Diagnostic and Molecular Challenges in Hantavirus Identification

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Introduction

Hantavirus diagnosis is difficult because the clinical syndromes begin with nonspecific symptoms, the viruses are genetically diverse, and laboratory methods do not all answer the same question. Fever, fatigue, muscle pain, headache, nausea, and thrombocytopenia can resemble influenza, leptospirosis, sepsis, dengue, or other infections. By the time hantavirus pulmonary syndrome (HPS) develops into severe cardiopulmonary disease, clinical deterioration can be rapid. Hemorrhagic fever with renal syndrome (HFRS) has a different dominant organ pattern but presents similar early uncertainty (Centers for Disease Control and Prevention, 2026a; Centers for Disease Control and Prevention, 2026b).

The molecular description also requires correction. Hantaviruses are no longer classified simply as a genus in the family Bunyaviridae. The International Committee on Taxonomy of Viruses places the relevant human pathogens in the genus *Orthohantavirus*, subfamily Mammantavirinae, family Hantaviridae. Their tripartite negative-sense RNA genomes encode the nucleoprotein, glycoprotein precursor, and RNA-dependent RNA polymerase. This essay explains the pathogen's biology, diagnostic workflow, cross-reactivity, molecular identification, biosafety, and research limitations while distinguishing clinical confirmation from precise species attribution (International Committee on Taxonomy of Viruses, 2024).

Taxonomy and Natural Hosts

The family Hantaviridae contains several genera associated with rodents, shrews, moles, bats, fish, and reptiles. Human disease is linked to orthohantaviruses. Many medically important viruses are associated with a principal small-mammal host, and long-term host–virus relationships influence geographic distribution. Sin Nombre virus is strongly associated with the deer mouse in North America; Puumala virus with the bank vole in Europe; Hantaan virus with the striped field mouse; and Seoul virus with rats that occur worldwide.

Rodent hosts generally carry infection without the severe human syndromes seen in people, although the statement that infection is always completely asymptomatic in every host is too absolute. Virus can be shed in urine, feces, and saliva. Human infection usually occurs through inhalation of contaminated aerosols during activities that disturb rodent excreta, although direct contact, bites, and contaminated food are possible routes. Most orthohantaviruses are not transmitted between

people. Andes virus is the important exception for which person-to-person transmission has been documented.

Genome Organization

The genome consists of three RNA segments conventionally called small (S), medium (M), and large (L). The S segment encodes the nucleoprotein, which binds viral RNA and participates in replication and immune interactions. The M segment encodes a glycoprotein precursor that is processed into the envelope glycoproteins Gn and Gc. The L segment encodes the viral RNA-dependent RNA polymerase.

These segments are negative-sense RNA, so they cannot be translated directly as messenger RNA. The polymerase must transcribe viral mRNAs after entry. Hantaviruses use “cap snatching,” acquiring capped fragments from host transcripts to initiate viral mRNA synthesis. Complementary RNA intermediates then serve as templates for new genomic RNA.

Segmented genomes permit reassortment when compatible viruses infect the same cell, although ecological separation and host specificity limit many opportunities. Recombination and reassortment claims require careful sequence analysis because laboratory contamination, assembly errors, and convergent evolution can mimic genuine events.

Cell Entry and Replication

Older accounts often present particular integrins as universal receptors for pathogenic hantaviruses. Entry biology is more complex, and receptor use can differ among viruses and cell types. Attachment is followed by endocytosis and fusion within an acidified compartment. The released ribonucleoprotein complexes remain in the cytoplasm, where transcription and replication occur.

Gn and Gc are synthesized through the host secretory pathway and assemble with nucleocapsids at cellular membranes. Some orthohantaviruses assemble predominantly in the Golgi region, while others show important plasma-membrane assembly. This variation is one reason a single replication diagram should not be treated as identical for every species.

Clinical Syndromes

Hantavirus Pulmonary Syndrome

HPS, also called hantavirus cardiopulmonary syndrome in much of the literature, is associated mainly with viruses in the Americas. A prodromal phase with fever and myalgia can progress to capillary leak, pulmonary edema, shock, and respiratory failure. The rapid transition from a nonspecific viral illness to critical disease creates a narrow window for recognition.

Hemorrhagic Fever with Renal Syndrome

HFRS occurs primarily in Europe and Asia, although Seoul virus is globally distributed. Its severity varies by virus. Kidney injury, thrombocytopenia, vascular leakage, and hemorrhagic manifestations can occur. Puumala virus often produces a milder form historically called nephropathia epidemica, while Hantaan and Dobrava-Belgrade viruses can cause more severe illness.

The syndromes overlap. Pulmonary findings can occur in HFRS, and renal abnormalities can occur in HPS. Diagnostic reasoning should not rely on a rigid geographic or organ-based division.

Why Early Clinical Recognition Is Difficult

Early symptoms are not specific enough to confirm hantavirus. Exposure history becomes essential. Clinicians should ask about cleaning rodent-infested buildings, sleeping in cabins, agricultural work, handling rodents, or travel to an endemic region. The absence of a remembered rodent encounter does not exclude infection because exposure may be unnoticed.

Laboratory patterns such as thrombocytopenia, hemoconcentration, leukocytosis with immunoblasts, or renal impairment can increase suspicion in the appropriate setting. They are supportive rather than pathognomonic. Imaging may show pulmonary edema in HPS, but imaging cannot identify the virus.

Serological Diagnosis

Detection of hantavirus-specific IgM by enzyme-linked immunosorbent assay is a common method for diagnosing acute infection. A rising IgG titer in paired sera can also support recent infection. Serology is useful because antibodies are often detectable near clinical presentation, whereas the period of detectable viral RNA may be limited.

Cross-reactivity is a major challenge. The nucleoprotein is immunogenic and contains conserved regions, so antibodies can react with related viruses. A positive screening assay may establish hantavirus infection without identifying the exact species. Assay antigen, local epidemiology, timing, and validation population influence interpretation.

IgM assays can also produce false-positive results through nonspecific reactivity. Confirmation may require an alternative assay, paired specimens, or testing at a reference laboratory. A single IgG-positive result may reflect past exposure rather than acute disease.

Molecular Detection

Reverse-transcription polymerase chain reaction can detect viral RNA in blood or tissue and can provide sequence information for identification. It is particularly useful early in illness, but sensitivity declines as viremia resolves. Specimen timing, RNA degradation, primer design, and viral diversity affect performance.

Targeted PCR assays are efficient when the likely virus is known. Broad-range or pan-hantavirus primers can detect a wider set of viruses but may sacrifice sensitivity. Sequence mismatch in primer or probe regions can cause false-negative results, especially for newly discovered lineages.

A positive PCR result should be interpreted with contamination controls and clinical context. Molecular detection from a rodent or environmental sample does not prove that the same virus caused a human patient's illness unless the epidemiological and sequence evidence connect them.

Neutralization Testing

Plaque-reduction neutralization testing can help distinguish closely related viruses by measuring whether patient antibodies prevent infection in cell culture. It is more specific than many binding-antibody assays, but it is labor-intensive, slow, and dependent on access to appropriate live viruses and containment facilities.

Neutralization is often most valuable for epidemiology or retrospective species attribution rather than urgent bedside decisions. Cross-neutralization can still occur, and suitable virus isolates may be unavailable for a newly identified species.

Immunohistochemistry and Pathology

Immunohistochemical detection of hantavirus antigen in tissues can support diagnosis, particularly in fatal cases. Pathology may show pulmonary edema and vascular leakage without the type of endothelial destruction that alone would explain the severity. Tissue handling requires appropriate biosafety and coordination with public-health laboratories.

Autopsy findings should be integrated with serology, PCR, exposure history, and clinical course. Morphology alone cannot reliably distinguish hantavirus from all competing causes of acute respiratory or renal failure.

Next-Generation Sequencing

Metagenomic and targeted next-generation sequencing can identify divergent viruses when routine assays fail. Sequencing can characterize all three segments, detect mixed infection, and support phylogenetic analysis. It is especially valuable for discovery and outbreak investigation.

The method has limitations. Clinical samples may contain little viral RNA compared with host RNA. Results can be affected by index hopping, reagent contamination, incomplete genome coverage, and bioinformatic classification errors. Discovery of a sequence does not automatically establish causation; independent confirmation and epidemiological evidence are required.

The Challenge of Species Attribution

Clinical management usually depends more on recognizing severe hantavirus disease than on naming the exact virus. Species attribution matters for surveillance, ecology, transmission assessment, and public-health communication. It becomes difficult when serological cross-reactivity is high or only a short sequence is available (Jonsson & Vapalahti, 2010).

Phylogenetic analysis should include appropriate reference sequences and all available segments. Because taxonomy changes as knowledge grows, laboratories should use current ICTV names while also reporting familiar virus names where helpful for clinicians.

Biosafety and Laboratory Coordination

Routine clinical specimens can be handled under established laboratory precautions, but virus culture and neutralization with live pathogenic strains require specialized containment and trained personnel. Suspected cases should prompt consultation with local or state health departments and, where appropriate, national reference laboratories.

Packaging, shipping, specimen type, timing, and required authorization should be confirmed before submission. Delays occur when a laboratory receives an unsuitable specimen or lacks the exposure and clinical information needed to prioritize testing.

Reverse Genetics and Functional Research

Earlier literature correctly identified the lack of reliable reverse-genetics systems as an obstacle. Researchers need systems that can generate infectious virus from defined genetic components to test how mutations affect replication, host range, immune evasion, or virulence. Progress has been made for some hantaviruses, but reverse genetics remains technically demanding and is not equally

established across the diverse family.

Functional claims must also separate correlation from causation. A sequence difference associated with severe disease may simply mark a lineage or outbreak. Demonstrating a virulence mechanism requires controlled experiments, relevant models, and attention to host factors.

Integrated Diagnostic Strategy

The most reliable approach combines clinical suspicion, exposure history, serology, and molecular testing. In an acutely ill patient, an IgM-positive result may provide rapid confirmation, while PCR and sequencing can identify the virus. Paired IgG testing can resolve uncertain timing. Reference-laboratory methods can clarify cross-reactive or unusual results.

Testing should not delay urgent supportive care. HPS can deteriorate quickly and may require intensive respiratory and hemodynamic support. Diagnosis is valuable not only for the patient but also for exposure investigation and prevention of additional rodent contact.

Conclusion

Hantavirus identification is challenging because early disease resembles many infections, viremia can be brief, antibodies cross-react among related viruses, and the family contains extensive genetic diversity. Modern taxonomy places human-pathogenic hantaviruses in the genus *Orthohantavirus* within Hantaviridae, correcting the older Bunyaviridae classification.

Serology remains central to acute diagnosis, while RT-PCR, sequencing, immunohistochemistry, and neutralization provide complementary evidence. No single test is ideal in every phase of illness or for every epidemiological question. Accurate diagnosis depends on specimen timing, validated assays, clinical context, biosafety, and collaboration among clinicians, public-health agencies, and reference laboratories (Vial et al.).

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