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Does Size Matter? Examining Virulence Of Eastern Equine Encephalitis Virus In Nonhuman Primates Exposed To Small And Large Aerosol Particles

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Eastern equine encephalitis virus (EEEV) is the most virulent of the alphaviruses causing 35-70% mortality in humans, and there are currently no FDA licensed medical countermeasures (MCM) such as vaccines or therapeutics available. Prior to 2019, the largest outbreak in the past decade occurred in 2012 with 15 people reported infected; however, in 2019 a reported 38 individuals were infected, with 19 succumbing to disease. The low case rate of human EEEV infections each year will likely require the licensure of a vaccine or therapeutic under the FDA Animal Rule. EEEV is naturally transmitted by mosquitoes but has been weaponized and can also cause disease from an aerosol exposure, thus is of interest to the DOD as a potential biowarfare/terrorism (BWT) agent. Classically, aerosol exposure has been generated with a Collison nebulizer that generates a ~1.0 µm aerosol particle. There has been one report examining a slightly larger particle delivery with EEEV in crystal.w.burke.civ@mail.milmice and guinea pigs where the researchers compared aerosol deliver of EEEV from a Collison nebulizer to that of a spinning-top aerosol generator that generates a slightly larger aerosol particle of >6.0 µm (PMID: 19852871). They found no difference in the overall virulence of EEEV between the two particle sizes. Here, we examined if the delivery of EEEV in large aerosol particles (>30 µm) altered pathogenesis of EEEV in nonhuman primates, as particles emitted from a BWT attack would likely originate from a large-particle generator, such as a crop duster. To generate a large particle, we utilized the intranasal Mucosal Atomization Device (MAD), which generates aerosol particles between 30 and 100 µm. Five cynomolgus macaques (Macaca fascicularis) were exposed to 1E8 PFU of EEEV V105-00210 per nare using the MAD device and monitored for clinical signs of disease. Four of the five animals became moribund and were humanly euthanized between days 5 and 7 post-exposure, consistent with the mean time to death of NHPs exposed to EEEV by small-particle generating Collison nebulizer. Interestingly, preliminary results show that all five of the animals exposed to EEEV with the MAD device had a spike in WBCs at 3 days post-exposure, which returned to normal levels, except for the one survivor who had a an additional spike in WBCs, at end of study. This is in contrast to animals who were exposed to EEEV using the Collison nebulizer in which moribund animals had an increase in WBCs 1-3 days before humane euthanasia. Overall, our data suggest that EEEV exposure delivered by either a small-particle or large-particle generator results in a lethal disease. Analyses of hematology and viremia data are ongoing to determine any additional differences in pathogenesis between the two particle delivery sizes. Understanding how small- versus large- particle aerosol exposure influences EEEV disease is important because in the event of a BWT attack either particle size delivery device could be utilized. Ensuring MCMs are available that are effective against both small- and large- particle exposures is imperative to protect the warfighter.