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Overview

Hantaviruses: An Overview

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Hantaviruses are a newly emerging group of rodent-borne viruses that have significant zoonotic potential. Human infection by hantaviruses can result in profound morbidity and mortality, with death rates as high as 50%, and potentially long-term cardiovascular consequences. Hantaviruses are carried by peridomestic and wild rodents worldwide and have occasionally been linked to infections in laboratory rodents. Because these viruses have been associated with significant human disease, they have become the subject of intense scientific investigation. In this review the reader is introduced to the hantaviruses, including hantavirus diseases and their pathogenesis. A review of the biology, morphology, and molecular biology of the hantaviruses with a brief overview of the ecology and biology of hantavirus-rodent pairs is also included. The risks of occupational exposure to hantaviruses, diagnosis of hantavirus infections, and methods for handling potentially infected rodents and tissues are discussed as well.

Introduction

The genus *Hantavirus* belongs to the family *Bunyaviridae*; other genera in the family include *Bunyavirus, Nairovirus, Phlebovirus,* and *Tospovirus.* All of the members of the family are enveloped, with tri-segmented, negative sense RNA genomes. *Bunyaviruses, Nairoviruses, Phleboviruses,* and *Tospoviruses* are classical arboviruses in that they are all transmitted through obligate intermediate arbovirus vectors such as mosquitoes, ticks, phlebotomine flies, other arthropods, or thrips (Table 1). Hantaviruses, however, are transmitted directly from an infected rodent to a naïve rodent via aerosolized urinary, fecal, or salivary secretions without the aid of an intermediate vector (1, 2). There are currently about 20 well-described hantaviruses, each of which is closely associated with a single rodent or insectivore host (3). Specific rodent-hantavirus pairs are so closely associated that it is generally believed that the rodent and its associated hantavirus have co-evolved (4). As a result of the long-standing co-evolution of rodent-hantavirus pairs, hantaviruses do not cause overt clinical disease in their well-adapted rodent hosts (5). "Spillover" of hantaviruses from their normal hosts to other animals can occur between closely related, closely associated, sympatric rodents (4). Occasionally, hantaviruses spillover from their normal rodent hosts to humans as well. When this occurs, it can result in asymptomatic infection with seroconversion or overt clinical disease, depending upon which hantavirus is involved. Hantaviruses can be divided into two general categories: Old World hantaviruses and New World hantaviruses. Old World hantaviruses are carried by *Arvicolinae* and *Murinae* subfamily rodents that are generally found in Europe and Asia; however, the Norwegian rat, the natural reservoir host for Seoul virus, has been distributed worldwide by international maritime commerce. When Old World hantaviruses, including Hantaan virus (HTNV), Seoul virus (SEOV), Puumala virus (PUUV), and Dobrava virus (DOBV), infect humans, they typically affect the urinary system and cause a disease syndrome known as hemorrhagic fever with renal syndrome (HFRS). A more recently described hantavirus disease emerged in the New World in May 1993 when a cluster of deaths occurred in the Four Corners area of the U.S. desert Southwest among a group of young, previously healthy individuals (6, 7). Because nearly one-half of the individuals died acutely from fulminant pulmonary edema, the disease syndrome was called hantavirus pulmonary syndrome (HPS) (7). New World hantaviruses are carried by *Sigmodontinae* and *Arvicolinae* subfamily rodents from North, Central, and South America. Numerous HPS-causing hantaviruses have been identified since 1993 in North and South America, including Sin Nombre virus (SNV), Andes virus (ANDV), Black Creek Canal virus (BCCV), New York virus (NYV), and Bayou virus (BAYV), to name a few.

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Human hantavirus diseases

History. Human hantavirus diseases were first recognized, and for a long time were only believed to occur, on the Eurasian landmass (8). Numerous HFRS-like disease outbreaks have been described beginning with reports starting in the 10th century in the Chinese literature, and extending through World War II (9). Historically, these diseases were referred to as epidemic hemorrhagic fever, epidemic nephroso-nephritis, field nephritis, Korean hemorrhagic fever, or nephropathia epidemica. During the Korean conflict approximately 3,200 United Nations soldiers were diagnosed with Korean hemorrhagic fever based on clinical presentation, and intense research efforts were undertaken to identify the etiologic agent (10). Carlton Gajdusek was the first clinician to recognize that all of these clinical disease entities were closely related and most likely the result of a common agent (11). Despite the tremendous efforts expended between 1950 and 1980, hantaviruses were not isolated, cell cul-

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Table 1. Members of the family *Bunyaviridae*

Genus	Type species	Principal vector(s)/Reservoir	Affected host species
Bunyavirus	Bunyamwera virus	Mosquitoes, culicoid flies, ticks	Human, sheep, cattle
Nairovirus	Dugbe virus	Ticks, culicoid flies, mosquitoes	Human, cattle, seabirds
Phlebovirus	Rift Valley fever virus	Phlebotomine flies, mosquitoes, ticks	Human cattle, seabirds
Tospovirus	Tomato spotted wilt virus	Thrips	Plants
Hantavirus	Hantaan virus	Rodents	Human

Table 2. Review of *Hantavirus*-associated diseases

	Disease severity	*Hantavirus*	Death Rate	Number of cases
HFRS[a]	moderate to severe	Hantaan (HTNV) Seoul (SEOV) Dobrava (DOBV)	1%-15%	~200,000 annually
	mild	Puumula (PUUV)	< 1%	
HPS[b]	prototypical	Sin Nombre (SNV) New York (NYV)	> 50%	~250-300 cases since their discovery
	renal variant	Bayou (BAYV) Black Creek Canal (BCCV) Andes (ANDV)	> 40%	
None		Prospect Hill (PHV)	None	None
None Reported		Tula (TULV) Thailand (THAIV) El Moro Canyon (ELMCV) Thottapalayam (TMPV)	None Reported	None Reported

[a]HFRS, hemorrhagic fever with renal syndrome.
[b]HPS, hantavirus pulmonary syndrome.

Table 3. Officially recognized members of the genus *Hantavirus*[a], family *Bunyaviridae*

Species	Disease	Reservoir	Distribution of virus
Order *Rodentia*, Family *Muridae*			
Subfamily *Murinae*			
Hantaan virus (HTNV)	HFRS[b]	*Apodemus agrarius*	China, Russia, Korea
Dobrava virus (DOBV)	HFRS	*Apodemus flavicolis*	Balkans
Seoul virus (SEOV)	HFRS	*Rattus norvegicus, Rattus rattus*	Worldwide
Thailand virus (THAIV)	ND[c]	*Bandicota indica*	Thailand
Subfamily *Arvicolinae*			
Puumala virus (PUUV)	HFRS	*Clethrionomys glareolus*	Europe, Scandinavia, Russia
Prospect Hill virus (PHV)	ND	*Microtus pennsylvanicus*	U.S., Canada
Khabarovsk virus (KHAV)	ND	*Microtus fortis*	Russia
Isla Vista virus (ISLAV)	ND	*Microtus californicus*	California
Topografov virus (TOPV)	ND	*Lemmus sibiricus*	Siberia
Tula virus (TULV)	ND	*Microtus arvalis*	Europe
Subfamily *Sigmodontinae*			
Sin Nombre virus (SNV)	HPS[d]	*Peromyscus maniculatus*	U.S., Canada, Mexico
New York virus (NYV)	HPS	*Peromyscus leucopus*	U.S.
Andes virus (ANDV)	HPS	*Oligoryzomys longicaudatus*	South America
Bayou virus (BAYV)	HPS	*Oryzomys palustris*	U.S.
Black Creek Canal virus (BCCV)	HPS	*Sigmodon hispidus*	U.S.
Caño Delgadito virus (CADV)	ND	*Sigmodon alstoni*	Venezuela
Laguna Negra virus (LANV)	HPS	*Calomys laucha*	Paraguay, Bolivia
Muleshoe virus (MULV)	ND	*Sigmodon hispidus*	U.S.
Rio Mamore virus (RIOMV)	ND	*Oligoryzomys microtis*	Bolivia
El Moro Canyon virus (ELMCV)	ND	*Reithrodontomys megalotis*	California
Rio Segundo virus (RIOSV)	ND	*Reithrodontomys mexicanus*	Costa Rica
Order *Insectivora*			
Thottapalayam virus (TPMV)	ND	*Suncus murinus*	India

[a](3).
[b]HFRS, hemorrhagic fever with renal syndrome.
[c]ND, no disease documented.
[d]HPS, hantavirus pulmonary syndrome.

ture-adapted, and characterized until the early 1980's (12, 13, 14). Human hantavirus diseases manifest themselves in two distinct clinical disease syndromes: HFRS and HPS.

Hemorrhagic fever with renal syndrome (HFRS). HFRS is most commonly a disease found in the human population on the Eurasian landmass, is caused by Hantaan, Seoul, Dobrava, and Puumala viruses, and is associated with rodents of the subfamilies *Murinae* and *Arvicolinae* (Tables 2 and 3). It is estimated that 150,000 to 200,000 cases of HFRS are diagnosed in China annually (8). Additionally, a significant but unknown number of cases are diagnosed in Korea and Russia each year (8). Several thousand cases are also reported in the Balkans,

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Western Europe, and Scandinavia (15). Further, sporadic cases of HFRS are reported worldwide due to the global distribution of the Norwegian rat (*Rattus norvegicus*). Death rates from HFRS range from less than 0.1% for Puumala virus infection to nearly 15% for Hantaan virus infection (Table 2).

Patients affected by severe HFRS typically experience a sudden onset of "flu-like" symptoms, including fevers and chills with accompanying myalgia and prostration. Following a prodromal incubation period of 2-3 weeks, the disease has five distinct and well defined phases, including a febrile phase of 3-5 days duration, a hypotensive phase that lasts from hours to days, an oliguric phase that lasts from a few days to several weeks, a diuretic phase, followed by convalescence (15-17). Additional findings during the febrile phase can include abdominal pain, thirst, nausea and vomiting, dizziness, blurred vision which is characterized by a pathognomonic myopia (18), and photophobia (15). Retroperitoneal edema with accompanying petechial rash of the palate and axillary skin folds and lumbar back pain are common. Due to the vascular leak syndrome which accompanies the hypotensive phase, thirst, periorbital edema, hemoconcentration, and postural hypotension are commonly seen (15). Approximately one third of human deaths occur during the hypotensive phase due to vascular leakage and acute shock (17). A left shifted leukocytosis and thrombocytopenia are typical features of HFRS that can be accompanied by disseminated intravascular coagulopathies (19). Proteinuria with abnormal urine sediment and a urine specific gravity of 1.010 followed by oligouria is also seen. Nearly one-half of deaths occur in the oliguric phase due to the resulting hypovolemia (17). Patients that survive and progress to the diuretic phase generally show improved renal function, but may still die as a result of shock or pulmonary complications. The convalescent phase may last from weeks to months before recovery (15, 17). In most cases recovery is believed to be complete, however, some investigators have linked chronic hypertensive renal disease to a previous infection with an HFRS-causing hantavirus (16, 20). Interestingly, the primary site of virus replication for both HFRS and HPS causing hantaviruses appears to be the pulmonary endothelium (21).

Hantavirus pulmonary syndrome (HPS). In contrast to the renal disease that is seen in HFRS cases, a novel hantavirus disease syndrome, known as hantavirus pulmonary syndrome, has recently emerged in the New World and is associated with severe pulmonary disease. Since the initial outbreak of hantavirus pulmonary syndrome was recognized in the Four Corners area of New Mexico, Utah, Arizona, and Colorado in May 1993, 288 cases of HPS have been documented in 31, primarily western, U.S. states (Fig. 1). Approximately 400 cases of HPS have been documented throughout the New World. Interestingly, reverse transcriptase polymerase chain reaction has been used for retrospective diagnosis of HPS caused by hantaviruses as early as 1978 (22). Further, serologic analysis and patient records have been used to suggest a U.S. HPS case as far back as 1959 (23). This clearly indicates that HPS is not a new disease, simply that its etiology and case presentation were previously under appreciated. HPS is caused by a newly recognized clade of hantaviruses that have only been found in New World rodents of the *Sigmodontinae* subfamily.

HPS has many similarities to HFRS, including a febrile prodrome, thrombocytopenia, and leukocytosis. However, in HPS

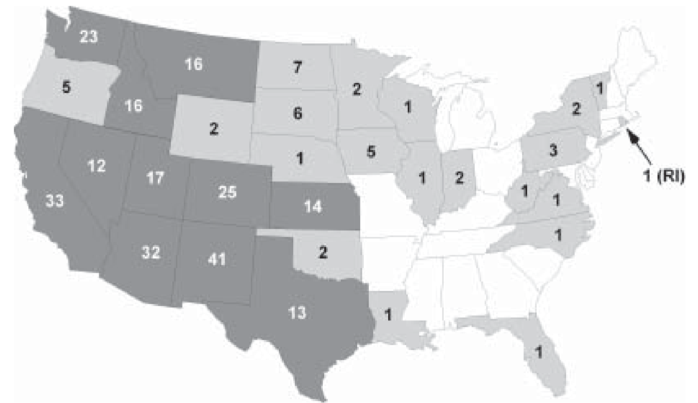

Figure 1. As of 30 November 2001, 288 cases of HPS have been confirmed in 31 primarily western U.S. states. Data taken from the Centers for Disease Control and Prevention website (http://www.cdc.gov/ncidod/diseases/hanta/hps/noframes/caseinfo.htm).

the site of vascular leakage is primarily in the lungs rather than the retroperitoneal space. In contrast to HFRS disease, the urinary system, including the kidneys, is largely unaffected in HPS (15-17). Death generally results from shock, pulmonary edema, and cardiac complications with oxygen saturation rates frequently less than 90% (17, 24, 25). However, within this group of viruses there is a spectrum of clinical disease syndromes that are seen frequently (17) including renal insufficiency (26, 27) and myositis (28).

Interestingly, as we learn more about human hantavirus diseases we find that they do not truly represent two clinically distinct diseases, rather they represent a continuum from renal disease in HFRS to pulmonary disease in HPS. For example, there are subclinical and clinical pulmonary sequelae to HFRS infection in humans (15, 29), as well as the previously described renal complications that arise from HPS (26-28). This is most likely a result of the fact that hantaviruses infect and replicate primarily within endothelial cells, which are widely distributed. The particular cellular tropisms of individual hantaviruses are most likely responsible for the varying course and severity of human diseases that are seen.

Pathogenesis of hantavirus diseases

It has been shown that NYV, SNV, HTNV, SEOV, and PUUV, all viruses which cause either HPS or HFRS (Table 2), use β_3 integrin receptors to infect platelets and endothelial cells. In contrast, Prospect Hill virus (PHV), Tula virus (TULV), and Thottapalayam virus (TPMV), all non-pathogenic hantaviruses, use β_1 integrin receptors to mediate infection of similar cell types (21, 30, 31). These data indicate that cellular tropisms play an important role in virus pathogenesis. Further study of virus-receptor interactions will be necessary to illuminate fully the role of integrin receptors and cell types in the pathogenesis of hantavirus infections.

The specific mechanism by which hantaviruses induce cellular damage in and cause disease to the renal and pulmonary systems of humans is poorly understood (24, 25, 32, 33); however, hantaviruses are known to infect and divide within endothelial cells and macrophages (24, 25, 33-36). Autopsy specimens from humans who died of HPS revealed higher than normal numbers of cytokine-producing cells in the lungs and spleens as

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assessed by immunohistochemical staining for interleukin (IL)-1α, IL-1β, IL-2, IL-4, IL-6, tumor necrosis factor (TNF)-α, TNF-β, and interferon-γ (37). These data are consistent with the results of Zaki et al., who reported that tissue biopsies taken from the lungs and spleens of fatal human HPS cases have high levels of hantavirus antigens (24). Immunoassay of serum from patients hospitalized with HFRS revealed elevated plasma levels of TNF-α, soluble TNF receptors, IL-6, and IL-10 (38, 39). These findings have led to speculation that vascular endothelial injury associated with HPS and HFRS is not a direct result of viral replication, but rather is the result of the host inflammatory response to the virus (15, 35). Hantavirus-specific CD4+ and CD8+ cytotoxic T lymphocytes have been isolated from pulmonary tissues of patients who died from HPS (40). These results clearly indicate that activated T cells are intimately involved in the pathogenesis of hantavirus infection. Further, the major histocompatibility (HLA) type of affected patients has also been associated with the clinical disease course (41, 42, 43), as might be expected in an immunopathologic disease (15). Yet a clear understanding of how hantaviruses induce HPS and HFRS awaits further study and the development of a suitable animal model of human hantavirus diseases. Hooper and colleagues (44) have recently reported a preliminary disease model in which Syrian hamsters (*Mesocricetus auratus*) inoculated with Andes virus developed fatal pneumonia with pulmonary edema that resembled the disease seen in HPS affected humans. However, similar hamsters that were inoculated with HTNV, SEOV, DOBV, PUUV, and SNV demonstrated asymptomatic seroconversion and no deaths (44, 45). While the initial ANDV report is promising, additional studies will be needed to fully describe and validate this potential HPS model.

Hantaviruses: general overview

Currently, there are 23 described hantaviruses, of which 22 have been accepted as species by the International Committee on Taxonomy of Viruses (Table 3) (3). All hantaviruses are enveloped, single-stranded negative-sense RNA viruses. Their genome is composed of three segments known as the small (S), medium (M), and large (L) segments that are approximately 1,700, 3,600, and 6,500 nucleotides long, respectively. Each of the genomic segments has a slightly mismatched, conserved, inverted repeat at its 3' and 5' ends (46). This allows the genomic segments to form a panhandle structure that is believed to play an important role in virus morphogenesis (Fig. 2) (46, 47). The three genomic segments code for four proteins: the S segment codes for the 48 kDa genome-associated nucleocapsid protein; the M segment codes for the two virion surface, membrane associated glycoproteins G1 and G2 (combined molecular weight approximately 125 kDa); and the L segment codes for a 250 kDa protein that is the putative virus polymerase, replicase, and transcriptase (48, 49). No proof-reading functions have been associated with the hantavirus polymerase protein, thus the viruses within the genus display tremendous genomic heterogeneity with nucleotide identities that can vary by as much as 50% between viruses within the *Hantavirus* genus (Fig. 3) (49).

Virion structure

The organization of hantavirus virions has not been well described; however, a schematic diagram depicting the putative virus organization is shown in Fig. 2. Glycoproteins G1 and G2 are type I transmembrane proteins that are intimately associated with the lipid envelope. The glycoproteins are believed to interact with integrin receptors to facilitate infection of endothelial cells, macrophages, and platelets by hantaviruses (21, 30, 31). Nucleocapsid proteins are found closely associated with the hantavirus genome, coating each of the three genomic segments within the virion (48). The functions of the nucleocapsid protein have not been well defined; however, it is believed that interactions between the virus nucleocapsid protein and virus genomic RNA (vRNA), complementary RNA (cRNA), and messenger RNA (mRNA) play an important role in the regulation of virus replication and transcription (50). Nucleocapsid proteins associate with the genomic vRNA to form ribonucleocapsids (48), which are visible in negative stained thin sections by electron microscopy (51). Virion ribonucleocapsids are believed to form panhandles due to complementary base pairing at the vRNA's 3' and 5' ends (46). Several copies of the virus polymerase protein are believed to be packaged within each virion and associated with the virus ribonucleocapsids through non-covalent interactions; however, this is not well defined.

Negative stained electron micrographs of hantaviruses show pleomorphic, enveloped virions that are approximately 100 nm in diameter (Fig. 2 B and C). Virions have a short 5-10 nm fringe of peplomers, which gives them a "fuzzy" ultrastructural appearance. Hantaviruses have been described as having a uniquely square "grid-like" external structure that is visible in electron micrographs (49, 52-54). Others have described the hantavirus external structure as having the appearance of a "soccer ball" (16). While not unique, the ultrastructural appearance of hantaviruses is quite distinct.

Hantavirus growth

Hantaan virus was the first hantavirus to be tissue culture-adapted and characterized in vitro (12-14). It took many years of intense research efforts to culture HTNV from the striped field mouse (*Apodemus agrarius*) (13). To accomplish this task, serial sections of infected *A. agrarius* were placed in tissue culture until suitable conditions for virus growth were found (12, 13). Hantaviruses remain very difficult to propagate in cell culture and only half of known hantaviruses have been cell culture-adapted. Even when hantaviruses are cell culture-adapted, they grow very poorly yielding low virus concentrations. Preferred cell lines for culture of hantaviruses grow slowly and demonstrate contact inhibition, for example Vero E-6 cells. Cytopathic effects (CPE) are not typically seen in hantavirus-infected cell cultures.

Molecular biology of the hantaviruses

When a hantavirus infects a permissive cell, it first binds to a cell surface receptor and is taken into the cell where virus uncoating occurs (48). Since hantaviruses are negative-stranded RNA viruses, they must first transcribe viral mRNA, which occurs in a short burst called primary transcription, after which virus mRNA is translated into functional viral proteins. To do this, the virus RNA-dependent RNA polymerase (RdRp) must first make virus mRNA. Hantavirus mRNA is different from cellular mRNA in several respects. First, hantaviruses do not poly-adenylate their 3' tails, making them distinct from the eukaryotic mRNAs with which they coexist. Additionally, hantaviruses lack the endogenous machinery for capping their

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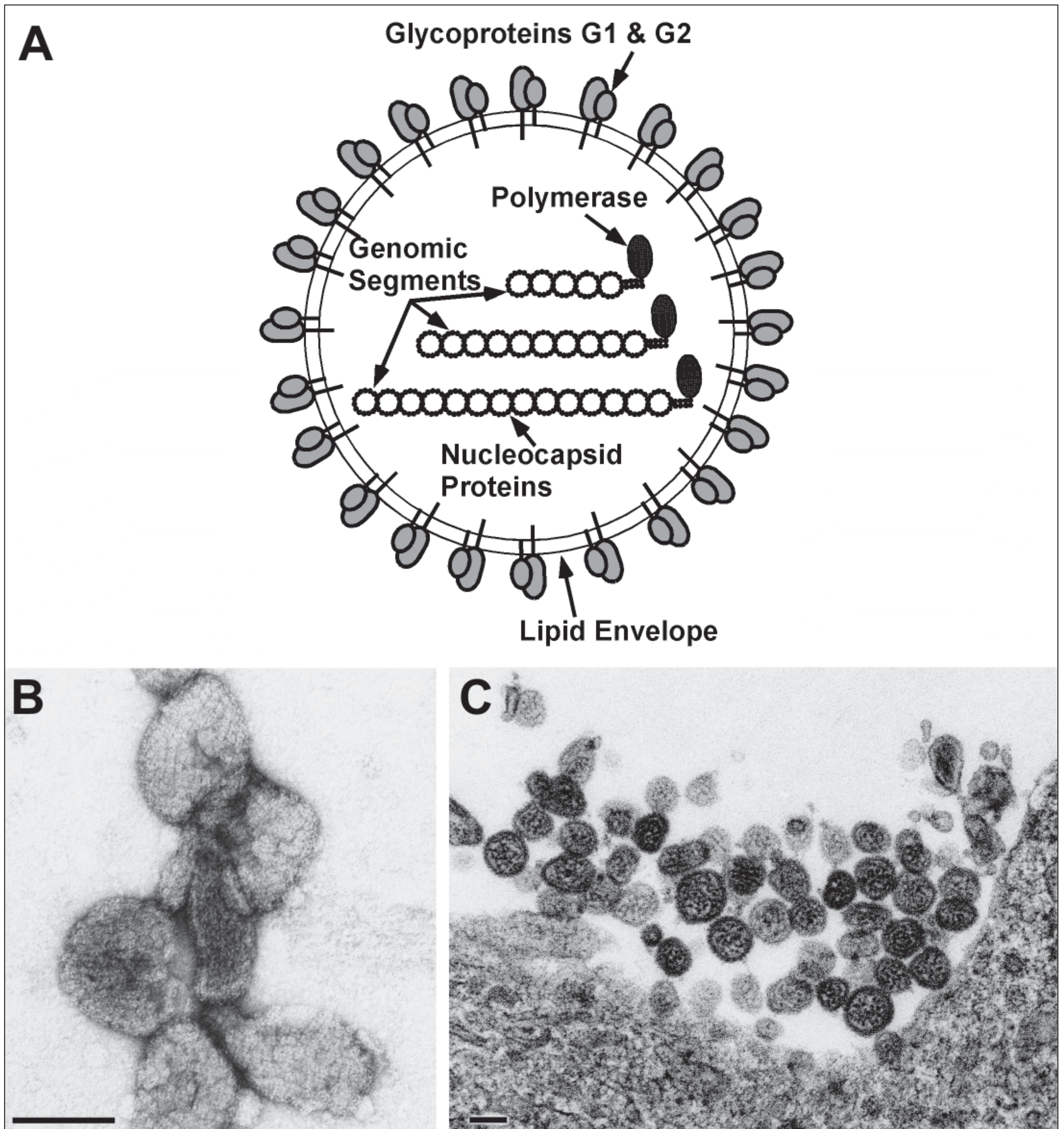

Figure 2. (A) Schematic diagram depicting the putative *Hantavirus* virion structure. Note the glycoproteins G1 and G2 protruding from the lipid envelope, the nucleocapsid proteins associated with the small (S), medium (M), and large (L) genomic segments, and the virus polymerase associated with the genomic segments. (B) Electron micrograph of negatively stained Hantaan virus particles. Note the uniquely square grid-like surface structure (bar = 100 nm). (C) Thin section electron micrograph of Puumala virus. The filamentous internal structure is believed to be due to the virus ribonucleocapsids (bar = 100 nm). Electron micrographs kindly provided by Dr. Tom Geisbert of the USAMRIID.

5' ends. Since a capped 5' end is necessary for translation in mammalian cells, hantaviruses rely on stealing mRNA caps from host mRNAs, which is colloquially referred to as "cap snatching." This results in heterogeneous 5' extensions of host cell origin on virus mRNAs that are typically less than 20 nucleotides in length. Virus mRNA is truncated at its 3' end, thus

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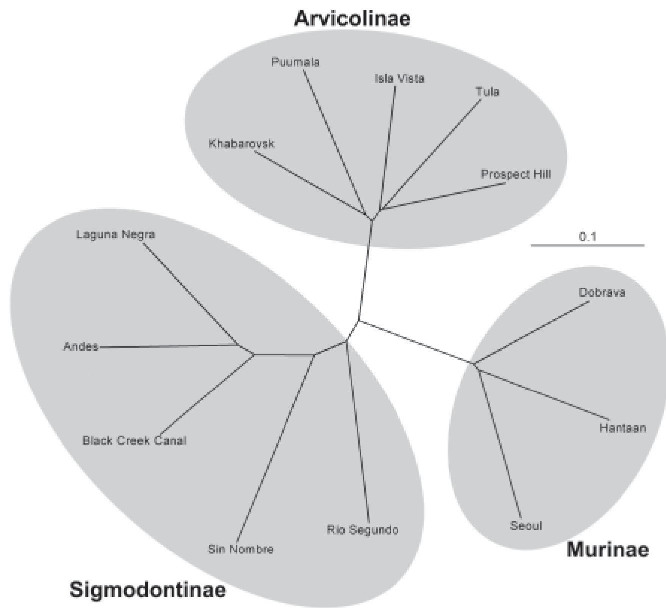

Figure 3. Unrooted tree demonstrating the three clades of the genus *Hantavirus* which are carried by three subfamilies of Muridae family rodents: *Arvicolinae, Murinae,* and *Sigmodontinae.* Rodents of the *Arvicolinae* are found in the northern hemisphere, the *Murinae* are Old World mice and rats, and the *Sigmodontinae* are New World mice and rats.

deleting the 3' genomic sequence that allows panhandle structures to form (49).

In addition to transcription, hantaviruses also must replicate their genomes. This is accomplished in a complex series of poorly understood events, diagramed in Fig. 4 (55). An undetermined signal allows the RdRp to read through the 3' truncation signals that are used to produce viral mRNA, resulting in exact complementary copies of the genomic virus RNA known as cRNA. Production of vRNA results in more viral genomes for packaging into progeny virions and it increases the number of templates available for virus mRNA synthesis. Cytoplasmic accumulation of virus nucleocapsid protein is postulated to be the mechanism for initiation of switching from mRNA synthesis to cRNA synthesis, and thus synthesis of more vRNA. While this has not been definitively demonstrated for hantaviruses, nucleocapsid proteins have been demonstrated to be very important in the regulation of transcription and replication of other negative stranded RNA viruses, including orthomyxoviruses and rhabdoviruses (56, 57).

As described earlier, hantaviruses lack a mechanism for capping their mRNA transcripts. Caps are "stolen" from host mRNAs by endonucleolytic cleavage of host cytoplasmic mRNAs 8-17 nucleotides downstream of their 5' caps (58, 59). These short capped oligonucleotides then serve as primers for virus mRNA synthesis. Thus, virus mRNA contains heterogeneous, non-templated 5' extensions of host cell origin. Often, one or more of the conserved 5' UAG triplets is deleted (59, 60). Despite the heterogeneous primers, hantavirus mRNAs commonly have a non-templated G residue contiguous with the 5' templated virus mRNA. This finding led Garcin, et al. (59) to propose a novel model of hantavirus transcription initiation, called "Prime and Realign" (Fig. 5). According to this model, the

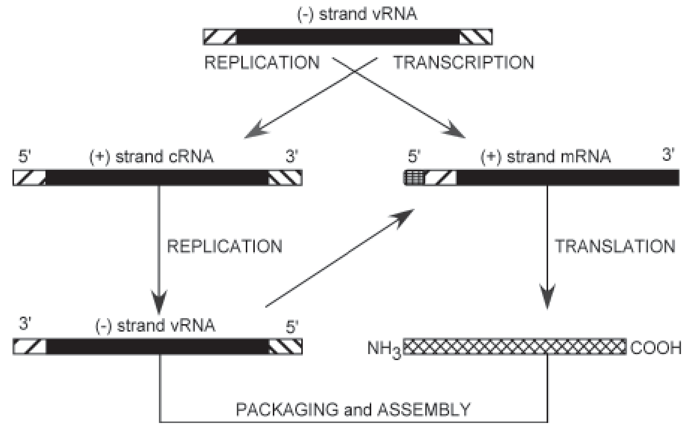

Figure 4. Hantavirus replication (left) and transcription (right) pathways. Note that in replication the genome must be faithfully transcribed twice, once from vRNA to cRNA and then back to vRNA. During translation the 3' end of the virus mRNA is cleaved, thus not allowing it to form panhandles or to serve as a vRNA template. Adapted from work by Jonsson and Schmaljohn (55).

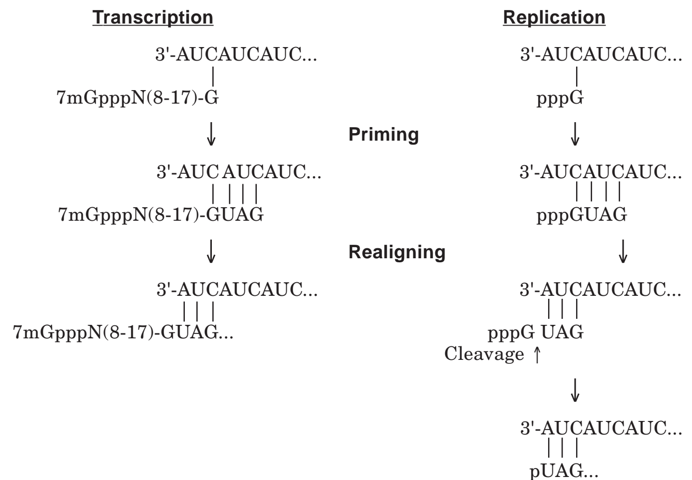

Figure 5. "Prime and Realign" mechanism of *Hantavirus* transcription and replication initiation as proposed by Garcin and colleagues (59). See text for a detailed description of the prime and realign process.

primer's terminal G residue aligns with the third nucleotide of the hantaviral vRNA template to initiate transcription. After a few nucleotides are added to the primer, the nascent virus mRNA then slips back several nucleotides on the repeated AUCAUCAUC sequence of the vRNA template and the transcript is extended (59).

A similar prime and realign model has been proposed for initiation of (+)stranded virus cRNA, which must serve as an identical template for faithful replication of genomic vRNA. In this model, transcription initiates with a pppG aligning at position three on the vRNA. As with viral mRNA synthesis, after several nucleotides are added to the nascent viral cRNA, polymerase slipping realigns the nascent cRNA so that the initiating pppG nucleotide is overhanging the 3' end of the template (Fig. 5) (59). In this instance, it is proposed that nucleolytic activity of the viral RdRp cleaves off the overhanging G nucleotide, leaving a monophosphorylated U nucleotide at the 5' end (59).

Mature genomic vRNA associates with nucleocapsid proteins

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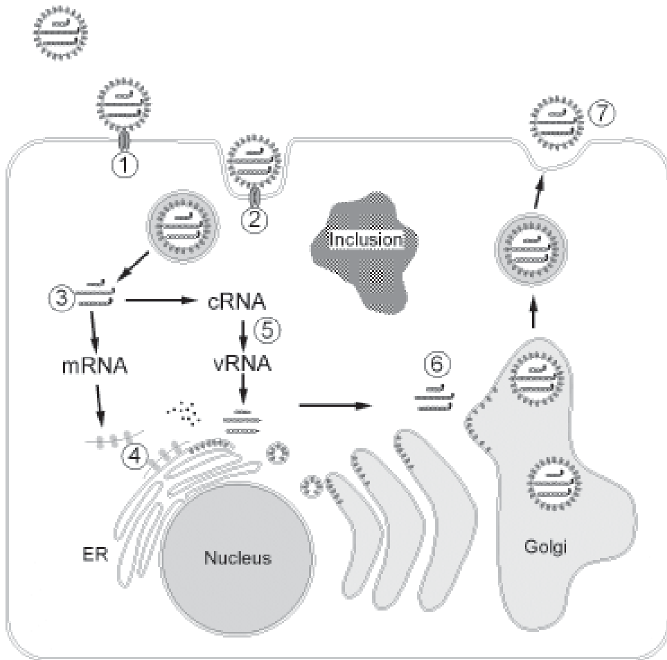

Figure 6. *Hantavirus* replication cycle. Steps in the replication cycle are as follows: 1, virion attachment to cell surface receptors; 2, receptor mediated endocytosis and virus uncoating; 3, primary transcription; 4, translation of virus proteins; 5, replication of virus genomic RNA through an intermediate virus cRNA; 6, assembly of virions at the Golgi apparatus; 7, virus release by exocytosis. Note the prominent intracytoplasmic nucleocapsid inclusion body that is commonly seen in many of the hantaviruses.

in the cytoplasm, forming the virus ribonucleocapsids. It is then believed that the viral ribonuclecapsids form a panhandle structure through complementary base pairing of the 3' and 5' ends of the viral genomes (46). Formation of complementary base paired, panhandle shaped, ribonucleocapsids is thought to be important to virus morphogenesis and budding (47).

Hantavirus morphogenesis

Since hantaviruses are enveloped viruses, they must bud through a host cell membrane to obtain their lipid envelope (Fig. 6). It is an unusual characteristic of the *Bunyaviridae* that virus budding occurs at the Golgi apparatus (47, 61). The mechanism by which Golgi budding occurs and the cellular signals that are responsible for initiation of the budding process have not been well described for the hantaviruses. However, it is known that the M segment gene is translated into a glycoprotein precursor that is co-translationally cleaved, most likely in the lumen of the endoplasmic reticulum, into glycoproteins G1 and G2 (47, 61). These proteins are type I transmembrane proteins that mature within the Golgi apparatus where glycosylation occurs. Golgi retention signals that are believed to result in the retention and accumulation of G1 and G2 glycoproteins in the Golgi have been identified in many of the *Bunyaviridae* (47, 61). Budding of virus ribonucleocapsids is thought to occur through the membranes of the Golgi apparatus. Virus filled vesicles are then thought to traffic from the Golgi to the plasma membrane where vesicle fusion leads to release of intact enveloped virions (48).

In contrast to other hantaviruses, ultrastructural studies have described Sin Nombre virus budding as occurring at the plasma membrane (51). This highly unusual method of budding, by a member of the *Bunyaviridae,* has not been confirmed by other scientific methods.

Hantavirus persistence

Hantaviruses establish persistent infections in both cell culture and their well-adapted rodent host species. In general, virus persistence has been proposed to occur by several mechanisms including immune surveillance avoidance and nonlytic viral replication (62, 63). Immune surveillance avoidance may include removal of recognition molecules from infected cells, abrogation of lymphocyte/macrophage function, or hiding in cells that lack MHC expression (such as neurons) (63). Mechanisms of nonlytic viral replication may involve generation of viral mutants or variants, or diminished expression of viral genes or their products (62). A consistent model by which hantaviruses establish persistence has not been developed. It is known that acutely infected rodents are transiently viremic with peak viremia occurring 7-14 days postinfection (1, 64-67). During the course of persistence, levels of infectious virus and the numbers of antigen-positive cells generally decrease. In some tissues, the amount of infectious virus may increase and decrease cyclically in a wave-like pattern (1, 12, 64, 65, 67, 68). These virus level alterations indicate that some stage of the viruses' life cycle is being downregulated (60). A similar scenario has been observed in Vero E-6 cells that were persistently infected with Seoul virus in which deletions accumulated in the 3' termini of the S, M, and L segments of the vRNAs during the acute phase of infection just prior to a decline in virus titer (60). Meyer and Schmaljohn proposed a model in which terminal nucleotide deletions result from the nuclease activity of the viral polymerase, which they believe causes down regulation of virus production (60). This model supports the theory that virus persistence can be established and maintained through a nonlytic viral replication and diminished expression of viral genes or their products (62). As natural infections of rodent-hantavirus pairs demonstrate a similar wave-like pattern of virus production it is tempting to speculate that this mechanism of persistence is occurring in vivo as well.

Hantaviruses and their rodent hosts: epidemiology and ecology

Muridae family rodents, with one notable exception, are reservoirs for hantaviruses (Table 3). Thottapalayam (TPMV) virus, which was isolated from an insectivorous shrew, *Suncus murinus*, in India is the exception. Curiously, TPMV was the first hantavirus to be isolated in cell culture (69); however, it was not thought to be a hantavirus until a partial genomic sequence was determined in 1992 showing genetic homology with known hantaviruses (70, 71). TPMV has not been well studied or described and there is considerable debate among scientists that study hantaviruses as to whether the shrew is truly the reservoir host for TPMV or if infection of the shrew by TPMV simply represents an inadvertent infection or spillover event (72). Because of this controversy, and the lack of information regarding TPMV, only the more thoroughly described rodent-borne hantaviruses will be discussed in this review.

Each of the currently recognized 21 species of rodent-borne hantaviruses is predominately associated with a single specific rodent host in which it establishes a persistent, most likely life-

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long, infection (72). Hantaviruses and their well adapted rodent reservoir hosts are believed to have co-evolved, thus hantavirus infections do not appear to cause any deleterious effects in their well-adapted rodent host species (4, 5, 17). Again, among closely related sympatric rodents, spillover infections can occur (4); however, if, and how well a spillover host can transmit the virus to other animals is poorly understood. Phylogenetic analysis demonstrates that hantaviruses break into three distinct clades, each of which closely coincides with the subfamily of its rodent host (Fig. 3 and Table 3). There are viral clades for the subfamily *Murinae* (Old World rats and mice), *Arvicolinae* (voles and lemmings of the Northern hemisphere), and *Sigmodontinae* (New World mice and rats) (72). It is interesting that the hantavirus phylogenetic tree closely resembles the phylogenetic tree of the host rodent species (72).

Hantavirus infection of the reservoir host is believed to be asymptomatic; however, slight histopathologic changes have been suggested by two authors. The changes include septal edema with enlargement and hyperchromasia of type 1 pneumocytes in New York-1 virus-infected *Peromyscus leucopus* (73), and hepatic triaditis in Sin Nombre virus-infected *Peromyscus maniculatus* (74). Additional experiments are needed to determine whether the subtle histopathologic changes described in these studies are associated with hantavirus infection. Regardless of the paucity of overt histologic changes in naturally infected rodent-hantavirus pairs, the host still mounts a vigorous antibody response to the virion envelope glycoproteins and the nucleocapsid core proteins (17).

It is currently estimated that there are approximately 2000 species of murid rodents (75), of which less than 100 have been thoroughly screened for hantaviruses (76), and only 22-23 different hantaviruses have been described (3). This means that roughly one quarter of rodents screened for hantaviruses are positive, and that approximately 1900 murid rodents have not been thoroughly tested for the presence of hantaviruses (76). Since hantaviruses are a newly emerging and recently recognized group of human pathogens, with case fatality rates as high as 50%, this certainly piques one's interest regarding how many species of hantaviruses might truly exist. As was experienced in the recent Sin Nombre outbreak in the U.S., many of these supposed hantaviruses may be an under-recognized causes of human disease.

Transmission

Hantaviruses are believed to be transmitted through aerosolized rodent exreta (2, 65). Transmission is thought to occur by close contact between infected and naïve rodents; however transmission has also been postulated to occur through fighting, biting, and sexual behavior (4, 77, 78). Interestingly, there is no evidence to support vertical transmission as a common means of transmitting hantaviruses from an infected dam to her pups. In fact, detailed catch and release studies performed with natural populations of SNV infected *Peromyscus maniculatus* (deer mice) demonstrate that SNV infection does not occur in newborn or weanling deer mice. Boruki and colleagues (79) clearly demonstrated that neonatal deer mice are antibody positive but virus RNA negative as assessed by RT-PCR. Further, they showed that the antibody prevalence in juvenile deer mice is inversely proportional to the weight of the mouse, which suggests that juvenile deer mice have maternally transferred antibodies that wane over time. In their study, ten deer mice that were antibody positive as juveniles were recaptured as adults and were found to be antibody negative and virus could not be detected in these mice by RT-PCR. The authors speculated that the disappearance and reappearance of antibodies in other deer mice in the study coincided with horizontal transmission of SNV later in life. The data from this natural infection study agree with data on maternal antibody production and transmission performed in laboratory rats infected with HFRS-causing hantaviruses (80, 81).

A statistically significant sex predilection has been documented in SNV infected deer mice (*Peromyscus maniculatus*). Adult male deer mice were more likely to be infected than were juvenile or adult female deer mice (79). This is curious, as female and male SEOV infected rats showed similar infection rates (77). This difference in the male-female seroprevalence rate is most likely a result of individual rodent species-specific behaviors and not a result of a strong sex predilection by the virus. For example, male deer mice are known to range widely within territories that cover those of many different female deer mice (79). This brings male deer mice into contact with a greater variety of deer mice than the female, giving the males a greater chance for encountering and contracting SNV. Further, since hantavirus infections have been documented to occur through bite wounds (78, 82), territorial fighting may increase the possibility of SNV transmission to previously unexposed males.

Occupational exposure to hantaviruses

There is significant risk of human occupational exposure to hantaviruses. Serologic analysis has indicated that mammalogists (83), and agricultural and forestry workers (18) are at the highest risk of occupational exposure to hantaviruses. There have been nine documented cases of hemorrhagic fever with renal syndrome caused by Hantaan virus in people that trapped wild rodents (84). Personnel that work in laboratory animal facilities or scientists that trap or work with wild rodents are also at risk, as hantaviruses have been documented in laboratory rats in Japan, Korea, China, Russia, Belgium, England, Malaysia, Hong Kong, Singapore, the United States, and Argentina (8). In Belgium, 39 staff members at a university were infected with a Hantaan-like virus following exposure to diseased rats, which resulted in three cases of acute renal failure (85). In England, four animal caretakers were admitted to hospitals with flu-like symptoms, abdominal and lower back pain, varying degrees of respiratory problems, proteinuria, oliguria, and renal failure after exposure to imported Louvain rats that were subsequently found to be carriers of a hantavirus (86). In Japan there have been sporadic outbreaks of HFRS among workers in laboratory animal facilities. To date, 149 cases of occupational zoonotic HFRS have been documented among animal caretakers in Japan with varying clinical disease courses and one documented fatality (8, 87). Cases of HFRS have been documented in animal caretakers after an occupational exposure to hantaviruses of less than five minutes (88). Biting appears to be a common method of hantavirus transmission among rodents (78). Human cases of hantavirus infection have also been associated with bite wounds (82). People that work with mammalian cell lines are also at risk as four laboratory workers were infected with cell culture-adapted Hantaan virus following exposure to hantavirus cultures (89), and several laboratory technicians that

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Table 4. General recommendations regarding hantaviruses in laboratory animal facilities

1. Facilities should be designed and maintained, and animals should be housed according to the standards set forth in "The Guide for the Care and Use of Laboratory Animals." (112)
   Specifically:
   a. Infestation by wild rodents should be avoided.
   b. Sanitation within animal facilities should be strictly maintained.
   c. A routine animal health monitoring program should be established and implemented.
   d. An occupational health and safety program should be established to monitor employee health.
2. Rodents brought into a facility should be from a hantavirus free source.
3. Wild caught rodents should be quarantined in a manner in which the spread of aerosolized excreta is strictly contained. Further, wild caught rodents should be appropriately tested for hantaviruses. This includes monitoring rodents for hantaviruses that are known to infect the rodent in use. If no hantaviruses have been specifically associated with the rodent under investigation, then more broadly reactive tests, which identify known hantaviruses that infect the rodent subfamily, should be used.
4. Known hantavirus infected rodents should be handled according to the guidelines as set forth by the Centers for Disease Control and Prevention, Table 5 (89).
5. Caesarean re-derivation has proven effective at eliminating hantavirus infections in laboratory rats (113). It is likely that caesarean re-derivation would be successful at eliminating hantaviruses from other rodent species as well, however, detailed studies have not been performed.

worked with hantavirus infected rat immunocytomas developed HFRS-like disease (90).

Since laboratory animal caretakers and scientists who work with hantavirus-infected rodents have a significant risk of infection and serious illness, the World Health Organization (WHO) has developed a series of guidelines to prevent and control the spread of hantaviruses in a laboratory setting (91). Our recommendations regarding hantaviruses and laboratory research facilities can be found in Table 4. A 1994 overview of hantaviruses in this journal contained a description of the Four Corners SNV outbreak and an initial series of recommendations regarding hantaviruses in the laboratory (92). In addition to the WHO guidelines, the Centers for Disease Control and Prevention has also issued a set of interim biosafety guidelines for working with agents that cause HPS (Table 5) (89).

Diagnosis of hantavirus infections

Diagnosis of hantavirus infections is very challenging due to the tremendous genomic heterogeneity among the hantaviruses. Genomic heterogeneity is in large part due to the fact that hantaviruses are negative-sense RNA viruses that must reverse transcribe their genome to initiate transcription and translation, and no proof-reading functions have been associated with the virus polymerase. As such, broadly reactive testing modalities must be employed in the diagnosis and monitoring of hantavirus infections. Serologic tests, particularly the immunofluorescent antibody test (IFAT), have historically been the diagnostic test of choice for subtyping and classifying arboviruses (93). Further, serological tests have historically been the method of choice for identifying new members of the family *Bunyaviridae* (93, 94). Molecular diagnostic tests, including amplification of viral genomic RNA by RT-PCR, are sensitive methods for assessing rodents for infection by hantaviruses; however, due to the tremendous genomic heterogeneity of the hantaviruses caution must be used in choosing appropriate primer sets and in interpreting results.

Serologic tests monitor antibody responses to a given agent; thus, actively replicating virus does not need to be present in

Table 5. The Centers for Disease Control and Prevention interim biosafety guidelines for working with agents that cause HPS (89)

1. Biosafety level 2 (BSL-2) facilities and practices are recommended for the laboratory handling of potentially infected sera.
2. Potentially infected tissue samples should be handled in BSL-2 facilities in accordance with BSL-3 practices. Cell-culture virus propagation should be carried out using BSL-3 facilities and practices. Large scale virus growth and handling viral concentrates, should be performed in BSL-4 facilities.
3. Experimentally infected rodents known not to excrete the virus can be housed in accordance with animal biosafety level 2 (ABSL-2) facilities and practices. Serum and tissue samples from HPS infected rodents should be handled according to BSL-3 practices in a BSL-2 facility. All work with infected natural host rodents or other permissive species should be conducted in an ABSL-4 facility.

the animals that are being tested to determine if exposure has occurred. A further benefit to using serologic tests is that antibody-negative carrier states have not been documented for the hantaviruses (4), and rodents mount a vigorous antibody response to hantavirus nucleocapsid and glycoproteins. Therefore, if an immunologically competent animal is infected it should develop a measurable antibody response.

Numerous serological tests have been developed to diagnose hantavirus infections (95). Among the most commonly employed are the IFAT and the enzyme-linked immunosorbent assay (ELISA). The IFAT is one of the most sensitive tests for diagnosing viral infections and, because the test uses virus-infected cells with a full complement of virus epitopes as antigen, it is broadly cross-reactive (96). Thus the IFAT is often the test of choice for screening laboratory rodent colonies for infection by hantaviruses (91) as it has proven to be both sensitive and specific (97); however, the IFAT is prone to false positive tests at serum dilutions of less that 1:32 (97). The enzyme-linked immunosorbent assay (ELISA) is commonly employed in diagnostic testing labs because it is rapid, sensitive and specific, and it is easily automated, making it a cost effective test (98). Many diagnostic ELISAs utilize recombinant nucleocapsid proteins as an antigen source, as the nucleocapsid protein is the most antigenic and cross-reactive of the four hantavirus proteins (99, 100, 101), and because use of a recombinant protein as the antigen source avoids the biohazard associated with handling intact infectious virus. A strip immunoblot assay has also been developed for the rapid diagnosis of SNV infections, which takes advantage of the immunogenicity of a recombinant, truncated, 59 amino acid N-terminal nucleocapsid protein (102). The strip immunoblot is both rapid and sensitive for the diagnosis of PUUV and SNV infections (102); however, it lacks sensitivity for detecting HTNV and SEOV infections (103).

Molecular diagnostic techniques are extremely sensitive and specific tests (104). As hantaviruses are RNA viruses, the molecular diagnostic test of choice is the RT-PCR assay (105). Since hantaviruses are believed to establish life-long persistent infections in their well-adapted reservoir host, virus genomic RNA should be present in the host's tissues throughout its life. A major disadvantage to RT-PCR assays is the tremendous genomic heterogeneity of the hantaviruses which hinders RT-PCR testing since multiple PCR primer sets must often be used, and specific information about the rodent species being tested must be considered. RT-PCR primer sets have been developed to amplify RNA from many of the hantaviruses carried by animals within a given Murid rodent subfamily (Table 6). Interpretation of RT-PCR results must be undertaken with caution, as negative test results do not necessarily mean that the animal is free

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Table 6. PCR primer sets for amplifying hantaviruses from the following rodent subfamilies

Subfamily	Primer	Primer sequence 5'-3'[a]	Reference
Sigmodontinae	SS143	TGG IIC CIG ATG AIG TTA ACA A	(114, 115)
	CSS1070R	GCC ATI ATI GTI TTI CTC AT	
	SS283C	CCA ACI GGG ITT GAI CCI GAT GA	
	PPT716R	AAI CCI ATI ACI CCC AT	
Murinae	MS120C	GGA TGC AGA AAA ICA GTA TGA	(116)
	MS1170R	AGT TGT ATI CCC ATI GAT TGT	
	MS364C	GAI ATT GAT GAA CCT ACA G	
	MS963R	ACC CAI ATT GAT GAT GGT GA	
Arvicolinae	PPT334C	TAT GGI AAT GTC CTT GAT GT	(114)
	PPT986R	GCA CAI GCA AAI ACC CA	
	PPT376C	CCI AGT GGI CAI ACA GC	
	PPT716R	AAI CCI ATI ACI CCC AT	

[a]Standard International Union of Biochemistry codes for bases and ambiguity.

of all hantaviruses. Another potential concern with molecular diagnostic tests is that positive control RNA is not readily available to validate RT-PCR assays for many of the hantaviruses.

Due to the great genomic heterogeneity of the hantaviruses and the very close co-evolutionary association between rodent-hantavirus pairs, the single most important issue in testing rodents for infection by hantaviruses is knowledge of the species of the hantavirus and rodent that are being tested. These two critical pieces of information allow rational application of appropriate diagnostic tests to assess the hantavirus infection status of laboratory rodents.

Specific recommendations regarding hantavirus testing of laboratory rodents

Hantaan virus. The natural reservoir host for Hantaan virus (HTNV) is the striped field mouse (*Apodemus agrarius*), which primarily inhabits eastern Asia. Hantaan virus has been documented in laboratory animal facilities among wild caught striped field mice. Laboratory mice and rats (*Mus musculus* and *Rattus norvegicus*) have been experimentally inoculated with HTNV; however, natural infection of these species by HTNV most likely does not occur due to the species-specificity of HTNV. Thus, testing laboratory maintained *Apodemus agrarius* or closely related rodent species for HTNV is warranted. However, routine testing of laboratory rats and mice for HTNV is generally unnecessary. Imported *Apodemus* sp. or closely related rodents should be quarantined and assessed for antibodies to HTNV.

Seoul virus. Both *Rattus norvegicus* and *Rattus rattus* have been found to be reservoirs for Seoul virus (SEOV), an HFRS-causing hantavirus. SEOV is the most likely candidate for the numerous Hantaan-like virus outbreaks that have been identified in laboratory rats in Asia, Europe, and North America. Since SEOV is a known human pathogen, laboratory-maintained rats should be monitored for Seoul virus as a part of a routine diagnostic testing and health maintenance program. Testing of wild caught rodents or laboratory rats in facilities that have been accessed by wild rodents is of particular importance as a global serosurvey for Hantaan-like viruses indicated that 8-64% of wild caught rodents in three U.S. cities had serum antibody to a hantavirus (106). The HTNV nucleocapsid ELISA has been found to cross react very well with antibodies to SEOV, thus the HTNV ELISA is commonly used in the serodiagnosis of SEOV infection. Seoul virus-specific ELISA antigens or whole SEOV IFATs are also appropriate diagnostic tests for SEOV infection. There is no specific evidence that laboratory mice can be naturally infected with SEOV; thus, testing of laboratory mice is probably not warranted.

Infection of laboratory maintained cell lines of rat origin by hantaviruses has been documented (90); thus, testing of rat cell lines for hantaviruses is appropriate. Since hantaviruses pose a significant human health risk, if a cell line is suspected of harboring a hantavirus it should be tested by IFAT or RT-PCR. If the rat antibody production test is used to assess suspect cell lines, then appropriate biological containment must be used to prevent exposure of personnel to hantaviruses in the event of a positive result.

Hantaviruses from uncommonly used or wild caught rodents. Uncommonly used or wild caught rodents should be speciated by an experienced mammalogist and tested for species-specific hantaviruses, if any are known. Due to the high human mortality rate associated with HPS-causing hantaviruses, appropriate diagnostic testing is particularly important when dealing with New World rodents of the species *Peromyscus, Oryzomys, Oligoryzomys, Sigmodon,* and *Calomys* as they have been associated with zoonotic, HPS-causing, hantaviruses. If no species-specific hantaviruses have been identified in the rodents to be tested, then broadly reactive testing modalities should be employed, such as serologic testing with ELISA or IFAT antigens that are known to cross react with hantaviruses from similar rodent species. When testing rodents for cross-reactive hantaviruses, caution must be used in interpreting the results, especially when considering negative tests.

Rat Respiratory Virus (RRV). RRV is a newly recognized virus that was isolated from clinically normal laboratory rats with interstitial pneumonia (107, 108). The virus is enveloped, 80-120 nm in diameter and the in vitro cultured virus has been used to experimentally recreate pulmonary disease in naïve laboratory rats (109, 110). Serologic evidence indicates that there is unequivocal two-way cross-reactivity between RRV and HTNV in an IFAT format (110, 111). Serologic evidence, using both Hantaan virus and RRV IFAT slides, indicates that approximately 8% of laboratory rats have antibodies to RRV, while similar diagnostic testing indicates that approximately 22% of laboratory mice have antibodies to RRV (110, 111). Thus, studies to date suggest that RRV is a previously unrecognized Hantaan-like virus of laboratory mice and rats. Retrospective serologic analysis indicates that RRV has been present in laboratory rodents since 1991, and probably much longer. Currently there is no commercially available diagnostic assay for RRV; however, these assays are under development. Like one-half of the previ-

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ously described hantaviruses, there is no evidence to date indicating that RRV is a human pathogen, so it should not cause undue human health concerns among laboratory rodent users.

Summary

The *Bunyaviridae* are among the most common and widespread viruses in nature with niches ranging from common houseplants to humans. Members of the *Hantavirus* genus have developed a unique mechanism for aerosol transmission and have exploited one of the most common mammalian hosts, murid rodents, as reservoirs. There are currently 22 well described hantaviruses; however less than 5% of the estimated 2000 species of murid rodents have been tested for hantavirus infection. This certainly creates the possibility that there may in fact be many, many more species of hantaviruses awaiting discovery. This presents a potentially serious disease problem as nearly one-half of known hantaviruses cause human disease with death rates ranging from 0.1% to nearly 50%, depending upon the hantavirus species. Further, approximately 200,000 cases of hantavirus-related disease are diagnosed in humans each year, and it is likely that several times that number are undiagnosed or unreported. The idea that previously undiscovered hantaviruses could be lurking in the wild was brought to the forefront in May 1993, when Sin Nombre virus arose in the U.S. desert Southwest. Since 1993 the New World hantaviruses, those associated with rodents of the subfamily *Sigmodontinae*, have become the largest single group of hantaviruses, and the number of newly recognized virus species is still increasing. The fact that hantaviruses are a large and growing group of viruses that have been associated with human disease underscores the fact that little is known about the biology of the hantaviruses, their pathogenesis, or their host-virus relationships. Further, no well described animal models of human hantavirus diseases exist, which is one of the most important obstacles that must be overcome to facilitate development of therapeutic intervention strategies to mitigate human disease.

Previous hantavirus outbreaks in research labs resulting from inapparent exposure to hantavirus infected rodents have led to human morbidity and to an occasional fatality. This underscores the need for vigilance in screening laboratory rodents for hantavirus infections. This is of particular concern when non-traditional species or wild caught rodents are used in a confined laboratory setting. Since hantavirus infections can be very difficult to diagnose, a great deal of care and expertise are required in applying the appropriate diagnostic tests to ensure the safety of people working with rodents. Further, more thorough methods of screening rodents for hantavirus diseases must be developed and implemented.

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References

1. **Lee, H. W., P. W. Lee, L. J. Baek, C. K. Song, and I. W. Seong.** 1981. Intraspecific transmission of Hantaan virus, etiologic agent of Korean hemorrhagic fever, in the rodent *Apodemus agrarius*. Am. J. Trop. Med. Hyg. **30:**1106-1112.
2. **Nuzum, E. O., C. A. Rossi, E. H. Stephenson, and J. W. LeDuc.** 1988. Aerosol transmission of Hantaan and related viruses to laboratory rats. Am. J. Trop. Med. Hyg. **38:**636-640.
3. **Elliott, R. M., M. Bouloy, C. H. Calisher, R. Goldbach, J. T. Moyer, S. T. Nichol, R. Pettersson, A. Plyusnin, and C. S. Schmaljohn.** 2000. Family *Bunyaviridae*, p. 599-621. *In* M. H. V. van Regenmortel, C. M. Fauquet, D. H. L. Bishop, E. B. Carstens, M. K. Estes, S. M. Lemon, J. Maniloff, M. A. Mayo, D. J. McGeoch, C. R. Pringle, and R. B. Wickner (ed.), Virus taxonomy: seventh report of the international committee on taxonomy of viruses. Academic Press, San Diego.
4. **Hjelle, B., and T. Yates.** 2001. Modeling hantavirus maintenance and transmission in rodent communities. Curr. Top. Microbiol. Immunol. **256:**77-90.
5. **Childs, J. E., G. E. Glass, G. W. Korch, and J. W. LeDuc.** 1989. Effects of hantaviral infection on survival, growth and fertility in wild rat (*Rattus norvegicus*) populations of Baltimore, Maryland. J. Wildl. Dis. **25:**469-476.
6. **Nichol, S. T., C. F. Spiropoulou, S. Morzunov, P. E. Rollin, T. G. Ksiazek, H. Feldmann, A. Sanchez, J. Childs, S. Zaki, and C. J. Peters.** 1993. Genetic identification of a hantavirus associated with an outbreak of acute respiratory illness. Science. **262:**914-917.
7. **Duchin, J. S., F. T. Koster, C. J. Peters, G. L. Simpson, B. Tempest, S. R. Zaki, T. G. Ksiazek, P. E. Rollin, S. Nichol, E. T. Umland, R. L. Moolenaar, S. E. Reef, K. B. Nolte, M. M. Gallaher, J. C. Butler, R. F. Breiman, and The Hantavirus Study Group.** 1994. Hantavirus pulmonary syndrome: a clinical description of 17 patients with a newly recognized disease. The Hantavirus Study Group. N. Engl. J. Med. **330:**949-955.
8. **Lee, H. W.** 1996. Epidemiology and pathogenesis of hemorrhagic fever with renal syndrome, p. 253-267. *In* R. M. Elliott (ed.), The *Bunyaviridae*. Plenum Press, New York.
9. **Johnson, K. M.** 2001. Hantaviruses: history and overview. Curr. Top. Microbiol. Immunol. **256:**1-14.
10. **Earle, D. P.** 1954. Symposium on epidemic hemorrhagic fever. Am. J. Med. **16:**617-709.
11. **Gajdusek, D. C.** 1962. Virus hemorrhagic fevers. J. Pediatr. **60:**841-857.
12. **Lee, H. W., P. W. Lee, and K. M. Johnson.** 1978. Isolation of the etiologic agent of Korean hemorrhagic fever. J. Infect. Dis. **137:**298-308.
13. **French, G. R., R. S. Foulke, O. A. Brand, G. A. Eddy, H. W. Lee, and P. W. Lee.** 1981. Korean hemorrhagic fever: propagation of the etiologic agent in a cell line of human origin. Science. **211:**1046-1048.
14. **Schmaljohn, C. S., S. E. Hasty, S. A. Harrison, and J. M. Dalrymple.** 1983. Characterization of Hantaan virions, the prototype virus of hemorrhagic fever with renal syndrome. J. Infect. Dis. **148:**1005-1012.
15. **Peters, C. J., G. L. Simpson, and H. Levy.** 1999. Spectrum of hantavirus infection: hemorrhagic fever with renal syndrome and hantavirus pulmonary syndrome. Annu. Rev. Med. **50:**531-545.
16. **Mertz, G. J., B. L. Hjelle, and R. T. Bryan.** 1997. Hantavirus infection. Adv. Intern. Med. **42:**369-421.
17. **Schmaljohn, C., and B. Hjelle.** 1997. Hantaviruses: a global disease problem. Emerg. Infect. Dis. **3:**95-104.
18. **Vapalahti, O.** 1996. Ph.D. thesis. University of Helsinki.
19. **Lee, H. W.** 1989. Hemorrhagic fever with renal syndrome in Korea. Rev. Infect. Dis. **11:**S864-876.
20. **Glass, G. E., A. J. Watson, J. W. LeDuc, and J. E. Childs.** 1994. Domestic cases of hemorrhagic fever with renal syndrome in the United States. Nephron. **68:**48-51.
21. **Mackow, E. R., and I. N. Gavrilovskaya.** 2001. Cellular receptors and hantavirus pathogenesis. Curr. Top. Microbiol. Immunol. **256:**91-115.
22. **Zaki, S. R., A. S. Khan, R. A. Goodman, L. R. Armstrong, P. W. Greer, L. M. Coffield, T. G. Ksiazek, P. E. Rollin, C. J. Peters, and R. F. Khabbaz.** 1996. Retrospective diagnosis of hantavirus pulmonary syndrome, 1978-1993: implications for emerging infectious diseases. Arch. Pathol. Lab. Med. **120:**134-139.

http://prime-pdf-watermark.prime-prod.pubfactory.com/ | 2025-02-25

23. **Frampton, J. W., S. Lanser, and C. R. Nichols.** 1995. Sin Nombre virus infection in 1959. Lancet. **346:**781-782.
24. **Zaki, S. R., P. W. Greer, L. M. Coffield, C. S. Goldsmith, K. B. Nolte, K. Foucar, R. M. Feddersen, R. E. Zumwalt, G. L. Miller, A. S. Khan, P. E. Rollin, T. G. Ksiazek, S. T. Nichol, B. W. J. Mahy, and C. J. Peters.** 1995. Hantavirus pulmonary syndrome. Pathogenesis of an emerging infectious disease. Am. J. Pathol. **146:**552-579.
25. **Khan, A. S., R. F. Khabbaz, L. R. Armstrong, R. C. Holman, S. P. Bauer, J. Graber, T. Strine, G. Miller, S. Reef, J. Tappero, P. E. Rollin, S. T. Nichol, S. R. Zaki, R. T. Bryan, L. E. Chapman, C. J. Peters, and T. G. Ksiazek.** 1996. Hantavirus pulmonary syndrome: the first 100 U.S. cases. J. Infect. Dis. **173:**1297-1303.
26. **Khan, A. S., C. F. Spiropoulou, S. Morzunov, S. R. Zaki, M. A. Kohn, S. R. Nawas, L. McFarland, and S. T. Nichol.** 1995. Fatal illness associated with a new hantavirus in Louisiana. J. Med. Virol. **46:**281-286.
27. **Khan, A. S., M. Gaviria, P. E. Rollin, W. G. Hlady, T. G. Ksiazek, L. R. Armstrong, R. Greenman, E. Ravkov, M. Kolber, H. Anapol, E. D. Sfakianaki, S. T. Nichol, C. J. Peters, and R. F. Khabbaz.** 1996. Hantavirus pulmonary syndrome in Florida: association with the newly identified Black Creek Canal virus. Am. J. Med. **100:**46-48.
28. **Hjelle, B., D. Goade, N. Torrez-Martinez, M. Lang-Williams, J. Kim, R. L. Harris, and J. A. Rawlings.** 1996. Hantavirus pulmonary syndrome, renal insufficiency, and myositis associated with infection by Bayou hantavirus. Clin. Infect. Dis. **23:**495-500.
29. **Kanerva, M., A. Paakkala, J. Mustonen, T. Paakkala, J. Lahtela, and A. Pasternack.** 1996. Pulmonary involvement in nephropathia epidemica: radiological findings and their clinical correlations. Clin. Nephrol. **46:**369-378.
30. **Gavrilovskaya, I. N., M. Shepley, R. Shaw, M. H. Ginsberg, and E. R. Mackow.** 1998. β_3 Integrins mediate the cellular entry of hantaviruses that cause respiratory failure. Proc. Natl. Acad. Sci. U. S. A. **95:**7074-7079.
31. **Gavrilovskaya, I. N., E. J. Brown, M. H. Ginsberg, and E. R. Mackow.** 1999. Cellular entry of hantaviruses which cause hemorrhagic fever with renal syndrome is mediated by β_3 integrins. J. Virol. **73:**3951-3959.
32. **Cosgriff, T. M.** 1991. Mechanisms of disease in Hantavirus infection: pathophysiology of hemorrhagic fever with renal syndrome. Rev. Infect. Dis. **13:**97-107.
33. **Nolte, K. B., R. M. Feddersen, K. Foucar, S. R. Zaki, F. T. Koster, D. Madar, T. L. Merlin, P. J. McFeeley, E. T. Umland, and R. E. Zumwalt.** 1995. Hantavirus pulmonary syndrome in the United States: a pathological description of a disease caused by a new agent. Hum. Pathol. **26:**110-120.
34. **Hughes, J. M., C. J. Peters, M. L. Cohen, and B. W. Mahy.** 1993. Hantavirus pulmonary syndrome: an emerging infectious disease. Science. **262:**850-851.
35. **Pensiero, M. N., J. B. Sharefkin, C. W. Dieffenbach, and J. Hay.** 1992. Hantaan virus infection of human endothelial cells. J. Virol. **66:**5929-5936.
36. **Yanagihara, R., and D. J. Silverman.** 1990. Experimental infection of human vascular endothelial cells by pathogenic and nonpathogenic hantaviruses. Arch. Virol. **111:**281-286.
37. **Mori, M., A. L. Rothman, I. Kurane, J. M. Montoya, K. B. Nolte, J. E. Norman, D. C. Waite, F. T. Koster, and F. A. Ennis.** 1999. High levels of cytokine-producing cells in the lung tissues of patients with fatal hantavirus pulmonary syndrome. J. Infect. Dis. **179:**295-302.
38. **Yang, C. W., and B. K. Bang.** 1992. Changes of serum levels of tumor necrosis factor-α in patients with hemorrhagic fever with renal syndrome. The Journal of Catholic Medical College. **45:**819-830.
39. **Linderholm, M., C. Ahlm, B. Settergren, A. Waage, and A. Tarnvik.** 1996. Elevated plasma levels of tumor necrosis factor (TNF)-α, soluble TNF receptors, interleukin (IL)-6, and IL-10 in patients with hemorrhagic fever with renal syndrome. J. Infect. Dis. **173:**38-43.
40. **Ennis, F. A., J. Cruz, C. F. Spiropoulou, D. Waite, C. J. Peters, S. T. Nichol, H. Kariwa, and F. T. Koster.** 1997. Hantavirus pulmonary syndrome: CD8+ and CD4+ cytotoxic T lymphocytes to epitopes on Sin Nombre virus nucleocapsid protein isolated during acute illness. Virology. **238:**380-390.
41. **Mustonen, J., J. Partanen, M. Kanerva, K. Pietila, O. Vapalahti, A. Pasternack, and A. Vaheri.** 1996. Genetic susceptibility to severe course of nephropathia epidemica caused by Puumala hantavirus. Kidney Int. **49:**217-221.
42. **Mustonen, J., J. Partanen, M. Kanerva, K. Pietila, O. Vapalahti, A. Pasternack, and A. Vaheri.** 1998. Association of HLA B27 with benign clinical course of nephropathia epidemica caused by Puumala hantavirus. Scand. J. Immunol. **47:**277-279.
43. **Plyusnin, A., J. Horling, M. Kanerva, J. Mustonen, Y. Cheng, J. Partanen, O. Vapalahti, S. K. Kukkonen, J. Niemimaa, H. Henttonen, B. Niklasson, A. Lundkvist, and A. Vaheri.** 1997. Puumala hantavirus genome in patients with nephropathia epidemica: correlation of PCR positivity with HLA haplotype and link to viral sequences in local rodents. J. Clin. Microbiol. **35:**1090-1096.
44. **Hooper, J. W., T. Larsen, D. M. Custer, and C. S. Schmaljohn.** 2001. A lethal disease model for hantavirus pulmonary syndrome. Virology. **289:**6-14.
45. **Hooper, J. W., D. M. Custer, E. Thompson, and C. S. Schmaljohn.** 2001. DNA vaccination with the Hantaan virus M gene protects hamsters against three of four HFRS hantaviruses and elicits a high-titer neutralizing antibody response in rhesus monkeys. J. Virol. **75:**8469-8477.
46. **Raju, R., and D. Kolakofsky.** 1989. The ends of La Crosse virus genome and antigenome RNAs within nucleocapsids are base paired. J. Virol. **63:**122-128.
47. **Pettersson, R. F., and L. Melin.** 1996. Synthesis, assembly, and intracellular transport of *Bunyaviridae* membrane proteins, p. 159-188. *In* R. M. Elliott (ed.), The *Bunyaviridae*. Plenum Press, New York.
48. **Schmaljohn, C. S.** 1996. *Bunyaviridae:* the viruses and their replication, p. 1447-1471. *In* B. N. Fields, D. M. Knipe, P. M. Howley, R. M. Chanock, T. P. Monath, J. L. Melnick, B. Roizman, and S. E. Straus (ed.), Fields virology. Lippincott-Raven Publishers, Philadelphia.
49. **Schmaljohn, C. S.** 1996. Molecular biology of hantaviruses, p. 63-90. *In* R. M. Elliott, (ed.), The *Bunyaviridae*. Plenum Publishers, New York.
50. **Severson, W., L. Partin, C. S. Schmaljohn, and C. B. Jonsson.** 1999. Characterization of the Hantaan nucleocapsid protein-ribonucleic acid interaction. J. Biol. Chem. **274:**33732-33739.
51. **Goldsmith, C. S., L. H. Elliott, C. J. Peters, and S. R. Zaki.** 1995. Ultrastructural characteristics of Sin Nombre virus, causative agent of hantavirus pulmonary syndrome. Arch. Virol. **140:**2107-2122.
52. **Murphy, F., C. Fauquet, D. Bishop, S. Ghabrial, A. Jarvis, G. Martelli, M. Mayo, and M. Summers (ed.).** 1995. Virus taxonomy-the classification and nomenclature of viruses: sixth report of the international committee on taxonomy of viruses, Springer-Verlag, Vienna.
53. **McCormick, J. B., D. R. Sasso, E. L. Palmer, and M. P. Kiley.** 1982. Morphological identification of the agent of Korean haemorrhagic fever (Hantaan virus) as a member of the *Bunyaviridae*. Lancet. **1:**765-768.
54. **White, J. D., F. G. Shirey, G. R. French, J. W. Huggins, O. M. Brand, and H. W. Lee.** 1982. Hantaan virus, aetiological agent of Korean haemorrhagic fever, has *Bunyaviridae*-like morphology. Lancet. **1:**768-771.
55. **Jonsson, C. B., and C. S. Schmaljohn.** 2001. Replication of hantaviruses. Curr. Top. Microbiol. Immunol. **256:**15-32.
56. **Honda, A., K. Ueda, K. Nagata, and A. Ishihama.** 1988. RNA polymerase of influenza virus: role of NP in RNA chain elongation. J Biochem (Tokyo). **104:**1021-1026.
57. **Patton, J. T., N. L. Davis, and G. W. Wertz.** 1984. Role of vesicular stomatitis virus proteins in RNA replication, p. 147-152. *In* D. H. Bishop, and R. W. Compans (ed.), Nonsegmented negative strand viruses: paramyxoviruses and rhabdoviruses. Academic Press, Orlando.

http://prime-pdf-watermark.prime-prod.pubfactory.com/ | 2025-02-25

58. **Rossier, C., J. Patterson, and D. Kolakofsky.** 1986. La Crosse virus small genome mRNA is made in the cytoplasm. J. Virol. **58:**647-650.
59. **Garcin, D., M. Lezzi, M. Dobbs, R. M. Elliott, C. Schmaljohn, C. Y. Kang, and D. Kolakofsky.** 1995. The 5' ends of Hantaan virus (*Bunyaviridae*) RNAs suggest a prime-and-realign mechanism for the initiation of RNA synthesis. J. Virol. **69:**5754-5762.
60. **Meyer, B. J., and C. Schmaljohn.** 2000. Accumulation of terminally deleted RNAs may play a role in Seoul virus persistence. J. Virol. **74:**1321-1331.
61. **Spiropoulou, C. F.** 2001. Hantavirus maturation. Curr. Top. Microbiol. Immunol. **256:**33-46.
62. **Oldstone, M. B.** 1991. Molecular anatomy of viral persistence. J. Virol. **65:**6381-6386.
63. **Oldstone, M. B.** 1998. Viral persistence: mechanisms and consequences. Curr. Opin. Microbiol. **1:**436-441.
64. **Yanagihara, R., H. L. Amyx, and D. C. Gajdusek.** 1985. Experimental infection with Puumala virus, the etiologic agent of nephropathia epidemica, in bank voles (*Clethrionomys glareolus*). J. Virol. **55:**34-38.
65. **Lee, H. W., G. R. French, P. W. Lee, L. J. Baek, K. Tsuchiya, and R. S. Foulke.** 1981. Observations on natural and laboratory infection of rodents with the etiologic agent of Korean hemorrhagic fever. Am. J. Trop. Med. Hyg. **30:**477-482.
66. **Lee, P. W., R. Yanagihara, C. J. Gibbs, Jr., and D. C. Gajdusek.** 1986. Pathogenesis of experimental Hantaan virus infection in laboratory rats. Arch. Virol. **88:**57-66.
67. **Hutchinson, K. L., P. E. Rollin, and C. J. Peters.** 1998. Pathogenesis of a North American hantavirus, Black Creek Canal virus, in experimentally infected *Sigmodon hispidus*. Am. J. Trop. Med. Hyg. **59:**58-65.
68. **Tanishita, O., Y. Takahashi, Y. Okuno, M. Tamura, H. Asada, J. R. Dantas, Jr., T. Yamanouchi, K. Domae, T. Kurata, and K. Yamanishi.** 1986. Persistent infection of rats with haemorrhagic fever with renal syndrome virus and their antibody responses. J. Gen. Virol. **67:**2819-2824.
69. **Carey, D. E., R. Reuben, K. N. Panicker, R. E. Shope, and R. M. Myers.** 1971. Thottapalayam virus: a presumptive arbovirus isolated from a shrew in India. Indian J. Med. Res. **59:**1758-1760.
70. **Zeller, H. G., N. Karabatsos, C. H. Calisher, J. P. Digoutte, C. B. Cropp, F. A. Murphy, and R. E. Shope.** 1989. Electron microscopic and antigenic studies of uncharacterized viruses. II. Evidence suggesting the placement of viruses in the family *Bunyaviridae*. Arch. Virol. **108:**211-227.
71. **Arthur, R. R., R. S. Lofts, J. Gomez, G. E. Glass, J. W. Leduc, and J. E. Childs.** 1992. Grouping of hantaviruses by small (S) genome segment polymerase chain reaction and amplification of viral RNA from wild-caught rats. Am. J. Trop. Med. Hyg. **47:**210-224.
72. **Plyusnin, A., and S. P. Morzunov.** 2001. Virus evolution and genetic diversity of hantaviruses and their rodent hosts. Curr. Top. Microbiol. Immunol. **256:**47-75.
73. **Lyubsky, S., I. Gavrilovskaya, B. Luft, and E. Mackow.** 1996. Histopathology of *Peromyscus leucopus* naturally infected with pathogenic NY-1 hantaviruses: pathologic markers of HPS viral infection in mice. Lab. Invest. **74:**627-633.
74. **Netski, D., B. H. Thran, and S. C. St Jeor.** 1999. Sin Nombre virus pathogenesis in *Peromyscus maniculatus*. J. Virol. **73:**585-591.
75. **Wilson, D. E., and D. M. Reeder (ed.).** 1993. Mammal species of the world: a taxonomic and geographic reference. Smithsonian Institution Press, Washington.
76. **Henttonen, H., O. Vapalahti, and A. Vaheri.** 1995. How many kinds of hantaviruses? Trends Ecol. Evol. **11:**7-8.
77. **Childs, J. E., G. E. Glass, G. W. Korch, and J. W. LeDuc.** 1987. Prospective seroepidemiology of hantaviruses and population dynamics of small mammal communities of Baltimore, Maryland. Am. J. Trop. Med. Hyg. **37:**648-662.
78. **Glass, G. E., J. E. Childs, G. W. Korch, and J. W. LeDuc.** 1988. Association of intraspecific wounding with hantaviral infection in wild rats (*Rattus norvegicus*). Epidemiol. Infect. **101:**459-472.
79. **Borucki, M. K., J. D. Boone, J. E. Rowe, M. C. Bohlman, E. A. Kuhn, R. DeBaca, and S. C. St Jeor.** 2000. Role of maternal antibody in natural infection of *Peromyscus maniculatus* with Sin Nombre virus. J. Virol. **74:**2426-2429.
80. **Dohmae, K., U. Koshimizu, and Y. Nishimune.** 1993. In utero and mammary transfer of hantavirus antibody from dams to infant rats. Lab. Anim. Sci. **43:**557-561.
81. **Dohmae, K., and Y. Nishimune.** 1998. Maternal transfer of Hantavirus antibodies in rats. Lab. Anim. Sci. **48:**395-397.
82. **Douron, E., B. Moriniere, S. Matheron, P. M. Girard, J. P. Gonzalez, F. Hirsch, and J. B. McCormick.** 1984. HFRS after a wild rodent bite in the Haute-Savoie--and risk of exposure to Hantaan-like virus in a Paris laboratory. Lancet. **1:**676-677.
83. **Childs, J. E., J. N. Mills, and G. E. Glass.** 1995. Rodent-borne hemorrhagic fever viruses: a special risk for mammalogists? J. Mammal. **76:**664-680.
84. **Lee, H. W., and K. M. Johnson.** 1982. Laboratory-acquired infections with Hantaan virus, the etiologic agent of Korean hemorrhagic fever. J. Infect. Dis. **146:**645-651.
85. **Desmyter, J., J. W. LeDuc, K. M. Johnson, F. Brasseur, C. Deckers, and C. van Ypersele de Strihou.** 1983. Laboratory rat associated outbreak of haemorrhagic fever with renal syndrome due to Hantaan-like virus in Belgium. Lancet. **2:**1445-1448.
86. **Lloyd, G., E. T. Bowen, N. Jones, and A. Pendry.** 1984. HFRS outbreak associated with laboratory rats in UK. Lancet. **1:**1175-1176.
87. **Kawamata, J., T. Yamanouchi, K. Dohmae, H. Miyamoto, M. Takahaski, K. Yamanishi, T. Kurata, and H. W. Lee.** 1987. Control of laboratory acquired hemorrhagic fever with renal syndrome (HFRS) in Japan. Lab. Anim. Sci. **37:**431-436.
88. **Tsai, T. F.** 1987. Hemorrhagic fever with renal syndrome: mode of transmission to humans. Lab. Anim. Sci. **37:**428-430.
89. **Centers for Disease Control and Prevention.** 1994. Laboratory management of agents associated with hantavirus pulmonary syndrome: interim biosafety guidelines. Centers for Disease Control and Prevention. MMWR. Morb. Mortal. Wkly. Rep. **43:**1-7.
90. **Lloyd, G., and N. Jones.** 1986. Infection of laboratory workers with hantavirus acquired from immunocytomas propagated in laboratory rats. J. Infect. **12:**117-125.
91. **Kawamata, J.** 1999. Laboratory rodent colonies, p. 200-203. *In* H. W. Lee, C. H. Calisher, and C. S. Schmaljohn (ed.), Manual of hemorrhagic fever with renal syndrome and Hantavirus pulmonary syndrome. WHO Collaborating Center for Virus Reference and Research (*Hantaviruses*) Asan Institute for Life Sciences, Seoul.
92. **Rand, M. S.** 1994. Hantavirus: an overview and update. Lab. Anim. Sci. **44:**301-304.
93. **Calisher, C. H., T. P. Monath, N. Karabatsos, and D. W. Trent.** 1981. Arbovirus subtyping: applications to epidemiologic studies, availability of reagents, and testing services. Am. J. Epidemiol. **114:**619-631.
94. **Gonzalez-Scarano, F., and N. Nathanson.** 1996. *Bunyaviridae*, p. 1473-1504. *In* B. N. Fields, D. M. Knipe, and P. M. Howley (ed.), Fields virology. Lippincott-Raven, Philadelphia.
95. **Lee, H. W., C. H. Calisher, and C. S. Schmaljohn (ed.).** 1999. Manual of hemorrhagic fever with renal syndrome and hantavirus pulmonary syndrome. WHO Collaborating Center for Virus Reference and Research (*Hantaviruses*) Asan Institute for Life Sciences, Seoul.
96. **Kendall, L. V., E. K. Steffen, and L. K. Riley.** 1999. Indirect fluorescent antibody (IFA) assay. Contemp. Top. Lab. Anim. Sci. **38:**23.
97. **LeDuc, J. W., G. A. Smith, and K. M. Johnson.** 1984. Hantaan-like viruses from domestic rats captured in the United States. Am. J. Trop. Med. Hyg. **33:**992-998.
98. **Kendall, L. V., and L. K. Riley.** 1999. Enzyme-linked immunosorbent assay (ELISA). Contemp. Top. Lab. Anim. Sci. **38:**46-47.
99. **Rossi, C. A., C. S. Schmaljohn, J. M. Meegan, and J. W. LeDuc.** 1990. Diagnostic potential of a baculovirus-expressed nucleocapsid protein for hantaviruses. Arch. Virol. **Suppl 1:**19-28.

http://prime-pdf-watermark.prime-prod.pubfactory.com/ | 2025-02-25

100. **Schmaljohn, C. S., Y. K. Chu, A. L. Schmaljohn, and J. M. Dalrymple.** 1990. Antigenic subunits of Hantaan virus expressed by baculovirus and vaccinia virus recombinants. J. Virol. **64:**3162-3170.
101. **Vapalahti, O., A. Lundkvist, H. Kallio-Kokko, K. Paukku, I. Julkunen, H. Lankinen, and A. Vaheri.** 1996. Antigenic properties and diagnostic potential of Puumala virus nucleocapsid protein expressed in insect cells. J. Clin. Microbiol. **34:**119-125.
102. **Hjelle, B., S. Jenison, N. Torrez-Martinez, B. Herring, S. Quan, A. Polito, S. Pichuantes, T. Yamada, C. Morris, F. Elgh, H. W. Lee, H. Artsob, and R. Dinello.** 1997. Rapid and specific detection of Sin Nombre virus antibodies in patients with hantavirus pulmonary syndrome by a strip immunoblot assay suitable for field diagnosis. J. Clin. Microbiol. **35:**600-608.
103. **Koster, F. T., and S. A. Jenison.** 1998. Hantaviruses, p. 2140-2147. *In* S. L. Gorbach, J. G. Bartlett, and N. R. Blacklow (ed.), Infectious diseases. W.B. Saunders, Philadelphia.
104. **Compton, S. R., and L. K. Riley.** 2001. Detection of infectious agents in laboratory rodents: traditional and molecular techniques. Comp. Med. **51:**113-119.
105. **Kendall, L. V., and L. K. Riley.** 2000. Reverse transcriptase polymerase chain reaction (RT-PCR). Contemp. Top. Lab. Anim. Sci. **39:**42.
106. **LeDuc, J. W., G. A. Smith, J. E. Childs, F. P. Pinheiro, J. I. Maiztegui, B. Niklasson, A. Antoniades, D. M. Robinson, M. Khin, K. F. Shortridge, M. T. Wooster, M. R. Elwell, P. L. T. Ilbery, D. Koech, E. S. T. Rosa, and L. Rosen.** 1986. Global survey of antibody to Hantaan-related viruses among peridomestic rodents. Bull. World Health Organ. **64:**139-144.
107. **Farrar, P., and M. LaRegina.** 1997. Diagnostic exercise: interstitial pneumonia in viral and mycoplasmal antibody-free Sprague Dawley rats. ACLAD Newsletter. **18:**5-9.
108. **Elwell, M. R., J. F. Mahler, and G. N. Rao.** 1997. "Have you seen this?" Inflammatory lesions in the lungs of rats. Toxicol. Pathol. **25:**529-531.
109. **Riley, L. K., J. H. Simmons, G. Purdy, R. S. Livingston, C. L. Franklin, C. L. Besch-Williford, and R. J. Russell.** 1999. Research update: idiopathic lung lesions in rats. ACLAD Newsletter. **20:**9-11.
110. **Simmons, J. H.** 2001. Ph.D. thesis. University of Missouri-Columbia.
111. **Simmons, J. H.** 2001. Rat respiratory virus (RRV): an update. Paper presented at: 52nd AALAS national meeting. Baltimore, Maryland.
112. **Committee on Care and Use of Laboratory Animals.** 1996. Guide for the care and use of laboratory animals. National Academy Press, Washington, D.C.
113. **McKenna, P., G. van der Groen, G. Hoofd, G. Beelaert, H. Leirs, R. Verhagen, J. P. Kints, F. Cormont, F. Nisol, and H. Bazin.** 1992. Eradication of hantavirus infection among laboratory rats by application of caesarian section and a foster mother technique. J. Infect. **25:**181-190.
114. **Bowen, M. D., W. Gelbmann, T. G. Ksiazek, S. T. Nichol, and N. Nowotny.** 1997. Puumala virus and two genetic variants of Tula virus are present in Austrian rodents. J. Med. Virol. **53:**174-181.
115. **Johnson, A. M., M. D. Bowen, T. G. Ksiazek, R. J. Williams, R. T. Bryan, J. N. Mills, C. J. Peters, and S. T. Nichol.** 1997. Laguna Negra virus associated with HPS in western Paraguay and Bolivia. Virology. **238:**115-127.
116. **Papa, A., A. M. Johnson, P. C. Stockton, M. D. Bowen, C. F. Spiropoulou, S. Alexiou-Daniel, T. G. Ksiazek, S. T. Nichol, and A. Antoniadis.** 1998. Retrospective serological and genetic study of the distribution of hantaviruses in Greece. J. Med. Virol. **55:**321-327.

http://prime-pdf-watermark.prime-prod.pubfactory.com/ | 2025-02-25