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Review Article

Chikungunya: An emerging threat

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Introduction:

Chikungunya fever is a mosquito-borne illness of humans caused by the chikungunya virus (CHIKV) that belongs to the *Alphavirus* genus of the family T*ogaviridae. Aedes aegypti* and *Aedes albopictus* mosquitoes are the main vectors of chikungunya in Asia and the Indian Ocean islands. The disease typically consists of an acute illness characterized by fever, rash, and incapacitating arthralgia.[1] The name "Chikungunya" derives from a word in the Kimakonde language, meaning "to become contorted", and describes the stooped appearance of sufferers with joint pain (arthralgia). The disease was first described during an outbreak in southern Tanzania in 1952.[2]

Chikungunya virus was first isolated by Ross in 1953 from the serum of a febrile human during an epidemic in Newala district of Tanzania.[3] The virus is believed to have originated in Africa, where it still circulates enzootically among nonhuman primates, and is transmitted by arboreal Aedes mosquitoes.[4] These cycles lead to regular outbreaks of infection in Africa, but most human cases result from CHIKV emergence into a human-mosquito cycle in urban areas of Africa, followed sometimes by spread beyond Africa.[5] Since then outbreaks have been reported from other countries in Africa, Asia, Europe, and the Indian and Pacific Oceans.[6]

Chikungunya was subsequently introduced in Asia where it has been transmitted from human to human mainly by *Aedes aegypti* and, to a lesser extent by *Aedes albopictus* through an urban transmission cycle.[7] Since 1953, CHIK virus has caused numerous well-documented outbreaks and epidemics in Southeast Asia, involving hundreds of thousands of people.[5]

An Asian epidemic was reported in Bangkok, Thailand, in 1958, continued until 1964, and reappeared after a hiatus in the mid-1970s and declined again in 1976. Major outbreaks were also reported from northwestern and southern parts of India, Sri Lanka and Myanmar in the early 1960s. The cases then declined before sporadic outbreaks were later reported in the Philippines and Indonesia in 1980s and Malaysia in the 1990s.[1] A major outbreak occurred in 2001 on islands in the Indian Ocean (Mauritius, Mayotte, Madagascar, Reunion Island).[8]

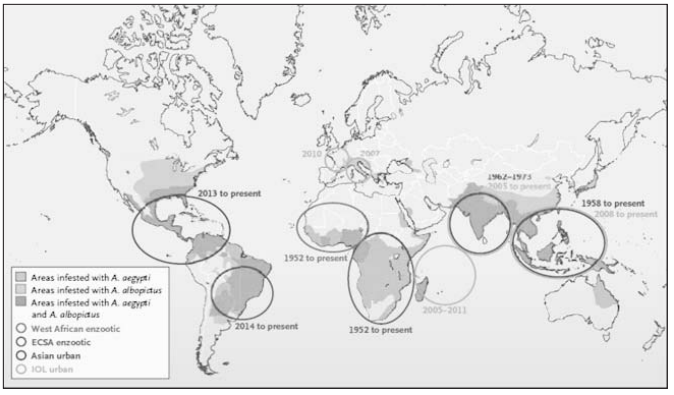

Fig-1: Origin, Spread, and Distribution of Chikungunya Virus and Its Vectors. The map shows the African origins of enzootic chikungunya virus strains and the patterns of emergence and spread of the Asian lineage and Indian Ocean lineage (IOL) of the virus during epidemics since the 1950s, based on phylogenetic studies. The distributions of the peridomestic vectors, *Aedes aegypti* and *A. albopictus*, are also shown. ECSA denotes eastern, central, and southern African.[9]

In 2004, CHIKV outbreak was reported from coastal Kenya and in 2005 in the Comoros, La Reunion, and other islands in the southwest Indian Ocean. Later, autochthonous transmission occurred in Italy, France and in America.[5]

The most severe chikungunya fever outbreak was reported in 2006 on Reunion Island, where one-third of the population was infected, resulting in 237 deaths. Around the

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same time, an historical outbreak on the Indian subcontinent involved 1.42 million people, with high morbidity rates.[10] By 2007, the disease was no longer considered a tropical illness, as it had spread to several nontropical and temperate areas, including Singapore.

In December 2013, the World Health Organization (WHO) reported the first local transmission of CHIKV on the Caribbean island of Saint Martin in the Western Hemisphere.[11] In late 2014, outbreaks were reported in the Pacific islands and small outbreaks of chikungunya in the city of Dakar, Senegal, and the state of Punjab, India in late 2015. In 2016, countries reporting most cases were Brazil, Bolivia and Colombia. In Argentina first autochthonous transmission of chikungunya was reported in 2016. In 2017, Pakistan continues to respond to an outbreak which started in 2016.[2] CHIKV has spread from the coast of Kenya throughout the Indian Ocean, Pacific, and Caribbean regions, causing millions of cases of disease in over 50 countries in less than 10 years. In other words, CHIKV has reemerged as a true global pathogen.[8]

Chikungunya infection: Bangladesh perspective

In Bangladesh, the first recognized outbreak of chikungunya was identified in Rajshahi and Chapai Nawabganj districts in 2008, the northwest area of the country where 32 cases were identified. Transmission appeared to be geographically limited to two villages bordering India in northwestern Bangladesh.[12] The second outbreak was in Shathiya upazilla of Pabna in 2009. The outbreak of chikungunya virus was detected in Dhaka, the capital and its suburbs Nababgonj, Dohar and Shibganj upazilla of Chapai Nawabganj in November 2011. According to Institute of Epidemiology Disease Control & Research (IEDCR), 250 samples were collected, among them the virus was identified in 46 persons, which was in fact the third outbreak.[13,14] Since then sporadic cases were reported from different parts of Bangladesh. Chikungunya fever were also diagnosed in a tertiary teaching hospital in Dhaka city in 2012.[15] Most of the cases of chikungunya remain undiagnosed or misdiagnosed due to the self-limiting nature of the disease, the prevalence of another arthropod-borne disease, dengue fever, lack of awareness and diagnostic facilities.[16]

In 2013, more than 600 blood samples were tested for chikungunya from various areas of Dhaka in IEDCR. Among the tested blood 33 percent people had recent infections and 3 percent had past infections, which confirmed its presence in the capital.[17] Chikungunya spread in the densely populated Dhaka, has raised a health alarm for the physicians.

The virus

The CHIKV is a small (about 60-70 nm-diameter), spherical, enveloped, and positive-strand RNA virus.[7] The genome approximately 11.5 kb in length that encodes four nonstructural proteins and three main structural proteins: the capsid and two envelope glycoproteins, E1 and E2, which form spikes on the virion surface. E2 binds to unknown cellular receptors to initiate cell entry through endocytosis, and E1 includes a fusion peptide, exposed at low pH in endosomes, which initiates the release of nucleocapsids into the host-cell cytoplasm.[9]

Transmission

Chikungunya virus is transmitted by bite of infected female culicine mosquitoes and can alternatively affect vertebrates and arthropods. The arthropods remain infected throughout its life. Its transmission to humans is mainly through Aedes species mosquitoes.[18] *Aedes aegypti* and *Aedes albopictus* are commonly involved in the transmission although Culex has also been reported for the transmission in some cases.[18]

The Aedes mosquito breeds in domestic settings such as flower vases containing water, water-storage containers, air coolers tray containing water and peri-domestic areas such as construction sites, coconut shells, discarded household junk items (tyres, plastic and metal cans, etc.) where water stagnate inside.[20] It bites throughout daylight hours, though there may be peaks of activity in the early morning and late afternoon. *Aedes aegypti* readily feed indoors but both species are found biting outdoors. In the rainy season when numbers of mosquitoes are at their greatest, the risk of transmission is highest. Chikungunya virus is transmitted rarely from mother to newborn around the time of birth.21 The bite of only the female mosquito is considered to be infective as blood meal is required for the formation of the egg. The same vectors can sometimes transmit several arboviruses, and mixed epidemics of chikungunya fever and dengue fever have been reported.[1,20] Human beings serve as the chikungunya virus reservoir during epidemic periods. During interepidemic periods, a number of vertebrates such as monkeys, rodents, birds and others have been implied as the reservoir.[22]

Pathogenesis

Following a mosquito (*Aedes aegypti* or *Aedes albopictus*) bite, CHIKV replicates in the skin, in fibroblasts, and disseminates to the liver, muscle, joints, lymphoid tissue (lymph nodes and spleen) and brain presumably through the blood.[23] Infected individuals experience an acute onset of disease 2 - 4 days after infection. Symptoms include high fever, rigors, headache and severe joint pain that is often

incapacitating. In addition, some of the infected individuals complain of a petechial or maculopapular rash.[24]

Disease onset coincides with rising viral titre, which triggers the activation of an innate immune response, the hallmark of which is the production of type I interferon (IFN).[25] Patients successfully clear the virus approximately 1 week after infection, and only at this time there is evidence of CHIKV-specific adaptive immunity. About 30% of individuals experience long-term sequellae that include arthralgia and, in some cases, arthritis.[26]

The cellular tropism of CHIKV in humans was characterized as in tissue culture experiments, the virus replicates in various human adherent cells, such as epithelial and endothelial primary cells and cell lines, fibroblasts and, to a lesser extent, monocyte-derived macrophages.[27] CHIKV also replicates in human muscle satellite cells, but not in differentiated myotubes.[28] In contrast to adherent cells, B cells and T cells are not susceptible to CHIKV infection in vitro. CHIKV is highly cytopathic in human cell cultures and infected cells rapidly undergo apoptotic cell death.[27]

The exact pathophysiology of Chikungunya virus remains to be investigated. A study from the Gabonese outbreak in 2007 concluded that Chikungunya virus infection elicited strong innate immunity with an abundant production of proinflammatory markers and cytokines, including high levels of alpha interferon, interleukin (IL-4, IL-10) and gamma interferon. They also demonstrated that humans show a CD8+ lymphocytic response in the early stage and a CD4+ predominant response in the later stage. In addition there is CD95-based apoptosis of CD4+ lymphocytes that partially explains the lymphopenia in patients.[29] Another study in murine model showed that anti-Chikungunya virus antibodies were elicited early in the course of the illness and were directed against the C-terminus of the viral E2 glycoprotein. Both natural and Chikungunya virus infection-induced specific antibodies were essential for controlling Chikungunya virus infections.[30]

The mechanism of entry of the virus into mammalian cells is under investigation.[31] The entry mechanism was evaluated and it was found that Chikungunya virus enters mammalian epithelial cells via a clathrin-independent, Esp-15-dependent, dynamin 2-dependent route and requires an endocytic pathway in combination with other unknown pathways.[32] Evaluation of the T-cell- and B-cell-mediated immunity has shed light on some possible mechanisms. Several murine models with Chikungunya virus-related joint and neurological diseases are being investigated.[33,34]

Clinical Manifestations

Chikungunya fever affects all age groups, and both genders are equally affected. In the acute stage, the onset is usually abrupt and sudden with high-grade fever (usually 102-105°F), severe arthralgias, myalgias, and skin rash.[1,35] Prodromal symptoms are rarely reported. Headache, throat discomfort, abdominal pain, and constipation may also be evident. Conjunctival suffusion, persistent conjunctivitis, cervical, or sometimes generalized lymphadenopathy may be present.[36]

The chikungunya viral polyarthropathy frequently involves the small joints of the hand, wrist, and ankles and the larger joints such as knee and shoulder.[1,36] The acute bilateral and symmetrical rheumatism is typically extensive and progressive within a few days. Peripheral joints are frequently very painful and swollen, especially interphalangeal joints, wrists, and ankles. A transient petechial or maculopapular rash of the trunk and occasionally the limbs can also develop in half of the patients.[37] Several mucocutaneous manifestations, such as morbilliform eruption, scaling, macular erythema, intertrigo, hypermelanosis, xerosis, excoriated papules, urticaria, and petechial spots have been described in patients with chikungunya fever.38 Ocular inflammation from chikungunya may present as iridocyclitis. Retinal lesions may also occur.[39]

In a majority of the patients, the joint pains resolve in 1 to 3 weeks. However, the arthritis can persist in about 33% of patients for 4 months, 15% for 20 months, and in 12% for 3-5 years.[1,40-42] The chronic stage is characterized by unpredictable relapses that include sensation of fever, asthenia, and exacerbation of arthralgias and stiffness. Affected patients may manifest inflammatory polyarthritis, severe subacute tenosynovitis/bursitis (consequently nerve tunnel syndromes) in hands, wrists, and exacerbation of pain on movement in previously injured joints.[37,43]

Chikungunya infection in children resembles that of adults, but can occasionally be complicated with neuropsychological changes, lethal meningoencephalitis, myocarditis or extensive epidermolysis.[44] In children, usually rheumatological manifestations are less frequent however sometimes it can be quite severe.[45] They are more likely to have rashes than adults have. Neonatal chikungunya virus infection become symptomatic after 4 days in one in two cases, with fever, pain, prostration, poor feeding, diffuse pain, distal joint edema, and miscellaneous skin alterations (petechiae, exanthema).[44]

Diagnosis

The diagnosis of CHIKV infection in acute phase is based commonly on clinical criteria such as fever and arthralgia.[46] However, as the clinical manifestations of chikungunya fever

resemble those of dengue and other fevers caused by arthropod-borne viruses, confirmation of chikungunya fever should be based on: isolation of the virus, molecular methods, detection of IgM antibody, and demonstration of a rising titer of the IgG antibody.[47]

Samples collected during the first week after the onset of symptoms should be tested by both serological and RT-PCR.[2] Serological diagnosis can be made by demonstration of fourfold rise in antibody titre in acute and convalescent sera or by demonstrating IgM antibodies specific for CHIKV.[48] IgM antibody levels are highest 3 to 5 weeks after the onset of illness and persist about 2 months. Genotyping of the virus can also be performed from RT-PCR products of clinical samples allowing comparisons with virus samples from various geographical sources.[2]

The most definitive test is virus isolation that can be done from the blood during the first few days of infection. The CHIKV produces cytopathic effects in a variety of cell lines including BHK-21, HeLa and Vero cells. Virus isolation should only be carried in BSL-3 laboratories.[47]

Treatment

There is no specific antiviral drug treatment for chikungunya. Treatment is directed primarily at relieving the symptoms.[2] Management includes consuming plenty of water with electrolytes and taking paracetamol tablets during periods of fever in persons with no preexisting liver or kidney disease. Taking adequate rest in a warm environment and giving cold compresses may help in reducing joint damage. Refraining from exertion may help, but mild forms of exercise and physiotherapy are recommended in recovering persons. Antihistamines can be used for itching. Medications with aspirin or NSAIDs should be avoided.[20] The person should be admitted in hospital if the person is hemo-dynamically unstable, having oliguria, altered sensorium, bleeding manifestations, persons not responding or having persistent joint pain or disabling arthritis even after three days of symptomatic treatment, persons above sixty years and infants (below one year of age), in pregnancy and high risk group.[49] Serious complications such as bleeding disorders, hypotension, myo-pericarditis or meningoencephalitis, hyperpigmentation and papular eruptions, arthralgia refractory to other drugs should be treated accordingly.[20]

Prevention and control

There is no commercial vaccine for chikungunya virus.[40] Thus controlling its spread can be done by reducing mosquitoes populations and limiting human contact with this vector.[20] The proximity of mosquito vector breeding sites to human habitation is a high risk factor for chikungunya. House-to-house interventions should be done to eliminate breeding places, and to encourage community participation.[7] Prevention and control relies heavily on reducing the number of natural and artificial water-filled container habitats that support breeding of the mosquitoes. During outbreaks, insecticides may be sprayed to kill flying mosquitoes, applied to surfaces in and around containers where the mosquitoes land, and to treat water in containers to kill the immature larvae.[2]

For protection of the patient as well as other members of the household, full sleeves clothing to cover extremities, preferably bright colored clothes is advised which minimizes skin exposure to the day-biting vectors.[20] Repellents can be applied to exposed skin or to clothing.[2] Insecticide-treated mosquito nets afford good protection for patients and those who sleep during the daytime, particularly young children.[20] Mosquito coils or other insecticide vaporizers may also reduce indoor biting. Basic precautions should be taken by people travelling to risk areas and these include use of repellents, wearing long sleeves and pants and ensuring rooms are fitted with screens to prevent mosquitoes from entering.[2]

Conclusion

In recent years there have been explosive outbreaks of chikungunya fever in several parts of the SEARegion and elsewhere. CHIKV has reemerged as a major threat to global public health.[8] It appears that chikungunya fever is not uncommon in Bangladesh as evidenced by three outbreaks and reports of several sporadic cases. Unfortunately, specific treatments or vaccines against CHIKV infection are not yet available. Vector controlling is the only way for prevention and it would require a planned approach, besides knowledge and awareness of early warning signs. Integrated vector management through the elimination of breeding sites, use of anti-adult and anti-larval measures and personal protection will contribute to preventing an outbreak.

REFERENCES:

1. Pialoux G, Gauzere BA, Jaureguiberry S and Strobel M. Chikungunya, an epidemic arbovirus. Lancet Infect Dis 2007; 7: 319-327.
2. World Health Organization (WHO) fact sheet 2017. Available at URL: http://www.who.int/mediacentre /factsheets /fs327/en [Retrieved 6 May, 2017]
3. Ross RW. The Newala epidemic III. The virus: isolation, pathogenic properties and relationship to the epidemic. J Hyg 1956; 54: 177-191.
4. Powers AM, Brault AC, Tesh RB and Weaver SC. Re-emergence of Chikungunya and O'nyong-nyong viruses:

evidence for distinct geographical lineages and distant evolutionary relationships. J Gen Virol 2000; 81: 471-479.

5. Weaver SC. Arrival of Chikungunya Virus in the New World: Prospects for Spread and Impact on Public Health. PLoS Negl Trop Dis 2014; 8(6): 1-4.
6. Centers for Disease Control and Prevention (CDC). Chikungunya virus. 2015; Available at URL: https://www.cdc.gov/chikungunya/ [Retrieved 7 May, 2017]
7. Presti AL, Lai A, Cella E. Zehender G and Ciccozzi M. Chikungunya virus, epidemiology, clinics and phylogenesis: A review. Asian Pac J Trop Med 2014; 7(12): 925-932.
8. Morrison TE. Reemergence of Chikungunya Virus. J Virol 2014; 88(20): 11644-11647.
9. Campion EW, Weaver SC and Marc L. Chikungunya Virus and the Global Spread of a Mosquito-Borne Disease. N Engl J Med 2015; 372:1231-1239.
10. Renault P, Solet JL, Sissoko D, Balleydier E, Larrieu S, Filleul L. A major epidemic of chikungunya virus infection on Reunion Island, France, 2005-2006. Am J Trop Med Hyg 2007; 77(4):727-31.
11. Centers for Disease Control and Prevention (CDC). Chikungunya in the Caribbean. 2016; Available at URL: https://wwwnc.cdc.gov/travel/notices/watch/chikungunya-caribbean. [Retrieved 6 May, 2017]
12. ICDDR B: First identified outbreak of Chikungunya in Bangladesh, 2008. Health Sci Bull 2009; 7:1-6.
13. Chowdhury FI, Kabir A, Das A, Mukerrama SM and Masud S. Chikungunya fever: An emerging threat to Bangladesh. J Med 2012; 13: 60-64.
14. Communicable Disease Control Bangladesh. Annual Report 2012 Version. Disease control unit, Director General of Health Services, Bangladesh. Available at URL: http://dghs.gov.bd/ bn/licts_file /images/Other_publication/ CDC_ Annual_ Report. pdf. [Retrieved 7 May,2017]
15. Hassan R, Rahman MM, Moniruzzaman M, et al. Chikungunya - an emerging infection in Bangladesh: A case series. J Med Case Rep 2014; 8: 67-69.
16. Hoque MS and Ahmed ASMNU. Chikungunya fever and Bangladesh: Review and updates. DS (Child) H J 2012; 28(2): 115-122.
17. Chikungunya-spreads-in-dhaka. 30 april 2014. bdnews24.com. Available at URL: http://bdnews24.com/health/2014/04/30/chikungunya-spreads-in-dhaka [Retrieved 16 May, 2017]
18. Diallo M, Thonnon J, Traore LM and Fontenille D. Vectors of Chikungunya virus in Senegal: current data and transmission cycles. Am J Trop Med Hyg 1999; 60: 281-286.
19. Vanlandingham DL, Hong C, Klingler K, et al. Differential inactivities of o'nyong-nyong and Chikungunya virus isolates in Anopheles gambiae and Aedes aegypti mosquitoes. Am J Trop Med Hyg 2005; 72: 616-62.
20. World Health Organization. SEA-CD-180 Guidelines on Clinical Management of Chikungunya Fever. Available at URL: http:// www.wpro. who.int/ mvp/ topics/ ntd/ Clinical_Mgnt_Chikungunya_WHO_SEARO.pdf. [Retrieved 6 May 2017]
21. Centers for Disease Control and Prevention (CDC). Chikungunya Virus Transmission. 2016; Available at URL: http://www.cdc.gov/Chikungunya/transmission/ [Retrieved 5 May 2017]
22. Chahar HS, Bharaj P, Dar L, et al. Co-infections with chikungunya virus and dengue virus in Delhi, India. Emerg Infect Dis 2009; 15:1077-1080.
23. Talarmin F, Staïkowsky F, Schoenlaub P, et al. Skin and mucosal manifestations of chikungunya virus infection in adults in Reunion Island. Med Trop 2007; 67: 167-173.
24. Schwartz O and Albert ML. Biology and pathogenesis of chikungunya virus. Nat Rev Microbiol 2010; 8: 491-500.
25. Ng LF, Chow A, Sun YJ, et al. IL-1?, IL-6, and RANTES as biomarkers of chikungunya severity. PLoS One 2009; 4: e4261.
26. Morrison TE, Whitmore AC, Shabman RS, et al. Characterization of Ross River virus tropism and virus-induced inflammation in a mouse model of viral arthritis and myositis. J Virol 2006; 80: 737-749.
27. Sourisseau M, Schilte C, Casartelli N, et al. Characterization of reemerging chikungunya virus. PLoS Pathog 2007; 3: 804-817.
28. Ozden S, Huerre M, Riviere JP, et al. Human muscle satellite cells as targets of chikungunya virus infection. PLoS One 2007; 2: 1-7.
29. Wauquier N, Becquart P, Nkoghe D, et al. The acute phase of Chikungunya virus infection in humans is associated with strong innate immunity and T CD8 cell activation. J Infect Dis 2011; 204(1): 115-123.
30. Lum FM, Teo TH, Lee WW, et al. An essential role of antibodies in the control of Chikungunya virus infection. J Immunol 2013; 190(12): 6295-6302

31. Teo TH, Lum FM, Claser C, et al. A pathogenic role for CD4+ T cells during Chikungunya virus infection in mice. J Immunol 2013; 190(1): 259-269.

32. Bernard E, Solignat M, Gay B, et al. Endocytosis of chikungunya virus into mammalian cells: role of clathrin and early endosomal compartments. PLoS One 2010; 5(7): e11479.

33. Rulli NE, Suhrbier A, Hueston L, et al. Ross River virus: molecular and cellular aspects of disease pathogenesis. Pharmacol Ther 2005; 107(3): 329-342.

34. Ziegler SA, Nuckols J, McGee CE, et al. In vivo imaging of chikungunya virus in mice and Aedes mosquitoes using a Renilla luciferase clone. Vector Borne Zoonotic Dis 2011; 11(11): 1471-1477.

35. Mohan A. Chikungunya fever: clinical manifestations & management. Indian J Med Res 2006; 124: 471-474.

36. Sudeep AB and Parashar D. Chikungunya: an overview. J Biosci 2008; 33: 443-449.

37. Simon F, Parola P, Grandadam M, et al. Chikungunya infection: An emerging rheumatism among travelers returned from Indian Ocean islands. Report of 47 cases. J Med 2007; 86: 123-137.

38. Bandyopadhyay D and Ghosh SK. Mucocutaneous features of Chikungunya fever: a study from an outbreak in West Bengal, India. Int J Dermatol 2008; 47: 1148-1152.

39. Mahendradas P, Ranganna SK, Shetty R, et al. Ocular manifestations associated with Chikungunya. 2008; J Ophthal 115: 287-291.

40. Fourie ED and Morrison JG. Rheumatoid arthritic syndrome after chikungunya fever. S Afr Med J 1979; 56:130-132.

41. Kennedy AC, Fleming J and Solomon L. Chikungunya viral arthropathy: A clinical description. J Rheumatol 1980; 7: 231-236.

42. Brighton SW, Prozesky OW and de la Harpe AL. Chikungunya virus infection. A retrospective study of 107 cases. S Afr Med J 1983; 63: 313-315.

43. Simon F, Savini H and Parola P. Chikungunya: a paradigm of emergence and globalization of vector-borne diseases. Med Clin North Am 2008; 92:1323-1343.

44. Simon F, Javelle E, Oliver M, Leparc-Goffart I and Marimoutou C. Chikungunya Virus Infection. Curr Infect Dis Rep 2011; 13: 218-228.

45. Thiberville SD, Moyen N, Dupuis-Maguiraga L, et al. Review Chikungunya fever: Epidemiology, clinical syndrome, pathogenesis and therapy. Antiviral Res 2013; 99: 345-370.

46. Staikowsky F, Talarmin F, Grivard P, et al. Prospective study of Chikungunya virus acute infection in the Island of La Réunion during the 2005-2006 outbreaks. PLoS One 2009; 4:1-9.

47. World Health Organization: Chikungunya in South-East Asia-update. 2008; Available at URL: ://209.61.208.233/en/Section10/Section2246_13975.htm. [Retrieved 7 May, 2017]

48. World Health Organization. Communicable Diseases Branch. Chikungunya fever, laboratory diagnosis of Chikungunya fevers. Geneva: World Health Organization 2007. Available at URL: http:// www. wpro. who.int / mvp/ topics/ ntd/ Chikungunya_WHO_SEARO. pdf. [Retrieved 7 May, 2017]

49. Centers for Disease Control and Prevention (CDC). National Guideline on Clinical Management of Chikungunya Fever 2017. Available at URL: https://www.google.com/ url?q=http:// www. iedcr.gov. bd/images / PDF/ Health_Message/chikungunya 2520Final.pdf [Retrieved 20 May, 2017]