Hantavirus Laboratory Information: Guidelines for Removing Organs or Obtaining Blood from Rodents Potentially Infected

CDC information on Hantavirus Pulmonary Syndrome.

These recommendations are a supplement to **Hantavirus Infection**— Southwestern United States: Interim Recommendations for Risk Reduction, published in the Morbidity and Mortality Weekly Report, July 30, 1993, Volume 42, Number RR-11, Pages i-13 (available upon request, 404-639-1510).

Persons contemplating trapping, handling, or performing necropsies on rodents in areas of hantavirus transmission should contact their state public health agency for local recommendations which may complement these guidelines. Local training, including an orientation about hantavirus transmission and specific safety procedures, is recommended. The references at the end of these guidelines provide background information concerning hantaviruses and hantavirus disease.

The preferred method of obtaining tissue specimens is to capture rodents in live-capture traps such as Sherman®1 or Tomahawk® traps. Workers checking and retrieving traps should wear protective clothing, including coveralls and thick rubber gloves. Traps containing rodents should be placed in double plastic bags and transported as quickly as possible to the processing site, keeping the animals out of direct sunlight to protect them from overheating. Gloved hands should be washed with soap and water or a suitable disinfectant. [Suitable disinfectants include 1% household bleach, 5% hospital type bulk Lysol® (National Laboratories, Lehn and Fink Industrial Products Division, Montvale, NJ 07645), or any EPA approved hospital grade disinfectant, used according to the manufacturer's instructions. 1% household bleach is an adequate surface disinfectant but 10% bleach is more effective for heavily soiled items or areas contaminated with rodent feces or nesting materials.] Hands should be thoroughly washed with soap and water immediately after removing gloves. Bagged traps should be transported in the back of a pickup truck or other compartment isolated from passenger sections.

The field processing site should be in a secluded area, away from other humans, livestock, or domestic animals. Table and other work surfaces, chairs, and floor should be of a non-porous material that can be easily disinfected and cleaned. If outdoors, workers should sit with the wind from behind at about a 45° angle; captured animals should be located downwind, and vehicles and equipment should be upwind. Workers should wear protective clothing, including a surgeon's gown or coveralls (preferably disposable), shoe covers, latex gloves (two pairs), and a half-face respirator and goggles or powered air-purifying respirator (equipped with high-efficiency particulate air filters). If a negative-pressure respirator is being used, the worker must have a pulmonary function test and proper fitting. Establishment of a comprehensive respiratory protection program, including proper fitting, pulmonary function test, and instruction on respirator care and use is required before using any respirator (Ref. 29CFR1910.134, OSHA Respiratory Protection Standard). Necessary guidance can be provided by the local health department or the Area Office of the Occupational Health and Safety Administration.

Animals in live-capture traps may be anesthetized by placing the entire trap into a plastic bag containing gauze or cotton soaked with an inhalant anesthetic such as methoxyflurane (Metofane®) or halothane. Alternatively, animals in Sherman® traps may be shaken into a clear plastic bag containing the

anesthetic. Technicians should take care to minimize inhaling anesthesia during these procedures. Blood samples should be obtained from the retro- orbital sinus of profoundly anesthetized animals by using a heparinized capillary tube or Pasteur pipette. Blood can be allowed to drip from the capillary tube into a 2-ml cryovial (e.g., Nunc®, Sarstedt®, Evergreen®). At least four or five drops of blood are required for laboratory testing. Needles should not be used for cardiac puncture because of the risk of needle- stick injury and infection. The capillary tube or pipette should be discarded in a disposable "sharps" container; no attempt should be made to eject the remaining blood from the capillary tube. The bleeding should then be stopped by pinching the rodent's eye closed with a gauze square, and the gloves, working surfaces, and outside of the vial should be cleaned, if necessary, using a suitable disinfectant (as described above) and paper towels. The anesthetized animal can be euthanized by cervical dislocation before necropsy.

Dissection scissors and forceps should be thoroughly cleaned and flamed over an alcohol burner before use or reuse. The ventral surface of the animal should be disinfected with alcohol and the peritoneal cavity opened using blunt-end dissection scissors. The required organs should be removed with sterile, blunt-end forceps (use of scissors is unnecessary) and placed in labeled 2-ml cryovials. A single pair of forceps may be used to take all organs from each animal. Forceps should not touch pelage or working surfaces to avoid contamination. Scissors should not be used to take organs after they have been used to cut through the skin and peritoneum. Cryovials containing specimens should be tightly closed, wiped with disinfectant, and immediately placed on dry ice or in liquid nitrogen. The gloves and working surface should be disinfected between animals, and clean, sterile instruments (forceps and scissors) should be used for each animal. Used instruments should be placed into a disinfectant bath. Instruments should remain in the disinfectant for the duration of the processing, or for a minimum of 15 minutes. Each instrument should then be carefully cleaned with a scrub brush under a fresh disinfectant solution and rinsed in clean water. The clean instruments should then be flamed prior to reuse. The carcasses should be placed into double plastic bags for later incineration. If they are to be submitted as voucher specimens to a museum, they should be tagged (animal field number, date of collection, and processor) and placed into a tight- sealing plastic container of 10% formalin for at least 48 hours before handling. After this time, carcasses should be placed in 70% alcohol for permanent storage. Tags should be made of 100% rag paper of about 110 lb. weight, and should be attached to the right hind leg above the heel, using a strong, white cotton mercerized thread, size 10 – 12. Data on tags should be written with permanent black India ink, or, if this is not available, a hard lead pencil (e.g., #4).

After all animals have been processed, all instruments and other items on the processing table should be wiped with disinfectant. Traps can be disinfected by placing each into a 5-gallon plastic container of disinfectant after the animal is removed for anesthesia. Bleach can be used but is not recommended for this purpose as it may corrode metal traps. Dirt and fecal material in traps can be removed with a toilet brush while the trap is submerged in the container. After approximately 10 minutes in the disinfectant, the traps should be passed through one or two containers of clear rinse water. Heavy rubber gloves should be worn over latex gloves while handling traps to avoid tearing the latex gloves on sharp trap surfaces. All working surfaces, tables, chairs, and floor should be cleaned thoroughly with disinfectant. Disposable

gowns, latex gloves, and contaminated trash should be placed into biohazard bags and disposed of in accordance with local requirements for infectious wastes. Finally, hands should be thoroughly washed with soap and water after protective clothing is removed. If coveralls or gowns worn during processing are not disposable, they should be laundered on site or immersed in liquid disinfectant until they can be washed. Potentially contaminated clothing should be laundered in hot water and detergent. (Use rubber gloves when handling the soiled clothing and wash gloved hands in a disinfectant or soap and water, then remove gloves and wash hands with soap and water.) Machine-dry laundry on a high setting or hang it to air dry in the sun.

As a less desirable alternative to taking tissues from live-captured animals, samples may be obtained from animals freshly killed in snap traps. In this case tissues should be taken within two hours after capture, or sooner if the ambient temperature is greater than 21°C (70°F). Procedures for taking organs are the same as described above. A small amount of blood may be obtained from freshly kill-trapped rodents by opening the thoracic cavity and cutting the heart open with sterile scissors. Blood may then be taken into a Pasteur pipette with rubber bulb or micro-pipetter with disposable tip and expelled (carefully) into a labeled cryovial. Alternatively, blood may be wicked into labeled, calibrated filter-paper strips, dried, and shipped to the processing laboratory in plastic zip-lock bags contained in a sturdy box. Nobuto® filter strips for this purpose may be obtained from Microfiltration Systems, 6800 Sierra Court, Dublin, CA 94568, (510-828-6010). Directions provided with the strips should be followed.

In some situations, frozen whole carcasses may be used for hantavirus detection. Blood and tissues should be removed as explained for kill-trapped animals.

*Use of trade names is for identification only and does not imply endorsement by the Public Health Service or the U. S. Department of Health and Human Services.

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Laboratory Management of Agents Associated with Hantavirus Pulmonary Syndrome: Interim

Summary

This report provides interim biosafety guidelines for preventing laboratory-associated infections with agents that cause hantavirus pulmonary syndrome. The guidelines are based on extensive laboratory experience with the other hantaviruses– particularly work involving the use of permissive host animal species–and on the limited experience with a hantavirus recently isolated from deer mice. The guidelines address handling patient-derived specimens, propagating viruses in culture (including viral concentrate preparations), and housing and handling infected animals. These recommendations were developed with the assistance of expert consultants during a meeting of the American Society of Tropical Medicine and Hygiene, Subcommittee on Arbovirus Laboratory Safety, November 2, 1993, in Atlanta.

Introduction

One or more newly identified hantaviruses have been implicated as the cause of a new disease, hantavirus pulmonary syndrome (HPS) (1-3). HPS is characterized by a febrile prodrome, followed by the rapid onset of noncardiogenic pulmonary edema and hypotension or shock. More than half the identified patients have died. As of April 5, 1994, 68 cases had been reported from 17 states (4,5; CDC, unpublished data, 1994). Most of these cases have been associated with a single virus isolated from deer mice obtained in New Mexico (6); the deer mouse (Peromyscus maniculatus) is its principal reservoir (7). Three other new hantaviruses have been identified in the Americas. Two were inferred from genetic sequences detected by reverse transcriptase polymerase chain reaction (RT-PCR) in lung tissue from HPS patients. A third hantavirus was isolated from the cotton rat (Sigmodon hispidus) after antibodies and RT-PCR had identified a target rodent species. CDC recently published recommendations to assist residents of the endemic area in avoiding exposure to rodents (8).

Hantaviruses are a genus in the family Bunyaviridae, which are lipid-enveloped viruses with a negative-stranded RNA genome composed of three unique segments. Like other lipid-enveloped viruses, they are susceptible to most disinfectants (e.g., dilute hypochlorite solutions, phenolics, detergents, 70% alcohol, or most general-purpose household disinfectants) (9). The survival time of these viruses in the environment in liquids or aerosols or in a dried state is not known. Limited studies with Hantaan virus have shown sensitivity to a pH of less than or equal to 5. However, infectivity has been reported to persist in neutral solutions for several hours at 37 C (98.6ºF) and for several days at lower temperatures, as well as in dried cell-culture medium for up to 2 days (10; Huggins, unpublished data, 1994).

Human hantavirus infection has been associated most often with hemorrhagic fever with renal syndrome (HFRS). Several pathogenic viruses that have been recognized within the genus include Hantaan virus, which causes the most severe form of HFRS and is present primarily in Asia; Dobrava virus, which causes serious HFRS and has been identified in the Balkans; Puumala virus, which causes a milder form of HFRS and a higher proportion of subclinical infections and is prevalent in Europe; and Seoul virus, which results in a less severe form of HFRS when humans are infected and has a worldwide distribution.

Serious or fatal disease may follow infection with any of these viruses. The clinical consequences of infection with Prospect Hill virus, which has been identified in the United States, are unknown, but antibodies have been detected in humans who could not recall an illness typical of HFRS (11).

Each member of the genus is associated with a specific rodent host (e.g., the striped field mouse [Apodemus agrarius] for Hantaan virus; the urban sewer rat [Rattus norvegicus] for Seoul virus; and the meadow vole [Microtus pennsylvanicus] for Prospect Hill virus). Hantaviruses do not cause apparent illness in their reservoir hosts, which remain asymptomatically infected for life (12). Infected rodents shed virus in saliva, urine, and feces for many weeks, but the duration of shedding and the period of maximum infectivity are unknown (13). The demonstrated presence of infectious virus in the saliva of infected rodents, the sensitivity of these animals to parenteral inoculation with hantaviruses, and field observations of infected rodents indicate that biting is an important mode of rodent-to-rodent transmission (7,14).

Hantaviruses may be present in the blood, organs, saliva, feces, or urine of infected animals. In studies in the southwestern United States in 1993 (7), about one-third of trapped deer mice (P. maniculatus) had hantavirus antibodies. Viral RNA with hantavirus sequences was demonstrated by RT-PCR in the tissues of virtually all antibody-positive and some antibody-negative deer mice. Antibody prevalences and the proportions of animals tested that had viral RNA demonstrable by RT-PCR were lower in other species of rodents (1,7).

Human Infections

Aerosols from infective saliva or excreta of rodents have been clearly implicated in the transmission of hantaviruses to humans. Persons visiting animal holding areas in laboratories where infected rodents were housed have been infected after approximately 5 minutes of exposure (15-17). The relative importance of primary aerosols from freshly shed material compared with secondary aerosols from dried excreta in bedding or nests is not known. Similarly, the possibilities of infection associated with ingestion of food contaminated with the virus, contact with mucous membranes, or contamination of breaks in the skin barrier have not been clearly evaluated. However, humans have become infected as a result of rodent bites (18,19).

Most cases of human illness associated with hantaviruses have resulted from exposure to naturally infected wild rodents. Colonized laboratory rats also have been infected with Seoul virus, and animal colony employees and scientists working in disciplines other than microbiology (e.g., physiology and immunology) have become infected with Seoul virus after being exposed to these animals. Approximately 120 cases of Seoul virus infection transmitted from laboratory rats have been reported from Japan, and other instances of laboratory-acquired infection have been reported from Belgium and England (19-21). Arthropod vectors are not known to transmit hantaviruses (12,15). Person-to-person transmission has not been reported with any of the hantaviruses primarily associated with HFRS or with the recently identified cases of HPS in the United States.

The difficulty in assaying hantaviruses in material from human patients or wild-caught rodents has constrained efforts to measure concentrations of virus in environmental or clinical samples. Viral genetic

material has been detected by RT-PCR in whole blood, lymphocyte fractions, and occasionally in plasma from patients with acute cases of HPS (B. Hjelle, et al., unpublished data, 1994).

The consequences of infection with the currently recognized European-Asian hantaviruses in humans vary from subclinical seroconversions to severe HFRS. The overall mortality of HPS of 60% includes deaths among previously healthy young persons; subclinical infection appears to be uncommon.

In the southwestern United States, rodents occasionally act as hosts for the bacterium Yersinia pestis, the etiologic agent of plague. Although fleas and other ectoparasites are not known to transmit hantaviruses, rodent fleas do transmit plague. Rodent-feeding deer ticks may also transmit the etiologic agent for Lyme disease. Thus, persons who handle field-trapped rodents, rodent sera, rodent tissues, or traps contaminated with rodent excreta also should be aware of the risk for exposure to materials contaminated with hantaviruses and other disease agents.

Laboratory Hazards

Laboratory transmission of hantaviruses from rodents to humans via the aerosol route is well documented (15,16,20,21). Exposures to rodent excreta, fresh necropsy material, and animal bedding are presumed to be associated with risk. In animal holding areas, the period of exposure to infectious animal excreta required for transmission may be short (15-17,19-21). Other potential routes of laboratory infection include ingestion, contact of infectious materials with mucous membranes or broken skin, and, in particular, animal bites.

Four laboratory workers recently were infected while working with cell-culture-adapted Hantaan virus. Although the procedures associated with infection are unclear, all four persons worked repeatedly with hantavirus cultures and performed centrifugation of concentrated virus (C. Schmaljohn, unpublished data, 1994).

Extensive experience with the hantaviruses that cause HFRS indicates that infection has not been transmitted from patients or clinical laboratory specimens. Similarly, transmission has not been reported from patients with HPS or from related clinical laboratory samples. However, viral antigens have been detected in necropsy specimens, and RT-PCR readily detects viral genetic material (22). Viral RNA has been detected by RT-PCR in blood and plasma obtained early in the course of disease (B. Hjelle, et al., unpublished data, 1994). The implications of these findings for the infectivity of blood or tissues are unknown.

Recommended Precautions

The following recommended biosafety guidelines are based on information regarding known rodent-to-human transmission of hantaviruses, the potential for exposure to aerosolized virus under laboratory conditions, and the high mortality among patients infected with the recently identified U.S. virus (Tables 1 and 2). Biosafety guidelines for laboratories and animal facilities are described in detail in the CDC/ National Institutes of Health publication, Biosafety in Microbiological and Biomedical Laboratories (22), which specifies the combinations of facilities and safe work practices suitable for handling infectious microorganisms.

Precautions for Handling Specimens from Humans

On the basis of these guidelines, Biosafety Level 2 (BSL-2) facilities and BSL-2 practices are recommended for laboratory handling of sera from persons potentially infected with the agents of HPS (Table 1). CDC recommends that universal precautions be followed whenever human blood is handled. The use of a certified biological safety cabinet is recommended for all handling of human body fluids when potential exists for splatter or aerosol.

Precautions for Handling Tissue Samples and Viral Cultures

Potentially infected tissue samples should be handled in BSL-2 facilities in accordance with BSL-3 practices (Table 1). Cell-culture virus propagation should be carried out in BSL-3 containment facilities in accordance with BSL-3 practices. Large-scale growth of the virus, including preparing and handling viral concentrates, should be performed in BSL-4 containment facilities.

Precautions for Work with Host Species

Experimentally infected rodent species known not to excrete the virus can be housed in animal biosafety level 2 (ABSL-2) facilities in accordance with ABSL-2 practices (Table 2). Biological safety cabinets and other physical containment devices should be used whenever procedures with high potential for generating aerosols are conducted. Serum or tissue samples from potentially infected rodents should be handled in accordance with BSL-3 practices, although BSL-2 laboratories can be used.

Because of the virulent nature of the agents of HPS and because animal-to-human transmission of hantaviruses may readily occur, persons working with the natural host species should take special precautions. All work involving inoculation of virus-containing samples into P. maniculatus or other permissive species should be conducted at ABSL-4.

Conclusion These guidelines are based on the current knowledge of the agents of HPS. The recommendations outlined in Tables 1 and 2 will be reviewed and revised as new information becomes available. Any such revision will be included in the fourth edition of Biosafety in Microbiological and Biomedical Laboratories (22).

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Hantavirus Pulmonary Syndrome (HPS)

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