

**Institute for Antiviral Research**  
**Utah State University**  
**ANIMAL MODELS FOR ANTIVIRAL STUDIES**  
**SUPPORTED BY THE**  
**VIROLOGY AND HEPATITIS BRANCHES, NIAID, NIH**

Utah State University currently has contracts from the NIAID, NIH to use animal models for evaluating antiviral agents against the following diseases:

- Influenza
- Smallpox
- SARS
- Hepatitis B
- Diseases induced by members of the Bunyaviridae family of viruses (e.g., Rift Valley fever, sandfly fever, Crimean-Congo hemorrhagic fever, hemorrhagic fever with renal syndrome, hantavirus pulmonary syndrome) utilizing the related Punta Toro virus
- Diseases induced by members of the Arenaviridae family of viruses (e.g., Lassa fever, Argentine hemorrhagic fever) utilizing the related Pichinde virus
- Diseases induced by the Togaviridae family of viruses (e.g., eastern, western, or Venezuelan equine encephalitis[VEE]) using VEE virus and the related Semliki Forest virus
- Diseases induced by the Flaviviridae family (e.g., yellow fever, tick-borne encephalitis, West Nile, dengue, hepatitis C) using yellow fever, West Nile, and the related Banzi viruses.

In addition, an animal model is being used to study agents to treat prion disease (e.g., transmissible spongiform encephalopathies) using the scrapie prion.

The following describes the animal models used and the disease parameters which can be evaluated in the model.

**A. Influenza**

The influenza animal model consists of an infection of laboratory mice with various strains of influenza A (H1N1, H3N2, H5N1) and B viruses, with the employment of several parameters to measure disease severity. The parameters which may be used, at the discretion of our Project Officer, are the following:

- a) Monitoring of blood oxygen saturation (SaO<sub>2</sub>) levels in live animals at frequent intervals utilizing pulse oximetry.
- b) Measuring of infectious pulmonary virus titers using in vitro endpoint dilution assay of homogenates of lungs taken at designated intervals during the infection.

- c) Assay of the degree of pulmonary consolidation using lungs taken in b), as determined both by score of lung discoloration and by weight of the lung.
- d) Death of the animal due to viral pneumonia.
- e) Mean survival time of the animals.
- f) Selected histopathological analysis of lung sections.

Where appropriate, studies will be run to determine the development of viruses resistant to significant antiviral drugs.

## **B. Smallpox**

The smallpox animal model is an intranasal infection of laboratory mice by the cowpox and vaccinia viruses, which induce an infection of the nose and lungs resulting in a smallpox-like toxemia-associated death. Parameters used in evaluating test agents in this model include:

- a) Death of the animal.
- b) Mean survival time of the animals.
- c) Lung and nose virus titers.
- d) Host weight loss.

Other parameters currently under study include:

- a) Monitoring SaO<sub>2</sub> levels.
- b) Assay of degree of pulmonary consolidation both by lung score and lung weight increase.
- c) Selected histopathological analysis of lungs and other organs.

Also utilized is a cutaneous infection in immunocompromised hairless mice can be induced by vaccinia virus. This infection is progressive and leads to death of the mice. It is now also being used in selected antiviral experiments. Parameters used in evaluating test agents in this cutaneous infection model include:

- a) Death of the animal.
- b) Severity score in initially induced lesions.
- c) Size of initially induced lesions.
- d) Number of spontaneous "satellite" lesions.
- d) Virus titer in various organs in the animal

## **C. Severe Acute Respiratory Syndrome (SARS)**

The SARS virus animal model utilizes weanling mice infected intranasally with the virus. A moderate lung infection is manifested by occasional lung hemorrhaging but primarily by infectious virus recovered from the lungs. Inhibition of development of virus in the lungs of the mice is used as parameters for evaluation of test agents.

## **D. Hepatitis B**

Two hepatitis B virus animal models are used: 1) The hepatitis B virus animal model uses transgenic HBV mice obtained from Dr. Francis A. Chisari (Scripps Research Institute, La Jolla, CA). They were derived from founder 1.3.32. The animals replicate high levels of hepatitis B virus in their liver, kidneys, and blood. 2) Non-transgenic mice that express HBV following hydrodynamic injection with an infectious clone of HBV. Experimental protocols include efficacy, optimal dosage, pharmacokinetic, safety/toxicity and combination studies. The following assays are used with these mouse models to screen candidate drugs:

- a) Southern analysis to measure HBV DNA in the liver.
- b) Quantitative reverse transcriptase PCR (qRT-PCR) to measure HBV RNA in liver.
- c) Immunoassays to measure hepatitis e antigen (HBeAg) and HBV surface antigen (HBsAg) in the serum.
- d) Immunohistochemistry to measure HBV antigens in the liver.
- e) Quantitative PCR (qPCR) to measure serum HBV DNA.
- f) Automated chemistry panel (ALT, BUN, creatinine, total bilirubin, albumin, alkaline phosphatase, globulin, glucose, Na<sup>+</sup>, K<sup>+</sup>, phosphorous, total protein) and complete blood count (CBC) are used to measure overall health, liver and kidney functions.
- g) Gross- and microscopic pathological examinations are performed as needed.

## **E. Bunyaviridae Virus Diseases**

The Punta Toro virus infection is achieved in C57BL/6 mice and in Syrian golden hamsters, with a generalized disease resembling that induced by Rift Valley fever. Parameters used for antiviral testing include:

- a) Death of the animal.
- b) Hepatic icterus, seen as yellowed liver.
- c) Elevated ALT levels in serum.
- d) Virus titers in liver and serum.
- e) Host weight loss.

## **F. Arenaviridae Virus Diseases**

The Pichinde virus model utilizes Syrian golden hamsters. Parameters used for antiviral testing include:

- a) Death of the animal.
- b) Virus titers in brain, liver, spleen and serum.
- c) Elevated ALT levels in serum.

## **G. Togaviridae Virus Diseases**

The VEE virus animal model utilizes the TC-83 vaccine strain of virus administered intranasally to C3H/Hen mice; the virus progresses to the central nervous system causing high virus titers in the brain and death of the animal. The Semliki Forest virus model is very similar to

that described above for the Banzi virus, with the same disease parameters. The Semliki Forest virus is a BSL-3-rated pathogen which requires special handling. Parameters for evaluation include:

- a) Death of the animal.
- b) Prolongation in mean day to death.
- c) Virus titers in the brains.
- d) Host weight loss.

## **H. Flaviviridae Virus Diseases**

The West Nile virus animal model currently utilizes both mice and hamsters. In each, neurological signs are produced, leading to eventual death of the animals. Virus is recovered from various tissues. Other parameters such as functional abilities are under study. This virus is a BSL-3-rated pathogen which requires special facilities and handling to assure safety for those working with it. Disease parameters used for antiviral evaluation include:

- a) Death of the animal.
- b) Prolongation in mean day to death.
- c) Virus titers in the brain and other tissues.
- d) Host weight loss.

The Banzi virus model is induced in intraperitoneally injected BALB/C mice; full details of the viral pathogenesis are being studied. To date, disease parameters used for antiviral evaluation include:

- a) Death of the animal.
- b) Prolongation in mean day to death.
- c) Virus titers in the brain.
- d) Virus titers in the serum taken early in the infection.
- e) Host weight loss.

## **I. Prion Disease**

The prion transgenic mouse model utilizes knockout mice for endogenous mouse PrP<sup>sen</sup>. These express high levels of hamster PrP<sup>sen</sup> in a wide range of tissues, including the brain. The animals infected with hamster scrapie agent replace the Syrian hamster model. The latter animals require approximately 120 days to die of the scrapie infection, whereas the prion transgenic mice die in approximately 82 days when infected with the same agent. Death is used as the parameter for anti-prion evaluation.

## **J. Follow-up Determinations**

Follow-up determinations of promising antivirals seen in the original animal studies may include effect of the administered test substances on key immunologic components in infected and in uninfected (toxicity control) mice. The immunologic effects studied include:

- a) Cytotoxic T lymphocyte (CTL) activity.

- b) Natural killer (NK) cell activity.
- c) Total T, T-helper, T-suppressor/cytotoxic, and B cell enumeration.
- d) Response to the T-cell mitogen, phytohemagglutinin (PHA).
- e) Production of interferon (IFN).
- f) Production of neutralizing antibody.

Where appropriate, studies will be run to determine the development of viruses resistant to significant antiviral drugs.

#### **K. Toxicity Determinations**

One or more toxicity determinations will be performed in all experiments on the test substances under evaluation. These determinations are:

- a) Lethality.
- b) Host weight loss or failure to gain weight.

As needed and where applicable, the following additional parameters will be studied:

- a) Increase in circulating serum levels of glutamic oxalic acid transaminase (SGOT) and pyruvic acid transaminase (SGPT) in the serum as markers for possible liver damage.
- b) Increase in circulating creatinine (CT) level as indicator of possible renal impairment.
- c) Increase in circulating creatinine phosphokinase (CK) levels as indicator of general tissue damage.

#### **L. Other Studies.**

When appropriate, studies will be conducted to evaluate novel strategies for drug delivery and dosing, including sequential therapy, oral treatment, aerosol delivery, therapeutic index determinations, and combination drug dosing.

Various studies will be done to further develop and define all animal models used in these experiments.

