**Development of Venezuelan Equine Encephalitis Bioaerosol Models in Mice and Marmosets for Use in Therapeutic Evaluation**

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**ABSTRACT**

Objective: Venezuelan equine encephalitis is a potentially re-emerging pathogen that has been responsible for several epizootics and epidemics. Most notably is a major outbreak in 1995 affecting Venezuela and Colombia resulting in tens of thousands of human cases and 26 reported deaths. Venezuelan equine encephalitis virus (VEEV) is an NAIAD Category B priority pathogen and of significant interest due to its high infectivity when aerosolized, its historical development as a bioweapon and its potential abuse as a bio-terrorist or bio-warfare agent. Our objective was to develop and characterize murine and marmoset models for aerosolized VEEV using an efficient bioaerosolization system for evaluation of therapeutics and vaccines.

Methods: VEEV challenge was performed with IITRI's bioaerosol system that utilizes Pari LC Plus nebulizers operating at 20 psi for 10 minutes. Determination of spray factor and assessment of aerosol consistency were accomplished through aerosolization of serally-diluted VEEV at multiple concentrations ranging from 10¹⁰ to 10¹² PFU/mL. The spray factor, calculated over multiple runs at different concentrations was calculated to be 2.3±0.5.

Results: Inoculum parameters and lethality of the challenge material were determined with Swiss Webster mice exposed to estimated inhalated doses of 0, 10, 100, and 1000 PFU/animal. Using a 10 day study, LD₅₀ inhalaed dose was determined to be 51 PFU.

Conclusion: We conclude that the lethal VEEV mouse model is likely a useful model for early screening of medical countermeasures against VEEV infection. Non-human primate VEEV models have been shown to recapitulate human clinical disease. Therefore, future work will evaluate the progression of clinical disease in marmosets exposed to aerosolized VEEV.

**METHODS**

Virus: Venezuelan equine encephalitis virus, strain Trinidad donkey (VEEV TID) was obtained through the NIH Biodefense and Emerging Infections Research Resources Repository, NIAID, NIH: VEEV (TID); NR-332.

Mouse Experimental Design: Mice, Female C3H/HeJ mice from Charles River (Wilmington, MA) were divided into four groups of 10 mice each. Mice were challenged via the aerosol route of exposure with four challenge dosages of 1,000, 100, 10 and 0 PFU. Mice were observed twice daily for survival over the 10 day post-challenge observation period and body weights collected three times per week.

Mouse Inhalation Exposure: Female C3H/HeJ mice were challenged using the IITRI bioaerosol system which utilizes a 64-port, flow-past, nose-only inhalation exposure chamber (Lab Products Inc., Seaford, DE) and six Pari LC Plus jet nebulizers (Pari, Germany) running concurrently during the exposure at 20 PSI. Viable aerosol material was determined by two glass impingers connected in series containing 10 mL of DMEM.

Marmosets Experimental Design: Marmosets (World Wide Primates, Miami, FL) were divided into two groups of two (one male, one female per group). Under ketamine anesthesia animals were challenged with VEEV via aerosol exposure with two different challenge dosages of 1 x 10⁷ and 1 x 10⁶ PFU and were then monitored for weight loss, changes in body temperature and clinical scores for 14 days. Viral titer of blood collected on days 2, 4, 6 and 8 after challenge was determined by qPCR.

Marmoset Exposure Inhalation Exposure: Male and female marmosets were exposed to aerosolized VEEV (1-3 µm MMAD) that was generated by a single Pari LC Plus nebulizer operating at 20 PSI via a head only exposure box. The respiration rate was determined via whole body plethysmography (Buxco Max II plethysmograph system) immediately preceding the exposure. Intake air was HEPA filtered and the exhaust passed through a medical-grade HEPA filter and merged with the ABSL3 HEPA filtered air handling system prior to exiting the building. Viable aerosol material was determined by a glass impinge set connected in series containing a total of 20 mL of DMEM.

Spray Factors: Mouse inhalation exposure spray factor, 2.3 x 10⁻¹; marmoset inhalation exposure spray factor 1.4 x 10⁻¹.

**RESULTS**

**MOUSE ANIMAL MODEL**

![Figure 1a, Mouse Body Weights Post Exposure](image)

**Effect of challenge on relative body weight. Mice were challenged with VEEV (TID) and body weights were monitored for 10 days post-challenge. N=10 per group.**

![Figure 1b, Mouse Survival Curve](image)

**Survival of mice challenged with different doses of VEEV (TID), determined using Kaplan-Meier analysis and GraphPad Prism 6.0. N=10 per group.**

**MARMOSET ANIMAL MODEL**

![Figure 2, Marmoset Inhalation Experimental Setup](image)

**Effect of challenge on body temperature. Marmosets were challenged with VEEV (TID) and body temperatures were monitored 2/day via transponder (BioMedic data systems, Seaford, DE) implanted subcutaneously in each marmoset. Each line represents an individual animal.**

![Figure 3a, Marmoset Body Temperatures Post Exposure](image)

**SUMMARY AND CONCLUSIONS**

- In the mouse, exposure to VEEV resulted in a loss in body weight along with increased lethality.
- The VEEV TID disease that follows aerosol exposure in mice supports the use of the VEEV mouse lethal model for vaccine or therapeutic efficacy studies.
- In the marmoset, the VEEV TID is infectious by aerosol and leads to clinical disease, including loss of body weight and fever, hypothermia and hypoxia.
- The VEEV TID disease that follows aerosol exposure in marmosets resembles that reported for human exposures to VEEV, making the marmoset a useful model for vaccine or therapeutic efficacy studies.