HANTAVIRUS HOST/VIRUS INTERACTIONS WITHIN SOUTHEAST EUROPE

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Abstract

Viral studies have historically approached their phylogenetic analysis without consideration of the impact of the role the host plays in evolution. Our study examines host/viral interactions through analysis of the phylogenetic relationship between hantavirus genetic sequences and host cytochrome B sequences.
Phylogenetic analysis of known Hantavirus genetic sequences were performed using PAUP 3.1.1 (vers. 4.0.0d64). Only sequences available through GENBANK were analyzed.
Phylogenetic analysis of hantavirus sequences revealed distinct patterns based upon geographic area. These patterns coincided with the known ranges of reservoir hosts. Multiple hosts for individual viruses and multiple viruses in a single host species for hantaviruses have been described. This may be due to accidental exposure, host-switching, co-speciation, or broad co-accommodation. Since the host is the actual environment that the virus survives in, changes in the host over time could potentially directly influence changes in the virus. Multiple viruses and hosts collide in Southeastern Europe increasing the prospect of finding distinct viral/host relationships. Rodent Cytochrome B is very well conserved and can be used to tract host lineage. By tracking the relationship of infected hosts, we theorize that patterns in host DNA will emerge that will mirror patterns in viral sequences. This analysis of the host DNA could provide an understanding into the causes of variation in hantaviral sequences, pathogenicity, transmissibility, infectivity, viral range and expand our knowledge of viral/host interactions. Surveillance for viruses in the field should include analysis of the host DNA in combination with the viral analysis.

Introduction

Hantaviruses are enveloped viruses with a diameter of approximately 90-120 nm and a negative sense RNA genome. The genome is tripartite and consists of a small (S), a medium (M) and a large (L) segment, which are separately packed in helical nucleocapsids. The S genomic segment encodes the nucleocapsid while the M genomic segment encodes (1) two glycosylated envelope proteins (2). The L genomic segment is presumed to encode the L protein, which is assumed to be the RNA-dependent RNA polymerase based on its transciptase as well as replicase functions (3). Viruses replicate in the cell cytoplasm and virus particles mature by budding into vesicles near the Golgi apparatus. Hantaviruses are serologically related rodent viruses representing a genus within the family *Bunyaviridae*. Unlike other bunyaviruses, hantaviruses are excreted in the saliva, urine, and feces of infected rodents. Humans may become infected through inhalation of aerosols of dried excreta, or by bite of infectious rodents (4). The distribution of hantaviruses is worldwide and different viral species circulate among natural populations of rodents (5). Cases of hemorrhagic fever with renal syndrome were first documented within Croatia in 1954 (6). Periodic outbreaks have continued to arise sporadically, with the largest epidemic occurring in 1995 (7). Types of hantavirus identified from Croatia include Dobrava, Puumala (8) and Tula (9).
Although accidental infections by hantavirus of other mammalian hosts have been found, each viral species within the genus *Hantavirus* is believed to be primarily associated with a single rodent species (10). Recent evidence brings into question the validity of this one virus/one host concept. Since the 1993 outbreak of Sin Nombre virus in the western United States, great emphasis has been placed upon analysis of viruses found in rodents of the North and South American subfamily *Sigmodontinae*. The majority of this research has concurred with the occurrence of a single viral species or lineage within each rodent host species or race. Exceptions to this include Oran and Andes viruses, which have both been isolated from *Oligoryzomys longicaudatus* (11) and Black Creek Canal (12) and Muleshoe (13) isolated from *Sigmodon hispidus*. The presence of 2 distinct hantaviruses in this single South American rodent species suggests that the co-occurrence of multiple viruses in a single host species is possible. Considerably less is known about hantaviruses within other murid rodent subfamilies. One rodent subfamily, *Arvicolinae* has a distribution in both the old

and new world and hantavirus strains have been isolated from several of its genera, including *Apodemus* and *Microtus*.

Dobrava Analysis

Apodemus is restricted to the Old World and members of this genus are known to be infected with both Hantaan (HTN) and Dobrava (DOB) viruses. *Apodemus flavicollis* is thought to be the primary host of Dobrava virus in Europe. Recently however, DOB was isolated from *A. agrarius*, the primary host of HTN in Estonia (14), Russia (15) and Hungary (16). The presence of DOB in a second host over such a wide geographic range within Europe brings into question the generality of the one virus per single host species concept.

The nucleotide sequence identities between Hungarian (Tazar) DOB and other related viral lineages shown in Figure 1 included: Russian DOB from *A. agrarius* 88%; Estonian DOB from *A. agrarius* 86%-87%; Bosnia DOB from *A. flavicollis* 88%; Greek DOB from *A. flavicollis* 88%-85%; Sapporo Rat virus 70%; HTN 68%; Khabarousk 57%; PUU 56%; TUL 53%; Sin Nombre 48%. Although the sequence data obtained are limited, phylogenetic analyses linked DOB isolated from *A. agrarius* into a group with DOB previously isolated from *A. flavicollis* (16). The *A. agrarius* DOB obtained from Russia (15) did not support monophyly (common ancestry) for DOB isolated from *A. agrarius* populations in Hungary and in Russia. Other representative hantaviruses, including PUU, TUL, HTN and Sapporo rat virus, were more basal in the phylogeny (see Figure 1).

Hantaan virus is known to infect *A. agrarius* populations in Asia, whereas the virus has not been isolated in Europe. Direct enzyme-linked immunosorbent assay has demonstrated the presence of Hantaan-like antigens in *A. agrarius* in the former republic of Czechoslovakia [5.5%, (17)], the European regions of the former Soviet Union [5.3%, (18)] [28.5%, (19)], and Serbia [2.2%, (20)]. As there are no reports of HTN sequences from Europe, and given the similarities in immunological response between HTN and DOB, one might assume that earlier findings of Hantaan-like antigens in Europe may actually represent a more widespread occurrence of DOB in European populations of *A. agrarius*. Recent verification of DOB in populations of *A. agrarius* in Estonia (14), Russia (15) and Hungary (16) provide support for this conclusion.

The co-occurrence of DOB in both *A. agrarius* and *A. flavicollis* provides an opportunity for evaluating the hypothesis concerning distribution of hantaviruses in related rodent hosts. Phylogenetically different Sin Nombre-like viruses have been found in different populations within species of peromyscine rodents that demonstrate ecological and geographical variation throughout their range (21). Although evidence of cospeciation between the rodent host phylogeny and the host-borne hantavirus phylogeny has been found, evidence of host-switching was observed with *Peromyscus leucopus*-borne New York virus grouping with *P. maniculatus*-borne viruses rather than with other *P. leucopus*-borne viruses.

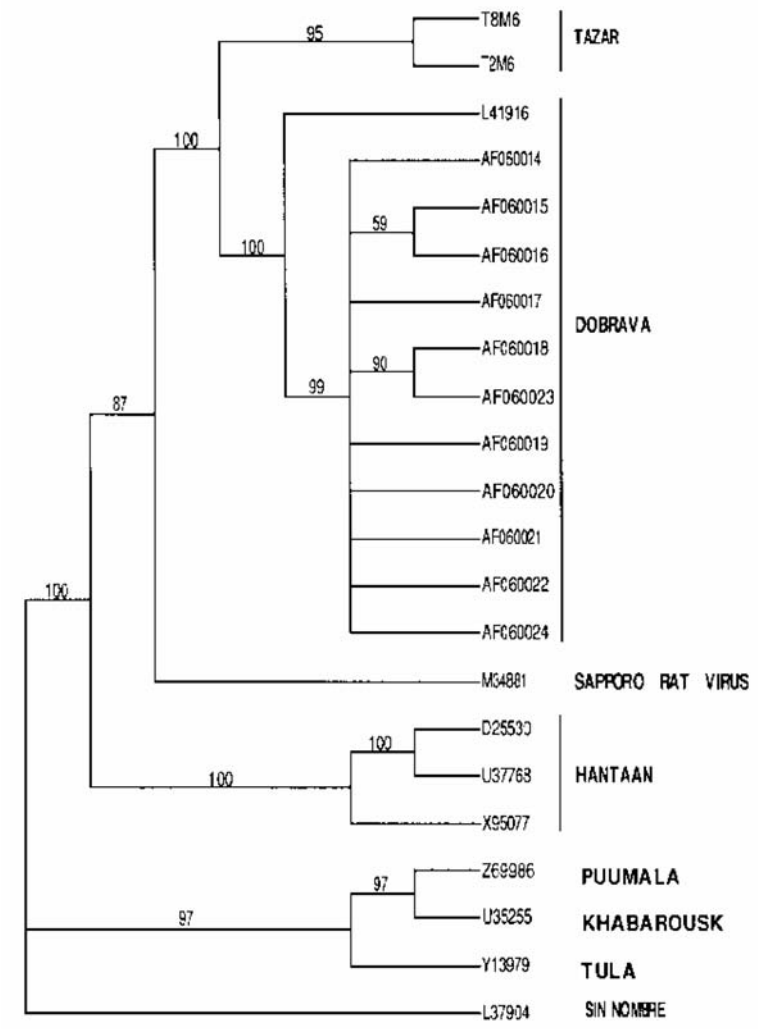
The phylogenetic analysis (Figure 1) indicates a closer relationship between the *A. agrarius* DOB from Hungary and *A. flavicollis* DOB, with Russian/Estonian DOB representing a sister-group to this clade. This lack of monophyly for the *A. agrarius* DOB may suggest the possibility of host-switching between *A. agrarius* and *A. flavicollis* similar to the switching observed for *P. leucopus* and *P. maniculatus*. Nevertheless, the relationships among the isolated DOB lineages suggests a more basal position for the *A. agrarius* DOB lineages, implying an older age than DOB in *A. flavicollis*.

Apodemus flavicollis ranges throughout much of Western Europe eastward to the Ural Mountains and *Apodemus agrarius* ranges from Eastern Europe eastward to the Pacific Ocean, covering the majority of the Asian continent (22). Given the extensive ranges of both rodent species, it would be interesting to examine other populations within each species as well as other species of *Apodemus*. This would allow one to relate the viral phylogeny to the rodent host phylogeny. It could well be that the pattern of divergence seen for hantaviruses in the New World peromyscine rodent species are mirrored in Old World arvicoline rodents. Therefore, more than one hantavirus in *Apodemus agrarius* may reflect either geographic variation within the species or host-switching in regions where two host species are potentially sympatric.

Tula Analysis

Tula Virus (TUL) within Southeastern Europe also provides an interesting opportunity to examine virus/host relationships. When TUL was initially described by Plyusnin et al. (23), the virus was found to infect both *Microtus arvalis* and *M. rossiaemeridionalis*, and Song et al. (Unpublished Genbank sequence) found TUL in *Pitymys subterraneus*. While most European systematists classify Pitymys as a separate genus, Nowak (22) classifies Pitymys as a subgenus of *Microtus*. Scharninghausen et al., (9) also found *M. agrestis* in Croatia infected with TUL. Using Nowak's classification, *M. agrestis* is the fourth species of Microtus found to be infected with TUL.

Sampling within Croatia has revealed TUL in two separate rodent species. The high percentages of the same TUL sequences in *M. agrestis* and *M. arvalis* from Croatia suggests that the co-occurrence of this virus in two *Microtus* species may not be the result of accidental infection. If *M. agrestis* were accidentally infected with TUL, the percentage of PCR positive animals should be lower than that of *M. arvalis*. Because the infection rate in *M. arvalis* (11.8%) was less than half of that found in *M. agrestis* (27.6%), it is unreasonable to assume that the predominance of positive *M. agrestis* could be due to accidental exposure. It is much more likely that the Croatian virus is circulating between both rodent species (9). While

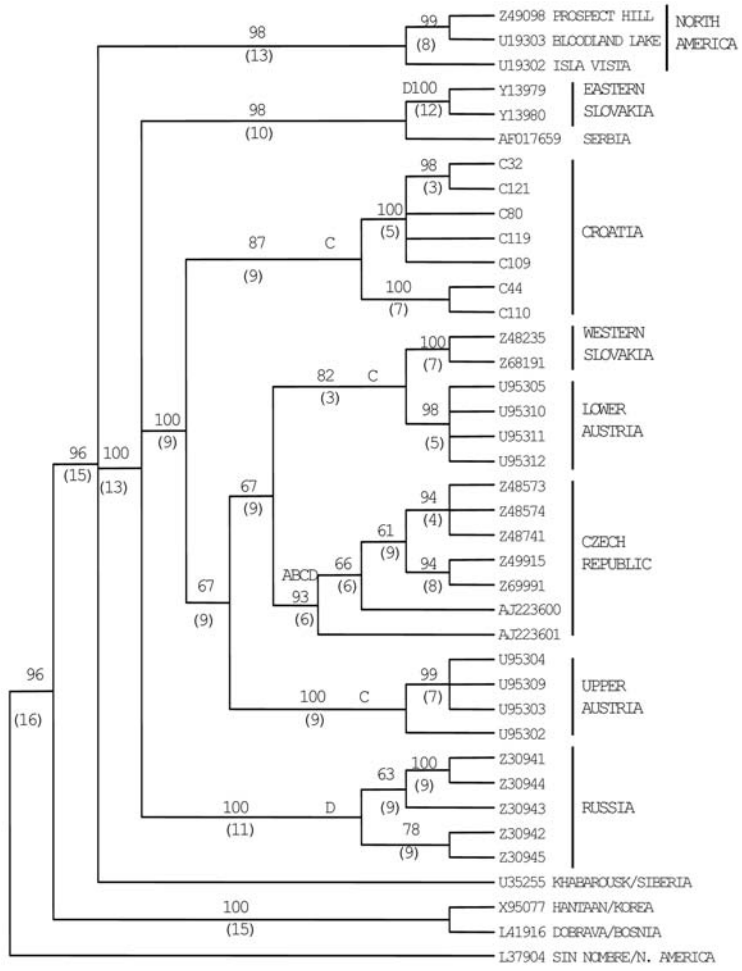


Legend to Fig. 1:
Cladogram derived from nucleotide sequences of DOB and various other hantaviruses. Numbers denote Genbank accession numbers. The cladogram was derived from the neighbor-joining estimated phylogeny and bootstrap analysis using p-distance estimates. The phylogenetic analysis was performed using PAUP 3.1.1 (vers. 4.0.0d64) (30). Numbers at each internode or bifurcation represent bootstrap support based on 1000 replicates. A Sin Nombre virus sequence (L37904) was used as the outgroup to root the tree. The sequences labeled Tazar were collected from the Military Base at Tazar, Hungary, (16).

it has been noted in numerous studies that several rodent species can be infected in with a single hantavirus (14; 15; 16; 21) circulation of viruses between closely related hosts has not been demonstrated. Scharninghausen et al. (16) found that although *Apodemus* agrarius was infected with DOB in Hungary, phylogenetic analysis of viral genetic sequences indicate that this was an ancient relationship and not evidence of host switching.

The presence of TUL in 2 different Croatian rodent species may be due to broad co-accommodation (24), where the same parasite establishes itself in an existing host without cospeciation between the host and the parasite occurring. Since no geographic isolation has occurred between *M. arvalis* and *M. agrestis* populations occupying the study area and viral exchange between the two species appears to be occurring, speciation of the virus in *M. agrestis* has not happened yet occurred.

Clustering of TUL sequences within the phylogenetic trees (Figure 2) roughly corresponds with the known historic range of *Microtus arvalis* subspecies. Subspecific ranges of *M. arvalis* are as follows: (1) *M. a. arvalis* ranges through the plains of southwest central Europe from northeast France to the western portion of the Czech Republic; (2) *M. a. gregarius* ranges through Germany and the Czech Republic, north through the Baltic states; (3) *M. a. levis* ranges through the mountains of central and eastern Europe, south to Bulgaria through northern Greece; (4) *M. a. duplicatus* ranges from the Oder River and northern Slovakia east to the Ural Mountains (25-27). Although the subspecies of the host can be estimated based upon the historic range, no host DNA was available from previously reported viral sequences. Therefore, it was not possible to determine weather or not rodents parasitized by each viral sequence were genetically and phylogenetically distinct as would be expected if viral distinction coincided with host subspecific distinction. Based on distributions, the predicted subspecies of *M. arvalis* analyzed in this and other studies are indicated in Figure 2. Samples from the Czech Republic are annotated with more than 1 subspecies, as the ranges of 4 subspecies come together in this area. It may be that viral differences observed in *M. arvalis* differentiate along subspecific lines of the host, but until the host has been properly surveyed, this remains an untested hypothesis.



Legend to Figure 2:
Cladogram of nucleotide sequences of Croatian Hantaviruses. Numbers denote Genbank accession numbers. The cladogram was derived from the neighbor-joining estimated phylogeny and bootstrap analysis using p-distance estimates (30). Numbers at each internode or bifurcation represent bootstrap support based on 1000 replicates. Bremer Support values are given in parenthesis below each internode. A Sin Nombre virus sequence (L37904) was used as the outgroup to root the tree. Host subspecies known to be present in the areas samples were collected from are indicated by a letter on the internode as follows; A: M. a. arvalis; B: M. a. gregarius; C: M. a. levis; D: M. a. duplicatus. Group C32 contained one M. arvalis (collection number JJS 888); Group C44 contained one M. arvalis (collection number JJS901); Group C80 contained one M. agrestis (collection number JJS 937); Group C109 contained two M. arvalis (collection numbers JJS 964 and JJS976) and one M. agrestis (collection number JJS 967); Group C110 contained one M. arvalis (collection number JJS922) and one M. agrestis (collection number JJS968); Group C119 contained four M. arvalis (collection numbers JJS863, JJS 902, JJS 975, JJS976) and one M. agrestis (JJS 954); and Group C121 contained three M. arvalis (collection numbers JJS892, JJS952 and JJS979) and four M. agrestis (collection numbers JJS911, JJS943, JJS 956 and JJS 997). All mammalian voucher specimens have been deposited at The Museum, Texas Tech University, Lubbock, Texas 79409 (9).

Discussion

Southeast Europe offers a unique opportunity to examine the relationship between hantaviruses and their hosts. Multiple viruses and hosts collide in this region, increasing the prospect of finding distinct viral/host relationships. Since the host is the actual environment that the virus survives in, changes in the host over time or across its range could potentially directly influence changes in the virus. To test the theories on the relationship of DOB and TUL with their hosts, direct comparisons between the phylogeny of the virus strains and the phylogeny of the infected rodents is necessary. Rodent DNA can be identified to species level by using standardized primers to examine Mitrochondrial DNA (28).

Using mitochrondrial DNA it is possible to trace the maternal lineage of the host and determine relationships between separate populations.

PCR may also be used to determine the sex of the host (29). Host tissue can be preserved in 95% ethanol for ease of storage. Mince a small piece of tissue approximately the size of a pea for suspension in the alcohol or mix 0.5 ml of blood in 1 ml of 95% ethanol. This will preserve the DNA indefinitely at room temperature. Analysis of the genetic sequence should follow standard phylogenetic methods. By tracking the relationship between infected hosts, we theorize that patterns in host DNA will emerge that will mirror patterns in viral sequences. The analysis of the host DNA could provide an understanding into the cause of hantaviral sequences, pathogenicity, transmissibility, infectivity, viral range and expand our knowledge of viral/host interactions. Surveillance for viruses in the field should include analysis of the host DNA in combination with the viral analysis

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