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Sexual Transmission of Ebola Virus in Liberia Confirmed Using Genomic Analysis

A suspected case of sexual transmission of Ebola virus disease (EVD) in Liberia was confirmed using genomic analysis, thanks to in-country laboratory capabilities established by U.S. Army scientists in collaboration with the Liberian Institute for Biomedical Research (LIBR).

The work, described in today's edition of the *New England Journal of Medicine*, provides molecular evidence of Ebola virus (EBOV) transmission between an EVD survivor and his female partner. It also demonstrates the value of real-time genomic surveillance during an outbreak, according to senior author Gustavo Palacios, Ph.D., of the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID).

CPT Suzanne Mate, Ph.D., of USAMRIID, said scientists working at the LIBR earlier this year analyzed blood samples from a female patient who tested positive for EBOV in March 2015 when there had been no new documented cases for 30 days. The patient was reported to have had recent sexual intercourse with a male partner who had survived EVD and had been declared EBOV negative in early October 2014.

Following the patient's death on March 27, Mate said, public health officials were able to secure the consent of the male survivor to obtain and test a semen sample from him. The semen sample tested EBOV positive by quantitative RT-PCR, but the assay indicated that the level of viral RNA was low and required a different sample preparation method than the one originally deployed to sequence EBOV RNA from acute samples.

"We implemented a new enrichment strategy in collaboration with scientists from Illumina, Inc. that was pivotal in obtaining the required coverage to complete downstream genomic analysis," said Michael Wiley, Ph.D, of USAMRIID. Next-generation sequencing of the enriched EBOV RNA extracted from the male survivor's semen was used to compare the genome for similarity to the virus RNA extracted from the female patient's blood sample.

"Ebola virus genomes assembled from the patient's blood and the survivor's semen were consistent with direct transmission," commented Jason Ladner, Ph.D., of USAMRIID. "The samples shared three genetic substitutions that have not been found in any other Ebola virus sequences in Western Africa."

In addition, said Ladner, these three genetic changes were distinct from the last documented transmission chain in Liberia prior to this case. Combined with epidemiologic data, the genomic analysis provides support for sexual transmission of Ebola virus and for the persistence of infective EBOV in semen for more than 179 days after disease onset. This caused both the Centers for Disease Control and Prevention and the World Health Organization to change their recommendations for convalescent patients regarding sexual contact until more definitive information is obtained about how long Ebola virus can persist in semen.

Mariano Sanchez-Lockhart, Ph.D., another member of the USAMRIID team, explained that some organs, including the testes, are considered to be "immune privileged" sites. This means that immune responses are tightly regulated in those sites to limit or prevent tissue damage. Other examples of "privileged" sites include the eye, central nervous system, and pregnant uterus.

"Within these sites, viruses might evade systemic immune responses and persist longer since the 'immune pressure' is more restricted than systemically," said Sanchez-Lockhart, adding that further studies are necessary to fully explore this mechanism.

CPT Jeffrey Kugelman, Ph.D., of USAMRIID, said the team's work has implications for development of Ebola virus therapeutics as well, since any potential treatments would need to be designed to reach immune privileged sites.

"For example, drugs that clear the virus in the bloodstream may not address the whole infection," he said.

Kugelman, who was instrumental in setting up the onsite genomic sequencing capability at LIBR, said the study illustrates why such a capability is needed.

"This work allowed for more informed decisions about how to manage and control the spread of the disease," he commented. "To be in the field, during an outbreak, and have the ability to make near real-time sequencing information available to health care providers and public health officials—that's a first."

Also contributing to the work were scientists from the Liberian Institute for Biomedical Research, the Centers for Disease Control and Prevention, the World Health Organization, the National Institutes of Health, and the Liberian Ministry of Health.

Ebola virus causes severe hemorrhagic fever in humans and nonhuman primates with high mortality rates and continues to emerge in new geographic locations, including Western

Africa, the site of the largest recorded outbreak to date. Over 28,000 confirmed, probable and suspected cases have been reported in Guinea, Liberia and Sierra Leone, with over 11,000 reported deaths, according to the World Health Organization.

USAMRIID's mission is to provide leading-edge medical capabilities to deter and defend against current and emerging biological threat agents. The Institute plays a key role as the lead military medical research laboratory for the Defense Threat Reduction Agency's Joint Science and Technology Office for Chemical and Biological Defense. USAMRIID is a subordinate element of the U.S. Army Medical Research and Materiel Command. For more information, visit www.usamriid.army.mil

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