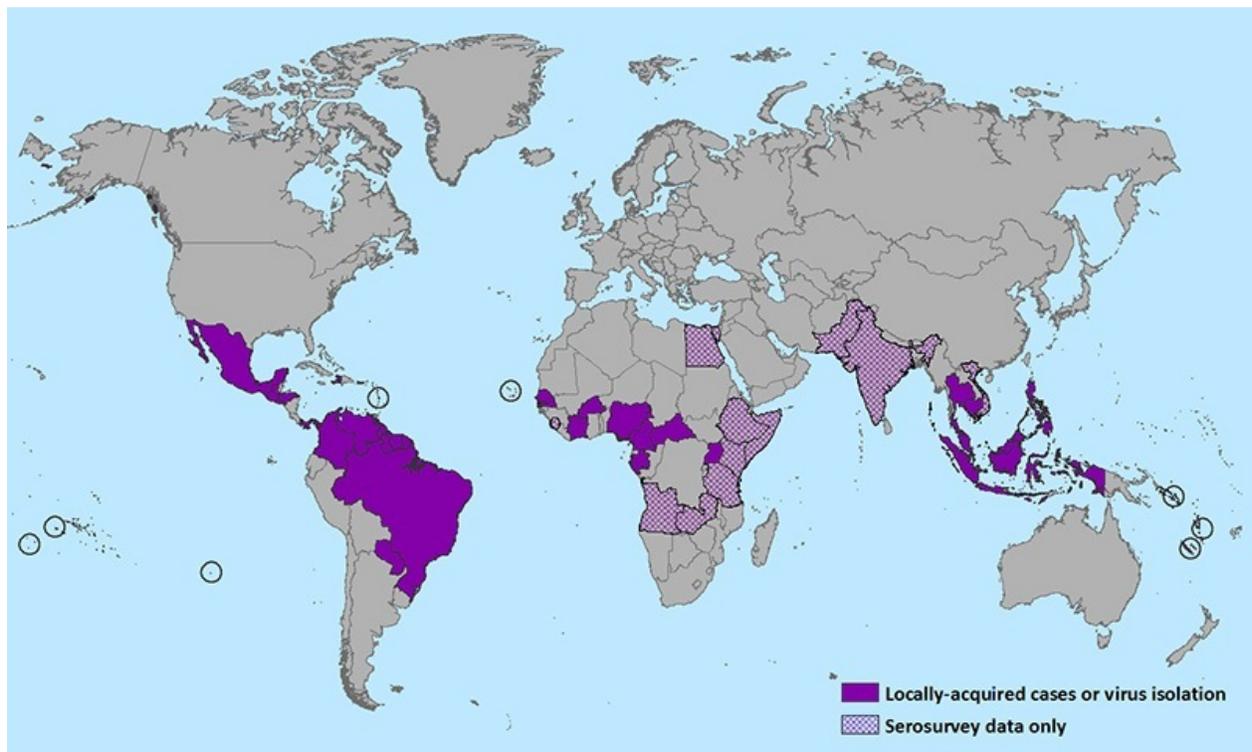


Figure 1. CDC map of Zika virus distribution in January 2016
https://commons.wikimedia.org/wiki/File:CD-C_map_of_Zika_virus_distribution_in_January_2016.jpg

In February 2014, not unexpectedly, following its spread through the Pacific, cases of the disease were reported in Easter Island, a Chilean territory in the Pacific. This event marked the introduction of the virus to the Americas (Musso et al., 2014).

ZIKV circulation was confirmed in Brazil in 2015, following its isolation from a suspected DENV patient. As mentioned previously, analysis of the ZIKV sequences obtained showed that the circulating strain in Brazil was of the Asian lineage.

The most likely paths of introduction are thought to be via a traveller during the Football World Cup of 2014, or the Va'a World Sprint Championship canoe race (Musso 2015 & Zanluca et al., 2015).

The Virus has since spread to many other countries in the Americas and must continue to be observed, especially given the growing association with GBS and microcephaly (Refer to PAHO.org for up-to-date information on situation).

VIROLOGY

The Zika virus belongs to the family Flaviviridae, and the genus Flavivirus. It is closely related to the Spondweni virus, with which it forms the Spondweni clade within the genus (Kuno et al., 2007). It is an RNA virus made up of 10,794 nucleotides encoding 3,419 amino acids. Similarly to other flaviviruses it has a non-segmented, single-stranded, positive sense RNA-genome. The virion structure of ZIKV has not yet been determined, however it is suspected to be similar to other known flaviviruses, in that it is encased by a host-cell endoplasmic reticulum-derived lipid envelope, surrounding a nucleocapsid made up of the protein C and the viral genome (Kuno et al., 2007).

RESERVOIRS

The reservoir most commonly suggested reservoir for ZIKV is non-human primates where the sylvatic cycle is mediated by *Aedes* mosquitoes. The virus has been detected in wild nonhuman primates in Africa (McCrae et al., 1982) and has been shown to exist in a sylvatic transmission cycle in orangutans in Borneo. This suggestion is supported by the fact that nonhuman primates are thought to play a key role in the sylvatic transmission cycles of other flaviviruses, including YFV (Monath et al., 1989), DENV (Rudnick 1965 & Fagbami et al., 1977 & De Silva et al., 1999) and CHIKV (McIntosh 1970 & McCrae et al., 1971).

Vertical transmission of ZIKV from mosquitoes to their offspring has also been observed (Diagne et al., 2015). Zika virus antibodies have even been observed in rodents suggesting them to play a potential role as reservoirs (Darwish et al., 1983)

TRANSMISSION

ZIKV transmission is most commonly associated with mosquito bites, generally via mosquitoes from the *Aedes* genus. It was initially isolated in mosquitoes of the Africanus species (Dick 1953), however the primary vector in the current outbreak is believed to be of the *Aedes Aegypti* species. Although vectorial transmission via mosquitoes is the most common path ZIKV will take, multiple cases of non-vector borne transmission have also been reported.

Non-human primates along with the *Aedes.spp.* mosquitoes, make up the sylvatic cycle of the virus in African and Asian forests.



Figure 2. All Countries and Territories with Active Zika Virus Transmission

Source: <http://www.cdc.gov/zika/geo/active-countries.html>

VECTORIAL

As mentioned, *Aedes Africanus* was the first observed vector of ZIKV. ZIKV would later be isolated in multiple other species of *Aedes*: *Aegypti* (Marchette et al., 1969), *hensilli*, (Lederman et al., 2014), *albopictus* (Grard et al., 2014), *Polynesiensis* (Cao-Lormeau et al., 2014) & others. The *aegypti* and *albopictus* are considered to pose the greatest health risk due to their competence for transmission (Grard et al., 2014 & Boorman et al., 1956). Both are highly invasive species, spanning tropical, subtropical and temperate areas, potentially infecting a large number of people. (Pinto Junior et al., 2015). Due to them co-existing in similar areas, however in differing conditions, vector control programs effective against one species may not affect the other.

Aedes aegypti are thought to have originated in Africa, before being globally distributed through global trade and shipping activities (Powell and Tabachnick 2013). Transmission of ZIKV to a rhesus monkey through infected *Ae. aegypti* mosquitoes (Boorman et al., 1956) has been demon-

strated in laboratory conditions (Boorman et al., 1956). In addition to this *Ae. Aegypti* collected in both Central Malaysia and Singapore have been shown to be carrying the virus.

The Asian tiger mosquito, *Aedes albopictus*, originated in Asia, before also being globally distributed through trade and shipping, primarily through international trade in used tires (Reiter and Sprenger 1987, Hawley 1988) was known previously as a potential vector of several significant pathogens, and was the first species other than *aegypti* to be observed as a vector for ZIKV outside of Africa (Wong et al., 2013). Great biological and ecological plasticity, along with increased intercontinental trade, have led to a global spread of *Ae. albopictus* (Paupy et al., 2009). It was the most frequently detected species of mosquito during the ZIKV outbreaks in Gabon (Grard et al., 2014). *Ae. albopictus* gained wider publicity during the recent CHIKV outbreak of 2013, when through a mutation in the viral envelope gene, the virus gained enhanced infectivity and transmissibility in *Ae. albopictus* (Tsetsarkin et al., 2007). The fact that this species acts as a vector for both CHIKV and ZIKV, suggests that similar mutations in ZIKV could lead to devastating consequences for human health.

NON-VECTOR BORNE TRANSMISSION

SEXUAL

In Senegal 2008, two American researchers working in Bandafassi as part of a mosquito-sampling project reported being bitten often by wild *Aedes spp.* mosquitoes. Upon returning home to Colorado, both patients fell ill after 6-9 days. Patient 1 exhibited extreme fatigue and headache, no fever was recorded, but developed signs of haemospermia a few days later. Patient 2's symptoms included a torsal macropopular rash, fatigue, headache, and swelling and pain in the wrists, knees and ankles. Patient 2 displayed no signs of haemtospermia. As patient 1 developed signs of haematospermia, the patient's wife (patient 3) developed similar clinical symptoms; malaise, chills, headache, photophobia and muscle pain. Again no fever was detected (Foy et al., 2011).

Serologic testing confirmed the presence of ZIKV in patients 1 and 2, as well as the wife of patient 1. Patients 1 and 3 reported engaging in sexual vaginal intercourse shortly after patient 1's return. This occurred before the appearance of clinical symptoms in patient 1. It is not unreasonable to suggest that that ZIKV may have spread between patients via seminal fluid during intercourse. Transmission could have occurred through the exchange of bodily fluids, however considering no illness developed in the 4 children of these two patients this route seems unlikely (Foy et al., 2011).

No sexual transmission of an arbovirus has been documented in humans. If transmission via this route could be confirmed in future studies, it would bear great ramifications for the potential epidemiology of ZIKV and other flaviviruses (Foy et al., 2011).

In 2015 ZIKV was observed to be detectable in the semen of patients following infection, strongly supporting the hypothesis that ZIKV can be transmitted by sexual intercourse (Musso et al., 2015).

Health officials in the US are currently investigating 14 reports of possible sexual transmission of ZIKV. In two of the cases, the only known risk was that the women had engaged in sexual contact with an ill male partner who had recently visited areas known to harbour ZIKV (BMJ 2016;352:i1180).

PERINATAL

Perinatal transmission has been observed for DENV (Tan et al., 2008), CHIKV (Fritel et al., 2006), WNV (Stewart et al., 2013), and YFV (Bentlin et al., 2009). Given the similarities in vectors and pathogenicity between these viruses and ZIKV, it is reasonable to suggest that it too has the potential for perinatal transmission.

Perinatal transmission can occur via many possible routes: transplacental transmission, during delivery or breastfeeding, or even via direct contact between mother and baby.

In November 2013, the first suspected case of perinatal transmission of ZIKV was seen in French Polynesia (Besnard et al., 2014). The newborn child of a mother displaying ZIKV-like symptoms, displayed a macropopular rash at delivery. Unfortunately no virological investigations were carried out on these patients.

In December, ZIKV RNA was detected by PCR in breast milk samples, highlighting the potential risk of transmission via breast-feeding. The ZIKV detected in the milk samples was not replicative, making contamination by this route less likely (Besnard et al., 2014). The risk of transmission via this route must however be considered, especially given the severe neonatal diseases known to be caused by other arboviruses, as well as the recently suggested link between ZIKV and microcephaly in new-born babies in Brazil.

BLOOD TRANSFUSION

During the ZIKV outbreak in French Polynesia, blood donor testing was implemented to prevent transmission of ZIKV through blood transfusion. 3% of blood donors were found to be positive for ZIKV by PCR. The capability for transmission through blood transfusion was suspected early

on, given its occurrence in other arboviruses (Musso et al., 2014).

Although an unexpectedly high number of blood donors tested positive, no recipients of ZIKV-positive blood have shown signs of infection. However, blood safety authorities are urged to be vigilant, and to consider deferral of blood donors returning from areas of ZIKV infection.

MONKEY BITE

In 2015, a traveller returning to Australia from Indonesia developed symptoms of fever, rash and conjunctivitis, five days after being reportedly bitten by a monkey in Bali. PCR was carried out on a nasopharyngeal swab from the patient and ZIKV RNA was detected. Although mosquito-borne transmission is another possibility, seeing as non-human primates are known to play a role in the sylvatic cycle of the virus, the monkey bite has been proposed as a plausible route of transmission (Leung et al., 2015).

URINE

It had been previously observed that other flaviviruses such as DENV (Hirayama et al., 2012) and WNV (Barzon et al., 2015) could be detected in urine over a longer period than in serum. It was observed in French Polynesia 2014 that ZIKV too is detectable at both a higher load, and with a longer duration, in urine than in serum. Attempts to isolate ZIKV from urine samples with the intent of cultivating infectious particles have failed. Further investigations are required to determine whether urine-borne transmission of ZIKV is a possibility, however urine samples have been suggested as being highly suitable for the detection and screening of ZIKV on a larger scale (Gourinat et al., 2015).

SALIVA

Zika virus has also been shown to be detectable in saliva samples, at a higher rate than in blood. Although ZIKV was detectable in saliva within the first week of symptoms appearing, use of saliva testing did however not increase the window of detection, as was reported for urine. Saliva samples will at least present an improved possibility of detection in patients where blood samples are not possible.

It is recommended that for diagnosis of acute phase ZIKV fever, both blood and saliva samples are taken to increase test sensitivity, whilst urine samples can be used for detection at a later stage (Musso et al., 2015).

PATHOGENESIS

Information regarding the pathogenesis of ZIKV is far from abundant, however in general mosquito borne flaviviruses are believed to first replicate in dendritic cells near the site of infection,

before spreading to the lymphatic and circulatory system (Diamond et al., 2003). In contrast to typical flaviviral replication, ZIKV has been suggested to replicate within the cell nuclei rather than in the cellular cytoplasm (Buckley et al., 1988).

CLINICAL MANIFESTATIONS

A ZIKV infection is typically represented by an onset of fever, headache, conjunctivitis, a macropopular rash, myalgias and arthralgias (Duffy et al., 2009). However, an estimated 80% of all patients with a ZIKV infection will display few clinical manifestations, or simply remain asymptomatic.

The first case of infection to be well documented was that of D. Simpson in 1964, who contracted the disease whilst working in the Zika forest in 1962-1963 (unpublished). Beginning with a slight headache, the next day the subject would develop a diffuse, pink, macropopular rash covering his face, neck, trunk and upper arms, as well as fever and back pains. One day later symptoms had subsided, and only the rash remained. ZIKV RNA was later isolated from serum collected at the time of the febrile symptoms (Simpson, 1964).



Figure 3 - ZIKV infection-related rash.

Source: By Fred - Own work, CC BYSA 3.0, <https://commons.wikimedia.org/wiki/File:Zika.Virus.Rash.Arm.2014.jpg#file>



Figure 4 - Presentation of conjunctivitis

Source: By Daemonanyndel - Own work, CC BY-SA 3.0, <https://commons.wikimedia.org/w/index.php?curid=22000222>

Although symptom duration for the virus has been observed as roughly 2-7 days, the macropopular rash and arthralgias have been shown to last for longer (Duffy et al., 2009).

Patients infected with ZIKV may often be misdiagnosed as having DENV or CHIKV, due to their similarities in clinical manifestation. Fortunately, some differences in presentation have been identified, which can hopefully act as a diagnostic aid in the future. Subjects infected with ZIKV are more likely to display conjunctivitis and peripheral edema, whilst leukopenia and thrombocytopenia are considered more common in DENV and CHIKV infections (Ioos et al., 2014).

In addition to DENV and CHIKV; there is a long list of differential diagnoses for patients infected with ZIKV, including herpes viruses, plasmodium-borne infections and, more commonly, WN and YF virus infections.

HEALTH COMPLICATIONS

MICROCEPHALY

The ZIKV outbreak in Brazil began in early 2015, being identified in a suspected DENV patient. By September there was an increase in reported cases of infants born with microcephaly in ZIKV affected areas. Two women whose foetuses had microcephaly were found to have ZIKV RNA in their amniotic fluid (Schuler-Faccini et al., 2015)

Reported findings on the association between ZIKV and microcephaly are however greatly limited. This may be due to under-ascertainment of the historical birth prevalence of microcephaly as recorded cases fell short of the estimated number (<http://www.eurocat-network.eu/accessprevalencedata/prevalencetables>).

Reported cases in 2015 appeared to show a sharp increase in birth prevalence, however it is suggested that this was caused by the enforcement of a special notification protocol. Additionally, infant head circumference has not been routinely historically recorded, and so may have been previously underreported. Finally, due to the lack of laboratory-confirmed cases in infants and mothers, unrelated rash illnesses may have been misidentified as ZIKV (Schuler-Faccini et al., 2015).

Further studies are required to confirm an association between ZIKV and microcephaly. Multiple health organisations have however issued travel warnings, especially aimed at pregnant women, advising against travelling through areas of known ZIKV activity.

GUILLAN-BARRÉ SYNDROME-

During the 2013 ZIKV outbreak in French Polynesia, an increase in Guillain-Barré syndrome (Cao-Lormeau et al., 2016) was reported suggesting an association between ZIKV and GBS.

Guillain-Barré syndrome often emerges following minor bacterial and viral infections, presenting itself as an acute, immune-mediated polyradiculoneuropathy.

It often effects general motor function, initially in the outer extremities before spreading inwards (Van den Berg et al., 2014). Guillain-Barré syndrome is the world's leading cause of non-traumatic paralysis.

Patient ethnicity was suggested to have a role in this outbreak, considering that all of the patients were of Polynesian origin. This theory was somewhat nullified by the more recently occurring increase in Brazil, El Salvador and Colombia, suggesting host ethnicity to be a less relevant factor (Samarasekera et al., 2016 , Cardoso et al., 2015)

88% of the reported GBS cases in French polynesia reported symptomatic ZIKV infection, a median of 6 days before the onset of neurological symptoms.

As previously mentioned ZIKV is spreading quickly through the Americas, and this recently discovered association should further highlight the potential harm to human health this virus may lead to (Cao-Lormeau et al., 2016).

DISCUSSION

At the emergence of any new epidemic, the potential threat to human health must always be analysed by international authorities, before then being communicated to the general public.

Following the first confirmed infection of ZIKV in Brazil in May 2015, the Pan American Health Organisation (PAHO) issued an epidemiological alert, highlighting the threat Zika was thought to pose. By Feb 1, 2016, the World Health Organisation (WHO) declared ZIKV a public health emergency of nation concern (PHEIC), mainly based on the increasing number of cases of neonatal and neurological disorders.

Although a link has been observed between development of Guillain-Barré Syndrome and ZIKV infection (Cao-Lormeau et al., 2016), there is still no demonstrated link between ZIKV infection during pregnancy and infant microcephaly. This highlights the challenges faced by organisations such as the WHO, as public health warnings may often need to be issued pre-emptively in order to avoid potential health-disasters. An over-reaction to an epidemic could have potential repercussions at later stages, due to, for example, overspending by public authorities, or an excessive travel ban. These organisations must do their best to correctly analyse any new threats, as their credibility will be vital in handling future epidemics.

In response to the ZIKV outbreak, the WHO has launched a global Strategic Response Framework and Joint Operations Plan to provide international guidelines on surveillance, community engagement and risk communication, vector control, care for those affected, research and coordination. WHO reportedly need \$56 million to complete this strategic response. Whether such a large economic response is warranted will not be apparent for some time to come.

Until further evidence is provided one cannot claim that the increasing rate of babies born with microcephaly in Brazil is due to ZIKV infection. The fact that earlier outbreaks have not been linked with the condition suggests the possibility for alternative causes. Alternative hypotheses are scarce, however doctors in Latin America have highlighted the possibility that the birth defects are being caused by drinking water contaminated with pyriproxyfen, a common industrial insecticide. Although this has been widely disregarded by Brazilian authorities as rumours and speculation, a literature review coordinated by SWETOX, Swedish Center for Toxicological Sciences, shows that few toxicological studies have been performed on human material, and states that a possible link between virus, insecticides and birth defects can not be ruled out (<http://swetox.se/en/2016/03/swetox-calls-for-potential-role-of-insecticides-in-zika-epidemic-to-be-examined/>). This research group recommends a series of further tests to be carried out: field surveys into the exposure of this and other insecticides, laboratory studies of bodily substance circulation

and interaction, additional literature studies and computer modelling of possible pathways for disease development.

The capability of ZIKV spreading beyond South America is already well-known. As mentioned in the case of sexual transmission (Foy et al., 2011), the virus has already been documented in the USA, and it is feared that there are many more infections than those reported. In addition to this both *Aedes Aegypti* and *Aedes Albopictus* mosquitoes are prominent in the USA, so vectorial transmission through this country would in no way be unexpected. A total of 147 cases of ZIKV infection have now been reported in the US and Puerto Rico. (BMJ 2016;352:i1212)

ZIKV cases have even been reported in Europe, generally in travellers returning from ZIKV infected areas. For example, two travellers returning to Italy from French Polynesia in 2014 were confirmed to be infected with ZIKV. It is feared returning travellers may ignite autochthonous transmission in countries such as Italy, where *Aedes albopictus* mosquitoes exist in abundance (Zammarchi et al., 2014). Mediterranean countries have already been clearly demonstrated to have a great affinity for autochthonous transmission of Aedes-borne arboviruses (20-24 imported to Italy), and so similar behaviour would be expected of ZIKV.

Further studies into ZIKV, and even other arboviruses, are required to fully understand the consequences of the current epidemic. It is understandable that historically both funding and interest in this subject have been relatively low, considering the common febrile symptoms ZIKV causes, and even sub-clinical manifestations it often displays. The recent occurrences on the global scene have led to a surge in research in this area, and although certainly beneficial, caution must be taken when assessing new research, as in times of global health scares there may be slightly more incentive to produce definitive results, and the possibility of experimental bias must always be considered.

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