Guidelines for Handling Animal Reservoirs of Hantavirus: Research and Educational Practices for Washington State University

PURPOSE

These guidelines are based on practices used by Center for Disease Control (CDC) personnel in areas known to have human cases of hantavirus infection. The guidelines have been modified for fieldwork in areas of undefined risk. They are intended to give you information about the best work practices to provide protection against hantavirus infection; the purpose of the document is to give investigators guidance about fieldwork and laboratory practices. The Department of Environmental Health and Safety (EH&S) is available to work with you in adapting the guidelines to your own fieldwork to help you protect yourself. Please contact EH&S or the Office of the Campus Veterinarian if you have comments or suggestions about the guidelines.

The guidelines will be reissued as additional information about fieldwork practices and about the epidemiology of hantavirus becomes available. In Washington at this time, the guidelines apply to handling deer mice, *Peromyscus maniculatus*, as well as the following species not native to Washington State: the white-footed mouse (Peromyscus leucopus), the cotton rat (*Sigmodon hispidus*), and the rice rat (*Orgonys palustris*). Other species may be included in the future if they are shown to be sources of human infection with viruses causing hantavirus pulmonary syndrome.

BACKGROUND

Hantaviruses are serologically related viruses of the family *Bunyaviridae*, including at least 14 different viruses. Old World rodents carry hantaviruses causing hemorrhagic fever with renal syndrome (HFRS), which is not addressed in this policy. Some New World rodents carry hantaviruses which cause hantavirus pulmonary syndrome (HPS), which is of particular concern due to its high case-fatality rate of greater than 40%. The most common cause of HPS is the *Sin Nombre* virus (SNV), also called *Four Corners* virus or *Muerto Canyon* virus, which is predominantly carried by deer mice, *Peromyscus maniculatus*. About 5 to 10 percent of deer mice tested in the western United States have antibodies to this virus. (This may be an overestimate of the true prevalence of hantaviruses, since most testing is done in areas where human exposure has occurred.) While all hantaviruses have apparently evolved to have one preferred rodent host, evidence of SNV has been found in other rodent species as well, including brush mice (*Peromyscus boylii*), pinion mice (*Peromyscus traei*), and western chipmunks (*Tamias species*). Other small mammals may also be infected, although to date the CDC has found evidence of infection only in the desert cottontail (*Sylvilagus auduboni*). Since there has been no evidence of hantavirus zoonosis originating from non-rodents, it is presumed that most non-rodents are "dead-end" hosts which shed little virus and are unlikely to infect people. Nosocomial transmission of
hantavirus pulmonary syndrome has not been documented in the United States, despite some cases of
needle stick exposure and mouth to mouth resuscitation attempts. Person to person transmission did
occur in Argentina in 1997. It is not known whether this represents a rare virus-host interaction of all
hantaviruses or a feature specific to the Argentine strain. Arthropod vectors have not been implicated in
hantavirus transmission.

Other HPS causing hantaviruses in North America include the New York-1 strain carried by the white-
footed mouse (Peromyscus leucopus), Black Creek Canal virus carried by the cotton rat (Sigmodon
hispidus), and Bayou virus carried by the rice rat (Oryzomys palustris). None of these reservoir hosts
species range into states west of Montana, and only a few cases of HPS are attributed to these strains of
hantavirus.

North American hantaviruses with no known association to human disease include the Prospect Hill virus
carried by the meadow vole (Microtus pennsylvanicus), El Moro Canyon virus carried by the western
harvest mouse (Reithrodontomys megalotis), Isla Vista virus carried by the California vole (Microtus
californicus), and Bloodland Lake virus carried by the prairie vole (Microtus ochrogaster). It is not known
whether lack of human disease associated with these hantaviruses is attributable to differing virulence or
to lower population density and lesser propensity for human contact by their reservoir host species.

Hantaviruses do not cause apparent illness in their reservoir hosts. Host animals remain infected for life,
carrying the virus in their blood and organs and shedding virus in saliva, feces, and urine. Duration of
shedding and the periods of maximum infectivity are not known. Aerosols from infective rodent excreta or
saliva, as well as rodent bites, have been implicated in transmission of hantaviruses to humans. Visitors
to laboratories housing infected rodents have become infected after about five minutes of exposure. The
highest risk appears to be associated with exposure to large amounts of rodent excreta in an enclosed
area, especially when the material is aerosolized by activity such as sweeping. With the current
knowledge about hantavirus transmission to humans, it is difficult to assess the degree of risk due to
exposure to a single infected rodent.

The incubation period for hantavirus is between 6 days and 5 weeks, with an average of about 2 to 3
weeks. The prodromal phase of HPS is difficult to distinguish from other viral diseases. Symptoms include
fever, myalgia, headache, chills, dizziness, non-productive cough, nausea, vomiting, and other
gastrointestinal symptoms, and sometimes shortness of breath. Cough and tachypnea usually develop by
the seventh day, followed by rapid progression to pulmonary edema and hypoxia.

GENERAL PRACTICES FOR FIELD STUDIES

It is suggested that undergraduate classes be accompanied by a well-trained staff member or graduate
student when engaged in field work that may lead to exposure to deer mice. Undergraduate students
should be cautioned to remain upwind, and allowed to handle traps only after inspection to determine that
they are free of deer mice. Using pitfall traps which are more likely to capture shrews and voles, rather
than a preponderance of deer mice, is recommended. Traps which did capture deer mice should be
handled by trained personnel, wearing the proper protective equipment and following the proper
procedures as outlined below. More detailed practical advice regarding adaptations of these procedures
for educational purposes may be obtained by consulting Dr. Terry Yates at the University of New Mexico
(see references).

• Workers in potentially high-risk settings should receive a thorough orientation about hantavirus
transmission and the symptoms of the disease. They should be given detailed guidance on
prevention measures and trained to safely perform the required activities.
• Practice good personal hygiene at all times. Wash hands with soap and water or with a disinfectant wipe before eating, drinking, smoking, or applying lip balm, sunscreen or cosmetics.

A baseline serum sample is recommended for workers in high risk settings.

• Workers who develop febrile or respiratory illness within 45 days of potential exposure should immediately seek medical attention and inform the physician of the occupational risk of hantavirus infection. The physician should contact local health authorities promptly if hantavirus-associated illness is suspected. If appropriate, a blood sample should be sent with available baseline sample to a laboratory for hantavirus antibody testing.

• Hantaviruses are lipid-enveloped viruses and are susceptible to most disinfectants, including dilute hypochlorite solutions (bleach), 70% alcohol, detergents, phenolics, and most general-purpose household disinfectants. The survival time of the virus in the environment in liquids, aerosols, or dried states is not known. In the field, carry a spray bottle of disinfectant or hand-wipes containing alcohol or detergent.

• Workers should wear rubber, plastic, or latex gloves when handling rodents or traps contaminated by rodents or whenever the worker has broken skin. Before removing the gloves, wash gloved hands in a disinfectant and then in soap and water. Thoroughly wash hands with soap and water after removing gloves. If this is not possible, then rinse gloves with water or use a disinfectant wipe; wash your hands thoroughly at end of the work period.

• Workers should wear EH&S-approved respirators when handling field-caught rodents or contaminated traps or when disturbing rodent burrows and nests. Contact EH&S for an evaluation of your work practices and for information about the Respiratory Protection Program. Until the infectivity of hantavirus is better understood, respirators should be used to minimize exposure to airborne particles of rodent excreta during procedures that generate aerosols. The proper use of respirators will provide protection against airborne particles of rodent excreta, which is the presumed cause of most hantavirus infections. However, the incorrect use or care of respirators may increase, rather than decrease, risk of exposure to harmful agents. Additionally, respirators must be used only under the guidance of the Respiratory Protection Program, conducted by EH&S, which includes fit-tests, training and medical use determinations.

• Disinfect all traps contaminated by rodent urine or feces or in which a rodent was captured. If this is not done until the end of the trapping run, wear a respirator whenever handling contaminated traps, and transport empty traps in closed plastic bags.

• Dispose of dead rodents by placing the carcasses in a plastic biohazard bag containing enough disinfectant to thoroughly wet the carcasses. Seal the bag and dispose of it by incineration at the WSU incinerator. (Refer to WSU Safety Policies and Procedures Manual, (SPPM) Section S80.12, Disposal of Biohazardous Waste.)

• Workers performing procedures with a high risk of contacting animal body fluids or creating aerosols, such as removing organs or obtaining blood from rodents in an affected area, should contact EH&S or the Washington State Department of Health for detailed safety precautions.

• Do not enter enclosed spaces or buildings known to be contaminated with rodents or rodent droppings. Contact the facility manager or EH&S for assistance.
SPECIFIC PRACTICES FOR FIELD STUDIES

A. VISUAL SURVEY OF AREA, WALKING, HIKING

No special precautions are needed for protection against hantavirus infection.

However, respiratory protection is advisable in a known affected area that is visually contaminated by rodents or has especially dusty conditions.

B. SETTING TRAP LINES

When setting disinfected traps, no special precautions are needed for protection against hantavirus. Respiratory protection is recommended if the traps have not been disinfected from prior use.

C. RECOVERY AND TRANSPORT OF TRAPS HOLDING LIVE ANIMALS

Wear protective clothing, including rubber or latex gloves. If using open-mesh traps, wear respiratory protection. Eye protection is recommended. Proper eye protection in this case may be achieved through the use of a full facepiece respirator, or a non-vented chemical-splash-type goggle which may be worn in conjunction with a half-facepiece respirator. Please note that if a half-facepiece respirator is worn, and the intent is that the employee/student will receive eye protection for field or laboratory work, the eye protection must be worn at the same time the person is fit tested for respirator use. This is so the hygienist performing the fit test can be determined if the eye protective device will interfere with proper respirator fit. Also, note that there are wipe applications for full facepiece respirators or goggles to reduce or prevent fogging. This information can be provided to the user at the time of fit testing. Stand upwind of the trap if possible. Put the trap into a plastic bag that is at least 4 mm thick and large enough to ensure a sufficient supply of air for the animals. When transporting animals in an enclosed vehicle to a processing site, isolate the trapped animals from the passenger compartment if possible.

1. Handling Live Animals

   a. Wear protective clothing, including gloves, eye protection and respiratory protection. Use appropriate methods to provide protection against both bites and urine contamination of the hands. Leather gloves in combination with rubber or latex gloves are advisable.

   b. Define a zone to exclude others who are not wearing appropriate protective equipment. Work with the wind coming from your back if possible. Perform all procedures in a manner to minimize the creation of aerosols and dust.

   c. When possible, anesthetize the animal before handling. Remove captured animal from the trap by shaking it into an anesthesia bag; or alternatively, pinch the animal's skin through the mesh of the trap with forceps and inject it with an anesthetic.

   d. Wear gloves to disinfect contaminated traps. The ideal method is to submerge them in a bucket of disinfectant for 10 minutes, rinse twice with water, and set in the sun to dry. Alternatively, spray the traps with disinfectant. If traps are not to be disinfected until end of the project, store them in closed plastic bags.

2. Field Dissection
a. Field dissection is strongly discouraged. Instead, transport animals to a laboratory with appropriate containment equipment in order to process them under safer working conditions.

b. If field dissection is done, wear protective clothing, including rubber or similar gloves, eye protection, and respiratory protection. Surgical gowns, shoe covers, and head coverings are recommended.

c. Process animals in an isolated area. Use the minimum number of workers to do the job safely. Define and mark a zone to exclude others not directly involved in the animal dissection. Work with the wind coming from your back if possible.

d. Perform all procedures carefully to minimize the creation of aerosols. Use extreme caution with any contaminated sharp items, including needles, syringes, slides, pipettes, capillary tubes and scalpels.

e. Perform all procedures carefully to minimize the creation of aerosols. Use extreme caution with any contaminated sharp items, including needles, syringes, slides, pipettes, capillary tubes and scalpels. Substitute plastic for glass whenever possible. Sharps should be properly disposed of in accordance with the WSU SPPM, sections 80.13 and 80.14.

d. Perform all procedures carefully to minimize the creation of aerosols. Use extreme caution with any contaminated sharp items, including needles, syringes, slides, pipettes, capillary tubes and scalpels. Substitute plastic for glass whenever possible. Sharps should be properly disposed of in accordance with the WSU SPPM, sections 80.13 and 80.14.

e. Use hypodermic needles and syringes only for gavage, parenteral injection, or aspiration of fluids from diaphragm bottles or well-restrained animals. Use only needle-locking syringes or disposable syringe-needle units. Do not bend, shear, break, recap or otherwise manipulate needles by hand before disposal; place used disposable needles and other sharps in a conveniently located puncture-resistant container. Decontaminate the sharps container before disposal. Refer to SPM Sections S80.13 and S80.14 for the proper disposal procedures for sharps and glass. Place sharps in a hard-walled closable container, preferably containing a suitable disinfectant. Do not handle broken glassware directly; use mechanical means such as brush and dustpan, tongs, or forceps.

f. Place tissues or specimens of body fluids in a container that prevents leakage during collection, handling, processing, storage, transport, or shipping. Carcasses may be preserved and transported in 10% formalin. Freezing tissue, body fluids, or carcasses with dry ice or liquid nitrogen subjects virus to a state of animation which does not deactivate the virus. Caution should be used when handling formalin, dry ice or liquid nitrogen; proper procedures for these materials should be followed and protective equipment should be worn, especially for eye, hand and body surface.

g. Dispose of unwanted carcasses in a plastic biohazard bag containing enough disinfectant to thoroughly wet the carcasses; seal the bag and dispose of it by incineration at the WSU incinerator. (See SPPM, Section S80.12.)

3. Clean Up:

a. Place used instruments into disinfectant for 10 minutes. Decontaminate all wastes appropriately before disposal.
b. Remove protective clothing in a well-ventilated area (such as outside). Put clothing in plastic bags for disposal or laundering.

c. Wash hands thoroughly with soap and water.

Additional Precautions:

Establish practical and effective protocols for handling emergency situations.

GUIDELINES FOR BRINGING WILD RODENTS OR THEIR TISSUES INTO WSU FACILITIES

A. QUARANTINE

Wild live deer mice brought into any campus facility must be quarantined and tested for the presence of Sin Nombre Virus. Wild live white-footed mice, cotton rats, or rice rats must be quarantined and tested for the presence of the strain of HPS-producing hantavirus which they are known to carry.

This quarantine must be accomplished in a facility designed and managed to comply with Biosafety Level 3 requirements (Centers for Disease Control Classification). For a group of rodents to be considered as being free of HPS-producing hantavirus, the entire group must prove to be serologically negative on two successive tests at least 21 days apart. The first test must include both ELISA and PCR methods. Any animal testing positive for antibodies to HPS-producing hantavirus, along with any cage mates, must be euthanized.

B. HANTAVIRUS CONTAINMENT & DISPOSAL

Rodent carcasses known or suspected to harbor Sin Nombre virus or other HPS producing hantaviruses must be double bagged in the field, the bags placed in a labeled and sealed container, the exterior of the container disinfected, and the container opened only under appropriate conditions in a Biosafety Level 3 facility, or a Biosafety Level 2 facility using CDC Biosafety level 3 protocols. All such carcasses will be disposed of by incineration at the WSU incinerator. (See SPPM, Section S80.12)

C. UNPRESERVED CARCASSES

Persons wishing to bring unpreserved carcasses of euthanized, wild-trapped deer mice, white-footed mice, cotton rats, or rice rats to campus facilities must collect serum and/or post-mortem tissue (i.e., lung, liver, kidney, or spleen) samples for ELISA or PCR testing, respectively. The samples must be double bagged and placed in a labeled and sealed container with the exterior of the container disinfected. Samples should be removed and processed only using established procedures under BL-3 guidelines. After the samples are obtained, the remainder of the unpreserved carcasses should be double bagged, placed in a labeled and sealed container, the exterior of the container disinfected, and the container placed in a freezer at the University. The samples must test negative for Sin Nombre virus antibodies before anyone works with the carcasses, unless all subsequent work is performed in BL-3 conditions.

D. TISSUES TO BE FORMALIZED

Rodents and/or rodent tissues that are to be formalized, heat treated (60°C for at least 1 hour), or placed in some other preservative (e.g., ethyl alcohol) in the field do not need to be tested.
E. BASELINE SEROLOGICAL TEST

Personnel who will handle wild rodents, or their tissues or bodily fluids, should obtain a baseline serological test for the presence of *Sin Nombre* virus antibody or have serum stored for use as a baseline in case testing is needed.

CONTACT PERSONNEL

* EH&S: (509) 335-3041
Exposure questions and protective measures: Mike Kluzik, email: mkluzik@wsu.edu
Respiratory Protection Program: Dennis Sasse email: dsasse@wsu.edu

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*Idaho Dept. of Health and Welfare
Bureau of Laboratories
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Boise ID 83712 (208)334-2235 ext. 228

Idaho Dept of Health and Welfare must be contacted in advance. They will also provide a filter paper strip in individual test tubes for the samples. They could do a few batches of rodent's samples at $20.00 / test, and human blood samples at $15.00 / test.

*CDC does not do this service routinely: only as requested by a state health dept.
1-800-532-9929

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SELECTED REFERENCES


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Revised: January 29, 2007