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MINI-REVIEW

Zika Virus and Sexual Transmission: A New Route of Transmission for Mosquito-borne Flaviviruses

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Beginning in 2015, concern over a new global epidemic has spread in the media, governmental agencies, legislative bodies and the public at large. This newly emerging threat has been reported to cause symptoms ranging from mild fever, rash, and body aches, to severe birth defects and acute onset paralysis. The causative agent of this disease, Zika virus, is closely related to two other important human pathogens, dengue and West Nile Virus (WNV†), but has some distinguishing features that has raised alarms from the scientific community. Like its two close relatives, this virus is a member of the Flaviviridae family, a class of single stranded RNA viruses with a positive sense genome and is spread primarily via the bite of an infected mosquito. However, this virus has demonstrated another route of transmission that is particularly concerning for people outside of the regions where the main mosquito vector for this virus is present. Sexual transmission of Zika virus has been increasingly reported, from both infected males and females to their partner, which has resulted in the World Health Organization (WHO) and the Center for Disease Control (CDC) issuing warnings to those living in or travelling to areas of Zika transmission to practice abstinence and/or avoid unprotected sexual contact for up to six months after infection with this virus. This perspective will outline the evidence for sexual transmission and persistence of viral infection in semen and vaginal secretions as well as review the animal models for sexual transmission of Zika virus.

INTRODUCTION

Zika virus, a member of the flavivirus family, was first isolated in 1947 from a sentinel monkey in the Zika forest of Uganda, and the next year, researchers were able to identify a group of the Yellow Fever vector *Aedes Africanus* mosquito carrying this virus in the same area [1]. Serologic evidence indicated human infection by Zika virus earlier than 1947 [2], but the first report of active infection came in 1954 during an outbreak of jaundice in Nigeria [3]. From that time until 2007, the virus was identified in many African and Asian countries [4-12], and has been shown to maintain periodic epizo-onotic cycles in sylvatic primate populations of Uganda in Africa [7,13-15]. In 2007, the first human epidemic of Zika virus occurred on Yap Island in the Federated States

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†Abbreviations: WHO, World Health Organization; CDC, Centers for Disease Control; GBS, Guillain-Barre Syndrome; WNV, West Nile Virus.

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of Micronesia, with 49 confirmed symptomatic cases and 73 percent of the population shown to be seropositive [16,17]. Since this first major outbreak, subsequent outbreaks have been reported throughout the Pacific area, in French Polynesia [18], New Caledonia, the Cook Islands, and Easter Island [19]. Most recently, a large epidemic in Brazil and spreading to other countries in South and Central America and the Caribbean, has affected well over two million people [20]. Interestingly, travel to Brazil from epidemic areas for the 2014 World Cup [21] and the 2014 Va'a World Sprint Championship canoe race [22] have been suggested as possible routes of transmission for this outbreak.

Before these recent epidemics, Zika virus has presented as a relatively mild Dengue-like disease, with fever, cutaneous rash, malaise and headache that usually resolves in around seven days [23,24], and up to 80 percent of infections appear to be asymptomatic [16]. These new epidemics, however, have come with significantly more severe symptoms including Guillain-Barre syndrome (GBS), marked by subacute flaccid paralysis [25,26], and congenital deformities and neurologic syndromes in newborns. Birth defects have been attributed to this virus in at least 28 countries according to the WHO [27-29]. This has led to warnings to pregnant women to be mindful of insect repellant use and to limit travel to epidemic areas [30]. Overall, this seeming increase in disease severity and rapid spread has led to increasing alarm across the globe.

For Zika virus, the main mosquito vector is the *Aedes aegypti* species, which is endemic in the southeastern United States [31], and to a lesser extent the *Aedes albopictus* species, which has a more temperate range [32]. With the increase of global trade and range expansion of important insect vectors that carry these viruses, the risk for global epidemics has greatly intensified over recent years and appears poised to continue increasing [33-36]. The vector capacity for Zika is very similar to dengue virus, which is estimated to infect close to 400 million people every year [37], so the potential for continued spread through mosquito transmission is extremely good. Perhaps more concerning to many though, is the ability for Zika to spread through sexual contact. This represents a new route of transmission for the flaviviruses, and could potentially increase the range for this virus to areas free from competent mosquito vectors. So far, according to the WHO, twelve countries have reported sexual transmission [27] of Zika virus, and it is likely that this phenomenon is even more widespread, yet underreported in areas with high levels of local vector-borne transmission.

TRAVEL RELATED CASES OF ZIKA VIRUS

The vast majority of individuals infected with Zika will never know they were infected, and even for those who do show symptoms, they are usually relatively mild, consisting of only a rash, headache, and general body aches, and these patients are often afebrile [38,39]. Therefore, many people who have contracted Zika while traveling abroad might never know that they are infected, and may not take appropriate steps to prevent sexual transmission of this virus. So far, the WHO reports that since 2015, 58 countries have reported local, mostly mosquito-borne, transmission of this virus in the following countries: American Samoa, Anguilla, Antigua and Barbuda, Argentina, Aruba, Bahamas, Barbados, Belize, Bolivia, Bonaire, Sint Eustatius and Saba – Netherlands, Brazil, British Virgin Islands, Cabo Verde, Cayman Islands, Colombia, Costa Rica, Cuba, Curaçao, Dominica, Dominican Republic, Ecuador, El Salvador, Fiji, French Guiana, Grenada, Guadeloupe, Guatemala, Guinea-Bissau, Guyana, Haiti, Honduras, Jamaica, Marshall Islands, Martinique, Mexico, Micronesia, Montserrat, Nicaragua, Palau, Panama, Paraguay, Peru, Puerto Rico, Saint Barthélemy, Saint Kitts and Nevis, Saint Lucia, Saint Martin, Saint Vincent and the Grenadines, Samoa, Singapore, Sint Maarten, Suriname, Tonga, Trinidad and Tobago, Turks and Caicos, United States of America, United States Virgin Islands, and Venezuela [27]. Travelers returning from these epidemic areas have been reported to carry Zika virus to numerous countries including: Israel [40], Italy [41], Germany [42], France [43], Norway [44], Japan [39,45], Australia [46], Denmark [47], Canada [48], and the United States [49]. It is likely though that this list is not complete and will continue to grow as more testing for Zika virus is performed. For this reason, both the CDC and the WHO have issued travel warnings for those traveling to areas where Zika transmission is endemic, indicating the need to use insect repellant while travelling and to use protection while engaged in sexual activity both in these areas and also for an extended period of time after return to their home country.

TRANSMISSION OF ZIKA THROUGH SEXUAL CONTACT

The dogma in the field has been that flaviviruses, such as Zika, dengue, and West Nile virus, are solely vector-borne diseases requiring an insect intermediary to pass these viruses from one person to another. Surprisingly, in 2008, a researcher at Colorado State University travelled to Senegal and after he returned, both he and his wife, who did not travel with him, began to experience symptoms that appeared to indicate an infection with dengue virus. When his serum was tested for antibodies against dengue, the test appeared to confirm this diagnosis, but, curiously, when clinicians tested his wife's serum she was negative for dengue antibodies. The mystery remained until two years later, when in a casual conversation about this case with a medical entomologist from the University of Texas Medical Branch, the possibility of a Zika virus infection was suggested. Upon testing the serum that was saved from 2008, it was revealed that, in fact, both the researcher and his wife had been infected with Zika and not dengue virus. This case was the first to suggest that Zika virus was capable of transmission via the sexual route, but at that time this virus had only been shown to be responsible for a small outbreak in Micronesia so the true impact of this phenomena was yet to be revealed [50,51].

During the latest outbreak in the Americas, the ability for Zika to be transmitted sexually has garnered much more attention, and many more reports of sexual transmission have been documented. These cases are mostly from individuals who have exhibited symptoms, but sexual transmission has also been shown from patients that are asymptomatic at the time of sexual contact [43]. Male to female transmission is the most common [52], but there have also been reports of sexual transmission from female to male [53] and from male to male [54]. The increasing number of cases of sexual transmission have led to a recommendation for abstinence or barrier protection during sexual contact for an extended period of time after infection with Zika virus, and further studies have suggested that this virus is capable of persisting in the genital tract of both males and females even after the virus has been cleared from other areas of the host.

PERSISTENCE OF ZIKA VIRUS IN THE GENITAL TRACT

The current recommendation by the CDC for the prevention of sexual transmission is that abstinence is practiced or condoms are used during sexual contact for those returning from a Zika endemic area for at least eight weeks for females and for at least six months for males. These suggestions stem from numerous studies showing persistence of Zika virus in the genital tract of both men and women. Commensurate with the CDC recommendations, this persistence has so far shown to be longer in males, with infectious Zika able to be cultured from semen up to 80 days after the onset of symptoms [55] and RNA from the virus detected for at least six months after symptoms and well after the virus could no longer be detected in the bloodstream [56]. This phenomenon of persistence in semen has been described previously for two other viruses, Ebola and Rift Valley Fever virus, which have both been detected for similar periods of time as Zika in the seminal fluid of patients well past clearance from the rest of the body [57,58]. For females infected with Zika virus, infectious virus has been demonstrated to be cleared from the vaginal tract by three weeks after symptoms, but to persist beyond the point at which virus can be detected in the bloodstream [59]. Further study is underway to determine if this persistence varies by population group or the immune status of the individual. For instance, in an animal model of Zika virus, it has been demonstrated that pregnant macaques show persistent infection despite mounting an immune response towards this virus [60].

ANIMAL MODELS OF SEXUAL TRANSMISSION

Other animal models of sexual transmission will be important for determining the mechanism of Zika virus using this route of infection and for the development of therapeutics against Zika infection through sexual contact. Similar to human infection, macaque monkeys infected with Zika virus cleared virus from the blood by 10 days after infection, but viral RNA was detected in the seminal fluid until the end of the study, three weeks after the resolution of symptoms [61]. Another study using macaques demonstrated that Zika virus could be detected in vaginal secretions even after clearance from the bloodstream, but again phenocopied human disease in that virus detection in the vaginal tract was not observed to persist for as long as in the genital tract of infected male animals [60].

The macaque model of Zika infection appears to mimic the disease process observed in humans very well, but is prohibitively expensive for many experiments and not ideal for the elucidation of viral mechanisms due to the lack of genetic manipulation. Mouse models, on the other hand, while not as close physiologically to humans, allow for the testing of hypotheses in an *in vivo* system and are a genetically tractable model for study. Researchers at Yale have shown that Zika can replicate in the vaginal tract of female mice, and that when pregnant mice are infected via this route the virus is capable of spreading to the brain of the developing fetal mice [62]. Zika infected male mice have also been shown by multiple studies to harbor replication of this virus in the testes. These male mice also demonstrate a loss of serum testosterone along with testicular atrophy and loss of sperm motility, which raises major implications for fertility in men infected with Zika virus [63,64].

CONCLUSION AND OUTLOOK

Sexual transmission of a virus with close relatives that infect almost half a billion people a year is frightening, and given the fact that this virus is capable of causing severe birth defects, you have a recipe for major headlines. Therefore, an important question becomes, "What is the contribution of sexual transmission in Zika virus outbreaks occurring across the globe?" This question can be partially answered using mathematical modeling to analyze the dynamics of disease spread for this virus. When researchers at the National Autonomous University of Mexico modeled recent Zika outbreaks, they found that sexual transmission was able to affect the magnitude and duration of the epidemics, perhaps adding only around 4 percent to the R_0 (the number of new cases of Zika generated from an infected individual), but that the main driver of the rapid spread of Zika across the globe was likely to be migration of people. This analysis indicates that the majority of transmission of this virus is likely to come via the mosquito route, although in certain population groups and in areas free from competent mosquito vectors sexual transmission may be responsible for a significant portion of locally transmitted cases [65]. Due to the low percentage of transmission attributable to sexual contact and the previously low levels of virus circulation observed before the most recent epidemics, it is also intriguing to speculate that sexual transmission could have occurred in earlier outbreaks of Zika virus without detection. Additionally, this route of transmission might actually be used by more viruses than is currently thought, but given the rarity of these occurrences relative to their main mode of transmission this phenomenon is overlooked. Important questions still remain about the mechanisms of Zika virus in the context of sexual transmission, so it will be important to continue to study infection through this route in both humans and animal models to determine the implications on this disease overall, particularly as it relates to pregnancy complications, viral persistence, and male fertility.

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