

## The Mousepox Model for Testing Antivirals

**Background:** Ectromelia virus is the causative agent of mousepox, an acute exanthematous disease of mouse colonies in Europe, Japan, China, and the USA. The natural reservoir for ECTV is unknown but one report provides evidence that wild mice may be involved. Laboratory studies have shown ECTV to have a very narrow host range, infecting only certain mouse species. A number of different strains of ECTV have been isolated which have been shown to differ in their virulence for the mouse. The Moscow, Hampstead, and NIH79 strains are the most thoroughly studied with the Moscow strain being the most infectious and virulent for the mouse. In the late 1940's mousepox was proposed as a model for the study of the pathogenesis of smallpox and generalized vaccinia in humans. Studies in the last five decades from a succession of investigators have resulted in a detailed description of the virologic and pathologic disease course in genetically susceptible (A, BALB/c, DBA/2, and C3H/He; death ~7 days post-infection) and resistant (C57BL/6 and AKR) inbred and out-bred mice; identification and characterization of the important cell-mediated and innate responses for recovery from infection; and the discovery of *rmp-1*, *rmp-2*, *rmp-3* and *rmp-4* loci which govern resistance to severe mousepox. Varying mouse genotype, virus strain, and dose of virus result in distinct disease patterns for a given route of infection.

**Mousepox, a Small Animal Model for Smallpox:** Mousepox differs from smallpox in at least two features following a respiratory tract infection. First, the disease course in mousepox is shorter as compared to smallpox. The eclipse period in mousepox and smallpox are 6 and 10 days, respectively. Fatal cases of mousepox usually occur 7 to 14 days post-infection (p.i.), whereas deaths in ordinary smallpox occur from ~18 to 22 days p.i. Second, the major lesions in mousepox are observed in the liver and spleen, whereas these organs are relatively uninvolved in smallpox. A feature of mousepox that is similar to smallpox is the relatively small dose of virus that is required to initiate disease in the upper and lower respiratory tract (Tables 1 and 2). Another similarity is the detection of virus in respiratory gases during the preexanthem period. And finally both diseases present with a characteristic exanthematous rash. In the case of mousepox, the development of rash is dependent on a number of parameters including mouse strain, virus strain, route of inoculation and virus dose.

**Antiviral Efficacy Testing:** An important use of the mousepox model is the evaluation of orthopoxvirus antivirals and vaccines. The ECTV aerosol model provides a broad dynamic range for evaluating antivirals. One can choose an aerosol lethal dose of 100 PFU, which is ~3-fold greater than the LD<sub>50</sub> value of 32 PFU (Table 2), and is likely in the range of the infectious dose for aerosolized smallpox as inferred from the Meschede Hospital incident and the 1971 smallpox epidemic in Aralsk, Kazakhstan. Alternatively, one could use a dose 1000 to 10,000 times the LD<sub>50</sub> to fully examine the robustness of the test antiviral.

Table 1. Intranasal Ectromelia Virus Infection of A/NCR mice

<b>Virus Dose (PFU/mouse)<sup>1</sup></b>	<b>Mean time to Death</b>	<b>Mortality</b>	<b>LD<sub>50</sub> (PFU)</b>
5,000	7±0.0	100%	0.3
500	7.3±0.5	100%	
50	8±0.0	100%	
5.0	9±0.0	100%	
0.5	10±1.4	50%	
0.05	10	25%	

<sup>1</sup>Mice were anesthetized by an intraperitoneal injection of a mixture of ketamine (90 mg/kg)/xylazine (10mg/kg). The mouse was held at a 45° angle and 3-5 µl of virus suspension was dispensed into one of the nares followed by a 10 min incubation to allow for complete aspiration of the drop. A second dose was then applied to the other nares followed by an additional 10 min aspiration period.

Table 2. Aerosol Ectromelia Virus Infection of A/NCR mice

<b>Presented Dose (PFU/mouse)<sup>1</sup></b>	<b>Mean time to Death</b>	<b>Mortality</b>	<b>LD<sub>50</sub> (PFU)</b>
1.9 x 10 <sup>4</sup>	8.1±0.4	100%	32
1.3 x 10 <sup>3</sup>	9.3±0.5	100%	
63	10.2±1.3	67%	
6.3	14	9%	
0	N/A	0%	

<sup>1</sup> Eight mice were exposed to the indicated presented dose of a small particle aerosol in a Nose-only Inhalation Exposure System (CH Technologies, Inc). Mice were observed for a period of 21 days.