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Review

Chikungunya in Southeast Asia: understanding the emergence and finding solutions

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SUMMARY

In the last few years, chikungunya has become a major problem in Southeast Asia, with large numbers of cases being reported in Singapore, Malaysia, and Thailand. Much of the current epidemic of chikungunya in Southeast Asia is being driven by the emergence of a strain of chikungunya virus that originated in Africa and spread to islands in the Indian Ocean, as well as to India and Sri Lanka, and then onwards to Southeast Asia. There is currently no specific treatment for chikungunya and no vaccine is available for this disease. This review seeks to provide a short update on the reemergence of chikungunya in Southeast Asia and the prospects for control of this disease.

1. Introduction

Chikungunya virus (CHIKV) is the causative agent of chikungunya fever, which is a global public health problem.[1–3] The disease was first formally described in 1955 following an outbreak in Tanzania, Africa in 1952,[4] and the virus was first isolated shortly thereafter.[5] CHIKV is a virus of the genus *Alphavirus* belonging to the family *Togaviridae*, and the genome consists of a linear, positive-sense single-stranded RNA molecule of approximately 11.8 kb, which encodes for four non-structural proteins (nsP1–nsP4), three structural proteins (C, E1, and E2), and two proteins of ill-defined function (E3 and 6K).

Three distinct lineages of CHIKV are believed to be circulating – two from Africa (West African and East Central and South African) and an Asian genotype.[6] The Asian lineage is believed to have diverged from the East Central and South African (ECSA) lineage either between 50 and 310 years ago[7] or between 61 and 124 years ago.[8]

In nature, CHIKV is maintained in two primary transmission cycles.[6] In Asia the virus is believed to be largely maintained in a mosquito–human–mosquito cycle, with the urban anthropophilic *Aedes aegypti* being the primary transmission vector. In Africa the virus is believed to be maintained primarily in a sylvatic cycle involving wild, non-human primates, such as macaques, and forest-dwelling *Aedes* mosquito species such as *Aedes furcifer*, *Aedes taylori*, *Aedes luteocephalus*, *Aedes africanus*, and *Aedes neoafricanus*.[6]

Classically, CHIKV infections are characterized by high fever, nausea, rash, and severe arthralgia, and symptomatically, chikungunya fever can be difficult to differentiate from dengue fever.[9] The association of CHIKV infection with severe joint pain is believed to have been the origin of the name chikungunya, which derives from the East African Makonde language and means 'that which bends up'.

Several studies have implicated a more severe pathobiology in recent years. In the 1963 outbreak in India, neurological and hematological complications were reported for the first time.[10,11] More pertinently however, in a 2005/6 outbreak on La Réunion Island in the Indian Ocean, chikungunya started to present with very complicated clinico-pathological manifestations, primarily associated with encephalopathy and hemorrhagic fever.[12,13] Arthralgia persisted for months or years in joints, causing severe pain, with the aged, particularly those suffering from diabetes, alcoholic hepatopathy, or impaired renal functions, suffering the most. This epidemic also marked the first chikungunya deaths, as well as the first cases of peripartum mother-to-child transmission.[14–16] Particularly significant was the extent of this outbreak,

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with some 220 000 people affected or nearly 40% of the population.[17]

More recent outbreaks in India have been characterized by high morbidity or extensive incapacitation. Again, neurological and renal involvement was reported in the disease pathology,[18–20] as well as ophthalmic involvement, hypokalemic paralysis, hearing loss, Guillain–Barré syndrome, and acute flaccid paralysis.[21–28] Significant abnormalities in the cerebrospinal fluid, raised hepatic enzymes, and altered renal function have all been reported in large numbers of patients in post-La Réunion outbreaks.[6]

As noted above, one of the most critical aspects of CHIKV infection is the severe arthralgia that can persist for months or years in joints,[29] causing severe pain. Understanding this process is one of the more urgent requirements in understanding the pathology of CHIKV infections. The earliest work on understanding the pathology of CHIKV infections came from studies in mice deficient for the type-1 interferon pathway.[14] While this animal model does not completely capture the pathology of human infections, it was shown that after an initial round of replication in the liver, CHIKV primarily targets muscle, joint, and skin fibroblasts. Markedly, dissemination of the virus to tissues in the central nervous system was also observed, which mirrors the neurological involvement seen in some cases of CHIKV infection. In a study on non-human primates,[30] macrophages were identified as a long-lasting reservoir of viral infection in monkeys, which is believed to explain the long-lasting symptoms in humans. Further studies in mice[31] have implicated proinflammatory factors released by alphavirus-infected macrophages as being critical mediators of arthralgia/arthritis, which is consistent with studies on human patients.[29] A more recent study has shown that levels of viremia during the acute phase determined the specific pattern of pro-inflammatory cytokines and that high levels of interleukin-6 and granulocyte macrophage colony-stimulating factor were associated with persistent arthralgia.[32]

The outbreak on La Réunion apparently marked a significant turning point in the pathobiology of chikungunya fever. Critically, the virus became more transmissible by *Aedes albopictus*, and possibly by *A. aegypti* as well. Examination of isolates from the La Réunion outbreak pinpointed a single mutation, a change of alanine to valine of amino acid 226 of the E1 protein (E1-A226 V) in the ECSA lineage,[33] which has been implicated as being the causative factor,[34] and it has been shown that this mutation was responsible for the enhanced infectivity in both *A. aegypti* and *A. albopictus*, although the reasons for this are less clear.[34] Some speculation has suggested that this mutation might reduce the cholesterol dependence of the virus[33] and might help in replication and transmission of the virus. Less clear at this point is whether the mutation is causatively associated with the changes in disease pathology seen since the 2005/6 outbreak on La Réunion. However, it is the ECSA lineage that has been driving the vast majority of the outbreak in Southeast Asia.

2. The road to Southeast Asia

The origin of the progenitor ECSA CHIKV strain that has led to the current outbreak sweeping across Southeast Asia remains open to debate. While some authors propose that the progenitor strain arose in the neighborhood of Uganda,[8] other authors feel this is not fully consistent with phylogenetic analyses and have proposed that a common ancestral strain arose around the time of an outbreak of chikungunya in Kenya in 2004.[35] It is however possible that the Ugandan-82 strain is the progenitor of the Kenyan 2004 isolates. This common ancestral strain of the ECSA lineage is proposed to have been wild-type for the critical E1-A226 V mutation,[35] and was subsequently responsible for the outbreaks on the Indian Ocean islands of Comoros and Seychelles in 2005,[36] with the outbreak being spread by *A. aegypti* mosquitoes.[37] This outbreak spread to the islands of La Réunion and Mauritius, where early infections were characterized as wild-type for the E1-A226 V mutation.[38] On La Réunion and Mauritius however, the principal mosquito vector species is *A. albopictus*, and it is believed that adaptation to *A. albopictus* led to the emergence of an E1-226 V strain of the ECSA lineage, and this lineage was predominantly responsible for the massive outbreak on La Réunion during the 2005/2006 period.[38]

CHIKV subsequently spread to India, where it was responsible for a massive outbreak between December 2005 and the end of 2008, with some 1.5 million affected cases. DNA sequence and phylogenetic analysis however show that this outbreak did not originate with the E1-226 V strain on La Réunion, as the early infections in India from around 2006 were characterized as being wild-type at E1-226,[39,40] and comprehensive phylogenetic analysis indicates that the Indian Ocean/La Réunion E1-226 V lineage forms a separate cluster from the Indian (and subsequently Southeast Asian) CHIKV strains.[35]

As noted, early reports from India characterized the CHIKV strain responsible for the massive outbreak as being wild-type for E1-A226 V and possibly arriving in India from East Africa or Comoros,[41] and it is believed that this was spread primarily by *A. aegypti* mosquitoes.[6] However, later infections (from 2007 onwards) were shown to originate from CHIKV carrying the E1-226 V mutation,[39,40] with transmission being driven by *A. albopictus*.[42] Remarkably, sequence analysis suggests that the E1-226 V mutation in the Indian lineage CHIKV strains originated independently from the event leading to the La Réunion E1-226 V CHIKV strains in an example of convergent evolution.[35,41] More remarkable still, is that the 2006 and 2007 chikungunya outbreaks in Cameroon and Gabon similarly likely arose from importation of a wild-type strain of the ECSA lineage (also possibly from the 2004 Kenya chikungunya outbreak), which independently acquired the E1-226 V mutation.[41] In all cases it seems to be the adaptation to *A. albopictus* that is associated with the emergence of this mutation.[41] The outbreak of chikungunya in Italy in 2007 that caused significant concern in Europe,[43] arose from the importation of an Indian ECSA strain, through an individual who was asymptomatic upon arrival in Italy, but developed symptoms 2 days after his arrival. Sequencing demonstrated the presence of the E1-226 V mutation, and local transmission was facilitated by high levels of *A. albopictus* in Italy at the time of arrival of the index patient.[44]

Sri Lanka experienced a significant outbreak of chikungunya starting from October 2006.[45] Again, as was seen in both La Réunion and India, early cases that arose predominantly in coastal and urban setting towns were wild-type for E1-226, while later cases (2008 onward) were predominantly in plantation areas and were characterized as carrying the E1-226 V mutation.[35] Again, data suggest that the E1-226 V mutation was acquired locally and that adaptation to *A. albopictus* was a significant factor,[35] although at this point it would seem unwise to rule out multiple importations from India, as chikungunya cases caused by ECSA carrying the E1-226 V mutation are temporally linked between India and Sri Lanka. Significantly however, the Sri Lankan CHIKV strains contain signature mutations characteristic of the prevalent CHIKV strains present in Southeast Asia.[35]

3. Chikungunya in Southeast Asia

Chikungunya, or at least a disease strongly suggestive of chikungunya, has historically been present in Southeast Asia since the late 1700s,[35,46] with the first regional isolation of the virus occurring in 1958 in Thailand.[47] While chikungunya was common in Southeast Asia in the late 1950s and 1960s, it was restricted to sporadic outbreaks from the late 1960s until the second half of the

first decade of the new millennium.[48] Until the most recent outbreak that has swept across Southeast Asia, chikungunya was caused by viruses belonging to the Asian lineage, which, as noted earlier, is believed to have diverged from the ECSA lineage between 50 and 300 years ago.[7,8] Interestingly, there are no data to suggest that the Asian lineage CHIKV has been re-introduced back into Africa. While the current outbreak in Southeast Asia results from the ECSA lineage, it is believed that Asian lineage CHIKV still circulates in Southeast Asia.[35,46]

3.1. Malaysia, Singapore, and Thailand

In the last 15 years, Malaysia has seen outbreaks of chikungunya fever caused by both the Asian and the ECSA lineage. An outbreak in 1998 in Klang in Selangor State (located some 30 km west of the capital of Kuala Lumpur), which affected 51 people,[49] was the first recorded outbreak of chikungunya in Malaysia, although low levels of CHIKV seropositivity have been recorded in the local population, suggesting a low level of transmission of the virus before the first outbreak.[50] A second outbreak of chikungunya was recorded between March and April 2006 in Bagan Panchor, Perak, which affected more than 200 people.[51] While this second outbreak coincided with the peak of the 2005/6 outbreak on La Réunion Island and with the start of the Indian subcontinent outbreak, evidence showed that this Malaysian outbreak as well as the previous outbreak resulted from transmission of the Asian lineage CHIKV.[52] A third, limited outbreak also occurred in Malaysia in December 2006 in Ipoh, Perak, which was caused by importation of an ECSA strain from India.[53] A major, nationwide outbreak of chikungunya started in April 2008 that spread to 14 out of 15 states and federal territories, and sequence analysis showed that this outbreak was driven by circulation of the E1-226 V mutation in the ECSA strain.[52] The association of the cases with primarily rural areas implicated transmission by *A. albopictus* mosquitoes.[52]

Singapore, the small city state located at the southern end of the Malay Peninsula, has frequently experienced imported cases of chikungunya in the past, but these have not resulted in local transmission of the virus.[46] The first cases of autochthonous transmission of CHIKV were reported from January 14, 2008 until February 21, 2008 in a localized outbreak that affected 13 patients.[54] A second limited outbreak of two cases was reported in May 2008, while a third case that only involved one patient was reported in June 2008.[54] Phylogenetic analysis determined that all of the causative viruses were of the ECSA lineage, most likely of Indian, Malaysian, and Sri Lankan origin, respectively.[35] From July 2008 a major outbreak was reported with more than 1000 patients affected,[48] with transmission of both the wild-type ECSA (E1-A226) and mutant (E1-226 V) being reported.[35,46] Transmission of the wild-type E1-A226 ECSA is believed to occur predominantly via *A. aegypti* mosquitoes in an urban setting, while the mutant E1-226 V is associated with more rural transmission by *A. albopictus*.[35,46] Phylogenetic analysis suggests that Sri Lankan, Singaporean, and Malaysian CHIKVs all share common signature mutations, indicative that they are closely phylogenetically related, although the presence of other unique signatures suggests that microevolution of CHIKV occurred in both Sri Lanka and Singapore/Malaysia.[35]

Thailand has suffered several significant outbreaks of chikungunya since the first reported case in 1960,[47] and past outbreaks in Thailand include those in the provinces of Prachinburi (1976), Surin (1988), Khon Kaen (July 1991), Loei and Phayao (1993), Nakhon Si Thammarat (July 1995), and Nong Khai (August 1995). In the most recent outbreak in the southern provinces of Thailand (2008/2009), some 22 000 cases of chikungunya were diagnosed in the first 5 months of 2009. The outbreaks prior to the current outbreak were the result of circulation of the Asian lineage,[7] while evidence has shown that the current outbreak is the result of the introduction of the ECSA lineage to Asia.[55] The first reported cases in Thailand during the current outbreak were reported in October 2008 in the province of Narathiwat, one of the southern-most provinces of Thailand, which is adjacent to the Malaysian state of Kelantan. Sequence analysis showed that the virus belonged to the ECSA lineage and had the E1-226 V mutation.[55] Consistent with other reports, *A. albopictus* was believed to be the primary transmission vector, and CHIKV was isolated from field-caught *A. albopictus* in the area of the outbreak.[56] From the initial outbreak that was focused around five villages in Narathiwat, chikungunya spread throughout much of Thailand, and by December 2009 chikungunya had been reported in 43 of the 75 provinces of Thailand, with cases reported in central, northeastern, and northern provinces in addition to its presence in the southern provinces;[57] more than 46 000 cases of chikungunya were reported. The virus isolated from both early and late outbreak cases grouped phylogenetically with the viruses from Singapore, Malaysia, and Sri Lanka, although the presence of some unique amino acid changes could indicate further evolution of the virus in Thailand.[57]

3.2. The rest of Southeast Asia

Details on CHIKV infections in other countries in Southeast Asia are scarce, with few if any detailed studies on CHIKV infections and the lineage of the virus that causes the infections. The first evidence for the presence of CHIKV in Indonesia comes from a seroconversion survey conducted in 1972[58] suggesting the widespread presence of the virus in Indonesia. Outbreaks of chikungunya were frequently recorded between 1982 and 1985 throughout Indonesia, after which there was a period of some 16 years until 2001, when a series of 13 outbreaks were reported between September 2001 and March 2003.[59] To date, there is little published work on the lineage of the virus responsible for the early outbreaks, although given the timeframe it is highly likely that these were a consequence of transmission of the Asian genotype CHIKV. Analysis of cases of CHIKV infection imported to Singapore in 2008 however, have shed some light on the current situation in Indonesia, with CHIKV isolates clustering with both the ECSA and Asian lineage being identified, suggesting that both lineages are currently circulating.[46] The ECSA Indonesian isolate imported into Singapore clustered with Malaysian/Singapore/Indian ECSA isolates from the recent outbreak, suggesting a common origin.[46] The Philippines similarly experienced scattered occurrences of chikungunya between the mid 1950s and later 1960s.[60,61] The infections in 1986 of three US Peace Corps Volunteers stationed in the Philippines[62] were the first reported cases of chikungunya in the Republic of the Philippines since the late 1960s. With the exception of a single report describing an outbreak of chikungunya in 1996,[63] there is no information as to the status of chikungunya in the Philippines. Similarly, we were unable to find formal published reports on the current status of chikungunya in Vietnam, Cambodia, Laos, and Myanmar.

4. Vector control strategies: lessons from Singapore

In the absence of a vaccine (see below), efforts at controlling the spread of chikungunya are primarily centered around vector control strategies that seek to reduce potential breeding sites, kill larvae through the action of larvicides such as temephos or Bacillus larvicidal toxins,[64] or reduce the numbers of adult mosquitoes through space spraying using pyrethroids or organophosphates. Singapore has a long history of vigorous vector control programs, which include public awareness programs aimed at reducing breeding sites for mosquitoes around the home.[65] However, the

vector control programs in Singapore were primarily aimed at controlling the emergence and spread of dengue, and the success of this program led to a shift in emphasis from vector surveillance to the early detection of cases of dengue coupled with emergency vector control programs at identified outbreak clusters.[65] In this way the chikungunya outbreak was largely unanticipated, and only with the appropriate identification of *A. albopictus* as the primary transmission vector were appropriate control measures put in place to target the exophilic and exophagic *A. albopictus* mosquito. These control measures effectively brought the outbreak under control, and underscored the necessity of combining mosquito control programs with comprehensive virological surveillance of mosquito populations.[66,67]

5. The prospects for a chikungunya vaccine

Although the causative agent of chikungunya fever was identified more than 50 years ago,[5] and despite its continued circulation in Africa and parts of Asia, there remains no commercially available vaccine, possibly due to the sporadic and limited nature of the outbreaks of this disease since the 1960s. Interestingly however, the attempts to develop a CHIKV vaccine can be traced back to about a decade after the virus was first isolated, when Harrison and colleagues compared the immunogenicities of formalin-inactivated CHIKV strain 168, an isolate from the 1952–53 Tanzanian outbreak, prepared from different tissue sources.[68] They showed that an inactivated CHIKV produced from green monkey kidney cells elicited the highest level of immune response while showing no neurovirulence in mice. The vaccine candidate also provided protection against lethal CHIKV challenge in a mouse model system and against viremia in a monkey model system.[68] Based upon these results, this group of investigators expanded their studies by applying a different method of inactivation (Tween–ether extraction) to the same strain of the virus[69] or using the same formalin inactivation approach to a different genotype of CHIKV, namely the Asian genotype strain 15561.[70] Both of these vaccine candidates were shown to be immunogenic and afforded protection to mice against three heterologous strains of CHIKV challenge. Vaccine development of the Asian genotype was carried through to an initial human trial in which significant levels of neutralizing antibody were detected in all subjects.[70]

An updated version of an inactivated virus vaccine candidate is being developed following the recent CHIKV outbreak in India. The vaccine was derived from a novel, Indian isolate of the ECSA genotype, which was Vero cell-adapted and formalin-inactivated and used in combination with aluminum hydroxide as an adjuvant. In a mouse models system, results from ELISA, a plaque reduction neutralization test, and a cytokine profile of isolated splenocytes, indicated the induction of both humoral and cellular immune responses after subcutaneous injection.[71]

Perhaps the most well studied CHIKV vaccine candidate is a live attenuated virus vaccine (LAV) developed by the US Army using a Thai isolate, CHIKV strain 15561, which underwent 18 plaque-to-plaque passages in the human lung fibroblast MRC-5 cell line. The resulting CHIK 181/clone 25 (181/25) candidate exhibited an attenuated phenotype both in vitro and in vivo. The vaccine candidate was able to induce neutralizing antibodies, protect mice against lethal challenge, and immunized Rhesus monkeys showed no viremia following challenge.[72] The lyophilized supernatant from CHIK 181/25-infected MRC-5 cells, designated as TSI-GSD-218, was shown to be safe and immunogenic in a phase I trial.[73] In a phase II trial, 95% of the 59 volunteers who received a single subcutaneous dose of the TSI-GSD-218 vaccine elicited neutralizing antibodies, and 85% remained seropositive 1 year after immunization; while transient arthralgia was experienced by 8% of the vaccinees, adverse reactions were generally similar in nature and extent to those experienced by the placebo group.[74]

As CHIK 181/25 is a live, albeit an attenuated virus, safety concerns about the possibility of natural transmission by a mosquito vector must always be considered, and indeed, in a series of experiments, Turell and Malinoski[75] showed that the CHIK 181/25 virus was able to replicate in female Aedes mosquitoes after intrathoracic inoculation, and was transmissible. Nevertheless, there was no evidence of reversion of the vaccine candidate strain to a more virulent phenotype upon replication in mosquitoes, and given the low viremia this vaccine produced in vivo, it is unlikely that mosquitoes would become infected via feeding on a vaccinee.[75]

The incidence of some adverse events in the TSI-GSD-218 clinical trial prompted another group of researchers to pursue an alternative LAV candidate strategy. Chimeric alphavirus vaccine candidates with backbones of either the attenuated Venezuelan equine encephalitis vaccine strain TC-83 or a naturally attenuated strain of eastern equine encephalitis virus or an attenuated Sindbis virus were constructed to contain the structural proteins of a CHIKV strain isolated during the La Réunion outbreak.[76] In addition to inducing a robust humoral immune response, the chimeras did not trigger reactogenicity in immunized adult mice as indicated by the lack of signs of neurological disease, febrile responses, or growth delays. In newborn mice, the chimeric alphavirus vaccine candidates showed better attenuation in terms of lower titers and a shorter duration of viremia as compared to the CHIK 181/25 vaccine candidate, and unlike mice immunized with CHIKV wild-type or CHIK 181/25, there was no detectable viral replication in the femoro-tibial joints or in the brains of mice immunized with the chimeras.[76] Taken together, the chimeric alphaviruses appear to be promising CHIKV vaccine candidates, yet further studies to determine the most appropriate chimeric construct and the vaccination regimen in another animal model are essential.

There will always be some risk concerns, including under-attenuation and pathogenic reversion related to LAVs, whether naturally attenuated or chimeric, and similarly, inactivated virus vaccines are of concern, particularly in immunocompromised individuals, with regards to incomplete inactivation. To address these issues, several alternate approaches to CHIKV vaccine development are under investigation.

In the first of these, novel consensus-based DNA vaccines for CHIKV are being developed. The vaccine candidates were designed based upon the consensus sequences of the E1, E2, and C proteins from 21 CHIKV isolates collected between 1952 and 2006. In addition, codon and RNA optimization were conducted, a Kozak sequence was added, and the signal peptide substituted with an immunoglobulin E leader sequence to improve vaccine efficacy. After three intramuscular immunizations with each plasmid DNA vaccine candidate, mice showed significant levels of anti-E1-specific, anti-E2-specific, or anti-C-specific IgG antibodies and interferon-gamma (IFN-γ) production, suggesting a strong humoral as well as cellular immune response.[77,78] More recently, a single plasmid that encodes the CHIKV envelope glycoproteins (E3, E2, and E1) was constructed using the same strategy. This new DNA vaccine induced a better immune response than the original constructs that expressed the CHIKV structural proteins individually. In addition, this DNA vaccine candidate was able to protect mice from challenge with a recent clinical isolate of CHIKV. An initial study in rhesus macaques showed the induction of hemagglutination inhibition antibodies, virus neutralizing antibodies, and IFN-γ.[79]

As an alternative to DNA vaccines in avoiding the problem associated with whole virus (inactivated or live attenuated) vaccines, virus-like particles (VLPs) are also being explored, as they are known to be safe while inducing a better immune

response than classical subunit vaccines. Recently, the first alphavirus VLPs were generated by expressing the structural proteins (C-E3-E2-6K-E1) from an old West African (strain 37997) lineage of CHIKV in 293T human kidney cells.[80] In animal model studies, serum from VLP 37997 vaccinated mice and monkeys showed comparable levels of neutralization against the homologous strain 37997 and a heterologous strain from the recent outbreak of the ECSA lineage (LR2006 OPY-1). Furthermore, passive transfer of purified IgG from an immunized monkey was able to completely protect type I interferon-defective mice from lethal challenge with LR2006 OPY-1, without detectable viremia.[14,80] In a similar manner, IgG isolated from CHIKV convalescent patients has been shown to prevent and cure CHIKV infection in mice,[81] suggesting that passive immunotherapy may be useful to control or treat CHIKV infection, and two human monoclonal antibodies with strong and specific neutralization activity have recently been described.[82]

Despite the potential of CHIKV vaccine candidates in the 1980s (such as the US Army vaccine candidate), the worldwide lull in the incidence of cases of chikungunya fever appears to have removed the driving force for bringing these products to commercial distribution, and to have stifled further CHIKV vaccine development. The massive resurgence of CHIKV around the Indian Ocean and elsewhere from 2005 onwards, as well as the introduction of CHIKV to Europe, therefore took place in the absence of a viable preventive vaccine. Clearly however, the outbreak has provided new impetus to the development of a safe, cheap, and effective CHIKV vaccine. While a number of significant questions remain to be addressed related to vaccine validation, such as the most appropriate animal models (species, age, immune status), the dose and route of immunization, the potential interference from multiple vaccinations against different viruses, and last but not least, the practical cost of the vaccine, since most of the epidemic geographical regions belong to the developing countries, there is real hope that a vaccine to prevent this disease will not be too long in arriving.

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