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# Hantaviruses: Rediscovery and new beginnings☆

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ABSTRACT

Virus and host gene phylogenies, indicating that antigenically distinct hantaviruses (family *Bunyaviridae*, genus *Hantavirus*) segregate into clades, which parallel the molecular evolution of rodents belonging to the *Murinae*, *Arvicolinae*, *Neotominae* and *Sigmodontinae* subfamilies, suggested co-divergence of hantaviruses and their rodent reservoirs. Lately, this concept has been vigorously contested in favor of preferential host switching and local host-specific adaptation. To gain insights into the host range, spatial and temporal distribution, genetic diversity and evolutionary origins of hantaviruses, we employed reverse transcription-polymerase chain reaction to analyze frozen, RNAlater®-preserved and ethanol-fixed tissues from 1546 shrews (9 genera and 47 species), 281 moles (8 genera and 10 species) and 520 bats (26 genera and 53 species), collected in Europe, Asia, Africa and North America during 1980–2012. Thus far, we have identified 24 novel hantaviruses in shrews, moles and bats. That these newfound hantaviruses are geographically widespread and genetically more diverse than those harbored by rodents suggests that the evolutionary history of hantaviruses is far more complex than previously conjectured. Phylogenetic analyses indicate four distinct clades, with the most divergent comprising hantaviruses harbored by the European mole and insectivorous bats, with evidence for both co-divergence and host switching. Future studies will provide new knowledge about the transmission dynamics and pathogenic potential of these newly discovered, still-orphan, non-rodent-borne hantaviruses.



## 1. Introduction

More than a decade before the seminal milestone demonstrating that Hantaan virus (HTNV) in the striped field mouse (*Apodemus agrarius*) was the prototype virus of hemorrhagic fever with renal syndrome (HFRS) (Lee et al., 1978), Thottapalayam virus (TPMV) was isolated from an Asian house shrew (*Suncus murinus*) captured in southern India in 1964 (Carey et al., 1971). But even after this formerly unclassified virus was shown to be a hantavirus (Zeller et al., 1989), the prevailing assumption was that TPMV represented a spillover event from a rodent host. Also, despite decades-old reports of HFRS antigens or antibodies in the Eurasian common shrew (*Sorex araneus*), Eurasian pygmy shrew (*Sorex minutus*), Eurasian water shrew (*Neomys fodiens*), European mole (*Talpa europaea*), Chinese mole shrew (*Anourosorex squamipes*) and northern short-tailed shrew (*Blarina brevicauda*) (Chen et al., 1986; Gavrilovskaya et al., 1983; Gligic et al., 1992; Lee et al., 1985a; Tkachenko et al., 1983), shrews and moles (order Soricomorpha, family *Soricidae* and *Talpidae*) had been largely ignored in the ecology and evolution of hantaviruses (family *Bunyaviridae* and genus *Hantavirus*). However, earlier serological tests and recent whole genome sequence analysis of TPMV, showing that it occupies an entirely separate evolutionary lineage, support an early divergence from rodent-borne hantaviruses (Chu et al., 1994; Song et al., 2007a; Xiao et al., 1994; Yadav et al., 2007).

Subsequent acquisition of new knowledge about the spatial and temporal distribution, host range and genetic diversity of hantaviruses in shrews and moles, and later in bats (order Chiroptera), was made possible largely through the generosity of museum curators and field mammalogists who willingly granted access to their archival tissue collections. The availability of such specimens provides strong justification for the continued expansion and long-term maintenance of archival tissue repositories for future investigations.

Phylogenetic analyses of these newfound hantaviruses indicate at least four distinct clades, with the most divergent lineage



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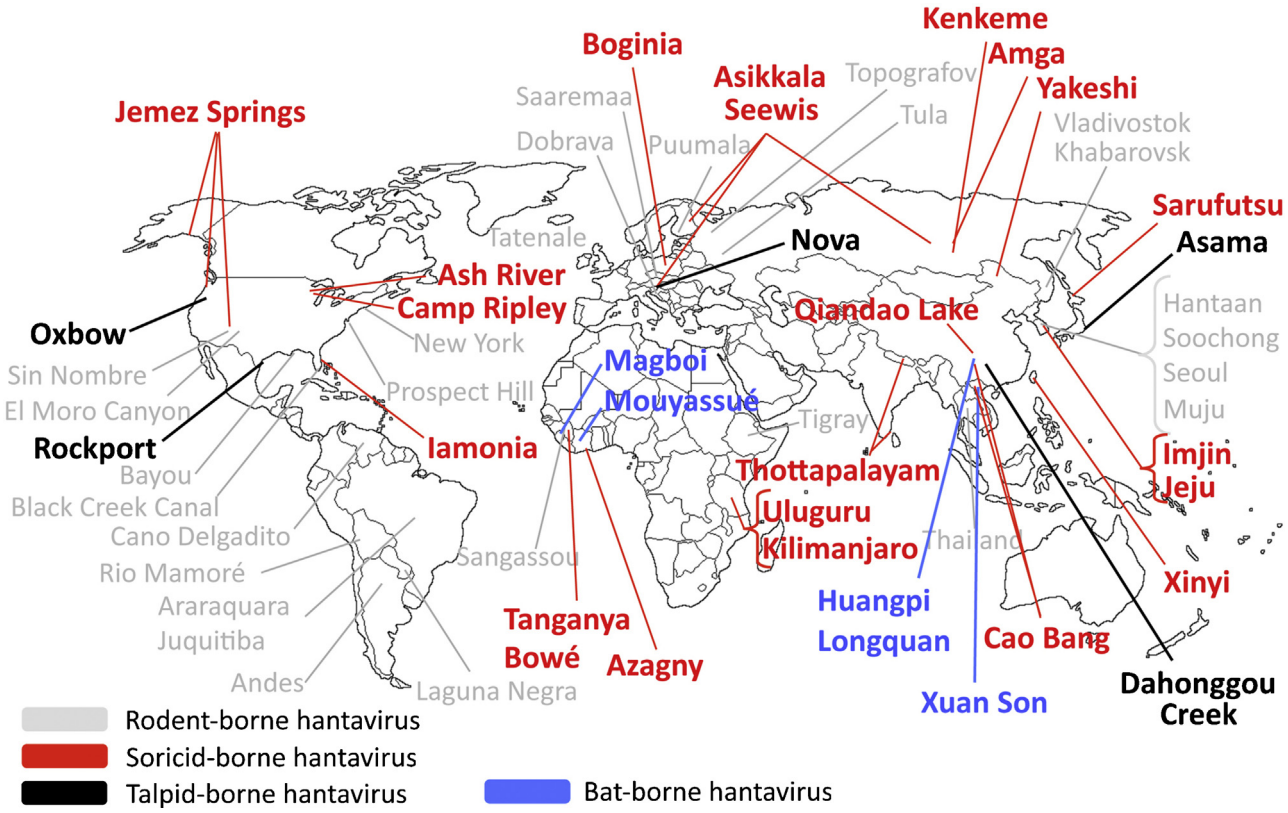


**Fig. 1.** Global landscape of representative rodent-borne hantaviruses and newfound hantaviruses harbored by shrews, moles and insectivorous bats. Lines indicate the approximate geographic locations where reservoir hosts were captured.

comprising hantaviruses harbored by the European mole (Kang et al., 2009c) and insectivorous bats (Arai et al., 2013; Guo et al., 2013; Sumibcay et al., 2012; Weiss et al., 2012). The realization that these soricomorph- and chiropteran-borne hantaviruses are genetically more diverse than those found in rodents suggests that the evolutionary history of hantaviruses is far more complex than previously conjectured. Thus, a new era in hantavirology is now focused on exploring the 'inconvenient' evidence that rodents may not be the original mammalian hosts of primordial hantaviruses. Also, the once-growing complacency and indifference toward rodent-borne hantaviruses is being supplanted by renewed zeal to fill major gaps in our understanding about the ecology, transmission dynamics and pathogenic potential of these newly discovered, still-orphan hantaviruses, before the next new disease outbreak is documented.

The history of hantaviruses has been marked by rediscovery and new beginnings. In this short review, the genetic diversity and phylogeography of hantaviruses from non-rodent small mammals are summarized in an attempt to gain insights into their evolutionary origins.

## 2. Hantavirus hunting

To gain insights into the host diversity of hantaviruses, we analyzed archival tissues from 1546 shrews (representing 9 genera and 47 species), 281 moles (8 genera and 10 species) and 520 bats (26 genera and 53 species), captured in Europe, Asia, Africa and North America during a more than three decade period, 1980–2012, using reverse transcription polymerase chain reaction (RT-PCR). Ironically, the availability of the TPMV whole genome was unhelpful, because of the rich genetic diversity of soricomorph- and chiropteran-borne hantaviruses. That is, for nearly every new hantavirus, time-consuming and labor-intensive hit-and-miss efforts of designing and redesigning oligonucleotide primers were necessary. This brute-force approach was rewarded by the identification of genetically distinct hantaviruses (likely representing new viral species) in multiple species of shrews belonging to three subfamilies (*Soricinae*, *Crocidurinae* and *Myosoricinae*) and moles of two subfamilies (*Talpinae* and *Scalopinae*) captured in widely separated geographic regions (Arai et al., 2007, 2008a,b, 2012; Gu et al., 2011, 2013a,b,c; Kang et al., 2009a,b,c, 2010, 2011a,b,c; Song et al., 2007b,c, 2009; Yashina et al., 2010).

Additional shrew-borne hantaviruses, including Asikkala virus in the Eurasian pygmy shrew (*Sorex minutus*) (Radosa et al., 2013), Tanganya virus in the Therese's shrew (*Crocidura theresae*) (Klempa et al., 2007) and Yakeshi virus in the taiga shrew (*Sorex isodon*) (Guo et al., 2013), and genetic variants of Seewis virus (SWSV) have been reported by other investigators (Resman et al., 2013; Schlegel et al., 2012b). And recently, the host range of hantaviruses has been further expanded by the detection of highly divergent hantavirus lineages in insectivorous bats (order Chiroptera) from Côte d'Ivoire (Sumibcay et al., 2012), Sierra Leone (Weiss et al., 2012), Vietnam (Arai et al., 2013) and China (Guo et al., 2013). As shown in Fig. 1, the emerging global landscape of hantaviruses, with the addition of soricid-, talpid- and chiropteran-borne hantaviruses against a backdrop of representative hantaviruses harbored by rodents, has become almost unrecognizable from just a few years ago.

Initially, our search for hantaviruses was limited to frozen tissues, in the belief that the probability of success would be highest. However, this self-imposed restriction reduced our virus-discovery opportunities, so we expanded the testing to include tissues preserved in RNAlater® RNA Stabilization Reagent with good success. More recently, because maintaining a cold chain under field conditions is not always feasible, we have found that archival tissues fixed in 90% ethanol are also suitable, as evidenced by the identification of a highly divergent hantavirus in ethanol-fixed liver

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