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Toward an Activist Agenda for Monitoring Virus Emergence

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The continuing emergence of arboviruses such as chikungunya virus requires thoughtful attention and approaches for risk management. Incorporating experimental evolutionary studies, as described in this issue by Stapleford et al. (2014), has the potential to move public health toward a more proactive agenda for predicting and responding to disease emergence.

In December 2013, chikungunya virus (CHIKV; Togaviridae, alphavirus) was detected in the Americas for the first time. Although CHIKV has been known since the 1950s (it was first isolated from the blood of a patient in Tanzania in 1953), it has received increased attention during the last 10 years due to its reemergence in coastal Kenya and spread throughout the Indian Ocean region. Although CHIKV is not frequently fatal, the current epidemic has resulted in over two million cases. Infected individuals experience severe morbidity. Symptoms include debilitating arthritis and painful swelling of the tissues that surround the joints: the word chikungunya is Makonde for "bent up," a reference to the posture of infected individuals. Patients require hospitalization and/or bed rest for an average of 7 days, but many are afflicted for up to several months with severe joint pain. The ongoing emergence of CHIKV in the Americas provides a timely reminder that arthropod-borne diseases remain some of the most difficult and complex problems facing public health and medicine.

Several factors contribute to the emergence of arthropod-borne diseases, including CHIKV. The emergence of Lyme disease was driven by the regrowth of the Eastern and Midwestern forests as farmlands were converted to wooded residential areas. This changing landscape provided an ideal habitat (and few predators) for white-tailed deer, favored hosts for adult deer ticks (*Ixodes scapularis*), the main vectors of the causative agent *Borrelia burgdorferi*. West Nile virus (WNV; Flaviviridae, flavivirus) was introduced into North America, likely via air travel and/or trade, and its ongoing health burden is facilitated by mosquitoes that thrive in urban or agricultural areas (*Culex pipiens* and *Cx. tarsalis*, respectively). The mosquito vectors of yellow fever, dengue virus, and CHIKV (*Aedes aegypti* and *Aedes albopictus*) prefer to breed in water containers and refuse that are frequently found in peridomestic areas and dumps. Climate change seems certain to further alter the distributions of all of these arthropod vectors and the diseases that they transmit. Most factors that lead to the emergence of new arthropod-borne diseases, therefore, are either directly or indirectly attributable to the human footprint on the environment.

But what role do the viruses themselves have in their emergence? Viruses, like other organisms, must adapt to available ecological niches or become extinct. Due to error-prone replication, RNA viruses, including arboviruses, possess an inherent ability to adapt to changing environments. Virally encoded RNA-dependent RNA polymerases lack error checking and mismatch repair mechanisms, leading to error rates several orders of magnitude greater than those of eukaryotic polymerases (Holland et al., 1982). The genetic variation present within arbovirus populations provides a continuously replenished pool of genetic variants (any of which may be most fit in a given environment) upon which natural selection can act. CHIKV provides an excellent example of this: its explosive emergence was mediated by seemingly trivial substitutions to the viral E1 (A226V) glycoprotein that conferred enhanced transmission by an aggressive and highly abundant mosquito vector, *Ae. albopictus* (the Asian tiger mosquito) (Tsetsarkin et al., 2007). The spread of CHIKV throughout the Indian Ocean region in the last decade, and its massive health burden, has largely been attributed to enhanced transmission by this mosquito (Tsetsarkin et al., 2011).

What if it were possible to precisely predict how arboviruses might evolve when introduced into a new region, a new host, or in response to changing environmental conditions? Stapleford et al. (2014) approach this question in a paper published in this issue of *Cell Host and Microbe*. The premise of the experiments is simple, intuitive, and elegant: expose *Ae. aegypti* (the "endemic" vector) and *Ae. albopictus* (the "epidemic" vector) to 226A CHIKV and monitor the virus population for 226V emergence. Remarkably, the 226V mutation that has facilitated the ongoing CHIKV epidemic was found in the expectorated saliva of most *Ae. albopictus* in varying proportions after a single round of infection, but not in the saliva from *Ae. aegypti*. Therefore, the E1 glycoprotein mutation that results in efficient transmission by *Ae. albopictus* might have been predicted well before the epidemic CHIKV strain emerged. Further, experiments could have been conducted to quantify the potential impact of the A226V mutant on CHIKV epidemiology. Next, the authors asked whether additional mutations might be predicted, on the basis of experimental transmission/evolution studies, to increase the overall transmission rate (i.e., the epidemic potential) of currently circulating CHIKV. Two linked mutations, V80I and A129V in the E1 glycoprotein, were identified in CHIKV present in the saliva of both *Ae. albopictus* and *Ae. aegypti*

mosquitoes. These substitutions resulted in (a) increased virus titers as the virus moved through the mosquitoes, particularly at a low input dose of $\sim 10^3$ genomes/ml of blood, and (b) increased viremia and pathogenesis in mice. These replication advantages are presumably due to predicted alterations in membrane fusion potential and to increases in virion stability in a cell-free milieu (i.e., mosquito saliva). Thus, the approaches described by Stapleford et al. (2014) both (a) correctly identify known mutations that increase virus transmission by a new mosquito vector and (b) identify mutations that could further enhance the epidemic potential of CHIKV. The ability to predict how RNA viruses might adapt to any given environment (i.e., their possible adaptive pathways) should be quite useful. In principle, Stapleford et al. (2014) have helped show what is required in order to move toward a more activist approach to monitoring arbovirus emergence.

Notably, the reported studies on CHIKV were facilitated by at least two critical factors. The first of these is the authors' ability to use laboratory systems to model CHIKV transmission in a manner that accurately mimics natural transmission; similar studies would have been difficult to carry out if, for example, the virus was a sexually transmitted pathogen of humans. Specifically, the increased fitness of the variants was not apparent in typical cell culture-based studies of virus replication. Only when the expectorated saliva of vector mosquitoes was examined was the 226V mutant identified. *Ae. albopictus* mosquitoes, the drivers of the ongoing epidemic, are generally abundant in nature and easy to collect and colonize. In fact, this study used several colonies made from individuals collected in endemic countries (e.g., Thailand, Cameroon, etc.). Further, several longstanding laboratory colonies are available and commonly shared among investigators.

The second key factor that facilitated this research is prior knowledge of what factors can shape the fitness landscape of CHIKV. Specifically, it had been clearly shown that transmission by *Ae. albopictus* would increase virus fitness in the global sense—nature had, in essence, performed the pilot in vivo experiment and found that A226V increased virus fitness in a species-specific manner. Logical, reasonable experiments could therefore be designed and conducted that would mimic this particular adaptive trait. Defining the factors that contribute to any given fitness landscape, however, is not usually straightforward due to the complexity of RNA virus transmission and replication. The situation is more complex for arboviruses, where multiple taxonomically divergent hosts are required for virus perpetuation. Several studies have indicated that arboviruses have the capacity to adapt to altered viremia production in particular vertebrate species (Brault et al., 2007), efficient vertical transmission (i.e., transmission from an infected female mosquito to her offspring) (Tesh and Gubler, 1975), and faster transmission by mosquitoes (Moudy et al., 2007). Moreover, the most critical determinants that impact "fitness," the reproductive success of a specific genotype within a particular set of environmental conditions, can be maddeningly hard to pin down. The trick, as the field moves forward, will be to know which factors shape the fitness landscape for any given virus.

In the meantime, arboviruses will continue to emerge in new regions and host populations. Less than 25 years ago, both WNV and CHIKV would have been considered "rare infections," a distinction currently bestowed on emerging arboviruses such as deer tick virus (DTV; Flaviviridae, flavivirus), eastern equine encephalitis virus (EEEV; Togaviridae, alphavirus), Zika virus (Flaviviridae, flavivirus), and many, many others. Integrating a detailed understanding of how vector, host, and human populations interact with changing environments and virus evolution to promote virus emergence remains a challenge, and surprises are undoubtedly in store. CHIKV, again, provides an example of how what we think we know is frequently incorrect. In light of the ongoing Indian Ocean epidemic, it was widely assumed that the strain introduced into the Caribbean region in 2013 would bear the *Ae. albopictus*-adaptive 226V mutation. Remarkably, this is not the case. Caribbean CHIKV belongs to the Asian lineage of CHIKV, and all strains sequenced thus far have the wild-type 226A codon (Lanciotti and Valadere, 2014; Leparc-Goffart et al., 2014). While the implications of this particular finding for the course of the ongoing CHIKV epidemic in the Americas are not clear, it highlights the complexity of the interactions that lead to arboviral emergence and points out the need for cross-disciplinary efforts to manage arbovirus disease outbreaks in an integrated manner, from surveillance to response.

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