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Zika Virus and its Association with Microcephaly

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Abstract: Zika virus, already widely distributed in Africa and Asia, was recently reported in America. This finding adds a potentially noxious virus to a list of several other viruses that are widely transmitted by vectors. Therefore, this seminar paper is aimed at giving an overview on Zika virus and its association with Microcephaly. Zika virus is a member of the Flaviviridae family that is spread by mosquitoes; in rare cases, the virus may be spread via blood transfusions, sexual contact and from mother to child in the womb. Most people who get Zika will not have any symptoms. Those who do get symptoms will generally have a fever, rash, joint pain, conjunctivitis (Red eyes) and, occasionally, muscle pain and a headache. Information regarding pathogenesis is scarce. Diagnostic tests for Zika virus infection include conventional or real-time RT-PCR and ELISA or immunofluorescence. There is no specific treatment or vaccine for Zika virus infection. Microcephaly is a condition where a baby's head is much smaller than expected. It can occur because a baby's brain has not developed properly. There have been reports of a serious birth defects including microcephaly, linked to Zika. Most of the mothers whose infants were diagnosed with microcephaly complained during their pregnancies symptoms of Zika virus infection. In conclusion until more is known, pregnant women are recommended to consider postponing travel to areas where Zika is being actively transmitted.

Key words: Congenital · Flaviviridae · Microcephaly · Mosquito · Zika

INTRODUCTION

Zika virus (ZIKV) is a mosquito borne *flavivirus,* related to yellow fever virus, dengue virus (DENV) and West Nile virus (WNV). It is a single-stranded positive RNA virus that is closely related to the Spondweni virus and is transmitted by many Aedes spp. mosquitoes, including *A. africanus*, *A. luteocephalus*, *A. hensilli* and *A. aegypti*. The virus was identified in rhesus monkeys during sylvatic yellow fever surveillance in the Zika Forest in Uganda in 1947 and was reported in humans in 1952 [1].

In 2007, an outbreak of ZIKV occurred on Yap Island in the Federated States of Micronesia. This was the first time that ZIKV was reported out with Africa and Asia. In October 2013, a large outbreak occurred in French Polynesia, followed by other outbreaks in the Pacific islands of New Caledonia, Cook and Easter occurred. In 2015, cases of ZIKV were reported in several Caribbean Islands and Brazil, progressing to an outbreak that spread to the Americas. The outbreak has continued in 2016, with ZIKV reported in an increasing number of countries [2]. The spread of ZIKV infections in the Americas and in the Caribbean constitutes a significant development in the epidemiology of this emerging vector borne disease [3].

An estimated 80% of persons infected with ZIKV are asymptomatic. Symptomatic disease is generally mild and characterized by acute onset of fever, maculopapular rash, arthralgia, or non-purulent conjunctivitis. Symptoms usually last from several days to one week. Severe disease requiring hospitalization is uncommon and fatalities are rare. Guillain Barre Syndrome has been reported in patients following suspected ZIKV infection. Pregnant women can be infected with ZIKV in any trimester. The incidence of ZIKV infection in pregnant women is not currently known and data on pregnant women infected with ZIKV are limited. No evidence exists to suggest that pregnant women are more susceptible to ZIKV infection or experience more severe disease during pregnancy [4].

The recent rise in microcephaly incidences in several northeastern states, with 1,248 cases reported in 2015 up through November 30th has been strongly suspected of

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being associated with ZIKV, with the virus being found in the amniotic fluid of two pregnant women whose fetuses presented a reduction in the circumference of the head [5]. This serious effect of ZIKV infection on fetuses, not previously reported, is unsurprising considering the perinatal transmission reported for two women from French Polynesia [6] and the strong neuro tropism of the virus [7]. Brazilian health authorities have reported adverse pregnancy outcomes and/or congenital Central nervous system (CNS) malformations with laboratory confirmation of ZIKV in amniotic fluid, placenta or fetal tissues. The evidence regarding a causal link between ZIKV infections during pregnancy and congenital CNS malformations is substantial [3].

Therefore, the objective of this paper is:

- To give an overview on Zika virus
- To highlight the association between Zika virus and Microcephaly.

Zika Virus

Etiology: Zika virus belongs to the Nondweni virus serogroup of mosquito borne viruses in the flavivirus genus. Phylogenies reveal the existence of two lineages: the African lineage which has showed no propensity to disseminate outside of Africa and the Asian lineage which continues to seed in previously unaffected regions of the world [8,9]. All strains having recently disseminated belong to the Asian lineage (With Cape Verde outbreak strain of unknown lineage). ZIKV genomes from patients infected in Surinam and Brazil in 2015 are closely related to the strain that circulated in French Polynesia in 2013, with more than 99.7% and 99.9% of nucleotide and amino acid identity, respectively [10].

Molecular characterization Virions of ZIKV are 40–60 nm in diameter, spherical in shape and contain a lipid envelope. Its Genome consists of a positive sense RNA of approximately 11 kb. The Virions consist of a single capsid (C) and two membrane associated envelope proteins (M, E). The nonstructural proteins (NS1NS5) contain sequence motifs characteristic of a serine protease, RNA helicase and RdRp (NS5).Translation initiation of genomic RNA is cap dependent. Viral proteins are synthesized as part of a polyprotein that is co and post translationally cleaved by viral and cellular proteases. RNA synthesis occurs in the cytoplasm in association with modified cellular membranes via synthesis of full length negative strand intermediates. Virions assembly, including acquisition of the glycoprotein containing lipid envelope, occurs by budding through intracellular membranes. Viral particles are transported in cytoplasmic vesicles through the secretary pathway before they are released by exocytosis [11,12].

Epidemiology: Host ranges ZIKV is most likely maintained in a sylvatic cycle involving nonhuman primates and mosquitoes, with cyclic epizootics in monkeys reported in Uganda. In the sylvatic transmission cycle, humans likely serve as incidental hosts. However, in areas without nonhuman primates, humans probably serve as primary amplification hosts and potentially as reservoir hosts if their viremia is sufficient in duration and magnitude. Although it is thought that enzootic ZIKV is maintained primarily in a monkey/mosquito transmission cycle, antibodies have been detected in numerous other animal species including water buffalo, elephants, goats, hippos, impala, kongoni, lions, sheep, rodents, wildebeest and zebras [13].

Geographic distribution ZIKV was first identified in Uganda in 1947. Since 2007, an increasing number of ZIKV infection outbreaks have occurred across multiple regions, including Southeast Asia, Polynesia and other Pacific regions, certain Caribbean islands. Locally acquired transmission has also been reported by Cape Verde [14]. Whereas, after October 2013, French Polynesia has experienced the largest outbreak of ZIKV infection ever reported, with an estimate of 28,000 ZIKV infections in early February 2014 (About 11% of the population) [15] However, it is likely that ZIKV had been circulating in Salvador de Bahia City prior to that, as an outbreak of exanthematous illness was reported there between 15 February and 25 June 2015. As of 19 January 2016, autochthonous cases of Zika virus infection were reported from 23 countries or territories worldwide within the past two months: Barbados, Bolivia, Brazil, Cape Verde, Colombia, Ecuador, El Salvador, French Guiana, Guadeloupe, Guatemala, Guyana, Haití, Honduras, Martinica, México, Panamá, Paraguay, Puerto Rico, Saint Martin, Samoa, Suriname, Thailand and Venezuela. The epidemic is still evolving in the Americas [3].

From 2007 to 5 February 2016, Zika viral transmission has been documented in a total of 44 countries and territories. This includes 33 countries that reported transmission in between 2015 and 2016, 6 countries with indirect evidence of transmission and 5 countries with a history of Zika transmission but no current reported transmission [10].

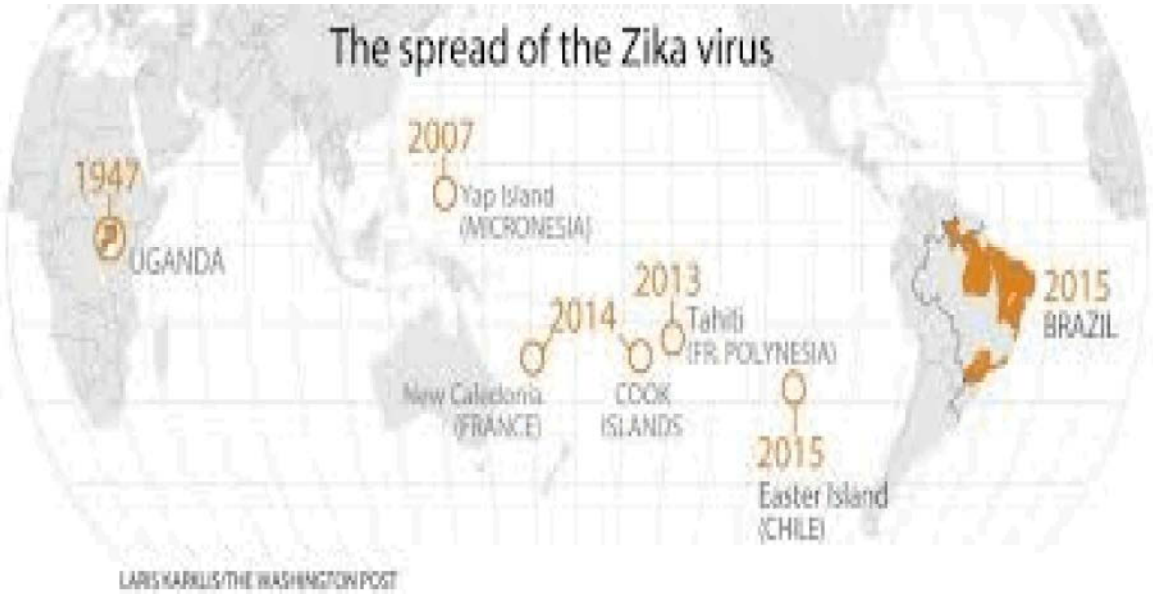

Fig. 1: Historical Timeline Map (1947 – 2015).
Source: WHO [10]

The first autochthonous cases of ZIKV in Brazil were confirmed in May, 2015 [16]. Since then, as of January 6, 2015, 21 states have confirmed virus circulation, with a higher prevalence in the Northeast Region [17]. Reports of microcephaly incidence in Brazil Geographically overlap with ZIKV reports; Most of the mothers whose infants were diagnosed with microcephaly complained during their pregnancies of clinical manifestations, such as low grade fever, headache and cutaneous rashes that might have been symptoms of ZIKV infection or infection with any other arbovirus species that is prevalent in the region [18].

Mode of transmission the virus is transmitted to people primarily through the bite of an infected Aedes species mosquito (*A. aegypti* and *A. albopictus*). These are the same mosquitoes that spread dengue and chikungunya viruses. These mosquitoes typically lay eggs in and near standing water in things like buckets, bowls, animal dishes, flower pots and vases. They prefer to bite people day time and live indoors and outdoors near people. Mosquitoes that spread chikungunya, dengue and Zika are aggressive daytime biters. They can also bite at night. Mosquitoes become infected when they feed on a person already infected with the virus. Infected mosquitoes can then spread the virus to other people through bites [4].

Perinatal transmission can occur most probably by trans placental transmission or during delivery when the mother is infected [6,19]. ZIKV has been isolated from semen collected 14 days post start of symptoms while detection of the virus genomes was described in semen 28 days post onset of symptoms. Sexual transmission was indicated in three case reports. There is also a potential risk of transfusion derived transmission and Brazilian authorities announced the first cases of blood transfusion mediated transmission on 5 February, 2016 [20].

Morbidity The 2007 Zika outbreak on Yap Island in the Federated States of Micronesia is so far the only well documented and investigated large outbreak. A total of 185 cases of suspected Zika disease were identified. Of the suspected cases, 49 (26%) were classified as laboratory confirmed and 59 as probable cases. These cases had onset between early April and mid July 2007 (13 consecutive weeks), the median age was 36 years and 61% were female. Clinical characteristics were documented for 31 confirmed cases and included: macular or popular rash (Frequency 90%, median duration of 6 days ranging from 2 to 14 days), fever either subjective or measured (Frequency 65%), arthritis or arthralgia (Frequency 65%, median of duration of 3.5 days ranging 1 to 14 days), nonpurulent conjunctivitis (Frequency 55%), myalgia and headache (both around 45% frequency) and retro orbital pain (Frequency 39%) [15].

Between 7 October 2013 and 6 April 2014, 28 750 suspected cases of ZIKV infection were reported by the syndromic surveillance sentinel network of French Polynesia, with 383 confirmed cases and an estimated 32 000 cases having consulted a healthcare facility for the

condition. During the outbreak, 74 individuals presented with neurological symptoms or autoimmune syndrome following a disease episode with symptoms consistent with ZIKV infection in previous days. Of these, 42 were confirmed as Guillain Barre Syndrome, with 37 cases having presented with a previous viral syndrome. The causal link between ZIKV infection and Guillain Barre Syndrome is still not established [21].

Pathogenesis Information regarding pathogenesis of ZIKV is scarce but mosquito borne *flaviviruses* are thought to replicate initially in dendritic cells near the site of inoculation then spread to lymph nodes and the blood stream [22]. Although flavivirus replication is thought to occur in cellular cytoplasm, study suggested that ZIKV antigens could be found in infected cell nuclei [23]. The reproductive cycle of ZIKV follows that of other known *flaviviruses*. First, the virion attaches to the host cell membrane receptors via the envelope protein which induces virion endocytosis. Next, the virus membrane fuses with the endosomal membrane and the ssRNA genome of the virus is released into the cytoplasm of the host cell. It is then translated into a polyprotein that is subsequently cleaved to form all structural and nonstructural proteins. Replication then takes place at intracellular compartments known as cytoplasmic viral factories in the endoplasmic reticulum resulting in a dsRNA genome. The dsRNA genome is then transcribed resulting in additional ssRNA genomes. Assembly then occurs within the endoplasmic reticulum and the new virions are transported to the Golgi apparatus and then excreted into the intracellular space where the new virions can infect new host cells [24].

Clinical Signs The incubation period of ZIKV appears to be 3 to 12 days for most people. About 80% of the population who are exposed to ZIKV will not get sick. If they do get sick, symptomatic disease is generally mild and characterized by acute onset of fever, maculopapular rash, arthralgia, or nonpurulent conjunctivitis. Symptoms usually last from several days to one week. Severe disease requiring hospitalization is uncommon and fatalities are rare. Guillain Barre Syndrome has been reported in patients following suspected Zika virus infection [4].

Diagnosis: Zika virus infections are sometimes hard to diagnose because the disease has symptoms similar to other arboviral diseases and few laboratories have molecular tests for ZIKV. A combination of assessments is used to determine presence of ZIKV associated disease. Outside areas of active transmission, preliminary diagnosis includes obtaining travel history [25]. There are no commercial serological assays available for detection of Zika specific antibodies [26].

The laboratory assessment of infection status involves detection of ZIKV nucleic acid in serum (By reverse transcriptase polymerase chain reaction) during the initial onset of symptoms. At the end of the first week with symptoms, IgM antibody against ZIKV (Detected by ELISA) and ZIKV plaque reduction neutralization test can be used to verify exposure to ZIKV [25].

Cross reactivity was more frequently noted with dengue virus than with yellow fever, Japanese encephalitis, Murray Valley encephalitis, or West Nile viruses, but there were too few samples tested to derive robust estimates of the sensitivity and specificity of the ELISA. IgM was detectable as early as 3 days after onset of illness in some persons; One person with evidence of previous *flavivirus* infection had not developed IgM at day 5 but did have it by day 8. Neutralizing antibody developed as early as 5 days after illness onset. The plaque reduction neutralization assay generally has improved specificity over immunoassays, but may still yield cross reactive results in secondary flavivirus infections. PCR tests can be conducted on samples obtained less than 10 days after illness onset; One patient from Yap Island still had detectable viral RNA on day 11 [26].

In case of pregnant women RT PCR testing can be performed on amniotic fluid. Currently, it is unknown how sensitive or specific this test is for congenital infection. Also, it is unknown if a positive result is predictive of a subsequent fetal abnormality and if so, what proportion of infants born after infection will have abnormalities. Amniocentesis is associated with an overall 0.1% risk of pregnancy loss when performed at less than 24 weeks of gestation. Amniocentesis performed =15 weeks of gestation is associated with lower rates of complications than those performed at earlier gestational ages and early amniocentesis = 14 weeks of gestation) is not recommended. Health care providers should discuss the risks and benefits of amniocentesis with their patients. A positive RT PCR result on amniotic fluid would be suggestive of intrauterine infection and potentially useful to pregnant women and their health care providers [4].

In general, diagnostic testing for *flavivirus* infections should include an acute phase serum sample collected as early as possible after onset of illness and a second sample collected 2 to 3 weeks after the first. However, in some patients with a probable previous history of flavivirus infection, a fourfold increase of neutralising antibodies to other flaviviruses has been observed [26].

Prevention and Control No specific antiviral treatment is available for Zika virus disease. Treatment is generally supportive and can include rest, fluids and use of analgesics and antipyretics. Fever should be treated with acetaminophen. Although aspirin and other nonsteroidal anti-inflammatory drugs are not typically used in pregnancy, these medications should specifically be avoided until dengue can be ruled out to reduce the risk for hemorrhage [4].

No vaccines for protection against Zika are currently available, so protecting against mosquito bites is the primary method of prevention. Protection against mosquito bites involves personal protection and mosquito population reduction. Personal protection against mosquito bites may be achieved through the use of mosquito repellents, protective clothing and avoiding areas where mosquitoes are abundant. The CDC provides a list of mosquito repellents with Environmental Protection Agency registered active ingredients, such as DEET, picaridin and oil of lemon eucalyptus that provide long lasting protection [27].

The mosquito species that carry ZIKV outside of Africa thrive in urban and suburban areas. Their larvae develop in water that collects in natural and manmade containers such as birdbaths, vases, animal water dishes, flower pots, discarded cans and old paint buckets, or other neglected/discarded water holding objects. Regularly removing and/or emptying such water holding containers are effective ways of reducing the number of mosquitoes that might transmit ZIKV [28].

Blood supply during a Zika virus outbreak should ideally be maintained by increasing blood collections in non-affected areas. In non-affected areas, consideration may be given to defer donors who have recently visited areas with ongoing transmission of Zika virus for a period of 28 days after their departure from the area twice the assumed maximum incubation period. It is crucial that public health authorities work with the blood transfusion service (BTS) to establish mechanisms to access regular, up-to-date epidemiological information on Zika virus transmission in the country [10]. Given the possibility that the virus may be sexually transmitted, couples should take precautions against direct transmission of the virus from their partners [28].

ZIKA AND MICROCEPHALY Microcephaly is a condition where a baby is born with a small head or the head stops growing after birth. Microcephaly is usually a rare condition, with one baby in several thousand being born with the birth defect. If this combines with poor brain growth, babies with microcephaly can have developmental disabilities [10]. Microcephaly usually results from abnormal brain development. The long-term consequences of microcephaly depend on underlying brain anomalies and can range from mild developmental delays to severe motor and intellectual deficits, like cerebral palsy. In addition to congenital infections, microcephaly can result from chromosomal abnormalities; Exposure to drugs, alcohol, or other environmental toxins; Premature fusion of the bones of the skull (Craniosynostosis) and certain metabolic disorders [4].

One of the causes of microcephaly involves abnormal function of centrosomes. Although normally associated with mitosis, these organelles are also involved in other cellular processes including migration, polarity and proper trafficking of vesicles. In reference to microcephaly, amplification of centrosome number has been revealed to be one of the inducers of this condition. Certain proteins have a dual role in autophagy as well as centrosome stability. One particular example is ultraviolet (UV) irradiation resistance associated gene (UVRAG). It is involved in initiation and maturation of autophagosomes as well as centrosome and chromosome stability. Another is Beclin1, which plays an integral role in autophagy and known to contribute to chromosomal stability in cancer cells. In the context of neural brain development, an increase in centrosomes in mice results in a delay in mitosis, an increase in apoptosis, improper neural stem cell orientation, premature neuronal differentiation and the reduced brain size indicative of microcephaly [29].

The pathological properties of Zika were first described in 1952, when Dick et al, demonstrated viral tropism to the brain in intraperitoneally infected mice and an increase in viral titers 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 who observed the progression of disease in directly infected mouse brains. Based on their observations, the virus infected neurons and glia, producing a variety of intracytoplasmic inclusions, which they termed, “Virus factories.” These factories originated from the endoplasmic reticulum and associated with other organelles including the nucleus and the mitochondria. It is not yet certain whether maternal ZIKV infection is responsible for the increase in cases of fetal microcephaly in Brazil. However, supporting determined that the amniotic fluid samples of two pregnant women were positive for evidence is mounting [29].

Historical tracking and diagnosis of microcephaly in Brazil has created limitations for determining a causal relationship. The historical birth prevalence of

microcephaly is lower in Brazil than expected (0.5 cases/10,000 live births as opposed to 12 cases/10,000 live births), implying a generally low diagnosis rate for microcephaly. This historical under discovery could have increased case reporting for microcephaly, over representing the increase in microcephaly during the Zika outbreak. Reported histories of nonspecific rash illness during pregnancy for mothers of children with microcephaly is also subject to recall bias and cannot confirm a definitive association between the virus and outcome [30].

There is a Temporal Association: The increase in cases of microcephaly started within nine months of the outbreak of ZIKV in northern Brazil. It has been demonstrated that ZIKV can cross the placental barrier and the virus has been detected in blood and tissues of at least seven affected fetuses/infants; The mothers of six of these cases presented with symptoms consistent with ZIKV infection during pregnancy. The gestation at which the infection is acquired may be important; One study of 35 cases of microcephaly in Brazil found that 26 (74%) of the women reported having had a rash, 21 in the first trimester, 5 in the second trimester and none in the third trimester. Based on this information and on experience from other congenital infections such as, rubella and toxoplasmosis, it is likely that ZIKV infection in early pregnancy poses the greatest risk [10].

Although the role of Zika virus in causing microcephaly has not been determined, recent research indicates a correlation. Vertical Transmission of Zika from mother to child in utero was suggested when it was seizures. Early intervention services to promote the child's development and support the family is required as well as medical treatment for seizure control. Specialized services such as physical therapy and child neurology may be available in some of the bigger c ka RNA while the mother's' urine and serum samples were negative for the virus [21]. A Brazilian Minister of Health task force investigation found that all mothers in their study sample (n=35) had either lived in or visited Zika affected areas during pregnancy and 74% reported a febrile rash during pregnancy. Further studies, however, are needed to determine causal association between Zika virus infection and microcephaly in infants [30].

The sharp increase in microcephaly during the Zika outbreak falls outside the range for simple diagnosis errors, however and a rapid risk assessment determined that a causal association between microcephaly in newborns and Zika virus infection during pregnancy is plausible [21]. The World Health Organization also issued an epidemiological alert about the association of Zika virus and congenital malformations or neurological syndromes [31].

Between 22 October 2015 and 30 January 2016, Brazilian authorities received 4,783 notifications of microcephaly or central nervous system (CNS) anomalies. Investigation and classification of these cases are in progress. So far, 404 cases from 156 municipalities in nine Brazilian States have been confirmed to have microcephaly and/or CNS anomalies suggestive of congenital infections. Pernambuco, the first state to identify an increase of microcephaly, has reported the highest number of confirmed cases (153, 37.9%), followed by Bahia (99, 24.5%), Rio Grande do Norte (63, 15.6%), Paraíba (37, 9.2%), Piauí (27, 6.7%), Alagoas (15, 3.7%), Ceará (7, 1.7%), For seventeen (4.2%) of the 404 cases, an infection with Zika virus was confirmed by serology or PCR. Studies to examine a possible causal association between Zika virus infection during pregnancy and congenital CNS malformations are ongoing [4].

CONCLUSION

Human Zika virus infection appears to have changed in character while expanding its geographical range. The change is from an endemic, mosquito borne infection causing mild illness across equatorial Africa and Asia, to an infection causing, large outbreaks, linked with neurological disorders including Guillain Barre Syndrome and microcephaly across the Pacific region and the Americas. The future transmission of Zika infection is likely to coincide mainly with the distribution of Aedes Mosquito vectors, although there may be rare instances of person to person transmission (Other than mother to child, e.g. through semen). Beyond the range of mosquitos, infection has been and will continue to be, carried widely by international travel. The possible association between Zika virus and microcephaly is one of the most severe potential consequences of the virus and the increase in microcephaly in Zika affected areas creates an emergency call. Based on the above conclusion the following recommendations are forwarded:

- Risk communications should be enhanced in countries with Zika virus transmission to address population concerns, enhance community engagement, improve reporting and ensure application of vector control and personal protective measures.

- In areas of known Zika virus transmission health services should be prepared for potential increases in neurological syndromes and/or congenital malformations.
- Research and development efforts should be intensified for Zika virus vaccines, therapeutics and diagnostics.
- The potential association of ZIKV with microcephaly should be investigated in detail.

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