# Discovery of hantaviruses in bats and insectivores and the evolution of the genus *Hantavirus*

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## ARTICLE INFO

## ABSTRACT

Hantaviruses are among the most important zoonotic pathogens of humans, causing either hemorrhagic fever with renal syndrome (HFRS) or hantavirus pulmonary syndrome (HPS). From the period 1964–2006 almost all hantaviruses had been identified in rodents, with the exception of Thottapalayam virus (TPMV) isolated from shrews sampled in India. As a consequence, rodents were considered as the natural reservoir hosts. However, over the past seven years, most of the newly found hantavirus genotypes have been from either shrews or moles. Remarkably, in recent years divergent hantaviruses have also been identified in bats sampled from both Africa and Asia. All these data indicate that hantaviruses have a broad range of natural reservoir hosts. Phylogenetic analyses of the available sequences of hantaviruses suggest that hantaviruses might have first appeared in Chiroptera (bats) or Soricomorpha (moles and shrews), before emerging in rodent species. Although rodent hantaviruses cluster according to whether their hosts are members of the Murinae and Cricetidae, the phylogenetic histories of the viruses are not always congruent with those of their hosts, indicating that cross-species transmission events have occurred at all taxonomic levels. In sum, both cross-species transmission and co-divergence have produced the high genetic diversity of hantaviruses described to date.

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## 1. Introduction

Hantaviruses (genus *Hantavirus*, family *Bunyaviridae*) are important (re)emerging zoonotic pathogens of humans. They can cause serious diseases in humans with mortality rates of up to 15% in hemorrhagic fever with renal syndrome (HFRS) and 40% in hantavirus pulmonary syndrome (HPS) (Schmaljohn and Hjelle, 1997; Vapalahti et al., 2003; Zhang et al., 2010a). In 1978, Lee and his colleagues determined the etiologic agent of HFRS (Hantaan virus, HTNV) and its reservoir hosts (*Apodemus agrarius* mice) (Lee et al., 1978). Since the discovery of HTNV, intense scientific efforts have focused on characterizing additional hantaviruses in rodents and humans, and which have led to the discovery of Puumala virus (PUUV) in the bank vole *Myodes glareolus* (Brummer-Korvenkontio et al., 1980), Seoul virus (SEOV) in Norway rats (*Rattus norvegicus*) (Lee et al., 1982), Dobrava virus in yellow-necked mouse (*A. flavicollis*) (Avsic-Zupanc et al., 1992), Sin Nombre virus (SNV) in *Peromyscus maniculatus* (Nichol et al., 1993), Andes virus (ANDV) in

*Oligoryzomys longicaudatus* (Padula et al., 1998). Notably, the identification of novel genotypes of insectivore-borne hantaviruses has increased rapidly since the discovery of the second shrew-borne hantavirus (Tanganya virus, TGNV) in 2007 (Klempa et al., 2007). More importantly, novel genotypes of hantaviruses have recently been found in bats sampled from Asia and Africa (Weiss et al., 2012; Sumibcay et al., 2012; Guo et al., 2013). All these data suggest that there are more unrecognized hantaviruses circulating in a wide range of animal hosts (Guo et al., 2013). The characterization of these novel viruses and their hosts not only provides information central to understanding their origin and phylogeny, but may ultimately assist in the prevention of their emergence in humans and animals.

In this review, I give an overview of novel genotypes of hantaviruses identified in bats, moles, and shrews, and then discuss recent progress in our understanding of the origins and evolutionary history of the hantaviruses, with an emphasis on the nature of the evolutionary association between hantaviruses and their hosts.

## 2. Like rodents, bats and insectivores are the important hosts of hantaviruses

Although researchers had focused on rodent reservoir hosts since the discovery of HTNV in 1978, the first hantavirus ever

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described was Thottapalayam virus (TPMV), which was isolated from the Asian house shrew (*Suncus murinus*) during a survey for Japanese encephalitis virus in India in 1964 (Carey et al., 1971), although the lack of a link with human disease meant that this virus was not studied further until recently. Interestingly, TPMV had not been classified as a bunyavirus until 1989 (Zeller et al., 1989). In addition, hantavirus antigens were also detected in the lung tissues of the Asian house shrews in China as early as 1980s (Chen et al., 1986), and the virus was isolated successfully from the Asian house shrew in China in 1985 (Tang et al., 1985). However, serological and RCR tests revealed that the virus isolated from the Asian house shrew was HTNV (Tang et al., 1985, 1990), suggesting the spillover of HTNV from the natural hosts or possible contamination during isolation.

The second shrew-bone virus (TGNV) was identified in *Crocidura theresae* sampled from Guinea in 2007 (Klempa et al., 2007). Remarkably, in the same year, other three novel genotypes of hantaviruses were detected in shrews; Camp Ripley virus (RPLV) in *Blarina brevicauda* from USA (Arai et al., 2007), Seewis virus (SWSV) in *Sorex araneus* from Switzerland (Song et al., 2007a,b), and Cao Bang virus (CBNV) in *Anourosorex squamipes* from Vietnam (Song et al., 2007a,b), respectively (Table 1). These studies reveal the potential of shrews as the natural hosts of hantaviruses, and have stimulated intense efforts in discovering more new genotypes of hantaviruses in insectivores. Asama virus (ASAV) was the first genotype of hantavirus identified in mole (*Urotrichus talpoides*) sampled from Japan (Arai et al., 2008). Interestingly, the mole-borne virus (Rockport virus, RKPV), identified in the eastern mole (*Scalopus aquaticus*) collected in USA, shared a common ancestor with cricetid-rodent-borne hantaviruses (Kang et al., 2011a). To date, a total of 24 genotypes of hantaviruses have been found in moles and shrews worldwide (Table 1). Of these, 20 genotypes of viruses have been found in shrews, and 4 from moles.

Like rodent-borne hantaviruses (Sironen et al., 2001; Zou et al., 2008a), insectivore-borne hantaviruses also display the high genetic diversity and the geographic clustering pattern. For example, SWSV has been identified in the Eurasian common shrew (*Sorex araneus*) sampled from many European countries and Russia (Kang et al., 2009; Yashina et al., 2010; Schlegel et al., 2012; Resman et al., 2013). Phylogenetic analyses indicated that the recovered SWSV sequences were highly divergent and were grouped according to their geographic origins (Kang et al., 2009; Schlegel et al., 2012). Additionally, TPMV has been identified in shrews (*Suncus murinus*) from China, India, Nepal so far (Carey et al., 1971; Guo et al., 2011; Kang et al., 2011c). TPMV also showed high genetic divergence, with three lineages divided according their geographic origins.

In 1994, Kim and colleagues found the bats (*Rhinolophus ferrum-equinum*, *Eptesicus serotinus*) collected in South Korea were serologically positive to HTNV, and they successfully isolated the virus from those bats (Kim et al., 1994). The subsequent serological and genetic analyses revealed that the viruses isolated from the bats were HTNV (Kim et al., 1994; Jung and Kim, 1995). Additionally, rodent-borne Araraquara virus was also found in two bat species (*Diphylla ecaudata*, *Anoura caudifer*) in Brazil in 2012 (de Araujo et al., 2012). Hence, these studies suggest the spillover of hantaviruses from rodents to bats. However, further studies are clearly needed to exclude the possibility of contamination.

Notably, two groups reported that they recovered the partial L segment sequences of hantavirus from the bats collected in Western Africa, respectively (Sumibcay et al., 2012; Weiss et al., 2012). Genetic analyses of the recovered partial L segment sequences indicated the presence of two novel genotypes of hantaviruses in bats in Western Africa: Magboi virus (MGBV) in *Nycteris hispida* bats in Sierra Leone (Weiss et al., 2012) and Mouyassué virus (MOUV) in *Neoromicia nanus* bats in Côte d'Ivoire (Sumibcay et al., 2012). More

importantly, the complete S and M segment sequences were recovered from bats (*Rhinolophus* spp. and *Pipistrellus abramus*) sampled from China (Guo et al., 2013). Phylogenetic analyses indicated that these sequences represent two novel genotypes of hantaviruses: Huangpi virus (HUPV) from *P. abramus* bats and Longquan virus (LQUV) from *Rhinolophus* spp. bats. Remarkably, LQUV was found in three species (*Rhinolophus affinis*, *R. onoceros*, and *R. sinicus*) from the same niche. Moreover, both HUPV and LQUV diverge early in the phylogeny (see below) and are most closely related to Nova virus (NVAV) identified in *Talpa europaea* moles from Hungary (Kang et al., 2009). Recently, the fifth novel genotype of bat-borne hantavirus (Xuan Son virus, XSV) was found in Pomona roundleaf bats (*Hipposideros pomona*) sampled from Vietnam (Arai et al., 2013). Hence, all these data demonstrate that bats are also the important natural reservoir hosts of hantaviruses. As their global distribution, abundance, ability to fly long distances, often large population densities, and sociality favor the efficient maintenance, evolution, and spread of viruses, it is clear that further efforts are needed to elucidate the potential importance of bats as hantavirus hosts, including the possible spread of bat-borne hantaviruses to other animals including humans and their pathogenicity (or the lack of it) for humans (Guo et al., 2013). Together, these data clearly show that bats and insectivores are the important hosts of hantaviruses.

Among the bat- and insectivore-borne viruses currently described, only TPMV has been defined as a separate species in the genus *Hantavirus* by the International Committee on Taxomony of Viruses (ICTV) (Plyusnin et al., 2012). However, for most of the viruses recently discovered in bats and insectivores, their whole genome sequences have not been obtained. Hence, it remains unknown whether these novel genotypes of viruses meet the species demarcation criteria in the genus *Hantaivirus* defined by ICTV (Plyusnin et al., 2012). Thus, they are referred to as "novel genotypes" in this review. Finally, further efforts are needed to isolate them or recover their whole genome sequences for the definition of their taxonomy.

## 3. Phylogenetic relationship among bat-borne, insectivore-borne, and rodent-borne hantaviruses

The discovery of more novel genotypes of hantaviruses from bats and insectivores over the past seven years help us to better understand the phylogeny of the genus *Hantavirus*. Phylogenetic analyses of the available S or M sequences reveal that all known hantaviruses could be divided into four 'phylogroups' (Figs. 1 and 2) (Guo et al., 2013). In the S segment tree (Fig. 1), the first phylogroup only comprised viruses from insectivore (*Soricidae*) species. It included TPMV, Imjin virus (MJNV) identified in the Ussuri white-toothed shrew (*Crocidura lasiura*) in South Korea (Song et al., 2009), and Uluguru virus (JX193695.1) identified in *Myosorex geata* and Kilimanjaro virus (JX193698.1) identified in *Myosorex zinki* both sampled from Tanzania. According to the Bayesian Maximum Cade Credibility (MCC) tree (which is automatically rooted on the assumption of a molecular clock such that basal viral lineages can be identified), this phylogroup occupied a basal position with respect to the remaining viruses (Guo et al., 2013). The second phylogroup included HUPV and LLQUV found in bats from China (Guo et al., 2013), XSV also found in bats from Vietnam (Arai et al., 2013), and the more divergent Nova virus (NVAV) identified in the European common mole (*Talpa europaea*) from Hungary (Kang et al., 2009). Phylogroup III contained all other known Soricomorpha-borne viruses as well as all known *Murinae*-borne viruses, forming two distinct clades. Finally, the fourth phylogroup included two monophyletic groups, corresponding to viruses sampled from the Arvicolinae, Neotominae, and Sigmodon-tinae subfamilies of rodents, respectively, along with the possibly