The Murine Model for Hantaan virus-Induced Lethal Disease Shows Two Distinct Paths in Viral Evolutionary Trajectory with or without Ribavirin Treatment. Dong-Hoon Chung¹², Åke Västermark³, Jeremy V. Camp¹, Carl Bruder², Susanna K. Remold⁴, Yong-Kyu Chu², Punya Mardhanan², Ryan McAllister⁵, and Colleen B. Jonsson¹²⁵* Department of Microbiology and Immunology, University of Louisville, KY 40202¹; Center for Predictive Medicine for Biodefense and Emerging Infectious Diseases, University of Louisville, Louisville, KY 40202²; Institutionen för Neurovetenskap, BMC, Box 593, 751 24 Uppsala, Sweden³; Department of Biology, University of Louisville, KY 40202⁴; Department of Pharmacology and Toxicology, University of Louisville, KY 40202⁵

In vitro, ribavirin acts as a lethal mutagen in Hantaan virus (HTNV)-infected Vero E6 cells resulting in increased mutation load and viral population extinction. Herein, we asked whether ribavirin treatment in the lethal, suckling mouse model of HTNV would act similarly. HTNV genomic RNA (vRNA) copy number and infectious virus were measured in lungs of mice in untreated and ribavirin-treated mice. In untreated, HTNV-infected mice, vRNA increased to 10 days post-infection (DPI) and thereafter remained constant to 26 DPI. Surprisingly, in ribavirin-treated, HTNV-infected mice, vRNA levels were similar to untreated between 10 and 26 DPI. Infectious virus levels, however, differed; in ribavirin-treated mice, infectious HTNV was significantly decreased relative to untreated mice, suggesting that ribavirin reduced the fitness of the virus (infectious virus produced per vRNA copy). Mutational analysis revealed a similar ribavirin-associated elevation in mutation frequency in HTNV vRNA as was previously reported in vitro. Codon-based analyses of rates of nonsynonymous (dN) and synonymous (dS) substitutions in the S-segment revealed a positive selection on amino acids in the HTNV N protein in the ribavirin-treated vRNA population. In contrast, the vRNA population in untreated HTNV-infected mice showed a lower diversity reflecting purifying selection for the wild-type genome. In summary, these experiments show two different evolutionary paths that Hantavirus may take during infection of a lethal murine model of disease, and the importance of the in vivo host environment in the evolution of the virus that is not apparent in our prior in vitro model system.