Zika virus in Asia

Veasna Duong, Philippe Dussart, Philippe Buchy

International Journal of Infectious Diseases 54 (2017) 121–128

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Review

Zika virus in Asia

Veasna Duong [a], Philippe Dussart [a], Philippe Buchy [b,*]

[a] Institut Pasteur du Cambodge, Phnom Penh, Cambodia
[b] GlaxoSmithKline Vaccines, R&D Asia-Pacific, 150 Beach Road, 189720, Singapore

ARTICLE INFO

Article history:
Received 14 October 2016
Received in revised form 26 November 2016
Accepted 29 November 2016

Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:
Zika virus
Asia

SUMMARY

Zika virus (ZIKV) is an emerging mosquito-borne virus that was first isolated from a sentinel rhesus monkey in the Zika Forest in Uganda in 1947. In Asia, the virus was isolated in Malaysia from *Aedes aegypti* mosquitoes in 1966, and the first human infections were reported in 1977 in Central Java, Indonesia. In this review, all reported cases of ZIKV infection in Asia as of September 1, 2016 are summarized and some of the hypotheses that could currently explain the apparently low incidence of Zika cases in Asia are explored.

1. Introduction

Zika virus (ZIKV) is an emerging mosquito-borne virus and member of the family *Flaviviridae*, which includes dengue virus (DENV), West Nile virus (WNV), Japanese encephalitis virus (JEV), and yellow fever virus (YFV) (Figure 1). The closest relative to ZIKV is Spondweni virus. ZIKV was first isolated from a sentinel rhesus monkey in the Zika Forest in Uganda in 1947 and from *Aedes africanus* mosquitoes in 1948.[1] ZIKV is transmitted by Aedes mosquitoes, with humans representing the amplifying host. A sylvatic cycle of transmission also exists and involves non-human primates and arboreal zoophilic Aedes mosquitoes from African and Asian forests.[2] ZIKV has also been isolated from *Culex* species mosquitoes, but their susceptibility to the virus seems low.[3]

The first human infection was recorded in Nigeria in 1954, where the virus was detected in a 10-year-old Nigerian female.[4,5] In Asia, the virus was isolated in Malaysia in 1966 from *Aedes aegypti* mosquitoes,[6] and the first human infections were reported in 1977 in Central Java, Indonesia.[7] Prior to the outbreak in Yap State (part of the Federated States of Micronesia) in 2007, where an estimated 75% of the residents were infected, only 14 human cases had been confirmed.[8,9] During 2013, an epidemic in French Polynesia affected approximately 28 000 people, and the possible association between ZIKV infection and Guillain–Barré syndrome was suggested for the first time.[9] As of August 31, 2016, 72 countries and territories had observed local ZIKV transmission.[10] In the Americas alone, over 500 000 locally acquired cases had been reported up to September 1, 2016; however the real number of infections is probably closer to several million, based on almost 2000 microcephaly and/or central nervous system malformation cases, which are suggestive of congenital ZIKV infection or potentially associated with ZIKV infection.[10,11]

2. The challenges of Zika diagnosis and detection by surveillance systems

In general, the clinical picture of natural human ZIKV infection is of a short duration, self-limiting, mild febrile illness that is accompanied by a maculopapular rash.[12] The most common clinical features reported during ZIKV infection are fever, rash, arthritis and/or arthralgia and/or myalgia, conjunctivitis, and fatigue.[13] Although it is difficult to clinically differentiate ZIKV infections from other arboviral diseases like dengue and chikungunya, symptoms such as oedema of the extremities, conjunctivitis, and the absence of leukopenia/thrombocytopenia are more common with Zika.[14]

To date, the literature describing the performances of Zika diagnostic tests remains relatively limited. The routine diagnosis of ZIKV infection can be determined by either direct methods, i.e., isolation and detection of viral genome by RT-PCR in blood, saliva, urine, and other body fluids (cerebrospinal fluid, amniotic fluid, semen, vaginal fluid, breast milk, pharyngeal secretions), or by indirect methods based on the identification of Zika antibodies in the blood.[2,15,16] Although a confirmation of the diagnosis is easily achieved with the direct diagnostic methods, the cross-reactivity between viruses of the *Flaviviridae* family makes the serological

* Corresponding author. Tel.: +6596173587.
E-mail address: philippe.x.buchy@gsk.com (P. Buchy).

http://dx.doi.org/10.1016/j.ijid.2016.11.420

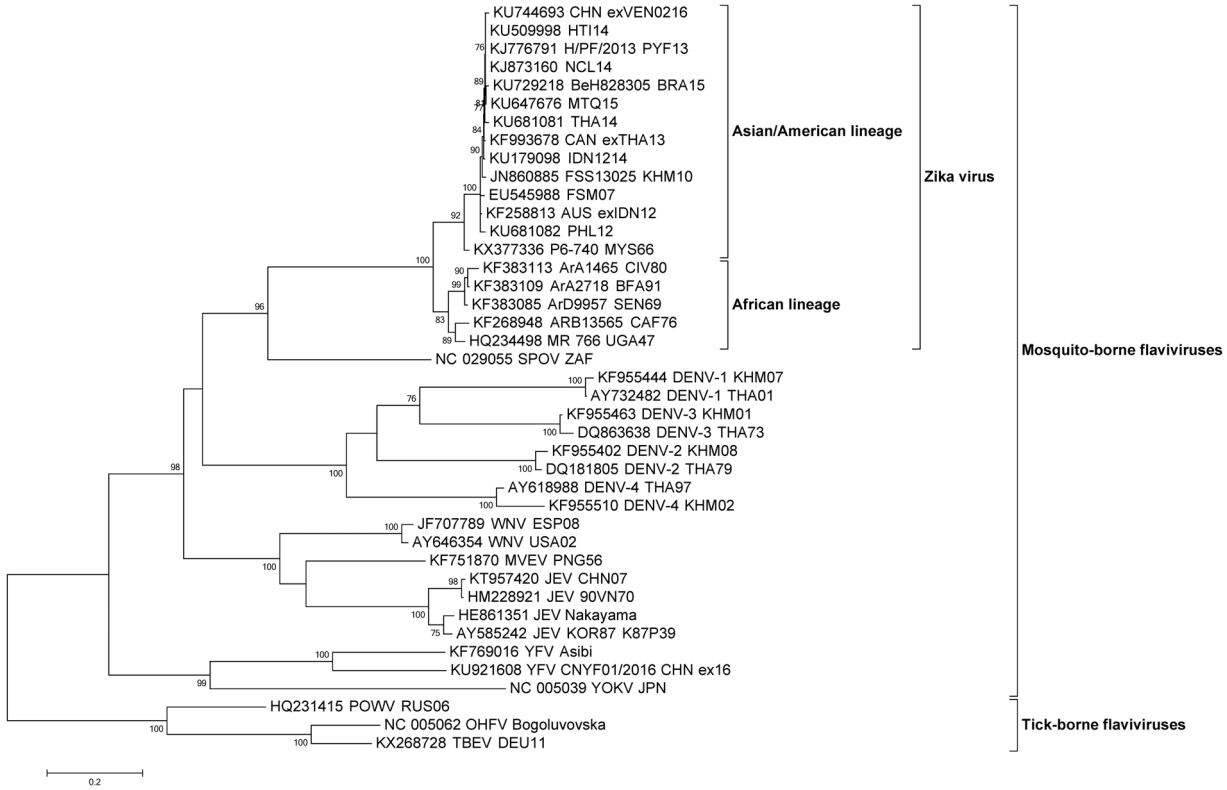

Figure 1. Phylogenetic tree of the NS5 gene of flaviviruses. The alignment includes 41 reference NS5 partial sequences from tick-borne and mosquito-borne flaviviruses available in GenBank. MEGA 6[99] was used to perform multiple sequence alignment and phylogenetic analyses using the maximum likelihood (ML) method and the GTR model with 1000 bootstrap re-sampling.

results very difficult to interpret, especially in those countries where more than one flavivirus circulates. The antibodies against ZIKV are often detected by ELISA and then confirmed by plaque reduction neutralization test (PRNT). Because of the potential for cross-reactivity, antigens from DENV and JEV should be tested in parallel and the results compared to identify the most likely aetiological agent. The Centers for Disease Control and Prevention (CDC) in the USA offer guidelines for the interpretation of serology results (http://www.cdc.gov/zika/pdfs/laboratory-guidance-zika.pdf).

There is a clear lack of a laboratory network with ZIKV diagnostic capacities, and access to standardized reagents remains difficult in many countries.[15,17] Using the existing national arbovirus surveillance systems in Asia to detect the circulation of ZIKV remains extremely challenging for several reasons.[15,17] First, the routine diagnosis of acute ZIKV infection relies essentially on the detection of viral nucleic acid by RT-PCR and the detection of IgM antibodies by IgM antibody capture (MAC)-ELISA. The detection of viral RNA by RT-PCR is often considered as a gold standard and provides a definitive diagnosis; however, this test remains unavailable in most clinical settings due to the associated costs and lack of experienced laboratory personnel. Virus isolation is useful for diagnostic and research purposes but is time-consuming and faces the same technical limitations as molecular assays. A number of ZIKV sero-epidemiological studies have been conducted in Asia; however because of the extensive cross-reactivity of anti-flavivirus antibodies and the large number of flaviviruses circulating in the region, the interpretation of serological results is complicated.[18–21] The antibodies against ZIKV antigen may be reactive to DENV, JEV, and/or YFV antigens to the same level, or with even higher titres in MAC-ELISA and haemagglutination inhibition (HI) assays.[16,22] PRNT can be used to differentiate antibodies of closely related viruses.[23] However, this test is labour-intensive and costly, requires handling of live virus, takes up to a week to perform, requires standardized reagents that are often not available, and is not widely performed. Due to the 'original antigenic sin' phenomenon, even the PRNT cannot reliably provide a diagnosis in patients who have previously been exposed to a heterologous *Flavivirus*,[24] which again poses some interpretational challenges in regions where dengue and/or other flaviviruses are co-circulating and more than 90% of the population may have had previous exposure to at least one of these flaviviruses.[18,25–27] In settings where PRNT is not available, specimens that test positive by ZIKV MAC-ELISA and negative by DENV and/or JEV MAC-ELISA may be interpreted as presumptive recent ZIKV infections (http://www.cdc.gov/zika/pdfs/laboratory-guidance-zika.pdf). However, the diagnostic accuracy of this approach remains to be validated.

Second, most of the surveillance programmes have a syndromic approach and they are often based on the report of hospitalized cases. Since ZIKV infection symptoms are rarely severe, poorly specific, and often misdiagnosed as dengue and chikungunya cases, the prevalence of Zika remains difficult to estimate through such surveillance systems.

3. Epidemiology of Zika in Asia

Tropical countries in Asia are believed to be endemic for many arboviral diseases including DENV, JEV, and chikungunya virus (CHIKV). In contrast to DENV, studies on ZIKV in Asia have been scarce, due to its apparent limited public health importance and the initial belief – prior to knowledge of the neurological

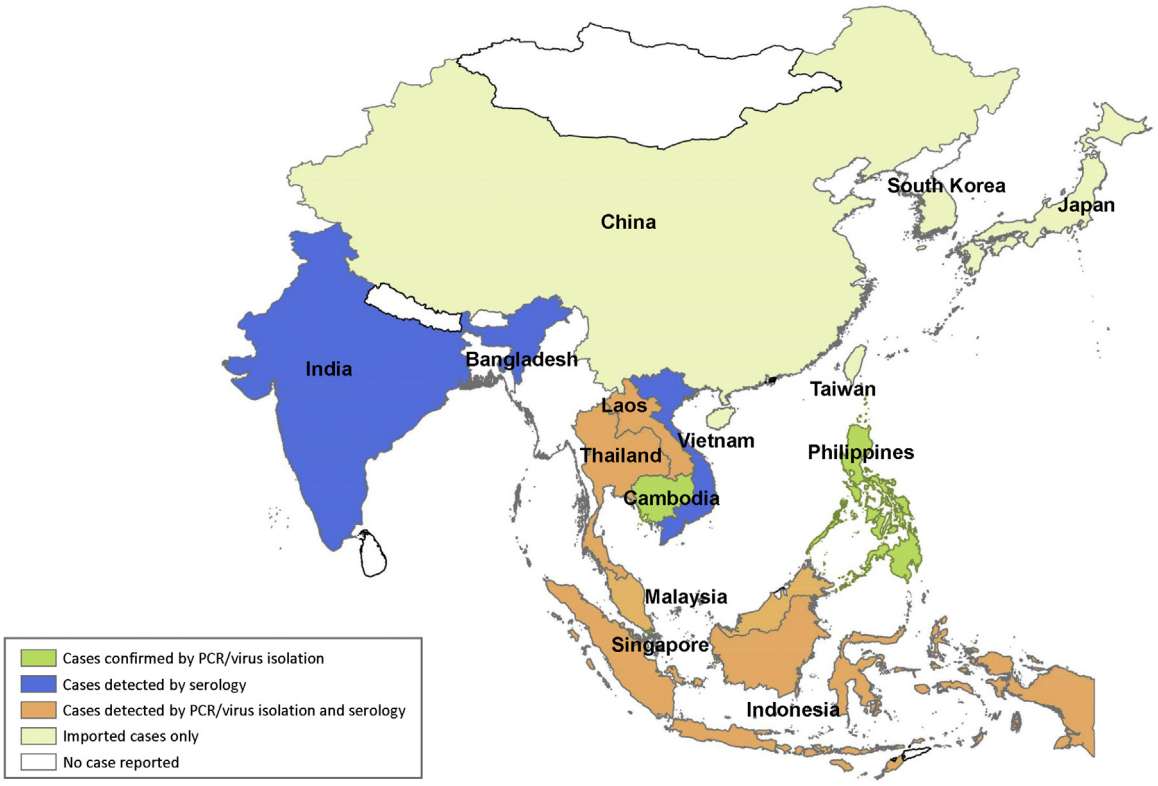

Figure 2. Map of Asian countries in which Zika virus circulation has been reported up to September 1, 2016.

complications – that the disease is mild in character.[28] Most of the survey results reported in the literature are dated in the 1950s and were based on serology using the HI test, complement fixation (CF) test, cross-neutralization test (NT), mouse protection test, haemagglutination assay (HA), and ELISA. Although these studies contain precious information regarding ZIKV infection in Asia, the specificity of these serological assays is questionable, especially when they are conducted in regions where other flaviviruses co-circulate. Prior to the outbreak in Yap in 2007 that drew some attention from the scientific community, little effort was made to describe ZIKV circulation in Asia. Now that Zika has become a public health concern, the infection is more closely monitored, and as of September 1, 2016 there have been reports of imported or autochthonous ZIKV infections in all Asian countries except Brunei, Hong Kong, Myanmar, and Nepal (Figure 2, Table 1).

3.1. Cambodia

Cambodia is endemic for a number of arboviruses including DENV,[25] CHIKV,[29] and JEV.[30,31] The serological and virological surveillance of arboviruses in Cambodia has been conducted through the National Dengue Control Program (NDCP) since 2000. Antigens from both *Flavivirus* (DENV and JEV) and *Alphavirus* genus (CHIKV) are included in the panel for MAC-ELISA and HI tests.[25]

The first ZIKV strain was detected in Cambodia in August 2010 in a febrile disease study that included approximately 10 000 patients.[32] A phylogenetic analysis of the full genome revealed that this strain belonged to the Asian cluster within the Asian/American lineage.[33] A recent retrospective study detected five human serum samples positive for ZIKV by RT-PCR among patients with dengue-like symptoms; these samples were collected in 2007 ($n = 1$), 2008 ($n = 1$), 2009 ($n = 2$), and 2015 ($n = 1$). Phylogenetic analysis of partial sequences of the non-structural protein 5 (NS5) gene from human samples (one from 2008 and two from 2009) demonstrated that all strains belonged to the Asian cluster within the Asian/American lineage.[34]

3.2. China

To date, confirmed ZIKV cases have only been reported in travellers returning to China and no autochthonous ZIKV infection has yet been reported. In February 2016, nine imported cases were documented in mainland China: five from Venezuela, three from Samoa, and one from Samoa/Fiji.[35] ZIKV RNA was detected by RT-PCR in serum ($n = 7$), urine ($n = 4$), and saliva ($n = 3$).[36–39]

In Hong Kong, one Zika case was detected in a traveler returning from the Caribbean region [40]

3.3. India

The only report on ZIKV in India is dated 1952 and was based on the results of NT assays;[41] the NT screening was conducted on 211 serum samples tested against 15 different arboviruses. Antibodies against ZIKV were detected in 33 out of 196 samples tested (16.8%) alongside many other viruses including, among others, DENV, WNV, JEV, Murray Valley virus, and St. Louis encephalitis virus.

3.4. Indonesia

The first human ZIKV infections were reported in Central Java, Indonesia in 1977, although the identification was only based on serology and no virus isolation confirmed the diagnosis.[7] Subsequently, additional serological surveys were conducted in 1977, 1978, and 1983.[7,42] Up to 12.7% of sera collected tested positive by HI testing.

Table 1
Asian countries with ZIKV autochthonous transmission and with ZIKV imported cases

Year	Country	Number of cases/total (%)	Diagnostic method		Reference
Autochthonous transmission					
1952	India	33/196 (16.8)	NT		41
1953	Philippines	19/153 (12.4)	NT		55
1953, 1954	Malaysia	75/100 (75)	NT		27
1953	Malaysia	15/79 (19)	NT		20
1953	Borneo	9/50 (18)	NT		20
1954	Thailand	8/50 (16)	NT		27
1954	Vietnam	2/50 (4)	NT		27
1977, 1978	Indonesia	7/219 (3.2)	HI		7
1983	Indonesia	9/71 (12.7)	HI		42
1983	Pakistan	1/43 (2.3)	CF		54
1996–1997	Borneo	49/11 (44.1)	NT		50
2014–2015	Indonesia	1	PCR		45
1969	Malaysia	Mosquito pool	Virus isolation		6
2010	Cambodia	1	PCR and virus isolation		32
2012–2014	Thailand	7/175 (4%)	PCR (4) and PRNT (3)		82
2001–2012	Thailand	2/21 (9.5)	Western Blot		21
2016	Thailand	97	Unknown		59
2016	Thailand	2	Unknown		81
2016	Singapore	115	PCR		61
2016	Lao PDR	1	Unknown		48
2016	Lao PDR	1.30%	Unknown		49
2012	Philippines	1/267 (0.37%)	PCR		56,57
2016	Vietnam	3	Unknown		75
Year	Country of origin of the patient	Number of cases/total (%)	Diagnostic method	Country of infection with ZIKV	Reference
Imported cases from and to Asia					
2012, 2015	Australia	2	PCR	Indonesia	43,44
2013	Canada	1	PCR	Thailand	76
2013, 2014	Germany	1	IgM, IgG, and NT	Thailand, Malaysia	51,77
2013, 2014, 2016	Japan	3	PCR	French Polynesia, Brazil, Thailand	47,78
2014	Italy	1	PRNT	Thailand	79
2015	Israel	1	PCR	Vietnam	83
2016	China	9	PCR	Venezuela, Samoa, Samoa/Fiji	35–39
2016	South Korea	9	Unknown	Brazil, Philippines, Vietnam, Dominican Republic, Guatemala	64
2016	Taiwan	6	PCR	St. Lucia, St. Vincent and the Grenadines, USA, Thailand, and Indonesia	74
2016	Hong Kong	1	PCR	Caribbean	40
2016	Singapore	1	PCR	Brazil	60
2016	Malaysia	1	PCR	Singapore	52
2016	USA	1	PCR	Philippines	48

ZIKV, Zika virus; NT, cross-neutralization test; HI, haemagglutination inhibition assay; CF, complement fixation test; PRNT, plaque reduction neutralization test.

ZIKV was detected in 2012 in an Australian traveller returning from Jakarta on the island of Java, and in 2015 in another traveller returning from Bali.[43,44] The latter traveller reported having been bitten by a monkey before the onset of symptoms, although mosquito bites might have occurred as well. Between December 2014 and April 2015, during an outbreak of dengue fever, the first autochthonous ZIKV infection was confirmed by RT-PCR in an Indonesian resident in Sumatra.[45] The phylogenetic analysis indicated that this strain belonged to the Asian cluster within the Asian/American lineage and clustered closely with strains isolated in Thailand and from the Australian traveller who had visited Bali.[45]

3.5. Japan

Three imported cases of ZIKV infection have been documented in Japan.[46,47] ZIKV was detected by RT-PCR in travellers returning from French Polynesia in December 2013 ($n = 1$) and January 2014 ($n = 1$), and from Thailand ($n = 1$) in August 2014.

3.6. Lao PDR

An autochthonous case of ZIKV infection was detected in Laos in March 2016.[48] Recently, a retrospective study investigating the presence of ZIKV in samples collected from suspected dengue cases detected the virus in a small proportion of samples (1.3%); in some cases ZIKV and DENV co-infections were detected.[49]

3.7. Malaysia

ZIKV was first isolated in Malaysia from *A. aegypti* in 1966. Using the NT assay, serological evidence of ZIKV infections were reported in 1953,[27] 1954,[20] 1996 and 1997[50] in humans as well as non-human primates in Malaysia. In 2014, a German traveller returning from Peninsular Malaysia and Sabah (Malaysian Borneo) was serologically diagnosed with ZIKV infection.[51] A confirmed ZIKV infection was reported in a woman who had recently visited Singapore in August 2016.[52]

3.8. Maldives

A ZIKV infection was detected by RT-PCR in a 37-year-old Finnish male who had returned to his country in June 2015 after spending several months in the Maldives.[53]

3.9. Pakistan

There is no recent report of ZIKV infection in Pakistan.[54] A serological study using a CF test was conducted in 1983 and

investigated the presence of antibodies to eight arboviruses in 42 human, 157 rodent, and 172 domestic animal sera. Antibodies against ZIKV antigen were detected in 2.3%, 3.8%, and 1.2% of human, rodent, and domestic animal sera, respectively.

3.10. Philippines

A serological study conducted in 1953 in the Philippines detected 19 Zika-positive sera out of 153 samples tested.[55]

A prospective cohort study of 267 acute febrile illnesses in Cebu City, Philippines, initiated in March 2012, detected one case of ZIKV infection by RT-PCR in a 15-year-old boy.[56,57] Phylogenetic analysis of the strain demonstrated that the virus belonged to the Asian cluster within the Asian/American lineage and was grouped closely with the strains isolated in 2007 in Micronesia.[56,57] ZIKV was also detected in US citizens returning from travels in the Philippines in March 2016[58] and in two Korean tourists travelling to the island of Boracay in the Visayas in June 2016.[48,59]

3.11. Singapore

Singapore declared its first imported case in May 2016.[60] The virus was detected in a Singapore Permanent Resident who had visited Sao Paulo, Brazil from March to May 2016. Subsequently, the country reported a dramatic rise in ZIKV cases after the Olympic games.[61] By August 31, 2016, a total of 115 ZIKV infections had been confirmed, including 41 cases that resulted from local transmission outside the original cluster.[62,63]

3.12. South Korea

South Korea has confirmed nine imported case of ZIKV infection in travellers[64] to countries where ZIKV circulation has been reported, including Brazil (n = 1; March 2016[65,66]), the Philippines (n = 3; April[67] and May 2016[68]), Vietnam (n = 3; May,[69] July,[70] and August 2016[71]), the Dominican Republic (n = 1; July 2016[72]), and Guatemala (n = 1; July 2016[73]).

3.13. Taiwan

By August 2016, the Central Epidemic Command Center (CECC) for Zika Virus had reported six imported Zika cases in Taiwan.[74,75] The first imported case was confirmed in a man travelling to Taipei from northern Thailand in January 2016. The other cases were detected in travellers returning from Thailand in January (n = 1) and in May 2016 (n = 1), from St. Lucia in July 2016 (n = 1), from St. Vincent and the Grenadines in August 2016 (n = 1), and from Miami, Florida, USA in August 2016 (n = 1).

3.14. Thailand

In Thailand, a serosurvey was conducted in 1954 using an NT assay. Sixteen percent of sera tested (n = 8/50; 25 from Bangkok and 25 from Chiang Mai) were reactive to ZIKV antigen.[27] However, HI testing showed that 80% of the 25 samples from Bangkok were also reactive to DENV-1 antigen, suggesting some potential cross-reactivity.

In early 2013, a Canadian woman who had returned from a vacation in southern Thailand developed a dengue-like symptom and tested positive for ZIKV RNA in blood, urine, and nasopharyngeal swab specimens.[76] The second case was reported in November 2013 in an adult German man who was diagnosed with acute ZIKV infection after visiting a number of islands in southern Thailand.[77] A third case was serologically suspected in a male Japanese traveller returning from southern Thailand.[47,78] A retrospective investigation by PRNT identified a probable Zika case in Florence, Italy involving a traveller returning from Thailand in May 2014.[79]

The first autochthonous cases were detected in a retrospective study of samples collected between 2012 and 2014. Seven cases of acute ZIKV infection were identified either by RT-PCR or PRNT assay in Thai residents in different provinces of central, northwest, and northeast Thailand.[56] Subsequently, ZIKV RNA was detected in the blood of a Thai national who was entering Taiwan Taoyuan International Airport.[80] The patient had no history of travel outside Thailand during the 3 months prior to his arrival in Taiwan. Another recent serosurvey reported anti-ZIKV antibodies in serum samples collected from northeast Thailand.[21] As of September 1st, 2016, approximately 99 Zika cases have been documented in the country. [59,81] All together, these studies suggest that ZIKV is endemic and widespread throughout the country. Analysis of the NS5 gene and full genome sequences demonstrated that the Thai ZIKV belonged to the Asian cluster within the Asian/American lineage and was clustered closely with sequences from the Cambodian strain detected in 2010 and to the sequences of the viruses isolated during the outbreak in French Polynesia.[56,82]

3.15. Vietnam

Little is known about ZIKV circulation in Vietnam. Historically, neutralizing antibodies against ZIKV were detected in 4% of 50 sera tested in residents of northern Vietnam in 1954.[27] Since then, the only reports of ZIKV infection have involved travellers to Vietnam from Israel in December 2015 (ZIKV RNA detected)[83] and South Korea (see above). The first cases of autochthonous infection in Vietnam were reported in March 2016 in two women living in Ho Chi Minh City and in August 2016 in a male living in the south-central coast region.[75]

4. Is the incidence of ZIKV in Asia actually low?

Following its first detection in humans in 1953 in Nigeria,[4,5] only sporadic human cases were detected over the next 55 years. There were no documented large outbreaks in Africa or Asia, and between the 1940s and 1990s only serological evidence of ZIKV circulation was provided in those regions.[28] An unprecedented outbreak of ZIKV then occurred on several islands of Yap state (Micronesia), resulting in an estimated 5000 infections among the total population of 6700 (attack rate over 74%).[13] Subsequently, outbreaks were reported in French Polynesia in 2013 and 2014, in which an estimated 11.5% of the population was infected,[84] in several Pacific Islands from 2014 to 2016, and in the Americas starting in March 2015.[85]

The outbreak in 2007 has led to several investigations, both retrospective and prospective, looking for ZIKV in Asia.[21,57,82] In stark contrast to these outbreaks, both serological and virological results from these studies suggest that ZIKV is endemically present with a low prevalence in those Asian countries.[21,57,82]

There is a clear difference in landscape of ZIKV transmission in different regions of the world. This marked heterogeneity in epidemiology has raised two questions: (1) Why has ZIKV presently circulating in Asia never been associated with outbreaks on the same scale as those in South America? (2) Would the strain causing outbreaks in the Pacific and the Americas cause the same extent of public health concern if introduced into Asia?

To date, there is no clear evidence to explain the relatively low prevalence of ZIKV infection in Asia and why ZIKV has not been associated with similarly large outbreaks in Asia to those seen in the Pacific and the Americas. The mild character and non-specific clinical presentation of ZIKV infection, coupled with the low burden to public health prior to 2007, led to ZIKV disease being of less interest in scientific studies. Additionally, ZIKV was not

recognized, was misdiagnosed, or was simply overlooked in clinical settings such as hospitals and private clinics, even during testing for arbovirus infections in the framework of national surveillance. This may explain why the circulation of ZIKV in some Asian countries is often documented based on cases in which travellers experiencing a febrile disease after returning to their home country are subsequently diagnosed following a broad investigatory panel of aetiology that includes Zika. Another challenge is in the laboratory diagnosis. The great extent of cross-reactivity between flaviviruses in the IgM and HI assays could lead to erroneous conclusions in Asian countries where DENV and JEV are predominantly circulating and where these assays are more widely available than molecular methods.[22]

Prior to the observation of the increase in microcephaly cases in Brazil and the confirmed link of neurological complications in neonates to ZIKV infection,[86–89] the prevalence of microcephaly due to ZIKV infection was never investigated in Asian and African countries even though ZIKV was endemic. Microcephaly due to ZIKV was reported retrospectively in French Polynesia,[28] and in late 2015 ZIKV of Asian lineage introduced from the Americas was associated with microcephaly in Cape Verde.[90] Since the present review was performed, further microcephaly cases linked to ZIKV infection have been reported, involving three babies in Thailand in September 2016[91] and one baby in Vietnam in October 2016.[92] However, it was not reported whether these microcephaly cases were associated with the endemic Asian strain or that from the Americas. At this stage, and considering that microcephaly is still a relatively rare event, it is difficult to establish whether the low numbers of microcephaly cases detected in Asia are the result of weak surveillance systems or whether the strains of the Asian cluster within the Asian/American lineage are associated with a lower risk of such birth defects.

The seroprevalence of ZIKV infection in the general population of Asian countries has not been assessed to estimate the burden of the disease; the large majority of studies conducted so far have included only symptomatic patients seeking medical care. Although the precise incidence and prevalence are not yet known, ZIKV infections may have occurred in many Asian peoples, and especially the youngest ones, and likely provided long-lasting natural immunity (although this remains to be explored). In French Polynesia and Micronesia,[13,15,84,93] pauci-symptomatic and asymptomatic infections were frequent. Among blood donors, 73.8% of the ZIKV infections were asymptomatic and the ratio of symptomatic to asymptomatic patients observed in Micronesia was approximately 1:5 to 1:6. It is therefore possible that in Asia a significant number of ZIKV infections do actually occur, but they are under-reported because of the absence of symptoms or the mild characteristics of the symptoms associated with this viral disease, while the clinically apparent infections are often wrongly classified as DENV or JEV infections, even after laboratory testing. In addition, although this may not necessarily be the case in Asia, recent studies in Latin America have reported that the frequency of ZIKV co-infections with DENV and CHIKV is even higher than that of mono-infections.[11] Considering the little attention that Asian countries have paid to Zika until recently, it is unlikely that patients who tested positive for DENV or CHIKV by first-line RT-PCR were further investigated for ZIKV as well.

The role of cross-protective immunity is also not well understood. We do not really know whether previous exposure to other flavivirus(es) may or may not be protective against ZIKV infection. DENV and JEV infections may play a role in the epidemiological dynamics of ZIKV by either providing protection or conversely enhancing the severity of ZIKV infection. The first concrete study on cross-reactivity between DENV and ZIKV demonstrated that plasma from DENV patients provided substantial cross-reaction to ZIKV and could not completely neutralize ZIKV; hence induced antibody-dependent enhancement (ADE) of ZIKV infection.[94] However, they also observed that some plasma from DENV-infected patients was able to neutralize ZIKV. They suggested that these patients might have high concentrations of cross-reactive neutralizing antibodies to DENV and ZIKV, such as those that recognize the EDE1 epitope.[95]

Interestingly, South American and Southeast Asian countries are endemic for dengue. If the hypothesis of ADE applies, Asia should experience similar or even larger outbreaks of ZIKV with severe infection and neurological complications. As of September 2016, only sporadic ZIKV cases have been reported throughout Asia. One potentially important difference between Asia and South America is that ZIKV has been endemic in Asia for at least several decades, while the population in the Americas was certainly fully naïve to Zika, providing the virus many opportunities to spread rapidly.[21,32,44,45,82,83] However, this does not mean that Asian populations do not face any risk of a Zika epidemic. Based on the volume of travellers arriving from airports in countries where ZIKV is circulating, the resident population at risk for ZIKV exposure, and health expenditure per capita, a recent model has suggested that India, the Philippines, Indonesia, Pakistan, and Bangladesh are at a high risk of ZIKV importation with a possible significant health impact on the population.[96] For example, in India, an estimated 1.2 billion people are susceptible to ZIKV exposure during the peak seasonal risk (August). Combined with the continuous growth of the population, globalization, urbanization, climate change, and the lack of effective vector control measures, Asian countries may well be exposed to Zika outbreaks in the near future.

ZIKV strains are divided into two major lineages: the African lineage and the Asian/American lineage. These two lineages differ in approximately 90% of their nucleotide sequence.[2] Strains belonging to the Asian/American lineage have been isolated in Southeast Asia, in the Pacific Islands, and in the Americas. Within the Asian/American lineage, the strains from the Americas have formed a new American cluster.[97–99] Molecular analysis of strains isolated in Brazil has suggested that there was a single introduction of the virus into the Americas between May and December 2013, but the study did not identify specific molecular markers associated with microcephaly.[98] Whether the rapid spread of ZIKV in the Americas is associated with some specific mutations remains to be determined. Considering the extremely complex interplay between virus, vector, and host, which characterizes such vector-borne diseases, it may take years before we really understand the differences in epidemic situations between Asia and the Americas.

Acknowledgements

We thank Stephen Griffiths (GSK Vaccines, Asia-Pacific) for his editorial assistance.

Conflict of interest: Philippe Buchy is an employee of GSK vaccines Asia-Pacific.

References

1. Dick GW, Kitchen SF, Haddow AJ. Zika virus. I. Isolations and serological specificity. *Trans R Soc Trop Med Hyg* 1952;**46**:509–20.
2. Barzon L, Trevisan M, Sinigaglia A, Lavezzo E, Palu G. Zika virus: from pathogenesis to disease control. *FEMS Microbiol Lett* 2016;**363**:1–17.
3. Huang YJ, Ayers VB, Lyons AC, Unlu I, Alto BW, Cohnstaedt LW, et al. Culex species mosquitoes and Zika virus. *Vector Borne Zoonotic Dis* 2016;**16**:673–6.
4. Fagbami AH. Zika virus infections in Nigeria: virological and seroepidemiological investigations in Oyo State. *J Hyg (Lond)* 1979;**83**:213–9.
5. MacNamara FN. Zika virus: a report on three cases of human infection during an epidemic of jaundice in Nigeria. *Trans R Soc Trop Med Hyg* 1954;**48**:139–45.
6. Marchette NJ, Garcia R, Rudnick A. Isolation of Zika virus from *Aedes aegypti* mosquitoes in Malaysia. *Am J Trop Med Hyg* 1969;**18**:411–5.

7. Olson JG, Ksiazek TG, Suhandiman, Triwibowo. Zika virus, a cause of fever in Central Java, Indonesia. *Trans R Soc Trop Med Hyg* 1981;**75**:389–93.
8. Faye O, Freire CC, Iamarino A, Faye O, de Oliveira JV, Diallo M, Zanotto PM, Sall AA. Molecular evolution of Zika virus during its emergence in the 20(th) century. *PLoS Negl Trop Dis* 2014;**8**:e2636.
9. Higgs S, Vanlandingham DL. Search and one will find: Zika virus everywhere. *Trans R Soc Trop Med Hyg* 2016;**110**:207–8.
10. World Health Organization. Zika virus, microcephaly and Guillain–Barré syndrome. Geneva: WHO; 2016, Available at: http://www.who.int/emergencies/zika-virus/situation-report/1-september-2016/en/ (accessed September 1, 2016)
11. Torres JR, Murillo J, Bofill L. The ever changing landscape of Zika virus infection. Learning on the fly. *Int J Infect Dis* 2016;**51**:123–6.
12. Simpson DI. Zika virus infection in man. *Trans R Soc Trop Med Hyg* 1964;**58**:335–8.
13. Duffy MR, Chen TH, Hancock WT, Powers AM, Kool JL, Lanciotti RS, et al. Zika virus outbreak on Yap Island. Federated States of Micronesia. *N Engl J Med* 2009;**360**:2536–43.
14. Ioos S, Mallet HP, Leparc Goffart I, Gauthier V, Cardoso T, Herida M. Current Zika virus epidemiology and recent epidemics. *Med Mal Infect* 2014;**44**:302–7.
15. Charrel RN, Leparc-Goffart I, Pas S, de Lamballerie X, Koopmans M, Reusken C. Background review for diagnostic test development for Zika virus infection. *Bull World Health Organ* 2016;**94**:574D–84D.
16. Waggoner JJ, Pinsky BA. Zika virus: diagnostics for an emerging pandemic threat. *J Clin Microbiol* 2016;**54**:860–7.
17. Zumla A, Goodfellow I, Kasolo F, Ntoumi F, Buchy P, Bates M, et al. Zika virus outbreak and the case for building effective and sustainable rapid diagnostics laboratory capacity globally. *Int J Infect Dis* 2016;**45**:92–4.
18. Fukunaga T, Rojanasuphot S, Pisuthipornkul S, Wungkorbkiat S, Thammanichanon A. Seroepidemiologic study of arbovirus infections in the north-east and south of Thailand. *Biken Journal* 1974;**17**:169–82.
19. Sekeyova M, Gresikova M. Haemagglutination-inhibiting antibodies to arboviruses in the human population in Thailand. *J Hyg Epidemiol Microbiol Immunol* 1969;**13**:288–92.
20. Smithburn KC. Neutralizing antibodies against arthropod-borne viruses in the sera of long-time residents of Malaya and Borneo. *Am J Hyg* 1954;**59**:157–63.
21. Wikan N, Suputtamongkol Y, Yoksan S, Smith DR, Auewarakul P. Immunological evidence of Zika virus transmission in Thailand. *Asian Pac J Trop Med* 2016;**9**:141–4.
22. Lanciotti RS, Kosoy OL, Laven JJ, Velez JO, Lambert AJ, Johnson AJ, et al. Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. *Emerg Infect Dis* 2008;**14**:1232–9.
23. Roehrig JT, Hombach J, Barrett AD. Guidelines for plaque-reduction neutralization testing of human antibodies to dengue viruses. *Viral Immunol* 2008;**21**:123–32.
24. Morens DM, Burke DS, Halstead SB. The wages of original antigenic sin. *Emerg Infect Dis* 2010;**16**:1023–4.
25. Huy R, Buchy P, Conan A, Ngan C, Ong S, Ali R, et al. National dengue surveillance in Cambodia 1980-2008: epidemiological and virological trends and the impact of vector control. *Bull World Health Organ* 2010;**88**:650–7.
26. Vong S, Goyet S, Ly S, Ngan C, Huy R, Duong V, et al. Under-recognition and reporting of dengue in Cambodia: a capture–recapture analysis of the National Dengue Surveillance System. *Epidemiol Infect* 2012;**140**:491–9.
27. Pond WL. Arthropod-borne virus antibodies in sera from residents of South-East Asia. *Trans R Soc Trop Med Hyg* 1963;**57**:364–71.
28. Musso D, Gubler DJ. Zika virus. *Clin Microbiol Rev* 2016;**29**:487–524.
29. Duong V, Andries AC, Ngan C, Sok T, Richner B, Asgari-Jirhandeh N, et al. Reemergence of chikungunya virus in Cambodia. *Emerg Infect Dis* 2012;**18**:2066–9.
30. Duong V, Sorn S, Holl D, Rani M, Deubel V, Buchy P. Evidence of Japanese encephalitis virus infections in swine populations in 8 provinces of Cambodia: implications for national Japanese encephalitis vaccination policy. *Acta Trop* 2011;**120**:146–50.
31. Touch S, Hills S, Sokhal B, Samnang C, Sovann L, Khieu V, et al. Epidemiology and burden of disease from Japanese encephalitis in Cambodia: results from two years of sentinel surveillance. *Trop Med Int Health* 2009;**14**:1365–73.
32. Heang V, Yasuda CY, Sovann L, Haddow AD, Travassos da Rosa AP, Tesh RB, Kasper MR. Zika virus infection, Cambodia, 2010. *Emerg Infect Dis* 2012;**18**:349–51.
33. Haddow AD, Schuh AJ, Yasuda CY, Kasper MR, Heang V, Huy R, et al. Genetic characterization of Zika virus strains: geographic expansion of the Asian lineage. *PLoS Negl Trop Dis* 2012;**6**:e1477.
34. Duong V, Ong S, Leang R, Huy R, Ly S, Mounier U, et al. Low circulation of Zika virus, Cambodia, 2007-2016. *Emerg Infect Dis* 2017;**23**. http://dx.doi.org/10.3201/eid2302.161432
35. Zhang J, Jin X, Zhu Z, Huang L, Liang S, Xu Y, et al. Early detection of Zika virus infection among travellers from areas of ongoing transmission in China. *J Travel Med* 2016;**23**. http://dx.doi.org/10.1093/jtm/taw047
36. Liao Y, Zeng ZL, Hu GL, Yang JP, Yuan H, Li JH, et al. Survey of the first case of Zika virus disease in the mainland of China. *Zhonghua Liu Xing Bing Xue Za Zhi* 2016;**37**:593–6.
37. Zheng CG, Xu Y, Jiang HQ, Yin YX, Zhang JH, Zhu WJ, et al. [Clinical analysis of two cases of imported children Zika virus infection in China]. *Zhonghua Er Ke Za Zhi* 2016;**54**:375–8.
38. Zhong YB, Liu XQ, Deng YC, Xu PH, Zhong GR, Zhang W. First case of laboratory-confirmed Zika virus infection imported into China. *Chin Med J (Engl)* 2016;**129**:2013–4.
39. Deng YQ, Zhao H, Li XF, Zhang NN, Liu ZY, Jiang T, et al. Isolation, identification and genomic characterization of the Asian lineage Zika virus imported to China. *Sci China Life Sci* 2016;**59**:428–30.
40. PRO/MBDS. Zika virus - China: Hong Kong, 1st case confirmed; 2016, Archive Number: 20160826.4442616 (Accessed September 01, 2016).
41. Smithburn KC, Kerr JA, Gatne PB. Neutralizing antibodies against certain viruses in the sera of residents of India. *J Immunol* 1954;**72**:248–57.
42. Olson JG, Ksiazek TG, Gubler DJ, Lubis SI, Simanjuntak G, Lee VH, et al. A survey for arboviral antibodies in sera of humans and animals in Lombok, Republic of Indonesia. *Ann Trop Med Parasitol* 1983;**77**:131–7.
43. Kwong JC, Druce JD, Leder K. Zika virus infection acquired during brief travel to Indonesia. *Am J Trop Med Hyg* 2013;**89**:516–7.
44. Leung GH, Baird RW, Druce J, Anstey NM. Zika virus infection in Australia following a monkey bite in Indonesia. *Southeast Asian J Trop Med Public Health* 2015;**46**:460–4.
45. Perkasa A, Yudhaputri F, Haryanto S, Hayati RF, Ma'roef CN, Antonjaya U, et al. Isolation of Zika virus from febrile patient. *Indonesia Emerg Infect Dis* 2016;**22**:924–5.
46. Kutsuna S, Kato Y, Takasaki T, Moi M, Kotaki A, Uemura H, et al. Two cases of Zika fever imported from French Polynesia to Japan, December 2013 to January 2014 [corrected]. *Euro Surveill* 2014;**19**. http://dx.doi.org/10.2807/1560-7917.ES2014.19.4.20683
47. Shinohara K, Kutsuna S, Takasaki T, Moi ML, Ikeda M, Kotaki A, et al. Zika fever imported from Thailand to Japan, and diagnosed by PCR in the urines. *J Travel Med* 2016;**23**. http://dx.doi.org/10.1093/jtm/tav011. pii: tav011.
48. ProMED. Zika virus (13): Americas, Asia, Europe, Pacific. ProMED-mail; 2016, Available at: http://www.promedmail.org/post/4086075 (accessed August 26, 2016)
49. ProMED. Zika virus (11): Americas, Europe, Asia. ProMED-mail; 2016, Available at: http://www.promedmail.org/post/4059896 (accessed August 26, 2016)
50. Wolfe ND, Kilbourn AM, Karesh WB, Rahman HA, Bosi EJ, Cropp BC, et al. Sylvatic transmission of arboviruses among Bornean orangutans. *Am J Trop Med Hyg* 2001;**64**:310–6.
51. Tappe D, Nachtigall S, Kapaun A, Schnitzler P, Gunther S, Schmidt-Chanasit J. Acute Zika virus infection after travel to Malaysian Borneo, September 2014. *Emerg Infect Dis* 2015;**21**:911–3.
52. Anonymous. Malaysia confirms first case of Zika infection. Channel NewsAsia; 2016.
53. World Health Organization. Zika virus infection—Maldives. Geneva: WHO; 2016, Available at: http://www.who.int/csr/don/8-february-2016-zika-maldives/en/ (accessed August 25, 2016)
54. Darwish MA, Hoogstraal H, Roberts TJ, Ahmed IP, Omar F. A sero-epidemiological survey for certain arboviruses (*Togaviridae*) in Pakistan. *Trans R Soc Trop Med Hyg* 1983;**77**:442–5.
55. Hammon WM, Schrack Jr WD, Sather GE. Serological survey for arthropod-borne virus infections in the Philippines. *Am J Trop Med Hyg* 1958;**7**:323–8.
56. Ellison DW, Ladner JT, Buathong R, Alera MT, Wiley MR, Hermann L, et al. Complete genome sequences of Zika virus strains isolated from the blood of patients in Thailand in 2014 and the Philippines in 2012. *Genome Announc* 2016;**4**. http://dx.doi.org/10.1128/genomeA.00359-16
57. Alera MT, Hermann L, Tac-An IA, Klungthong C, Rutvisuttinunt W, Manasatienkij W, et al. Zika virus infection, Philippines, 2012. *Emerg Infect Dis* 2015;**21**:722–4.
58. ProMED. PRO/EDR > Zika virus (13): Americas, Asia, Europe, Pacific. ProMED-mail; 2016, Available at: http://www.promedmail.org/post/4086075 (accessed August 31, 2016)
59. ProMED. Zika virus (46): Americas, Asia, Pacific, Europe. ProMED-mail; 2016, Available at: http://www.promedmail.org/post/20160905.4467034 (accessed August 31, 2016).
60. Ministry of Health. First case of Zika virus infection in Singapore. Singapore: Ministry of Health and National Environment Agency; 2016.
61. ProMED. Zika virus—Singapore: Alert, travel advisory. ProMED-mail; 2016, Available at: http://www.promedmail.org/ (accessed September 1, 2016)
62. Ministry of Health. First case of locally transmitted Zika virus infection. Singapore: Ministry of Health and National Environment Agency; 2016.
63. Ministry of Health. Localised community spread of Zika virus infection with more cases confirmed. Singapore: Ministry of Health and National Environment Agency; 2016.
64. ProMED. PRO/EDR > Zika virus (41): Americas, Asia, Europe. ProMED-mail; 2016, Available at: http://www.promedmail.org/post/4412646 (accessed August 31, 2016)
65. ProMED. Zika virus (16): Americas, Asia, Pacific, Atlantic. ProMED-mail; 2016, Available at: http://www.promedmail.org/post/4118019 (accessed September 1, 2016)
66. Jang HC, Park WB, Kim UJ, Chun JY, Choi SJ, Choe PG, et al. First imported case of Zika virus infection into Korea. *J Korean Med Sci* 2016;**31**:1173–7.
67. ProMED. Zika virus (26): Americas, Asia, Europe, Indian Ocean. ProMED-mail; 2016, Available at: http://www.promedmail.org/post/4202525 (accessed August 31, 2016)
68. ProMED. Zika virus (30): Americas, Asia, Atlantic, Europe. ProMED-mail; 2016, Available at: http://www.promedmail.org/post/4292221 (accessed August 31, 2016)
69. ProMED. Zika virus (27): Americas, Asia, Europe. ProMED-mail; 2016, Available at: http://www.promedmail.org/post/4214303 (accessed August 31, 2016)
70. Anonymous S. Korea confirms 9th Zika infection. yonhapnews; 2016.
71. Anonymous S. Korea confirms 11th Zika virus infection. yonhapnews; 2016.

72. ProMED. Zika virus (34): Americas, Asia, Africa, Europe. ProMED-mail; 2016, Available at: http://www.promedmail.org/post/4331999 (accessed September 1, 2016)
73. ProMED. Zika virus (35): Americas, Asia, Europe. ProMED-mail; 2016, Available at: http://www.promedmail.org/post/4346031 (accessed September 1, 2016)
74. Anonymous. Taiwan confirms sixth imported Zika case in Taiwanese traveler who visited Miami, Florida. Taiwan: Centers for Disease Control; 2016.
75. ProMED. Zika virus (43): Americas, Europe: Tampa Florida area, research. ProMED-mail; 2016, Available at: http://www.promedmail.org/post/4436991 (accessed August 31, 2016)
76. Fonseca K, Meatherall B, Zarra D, Drebot M, MacDonald J, Pabbaraju K, et al. First case of Zika virus infection in a returning Canadian traveler. *Am J Trop Med Hyg* 2014;**91**:1035–8.
77. Tappe D, Rissland J, Gabriel M, Emmerich P, Gunther S, Held G, et al. First case of laboratory-confirmed Zika virus infection imported into Europe, November 2013. *Euro Surveill* 2014;**19**. pii: 20685.
78. Shinohara K. Zika virus—Japan ex Thailand. ProMED-mail; 2014, Available at: http://www.promedmail.org/post/2716731 (accessed August 25, 2016)
79. Venturi G, Zammarchi L, Fortuna C, Remoli ME, Benedetti E, Fiorentini C, et al. An autochthonous case of Zika due to possible sexual transmission, Florence, Italy, 2014. *Euro Surveill* 2016;**21**. http://dx.doi.org/10.2807/1560-7917.ES.2016.21.8.30148
80. Huang AS, Shu PY, Yang CH. A new reportable disease is born: Taiwan Centers for Disease Control's response to emerging Zika virus infection. *J Formos Med Assoc* 2016;**115**:223–5.
81. ProMED. Zika virus (33): Americas, Asia, Europe. ProMED-mail; 2016, Available at: http://www.promedmail.org/post/4321150 (accessed September 1, 2016)
82. Buathong R, Hermann L, Thaisomboonsuk B, Rutvisuttinunt W, Klungthong C, Chinnawirotpisan P, et al. Detection of Zika virus infection in Thailand, 2012-2014. *Am J Trop Med Hyg* 2015;**93**:380–3.
83. Meltzer E, Lustig Y, Leshem E, Levy R, Gottesman G, Weissmann R, et al. Zika virus disease in traveler returning from Vietnam to Israel. *Emerg Infect Dis* 2016;**22**:1521–2.
84. Musso D, Nilles EJ, Cao-Lormeau VM. Rapid spread of emerging Zika virus in the Pacific area. *Clin Microbiol Infect Dis* 2014;**20**:O595–6.
85. Wikan N, Smith DR. Zika virus: history of a newly emerging arbovirus. *Lancet Infect Dis* 2016;**16**:e119–26.
86. Strafela P, Vizjak A, Mraz J, Mlakar J, Pizem J, Tul N, et al. Zika virus-associated micrencephaly: a thorough description of neuropathologic findings in the fetal central nervous system. *Arch Pathol Lab Med* 2016. http://dx.doi.org/10.5858/arpa.2016-0341-SA
87. Mlakar J, Korva M, Tul N, Popovic M, Poljsak-Prijatelj M, Mraz J, et al. Zika virus associated with microcephaly. *N Engl J Med* 2016;**374**:951–8.
88. Melo AS, Aguiar RS, Amorim MM, Arruda MB, Melo FO, Ribeiro ST, et al. Congenital Zika virus infection: beyond neonatal microcephaly. *JAMA Neurol* 2016;**73**:1407–16.
89. Calvet G, Aguiar RS, Melo AS, Sampaio SA, de Filippis I, Fabri A, et al. Detection and sequencing of Zika virus from amniotic fluid of fetuses with microcephaly in Brazil: a case study. *Lancet Infect Dis* 2016;**16**:653–60.
90. Pettersson JH, Eldholm V, Seligman SJ, Lundkvist A, Falconar AK, Gaunt MW, et al. How did Zika virus emerge in the Pacific Islands and Latin America? *mBio* 2016;**7**. http://dx.doi.org/10.1128/mBio.01239-16
91. ProMED. PRO/MBDS > Zika virus—Thailand (08). ProMed Mail; 2016, Available at: http://www.promedmail.org (accessed October 24, 2016)
92. ProMED. PRO/MBDS > Zika virus—Viet Nam (04). ProMed Mail; 2016, Available at: http://www.promedmail.org (accessed October 24, 2016)
93. Vorou R. Zika virus, vectors, reservoirs, amplifying hosts, and their potential to spread worldwide: what we know and what we should investigate urgently. *Int J Infect Dis* 2016;**48**:85–90.
94. Dejnirattisai W, Supasa P, Wongwiwat W, Rouvinski A, Barba-Spaeth G, Duangchinda T, et al. Dengue virus sero-cross-reactivity drives antibody-dependent enhancement of infection with Zika virus. *Nat Immunol* 2016;**17**:1102–8.
95. Barba-Spaeth G, Dejnirattisai W, Rouvinski A, Vaney MC, Medits I, Sharma A, et al. Structural basis of potent Zika–dengue virus antibody cross-neutralization. *Nature* 2016;**536**:48–53.
96. Bogoch II, Brady OJ, Kraemer MU, German M, Creatore MI, Brent S, et al. Potential for Zika virus introduction and transmission in resource-limited countries in Africa and the Asia-Pacific region: a modelling study. *Lancet Infect Dis* 2016;**16**:1237–45.
97. Fajardo A, Sonora M, Moreno P, Moratorio G, Cristina J. Bayesian coalescent inference reveals high evolutionary rates and diversification of Zika virus populations. *J Med Virol* 2016;**88**:1672–6.
98. Faria NR, Azevedo Rdo S, Kraemer MU, Souza R, Cunha MS, Hill SC, et al. Zika virus in the Americas: early epidemiological and genetic findings. *Science* 2016;**352**:345–9.
99. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol Biol Evol* 2013;**30**:2725–9.