ZIKA VIRUS: IMPLICATIONS FOR PUBLIC HEALTH

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Brief, 40-word summary of article's main point :

ZIKV is an emerging arbovirus whose undergoing pandemic is threatening public health. Sequelae of infection range from neonatal microcephaly to neurological disorders. Prevention of contagion and vaccine development are priorities for health systems.

Abstract

The World Health Organization has declared the current Zika virus (ZIKV) epidemic a public health emergency of international concern. Lack of vaccines and reliable diagnostic tests, broad geographical distribution of mosquito species that can transmit the virus, and absence of population immunity in newly affected countries are causes for concern. Although most infected persons are asymptomatic, ZIKV has been associated with a rise in cases of neurological complications and fetal central nervous system malformations. This defines such arbovirus as something whose transmission should be prevented. This review summarizes the current understanding of ZIKV biology and epidemiology, and possible interventions to prevent contagion and transmission.

The ongoing Zika virus (ZIKV) pandemic represents an emergency for general populations, especially pregnant women, transfusion recipients and immunosuppressed patients. Figure 1 depicts the current worldwide outbreak foci as of March 2016. Now, with the virus knocking at the doors of North America and Europe, we attempt here to summarize the implications for public health and the barriers we have against contagion.

Fundamental virology

ZIKV is an enveloped single-stranded, positive-polarity RNA virus belonging to the family of Flaviviridae, genus *Flavivirus*. It is antigenically closely related to other arboviruses of the family, and is grouped into 3 genotypes: East Africa, West Africa, and Asia[1]. The RNA genome is 11 Kb in size and includes a single open reading frame that encodes a polyprotein with 3 structural components (capsid [C], premembrane [prM] or membrane [M], and envelope [E]) and 7 non-structural proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b, NS5) [2]. Spread of the pandemic ZIKV lineage is associated with consistent NS1 codon usage adaptation in humans [3, 4].

Epidemiology

After West Nile (WNV) [5], dengue (DENV) [6], and Chikungunya viruses (CHKV)[7], ZIKV is the most recent arthropod-borne virus emerged with pandemic potential. ZIKV strain MR766 was first isolated in 1947 from a *Rhesus* monkey from the Zika forest (Uganda) [8]. In 1954, the first 3 human cases were reported during an epidemic of jaundice in Eastern Nigeria[9]. In 2007-2008, ZIKV epidemics occurred in the island of Yap (Micronesia) [10], Gabon [11] and Senegal [12]. Major epidemics broke out in French Polynesia in October 2013, New Caledonia [13] and Easter Island [13] in 2014, leading to imported cases worldwide [14-19]. The pandemic exploded at the end of 2014, and in March 2016 the virus is circulating in 26 countries. Brazil, where an estimated 1.5 million cases have occurred, heads the roster of affected countries, followed by Colombia (> 25,000 suspected cases) and by Cape Verde (> 7000 suspected cases) [20, 21]. Introduction of ZIKV into South America has been temptatively linked to Asian tourists attending the soccer 2014 World Cup in Brazil [22]. Airport connections and traveler volumes from Brazil mostly expose USA, Argentina, Chile, Italy, Portugal, France, China and Angola [23]. Scheduled international mass gatherings in 2016 could exacerbate the spread of ZIKV. In Brazil, the Rio Carnival on Feb 5–10 attracted more than 500 000 visitors, and on Aug 5–21 more than 1 million visitors are expected to go to the summer Olympics followed by Paralympic Games on Sep 7–18. Saudi Arabia expects to host > 7 million pilgrims from > 180 countries for the Umrah, between June and September, and the Hajj pilgrimage on Sep 8–13. On Feb 1, 2016 the WHO, according to International Health Regulations, declared that ZIKV "constitutes a public health emergency of international concern" [24].

Transmission

The first isolation of ZIKV from mosquito samples was made in 1948 from *Aedes africanus* in the Zika forest [25]. In 1956, 2 other strains were isolated from the same mosquito species [26], but these and many other following investigators neglected other mosquito species [27-29].

Aedes aegypti was considered the sole vector for the outbreaks prior to 2007, when *Ae. albopictus* was added [11]. Other *Ae.* species (*Ae. polynesiensis*, *Ae. hensellii, Ae. dalzieli*, etc.) have been reported as competent vectors of ZIKV, which might explain the possible recombination events and extensive variation of the viral envelope protein that occurred in the virus as a result of the adaptive response to the different hosts [4, 14].

Diallo and colleagues[30] surveyed mosquitoes from different environments from Senegal and detected by RT-PCR the presence of ZIKV in ten species from the genus *Aedes*, and *Mansonia uniformis*, *Anopheles coustani*, and *Culex perfuscus*. These mosquito species probably contribute to the zoonotic cycle of ZIKV transmission. Unfortunately, variety of species and rapidly increasing presence of these vectors worldwide could fuel the current epidemic in urban areas [10, 31] (Figure 1). However, the simple detection of a virus in a mosquito sample does not incriminate it as a vector [32].

In addition to transmission with blood-sucking insects of the *Aedes* species, numerous reports indicate perinatal [33, 34], sexual [12, 19, 35-37], and transfusion transmission [38]. Brazilian officers recently confirmed 2 cases of transfusion-transmitted ZIKV: the first recipient remained asymptomatic, while the second one died from other cause [39]. Infectious ZIKV particles have been reported in breastmilk, but evidences of transmission via breastfeeding remain poor[40]. Evidences of perinatal transmission are discussed in the following paragraph.

Clinical manifestations

The incubation period of flaviviruses, such as WNV and DENV, ranges from 3 to 14 days [41]. Similarly to other arbovirus infections, ZIKV infection is largely (80%) asymptomatic. When symptomatic, it is characterised by mild fever, arthralgia (small joints of hands and feet), myalgia, headache, asthenia, abdominal pain, oedema, lymphadenopathy, retro-orbital pain, conjunctivitis, and cutaneous maculopapular rash. This clinical picture can be misdiagnosed during the acute and viraemic phase because of non-specific influenza-like signs and symptoms. Moderate thrombocytopenia is not uncommon, with several cases developing profound thrombocytopenia and subcutaneous bleedings [42].

As with other arboviruses, neurological complications - including self-limiting meningoencephalitis [43] and Guillain-Barré syndrome [10, 31] - have been observed in humans. During the 2013–2014 outbreak in French Polynesia, the rise and fall of ZIKV infections preceded by 3 weeks a similar rise and fall in the incidence of anti-ganglioside antibodies-negative Guillain–Barré syndrom [44]. Nevertheless, an outbreak of

ZIKV infection in Cape Verde during 2015–2016 involving thousands of cases and possibly caused by an African strain of ZIKV has not been linked to any neurologic disorders [45].

In November, the Brazilian Ministry of Health released a report declaring a dramatic rise in the number of severe, isolated neonatal microcephaly cases [46-48]. The incidence varied from 2% to 8% according to the utilized classification criteria, with a seasonality reflecting that of *Ae. aegypti* [49]. The long-term consequences of abnormal brain development depend on underlying brain anomalies and can range from mild developmental delays to cerebral palsy. The association between maternal infections and congenital anomalies has long been recognized, especially when infection occurs during the first 12 weeks of pregnancy [50]. CDC scientists found ZIKV genome from brains of 2 miscarried fetuses and from 2 infants diagnosed with microcephaly who died shortly after birth : all 4 Brazilian mothers reported having ZIKV-like disease during their pregnancies [51]. Similarly, Mlakar *et al.* found ZIKV genome by RT-PCR, with consistent findings on electron microscopy, in a microcephalic fetal brain tissue born from a mother reporting ZIKV symptoms [52]. Calvet *et al.* later found ZIKV genome in the amniotic fluid of 2 pregnant women with microcephalic fetuses, although the virus was not detected in their urine or serum [34]. Bilateral macular and perimacular lesions as well as optic nerve abnormalities have been reported in most congenital cases [53-55]. Adverse fetal findings have been shown in 30% of infected pregnant women, and include fetal deaths, *in utero* growth restriction with or without microcephaly, ventricular calcifications or other central nervous system lesions, and abnormal amniotic fluid volume or cerebral or umbilical artery flow[56]. Similar findings have been recently retrospectively reported from the 2013-2014 French Polynesia outbreak [57].

Accordingly, in 1952 Dick *et al.* [58] reported viral tropism to the brain in intraperitoneally infected mice and an increase in viral titres over several days. This research suggested the virus could cross the blood brain barrier. The research findings were complemented in 1972 by Bell and colleagues[59] who observed an autophagy-like phenomenon in glia and neurons, later confirmed as real autophagy in experimentally-infected skin fibroblasts[60]. It remains unknown how ZIKV may gain access to the fetal brain moving from skin cells and fibroblasts, but immaturity of the blood-brain barrier in fetuses may facilitate migration. Tang *et al* recently showed that ZIKV infects, among various human cell lines, also human induced pluripotent stem cells (iPSCs)-derived forebrain-specific cortical neural progenitors (hNPC) and induces both increased cell death and cell-cycle dysregulation. Infected hNPCs also release infectious viral particles [61].

Laboratory examinations

ZIKV RNA can be detected by reverse transcription polymerase chain reaction (RT-PCR). RT-PCR pan-Flavivirus approaches targeting the conserved NS5 gene region across numerous species of the genus Flavivirus, but enabling subsequent discrimination via amplicon sequencing were designed by Ayers *et al.* in 2006 [62] and Moureau *et al.* in 2007 [63]. An envelope (E)-protein coding region-based, one-step RT-PCR was developed by Faye in 2008 [64]. A ZIKV-specific, quantitative real-time RT-PCR was later

developed by the same group in 2013 [65]. Nested PCR targeting ZIKV partial envelope and NS3 gene sequences has also been developed [66]. The variations in detection sensitivity encountered using different sets of primers may exist and may at least in part reflect the genetic diversity of the virus.

ZIKV RNA is present at high titer in the blood [67] only during the first 3-5 days after the onset of symptoms, while it is detected in urine for > 10 days in urine [68] and in semen for several weeks [12, 19, 35, 36, 69]. Although frequency is not known, at least one report suggests that the virus persists in semen for more than 2 months[36] and that, for unknown reasons, viral load in the semen can be roughly 100,000 times that of blood or urine [69]. ZIKV in such samples is viable and isolates have sometimes been achieved in cell culture [18]. Nasopharyngeal swabs have also tested positive by RT-PCR [18].

What clinicians and epidemiologists need is to determine whether a baby was exposed to ZIKV *in utero* months earlier. Currently available IgM ELISAs suffer from cross-reaction with other flaviviruses, so that positive results should be confirmed by neutralisation assay (i.e. PRNT) to document at least a 4-fold increase in ZIKV neutralising antibody titres [68]. At least 18 companies are working on laboratory tests for ZIKV : 10 are in Europe and the rest in Australia, Brazil, China, India, Israel, Japan, South Korea, and the USA [70]. Table 1 summarizes some of commercially available reagents for laboratory diagnosis of ZIKV infection, while Table 2 summarizes information regarding permissive cell lines.

Prevention

Fourteen vaccine developers (7 headquartered in the USA, 3 in France, 2 in Brazil, 1 in India, and 1 in Austria) are currently working on 23 projects of vaccine development[70], but timeline before clinical deployment can't be predicted.

As for DENV or CHKV, which are transmitted in the same fashion and there are no licensed vaccines, people in ZIKV-affected areas should protect themselves from mosquito bites by using air conditioning, screens, or nets when indoors, wearing long sleeves and pants, using permethrin-treated clothing and gear, and using insect repellents when outdoors. [71]. The insecticide-treated bednets (ITN) used to protect people against the night-biting *Anopheles* mosquitoes are poorly effective against the day-biting *Aedes* mosquitoes. Although pregnant and lactating women can use all registered insect repellents according to the product label, the US CDC and many national health ministries have recommended expectant mothers not to visit countries where ZIKV has become endemic. In mid-January, 2016, health ministers from different Latin American countries made public recommendations to women and couples to postpone pregnancy for 6 months to 2 years in the face of the ZIKV outbreak. These recommendations are very difficult to implement since up to 56% of pregnancies in the region are unintended [72]. In addition to poor quality of sex education, voluntary abortion is outlawed in several Latin America countries [73]: on February 5, 2016 the United Nations Human Rights Office of the High Commissioner asked for increasing women's rights in such countries [74].

Aedes mosquito control today relies on mechanical breeding-site reduction and chemical pesticides (insecticides and larvicides). Both *Ae. aegypti* and *Ae. albopictus* species thrive in close proximity to people, but while the former uses discarded containers and often breeds very close to or even in the home, *Ae. albopictus* often breeds in less accessible areas such as the water-filled leaf axils of plants. Furthermore, when populations of *Ae. aegypti* are reduced, the opportunistic invasive *Ae. albopictus* may rapidly move into an area [75]. The high pyrethroid resistance rates [76] are making the problem worse.

Oxitec is testing OX513A, a genetically-engineered, bisex RIDL® (Release of Insects with Dominant Lethality) strain of *Ae. aegypti*. During their rearing in insectaries, the mosquitoes are provided with dietary tetracycline to represses lethal gene activation. Before release, male and female pupae are separated mechanically, exploiting the fact that they are naturally significantly different in size. The strain contains the DsRed marker that is clearly visible in larvae, a useful tool for quality control in production and effective monitoring in the field. RIDL males released to mate with wild females generate progeny that die as late larvae or pupae (competing with wild-type larvae for resources) because they do not receive the dietary additive in the wild. Continual releases of sufficient numbers of RIDL males will reduce the target population [77]. Open field trials have taken place in both Grand Cayman Island and Malaysia [78], and are currently underway in Brazil : recent field release in Bahia, Brazil, reportedly achieved a 95% reduction in local *Ae.* populations[79].

Alternatively, researchers from the *Eliminate Dengue Program* have introduced bacteria of *Wolbachia* spp. into *Ae. aegypti*. Whereas RIDL® is a self-limiting approach (the genetic modification is not perpetuated in wild populations), *Wolbachia*-based control strategies rely on this endosymbiont successfully invading wild *Ae. aegypti* populations through a reproductive phenotype known as "cytoplasmic incompatibility". When uninfected female mates with a *Wolbachia*-infected male, females will have eggs but won't hatch due to cytoplasmic incompatibility. By contrast, *Wolbachia*-infected females can produce viable progeny when they mate with both infected and uninfected males (resulting in a reproductive advantage over uninfected females), and all the offspring will carry *Wolbachia* [80]. Open-field tests have been run in Australia [81] and releases are ongoing in DENV-endemic countries such as Indonesia, Vietnam, and Brazil. Mathematical models predict that one strain of *Wolbachia* (*w*Mel) would reduce the basic reproduction number, R_0, of DENV transmission by 70% [82].

Despite that fauna and food chain alterations carry potential risks and raise ethical concerns, these means are likely preferable than use of old insecticides (such as *p*-dichlorodiphenyltrichloroethane, DDT) known as teratogenic [83] and carcinogenic (e.g. malathion is classified as probably carcinogenic to humans [84]), and induce selective pressure favoring resistance. Despite negative results with pesticides against DENV [85], the Ministry of Health in Brazil has controversially intensified the same strategy to face the epidemics of ZIKV and CHKV.

Impact on donor selection

Possible strategies for blood donor selection will likely differ according to endemicity of vectors and occurrence of human cases, as summarized in the WHO Interim Guidance [86] :

- Countries that are both case and vector-free (e.g. New Zealand, Canada):
  - Laboratory testing for people who have recently travelled in case-endemic countries and have a clinical history of ZIKV infection;
  - Deferral of donors with a travel history in line with measures previously defined for WNV. The American Red Cross has asked donors returning from ZIKV-hit countries to postpone giving blood for at least 28 days, while Canadian Blood Services announced a temporary 21-day deferral period for anyone who has traveled outside of Canada or the continental USA or Europe[39].
- Countries case-free but vector-endemic (e.g. most Mediterranean countries): in addition to the above mentioned measures, such countries require early detection and response to imported and/or locally acquired cases of ZIKV;
- Countries that are case-endemic (e.g. French Polynesia [38]) : on the basis of protocols implemented for WNV NAT [87], blood donor samples are tested in minipools using a modified RT-PCR and in order to prevent virus transmission through blood transfusion without discontinuing blood donations. To increase sensitivity of detection and reduce the occurrence of false-negative results, sera from no more than three blood donors were included in each minipool. This approach suffers from a very low specificity, so that it is common for positive minipool to include donors who test negative individually. During the outbreak in French Polynesia, a serosurvey estimated that 38% of inhabitants had been infected : RT-PCR detected ZIKV in 3% of 1,505 asymptomatic blood donors [38]. Should autochthonous transmission of ZIKV occur in countries able to supply blood components to the affected areas, this would probably lead to suspension of blood collection and self-sufficiency measures similar to those adopted in the past to deal with a CHKV outbreak in Italy (2007) [88] and in Réunion Island (2005–07) [89]. Pathogen inactivation (PI) of platelet and plasma units is a sound alternative for such countries. However, PI increases the cost of the blood component, which is a significant obstacle in the current ZIKV affected countries [90], and to date there is no licensed pathogen inactivation technology for red cells or whole blood. Aubry *et al.* spiked fresh-frozen plasma units with ZIKV and measured viral titers and RNA loads before and after amotosalen and UVA photochemical treatment (Intercept®, Cerus). Inactivation led to a mean reduction of 1 log of RNA loads (from 10.25 log down to 9.51 log copies/ml): this apparent poor efficacy results from PCR detection of noninfectious ZIKV genome fragments generated by inactivation. Accordingly, and most importantly, cell cultures inoculated with inactivated plasma neither resulted in infected cells nor produced any replicative virus (> 6 logs drop) after one passage and detectable viral RNA from the second passage[91]. Unfortunately there are no data yet regarding

the efficacy of riboflavin and UVB (Mirasol®, TerumoBCT) against ZIKV, although such combination has proved effective against a wide range of arboviruses[92].

The abovementioned approaches should also apply to organ and tissue donations, with a special attention on gamete donation because of the aforementioned presence of ZIKV in semen [12, 19, 35, 36, 69] (and potentially in a woman's follicular fluid).

Tourism and trade have accelerated worldwide dissemination of mosquitoes, whose residency has been facilitated by global warming in previously noncompetent areas [93]. As for many other arboviruses, ZIKV has been shown to cause significant hazard to human health. Most importantly ZIKV affects reproductive health, with severe long-term sequelae for survivors that imply high costs for national health systems.

In conclusion, before an effective vector elimination method will be set in place, it is imperative to develop effective prophylactic measures and reliable diagnostic procedures to ensure safety of blood components, especially to pregnant recipients and immunocompromised patients.

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Table 1. Some reagents commercially available for ZIKV testing.

Use	Product	Product code	Manufacturer
Antibody screen	Qualitative Human ZIKV IgM (ZV-IgM) ELISA Kit	MBS109003	MyBioSource
	Qualitative Human ZIKV IgG (ZV-IgG) ELISA Kit	MBS109002	MyBioSource
Quick tests	Zika IgG/IgM Ab and Chikungunya IgG/IgM Ab rapid test	B826C CE	Biocan
	Dengue IgG/IgM ab and Zika IgG/IgM Ab rapid test	B828C CE	
	Zika IgG/IgM Ab rapid test	B815C CE	
	IIFT Arboviral Fever Mosaic 2 IgM/IgG Ab	FI 2668-1005-1 M and G	Euroimmun
Genomic RNA screen	Zika Real-time PCR kit	MBS598109	MyBioSource
	RealStar ZIKV RT-PCR Kit 1.0	591013	Altona Diagnostics
Viral strains for PRNT	ZICAV	1308258v	Public Health England
		ATCC® VR-84™	ATCC
		Ref: 143	EVA

Table 2. Summary of ZIKV infection of different cell types (modified from ref [61]).

Name	Type	ZIKV permissiveness
WA09	hESCs	+/-
DF19-9-11T.H.	hiPSCs	+/-
C1-2-NPC	hNPCs	+++
D3-2-NPC	hNPCs	+++
C1-2-N	Differentiated immature neurons from hNPCs	+
293T	Human embryonic kidney cell line	+/-
SNB-19	Human CNS cell line (glioblastoma)	+++
SF268	Human CNS cell line (astrocytoma)	+++
Vero	Monkey IFN- kidney cell line	+++
C6/C36	Mosquito (*Aedes albopictus*) cell line	+++

Legend : hESC : human embryonic stem cells; hiPSCs : human inducible pluripotent stem cells; hNPCs : human neuronal progenitor cells; CNS : central nervous system; +++: 65-100% cells infection after 3 days; +: 10-20% of the cells infected after 3 days; +/-: < 10% of cells infected after 3 days.
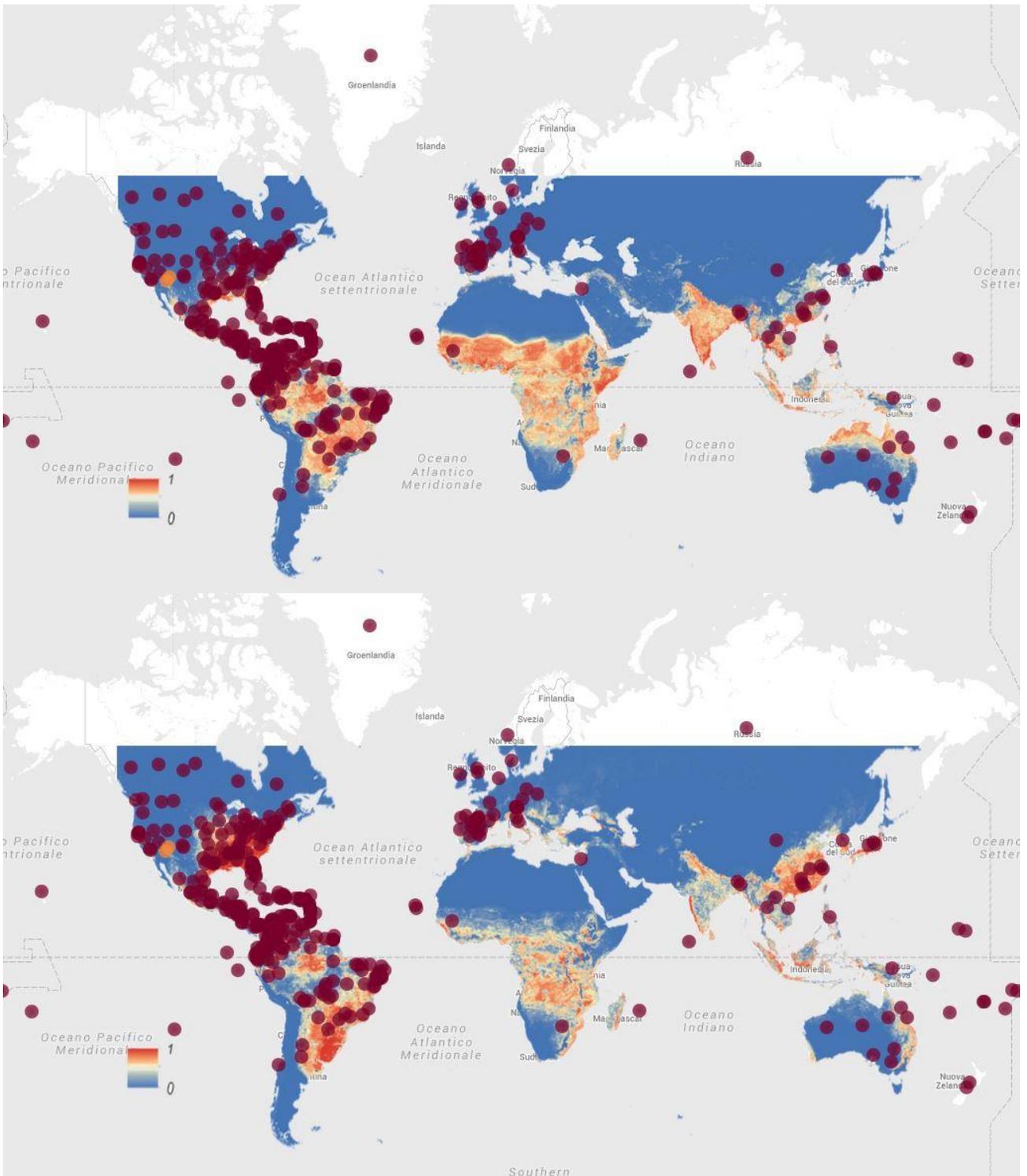
Figure 1. Map of confirmed cases of ZIKV infection (red circles) as of March 18, 2016. Upper and lower panels show heat map distribution of *Ae. aegypti* and *Ae. albopictus,* respectively (©HealthMap 2016) [94].