CDC
CENTERS FOR DISEASE CONTROL AND PREVENTION

May 13, 1994 / Vol. 43 / No. RR-7

MMWR

Recommendations and Reports

MORBIDITY AND MORTALITY WEEKLY REPORT

Laboratory Management of Agents Associated with Hantavirus Pulmonary Syndrome: Interim Biosafety Guidelines

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Centers for Disease Control
and Prevention (CDC)
Atlanta, Georgia 30333

The *MMWR* series of publications is published by the Epidemiology Program Office, Centers for Disease Control and Prevention (CDC), Public Health Service, U.S. Department of Health and Human Services, Atlanta, GA 30333.

SUGGESTED CITATION

Centers for Disease Control and Prevention. Laboratory management of agents associated with hantavirus pulmonary syndrome: interim biosafety guidelines. MMWR 1994;43(No. RR-7):[inclusive page numbers].

Copies can be purchased from Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20402-9325. Telephone: (202) 783-3238.

Contents

Single copies of the 3rd edition of *Biosafety in Microbiological and Biomedical Laboratories* can be obtained by contacting the Office of Health and Safety, CDC. Telephone: (404) 639-3883; FAX: (404) 639-2294.

Consultants*

Harvey Artsob, Ph.D.
Laboratory Center for Disease Control
Tunney's Pasture, Ottawa, Canada

Barry Beaty, Ph.D.
Colorado State University
Fort Collins, CO

Peter Gerone, Sc.D.
Delta Regional Primate Research Center
Covington, LA

Jean-Paul Gonzalez, Ph.D.
Institut Français de Recherche Scientifique en Cooperation
Yale School of Medicine
New Haven, CT

Peter Jahrling, Ph.D.
U.S. Army Medical Research Institute for Infectious Diseases
Frederick, MD

Michael Kiley, Ph.D.
National Institutes of Health
Bethesda, MD

James LeDuc, Ph.D.
World Health Organization
Geneva, Switzerland

Robert McKinney, Ph.D.
National Institutes of Health
Bethesda, MD

Robert Purcell, M.D.
National Institutes of Health
Bethesda, MD

Jean-François Saluzzo, Ph.D.
Pasteur Mérieux
Lyons, France

Walter Tabachnick, Ph.D.
U.S. Department of Agriculture
Laramie, WY

Robert Tesh, M.D.
Yale University
New Haven, CT

*Other consultants were selected guest editors of the CDC/National Institutes of Health publication *Biosafety in Microbiological and Biomedical Laboratories.*

The following CDC staff members prepared these recommendations:

Richard C. Knudsen, Ph.D.
Henry M. Mathews, Ph.D.
Jonathan Y. Richmond, Ph.D.
Office of Health and Safety

Robert B. Craven, M.D.
Julia S. Garner, R.N., M.N.
Nick Karabatsos, Ph.D.
Rima F. Khabbaz, M.D.
Thomas G. Ksiazek, D.V.M., Ph.D.
Goro Kuno, Ph.D.
Brian W.J. Mahy, Ph.D., Sc.D.
Clarence J. Peters, M.D.
Pierre E. Rollin, M.D.

Laboratory Management of Agents Associated with Hantavirus Pulmonary Syndrome: Interim Biosafety Guidelines

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 ≤5. However, infectivity has been reported to persist in neutral solutions for several hours at 37 C (98.6 F) 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 (*1*). 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*).

TABLE 1. Biosafety guidelines for agents of hantavirus pulmonary syndrome

Activity	Laboratory settings	Practices	Comments
Caring for patients with hantavirus pulmonary syndrome (HPS) or suspected hantavirus infection	N/A	N/A	Universal precautions apply. Additional protection may be necessary during aerosol-generating procedures. No evidence of nosocomial transmission has been reported to date.
Handling serum from potentially infected humans	BSL-2	BSL-2	Universal precautions apply. No evidence of laboratory-associated infections has been reported with other hantaviruses or in limited studies with the virus associated with HPS. Use a biological safety cabinet when splashes or aerosols are possible.
Examination of autopsy specimens obtained from humans	N/A	N/A	No diseases from hantaviruses causing hemorrhagic fever with renal syndrome and no disease or infection from the virus associated with HPS have been reported, although experience with the latter virus is limited. Observe universal precautions and protect mucous membranes. Use respiratory protection for aerosol-generating procedures.
Handling potentially infected tissue samples	BSL-2	BSL-3	Use a biological safety cabinet or respiratory protection when splashes or aerosols are possible.
Research-scale virus propagation in cell culture and handling infectious materials	BSL-3	BSL-3	Experience with other hantaviruses indicates that this combination of facilities and practices can provide safe conditions for this work.
Virus growth in large volumes; preparing and handling viral concentrates	BSL-4	BSL-4	Centrifugation and handling of other hantavirus concentrates have resulted in laboratory-associated infections.

TABLE 2. Animal biosafety guidelines for agents of hantavirus pulmonary syndrome

Activity	Animal rooms	Practices	Comments
Working with uninfected natural host species	N/A	N/A	Follow standard practices for handling wild-caught or colonized rodents.
Handling and necropsy of potentially infected rodents in the field	N/A	N/A	Provisional guidelines for work with rodents have been published (*4*).
Working with experimentally infected rodent species known not to excrete virus	ABSL-2	ABSL-2	Experimental studies to establish the range of permissive species should be conducted.
Handling serum or tissue samples from potentially infected rodents	BSL-2	BSL-3	Samples should be transported to laboratory in containment devices.
Working with infected natural host or other susceptible rodent species	ABSL-4	ABSL-4	Infected animals may be housed in Class III biological safety cabinets or in appropriate containment cages when personnel wear positive-pressure suits ventilated with a life-support system (*21*).

References

1. Nichol ST, Spiropoulou CF, Morzunov S, et al. Genetic identification of a hantavirus associated with an outbreak of acute respiratory illness. Science 1993;262:914–7.
2. Spiropoulou CF, Morzunov S, Feldmann H, Sanchez A, Peters CJ, Nichol ST. Genome structure and variability of a virus causing hantavirus pulmonary syndrome. Virology (in press).
3. Hjelle B, Jenison S, Torrez-Martinez N, et al. A novel hantavirus associated with an outbreak of fatal respiratory disease in the southwestern United States: evolutionary relationships to known hantaviruses. J Virol 1994;68:592–6.
4. CDC. Update: outbreak of hantavirus infection—southwestern United States, 1993. MMWR 1993;42:495–6.
5. CDC. Hantavirus pulmonary syndrome—United States, 1993. MMWR 1994;43:45–8.
6. Elliott LH, Ksiazek TG, Rollin PE, et al. Isolation of Muerto Canyon virus, causative agent of hantavirus pulmonary syndrome. Am J Trop Med Hyg (in press).
7. Childs JE, Ksiazek TG, Spiropoulou CF, et al. Serologic and genetic identification of *Peromyscus maniculatus* as the primary rodent reservoir for a new hantavirus in the southwestern United States. J Infect Dis (in press).
8. CDC. Hantavirus infection—southwestern United States: interim recommendations for risk reduction. MMWR 1993;42(No. RR-11).
9. Prince HN, Prince DL, Prince RN. Principles of viral control and transmission. In: Block SS, ed. Disinfection, sterilization, and preservation. 4th ed. Philadelphia: Lea & Febiger, 1991:411–44.
10. Lee HW, Baek LJ, Seong IW, Gwag TR. Physico-chemical properties of Hantaan virus, II: the effect of temperature and pH on infectivity of Hantaan virus. J Korean Soc Virol 1983;13:23.
11. Yanagihara R. Hantavirus infection in the United States: epizootiology and epidemiology. Rev Infect Dis 1990;12:449–57.
12. McKee KT Jr, LeDuc JW, Peters CJ. Hantaviruses. In: Belshe RB, ed. Textbook of human virology. 2nd ed. St. Louis: Mosby Year Book, 1991:615–32.
13. LeDuc JW. Epidemiology of Hantaan and related viruses. Lab Anim Sci 1987;37:413–8.
14. Nuzum EO, Rossi CA, Stephenson EH, LeDuc JW. Aerosol transmission of Hantaan and related viruses to laboratory rats. Am J Trop Med Hyg 1988;38:636–40.
15. Tsai TF. Hemorrhagic fever with renal syndrome: mode of transmission to humans. Lab Anim Sci 1987;37:428–30.
16. Umenai T, Lee HW, Lee PW, et al. Korean haemorrhagic fever in staff in an animal laboratory. Lancet 1979;i :1,314–6.
17. Lee HW, Johnson KM. Laboratory-acquired infections with Hantaan virus, the etiologic agent of Korean hemorrhagic fever. J Infect Dis 1982;146:645–51.
18. Dournon E, Moriniere B, Matheron S, et al. HFRS after a wild rodent bite in the Haute-Savoie—and risk of exposure to Hantaan-like virus in a Paris laboratory. Lancet 1984;1:676–7.
19. Kawamata J, Yamanouchi T, Dohmae K, et al. Control of laboratory acquired hemorrhagic fever with renal syndrome (HFRS) in Japan. Lab Anim Sci 1987:37;431–6.
20. Desmyter J, LeDuc JW, Johnson KM, Brasseur F, Deckers C, van Ypersele de Strihou C. Laboratory rat associated outbreak of haemorrhagic fever with renal syndrome due to Hantaan-like virus in Belgium. Lancet 1983;11:1,445–8.
21. Lloyd G, Bowen ETW, Jones N, et al. HFRS outbreak associated with laboratory rats in UK. Lancet 1984;i:1,175–6.
22. CDC/National Institutes of Health. Biosafety in microbiological and biomedical laboratories. 3rd ed. Washington, DC: US Department of Health and Human Services, Public Health Service, 1993. HHS publication no. (CDC)93-8395.

The *Morbidity and Mortality Weekly Report (MMWR)* Series is prepared by the Centers for Disease Control and Prevention (CDC) and is available on a paid subscription basis from the Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20402; telephone (202) 783-3238.

The data in the weekly *MMWR* are provisional, based on weekly reports to CDC by state health departments. The reporting week concludes at close of business on Friday; compiled data on a national basis are officially released to the public on the succeeding Friday. Inquiries about the *MMWR* Series, including material to be considered for publication, should be directed to: Editor, *MMWR* Series, Mailstop C-08, Centers for Disease Control and Prevention, Atlanta, GA 30333; telephone (404) 332-4555.

☆**U.S. Government Printing Office: 1994-533-178/05001 Region IV**