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DIANA M. BONTÁ, R.N., Dr. P.H.
Director

ARNOLD SCHWARZENEGGER
Governor

Guidelines for conducting surveillance for hantavirus in rodents in California

Introduction

In 1993, an outbreak of acute, severe respiratory disease occurred among residents of the Four Corners region of the southwestern United States. The etiology was identified to be a previously unrecognized hantavirus. A decade later, the Centers for Disease Control and Prevention reported a cumulative total of over 330 cases of hantavirus pulmonary syndrome (HPS), including over 100 deaths, in 31 states, including California.

The causative agent of HPS in the western United States is Sin Nombre virus (SNV). While other hantaviruses have been identified in California (e.g., El Moro Canyon, Isla Vista, Limestone Canyon), to date only SNV has been associated with human illness.

Hantaviruses are maintained in nature in rodents, with each virus typically associated with one rodent species. The reservoir for SNV is the deer mouse, *Peromyscus maniculatus*. Deer mice are believed to become infected as juveniles or young adults and to maintain infection for life. SNV is shed in urine, feces, and possibly saliva which if inspired or ingested can precipitate illness in humans. Evidence of infection with SNV in rodents can be determined through serologic testing. Rodent surveillance data compiled by the California Department of Health Services (CDHS) indicate that approximately 12 percent of deer mice in California have evidence of SNV infection, though the seroprevalence can vary dramatically between populations.

Objectives of rodent hantavirus surveillance

The ultimate goal of all vector-borne disease surveillance, including hantavirus, is to prevent or reduce human morbidity. Information gathered through surveillance can highlight specific avenues for control that might be pursued directly. The surveillance efforts may be in response to a recognized case of HPS, or may be directed at locations where the existing knowledge of rodent and human activity suggests that potential virus transmission is a reasonable concern. Also, by expanding the knowledge of the dynamics and distribution of rodent-virus relationships, public health agencies can better formulate and target personal protective recommendations to at-risk populations.

General scientific studies of hantavirus ecology can offer indirect information on disease prevention, or stimulate new areas for inquiry. However, these types of studies should be focused on a specific investigative hypothesis to be answered within a specified time and space. The objectives, methods, safety procedures, and anticipated relevance of the study should be described in a detailed written study protocol. The protocol should be reviewed periodically by the investigators and others in light of the data collected to date, as well as newly published

information. Significant changes in the general knowledge of hantavirus ecology should be incorporated into modifications of the study methodology.

Limitations of rodent hantavirus surveillance

1. Serology.

The laboratory method most frequently used to assess SNV infection in rodents is serology. An assay, either immunofluorescent assay or enzyme immunoassay, is used to detect antibody to SNV in the blood of rodents. Detection of antibody does not indicate necessarily that the rodent is currently infected or infectious, only that the rodent was at some previous time exposed to the virus. Nevertheless, as rodents are believed to remain infected for life, a seropositive rodent may be regarded in most circumstances as an actively infected rodent. Similarly, from an ecologic perspective, serologic results collectively offer a cumulative depiction of what has transpired in the rodent population in the past and only indirect evidence of the current level of virus transmission. Furthermore, while serologic titers provide an ordinal quantification of the level of circulating antibody, immunologic responses depend on a number of factors and can vary between and within rodents. The correlation between serologic titers and epidemiologically relevant measures such as time of exposure, level of virus load, and amount of virus shedding has not been reliably demonstrated.

2. Laboratory assay.

Most laboratories, including the CDHS Viral and Rickettsial Disease Laboratory, currently use an enzyme immunoassay that employs SNV as antigen to detect hantavirus antibody in rodent serum. While SNV is the only hantavirus known to be pathogenic to humans in California, several other hantaviruses circulate among rodent hosts. Among these other hantavirus-rodent relationships are Prospect Hill and Isla Vista viruses in voles (*Microtus* spp.), El Moro Canyon virus in harvest mice (*Reithrodontomys megalotis*), and the recently identified Limestone Canyon virus in brush mice (*Peromyscus boylii*). Serum from rodents with antibodies to these other nonpathogenic hantaviruses can test "positive" (i.e., cross-react) on the SNV assay. In areas where these and other rodent species share habitat with *P. maniculatus*, positive serologic results could represent incidental spillover of SNV. Thus, the detailed ecology of the surveillance area should be considered when evaluating the significance of serologic results.

3. Multifactorial risk.

The presence of deer mice with SNV antibody is only one of many factors to consider when evaluating risk of HPS. Agent, vector, environment, and host factors interact to determine whether effective transmission, infection, and disease are likely. Agent factors include strain and concentration of hantavirus. Vector factors include active infection (vs. serologic evidence of past infection), concentration of virus shed in excreta, density of rodents, and the frequency and distribution of excretion. Environmental factors include temperature, air turnover, ultraviolet penetration, and humidity. Finally, host factors include proximity of humans to rodents, likelihood of direct or aerosolized contact with contaminated excreta, and possibly individual susceptibility factors. All these factors should be considered when evaluating the significance of rodent serology data and designing an appropriate preventive plan.

Surveillance design and practice

<u>Developing a protocol</u>

1. Site selection.

The indispensable factors to consider when identifying sites for rodent hantavirus surveillance are 1) presence of appropriate rodents, 2) human activity, and 3) likelihood of human-rodent contact. Any site that does not have all these features is not an appropriate location for conducting hantavirus public health surveillance. Remote populations of deer mice isolated from significant human activity, or developed urban areas where sylvatic rodents are rare, are examples of areas generally inappropriate for hantavirus surveillance. Deer mice can be found in nearly any undeveloped area of California. However, deer mice are most abundant in disturbed habitats and often areas adjacent to human habitation (out buildings, wood and brush piles, etc.). Humans can encounter deer mice while at home, at their place of work, or while engaged in outdoor recreational activities. Surveillance sites should be selected following careful scrutiny of their potential for ongoing rodent activity at a human interface.

2. Frequency.

The dynamics of hantavirus transmission within rodent populations in a given area can vary from season to season and from year to year. Therefore, a single surveillance effort provides limited information on the ongoing risk. On the other hand, climatological conditions may restrict surveillance in some sites to only a few months of the year. Surveillance should be scheduled when rodents are active and, if possible, prior to increased human activity.

3.Timing.

As deer mice are principally nocturnal, surveillance should be conducted overnight. Traps should be placed late in the afternoon and retrieved in the early morning. Traps should be numbered and their location clearly marked to facilitate complete accounting when they are retrieved.

4. Ancillary data.

A complete surveillance plan includes collection and recording of information on habitat, weather, georeferencing coordinates, and age, sex, and biometrics of the rodents. The CDHS Mammal Collection Forms (attached) are designed for recording this information in an efficient and consistent manner. These data may later be transferred to an electronic database. Conscientious documentation of these data at the time of surveillance provides a permanent record of the surveillance activities for future reference by other public health officials.

<u>Rodent collection</u>

1. Target species.

Rodent surveillance should target known or suspected reservoirs of pathogenic hantaviruses. In California, the deer mouse, *P. maniculatus*, is the principal and possibly sole reservoir for SNV. Traps should be selected (e.g., Sherman live traps), baited (e.g., rolled oats), and dispensed (e.g., overnight) so as to maximize yield from this target species. In habitats where populations of different *Peromyscus* species coexist, SNV may spill over from *P. maniculatus*. While other *Peromyscus* species do not likely serve as viable reservoirs or vectors of SNV, limited serologic testing of these other species may provide some secondary information on the frequency of interaction between populations and the efficiency of inter-specific virus transmission. Persons conducting surveillance should be well trained in the identification and differentiation of rodent species so that serologic results can be properly attributed to the correct

species. It can be difficult to differentiate *Peromyscus* species without adequate training, field experience, and reference materials.

Other rodent species maintain hantaviruses that are not pathogenic to humans but will cross-react on standard SNV serologic assays. Surveillance among these rodent species should be conducted only under the aegis of a specific scientific research project and should not be included in a public health surveillance program. Collection and sampling should be avoided among rodent species that play no role in the maintenance or transmission of SNV. This would include most non-*Peromyscus* sylvatic rodent species (e.g., wood rats), all rodents belonging to the Sciuridae (squirrels, chipmunks), Heteromyidae (pocket mice, kangaroo rats), and Geomyidae (gophers) families. Certain species (e.g., salt-marsh harvest mouse, *Reithrodontomys raviventris*) are protected by California and/or federal law and may not be taken or disturbed in any manner. Finally, commensal (domestic) rodents (*Mus musculus*, *Rattus* spp.) are known reservoirs of some Old World hantaviruses and arenaviruses; however, there is no evidence that they play any part in maintenance or transmission of New World hantaviruses such as SNV. Therefore, collection and sampling of domestic rodents should be restricted to special studies with specifically targeted objectives, protocols, and laboratory processes. There is no scientific justification for collecting and sampling domestic rodents as part of a routine hantavirus surveillance program.

2. Number of specimens.

The appropriate number of rodents to collect is the minimum necessary to adequately achieve the surveillance objective. The number of rodents necessary to determine whether hantavirus is circulating in a particular population will be less than that for surveillance undertaken to estimate the proportion of rodents infected. Focused scientific research studies will typically necessitate collection of more specimens than routine public health surveillance.

The number of target rodents collected (trap success) is a function of the density of rodents in the surveillance area, the presence and number of nontarget competing rodent species, the number and location of traps placed, the duration over which traps are left in place, and ambient conditions (e.g., lunar phase, temperature, precipitation) that influence rodent activity.

3. Handling and disposition.

Rodents should be live-trapped, handled, and sampled in a manner that minimizes stress and trauma to the rodents. The Animal Care and Use Committee of the American Society of Mammalogists has prepared guidelines for the care and handling of field collected mammals (see References). In general, rodents should be collected, sampled, and handled in a careful and expeditious manner, while providing for their physiologic needs. Traps should be briefly examined at the site of capture and any nontarget species immediately released. Captured rodents awaiting sampling in traps should be provided adequate space for movement; larger rodents inadvertently captured in small traps should be processed immediately and/or transferred to a holding facility of adequate volume. Surveillance staff should be attendant to temperature fluctuations and extremes. In cold environs, additional food and cotton to serve as nesting material should be provided in traps at the time when they are set. In hot weather, traps should be provided with a fluid source (e.g., apple slice or grape), in addition to dry bait. Traps should not be located where they receive direct morning sun; if this is impractical, they should be retrieved very early in the morning. Rodents awaiting processing or release in traps should be kept in the shade to prevent heat stress. Traps that failed to capture a rodent should be removed or, if

multiple nights' trapping is planned, closed and reopened when remaining traps are re-set in the late afternoon.

Every effort should be made to conduct nonfatal collection and sampling of rodents. While rodents may occasionally succumb to stress or anaesthesia, careful adherence to protocols and constant monitoring of captured rodents will minimize accidental deaths. Rodents should be released as soon as possible after processing. Ideally, each rodent should be released at its place of capture. Rodents captured within or near occupied buildings and which may pose an immediate health risk should be humanely euthanized. Carcasses should be double-bagged, sprayed with disinfectant, and disposed of with other waste.

Rodent processing

Handling and sampling wild rodents presents a risk of disease transmission to persons conducting the surveillance. Rodent processing should be performed in a standardized manner that maximizes efficiency of equipment and personnel and minimizes potential exposure to pathogens, directly from rodents or indirectly through vector fleas, ticks, etc. All persons involved in the rodent surveillance activities should be knowledgeable of and abide by these safety guidelines.

1. Safety.

All wild-caught rodents should be regarded as potential sources of disease. Personal safety should be the overriding consideration during all surveillance efforts. All personnel should receive adequate training prior to processing and strictly adhere to appropriate safety and personal protective measures during all phases of rodent handling and specimen collection. Standard safety protocols have been developed and the reader is directed to these essential publications (see References).

2. Specimen collection.

Surveillance for hantavirus in rodents requires the collection of blood specimens. Blood specimens should be collected in a manner that minimizes trauma to the rodent and risk of injury to the surveillant. Retro-orbital bleeding is the preferred method as it induces the least trauma and risk of mortality to the rodent and avoids the additional safety hazard of hypodermic needles. Intracardiac puncture is an acceptable alternative for larger or expired rodents.

3. Specimen storage.

Blood specimens should be transferred to storage vials that are secure, do not compromise the integrity of the specimen, and can be efficiently handled by the testing laboratory. Plastic screw-top vials offer the best option for convenience and safety. Vials should be individually marked with unique identification numbers, using indelible ink or pre-printed labels, prior to initiating rodent processing. Vials should be placed sequentially in a rack that prevents spillage. Following transfer of blood specimens, vial lids should be secured. Any blood that is spilled on the exterior of the vial should be immediately wiped off. After all processing is completed, vials should be sprayed with disinfectant prior to being packaged for shipping.

4. Transporting specimens.

Packing and shipping of diagnostic specimens are strictly regulated, and should be done in accordance with the most current regulations. Contact the laboratory or agency designated to

receive the specimens for appropriate instructions prior to collecting specimens. Notify the laboratory prior to shipping the specimens so that they will be properly handled upon arrival. Avoid scheduling specimen shipments to arrive on weekends, as the delay in handling and processing may compromise the integrity of the specimens.

5. Site clean-up and disposal of biohazardous waste.

After specimen collection has been completed, the processing site, equipment, and personnel should be decontaminated in accordance with appropriate safety protocols (See RM Davis 2002 in References). Rodent tissues and fluids, as well as materials that have come in contact with them, are considered biohazardous waste. Strict regulations govern the disposition and disposal of biohazardous waste. Persons conducting surveillance should learn which state and local requirements apply and prepare ahead of time to practice them.

Summary

Rodent surveillance is an important part of a comprehensive public health program in the prevention of HPS. Information gathered through well-conducted rodent surveillance can help to direct education efforts to businesses, medical professionals, and the general public. A well-designed program can maximize useful information without squandering valuable resources or placing personnel at unnecessary risk.

Public health agencies considering developing a rodent hantavirus surveillance program should consult with experts to ensure that their efforts are adequate, appropriate, and conducted in a safe manner. The CDHS Vector-Borne Disease Section (VBDS) has extensive experience in conducting rodent hantavirus surveillance statewide and is available for consultation and training in surveillance methods. Interested agencies should contact their VBDS District Biologist or VBDS Headquarters (916-552-9730) for technical assistance or to schedule training sessions.

References

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Calisher CH, Root JJ, Mills JN, Beaty BJ. Assessment of ecologic and biologic factors leading to hantavirus pulmonary syndrome, Colorado, U.S.A. *Croat Med J* 2002; 43:330-7.

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Mammal Collection Form ☐ Plague ☐ Hantavirus ☐ Other: _____ Page _____ of _____

Part I. General Information & Habitat Description

Location				County	Collection Date
Jurisdiction ☐ USFS (Name of national forest: _____) ☐National Park/Monument ☐Military ☐University ☐ State Park/Recreation Area ☐ County Park ☐ City Park ☐ Wildlife Refuge Area ☐Private ☐Other (Specify):					
Name of Submitter	Participants				Phone
Collecting Agency		Address			Fax
Elevation (ft)	Latitude ° ‘ N		Longitude ° ‘ W	USFS Ecological Section	USFS Ecological Subsection
Primary Habitat (Check only one)	*Trees:* ☐ Hardwood ☐ Conifer ☐ Riparian *Shrubs*: ☐ Chaparral ☐ Sage ☐ Scrub *Herbaceous:* ☐ Grass ☐ Marsh ☐ Meadow *Developed:* ☐ Agriculture ☐ Urban ☐ *Other (Specify):*				
Total Captures (a)	Total Traps Set (b)	Trap Success (a/b x100) %	Trap Period ☐ Overnight ☐ Daytime		Total Hours
Comments					

Summary of Results	PLAGUE					HANTAVIRUS						OTHER				
						Specimens			Results							
Primary Mammal Species	# Hosts	# with Fleas	% Infest.	# Fleas	Flea Index	# Hosts	# S	# C	# Pos. Hosts	# Neg. Hosts	% Positive	# Hosts				

Send samples to:

PLAGUE - California Department of Health Services
Specimen Receiving
ATTN: A. Hom (VBDS)
850 Marina Bay Parkway
Richmond, CA 94804

HANTAVIRUS - California Department of Health Services
Specimen Receiving
ATTN: B. Enge (VRDL)
850 Marina Bay Parkway
Richmond, CA 94804

Part II. Mammal & Ectoparasite Record

Location									County	Collection Date		
	MAMMAL DATA								DISEASE DATA			
V NO.	FIELD NO.	GENUS & SPECIES	AGE	SEX	REPRO. STATUS	H & B	# ECTOS.	REMARKS	POOL NO.	SPECIMEN S, N, C	LAB NUMBER OR ECTOPARASITE ID	RESULT

KEY to Part II.

Age: A = adult, SA = subadult; Sex: M = male, F = female; Reproductive Status: Male, S = scrotal, Abd = abdominal; Female, Imp = imperforate, Per = perforate,

Lac = lactating, PL = postlactation; Head & Body Length: Measure tip of nose to base of tail at body in millimeters; Number of Ectoparasites: 0 = none; Remarks: E = escaped; Specimen: S = serum sample only, N = nobuto strip only, C = carcass only, N/C = nobuto and carcass, 0 = no sample