An endemic hantavirus in field voles in northern England

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Abstract

We report a PCR survey of hantavirus infection in the extensive field vole (*Microtus agrestis*) populations occurring in the Kielder Forest, northern England. A Tatenale virus-like lineage was frequently detected (~ 15% prevalence) in liver tissue. Such lineages are likely to be endemic in northern England.

Recently a new vole-associated hantavirus (Tatenale virus) was discovered in northern England (*1*), but only from a single individual of the field vole, *Microtus agrestis.* This finding was particularly notable as only hantaviruses from murine-associated lineages (Seoul virus, SEOV and SEOV-like viruses) had previously been reported in the U.K., despite the abundance of potential vole hosts in the mainland U.K. and the endemicity of vole-associated lineages (Puumala virus (PUUV) and Tula virus (TULV)) in mainland Europe (*2*). Here we present data establishing the endemicity in northern England of the Tatenale virus lineage.

European hantaviruses are of public health significance as a causative agent of haemorrhagic fever with renal syndrome (HFRS). In the U.K., HFRS cases have primarily been attributed to SEOV-like viruses on the basis of serological tests. Anti-SEOV antibodies have been detected in both humans and Norway rats (*Rattus norvegicus*) in Northern Ireland and Yorkshire (*3, 4*), seropositivity in humans correlating with domestic or occupational exposure to rats (*3, 5*). However, there have been U.K. cases of HFRS with serological cross reactivity to PUUV (*3*) which may share antigenic determinants with Tatenale virus.

To investigate the endemicity of hantavirus in U.K. field voles, we surveyed the extensive populations of this species occurring in the Kielder Forest, Northumberland (~230 Km distant from the locality where Tatanale virus was discovered). All sampled sites were grassy clear-cut areas (adjacent to forest stands) where field voles are the dominant component of rodent assemblages. Fieldwork was approved by the University of Liverpool Animal Welfare Ethical Review Board and conducted subject to U.K. Home Office project licence PPL 70_8210. Following the capture and processing of animals as previously described (*6*), viral RNA was extracted from 48 livers using a QIAamp Viral RNA Mini Kit (Qiagen, U.K.) and converted to cDNA using a High-Capacity RNA-to-cDNA Kit (Applied Biosystems, ThermoFisher Scientific, U.K.). Detection of hantaviruses was, following Klempa *et al.* (2006) (*7*), carried out by PCR amplification of a fragment of the genomic L segment encoding RNA-polymerase.

PCR positives were recorded for 14.6% of voles (7/48), at 3 of 5 sampled sites (Figure, panel A) and across the full survey period (March to September 2015). Three positive samples from different individuals were sequenced (in both directions from independent replicate PCR reactions) by Sanger sequencing (Source BioScience, U.K.). A 380bp sequence was determined (GenBank accession numbers: KY751731, KY751732) in all three positive vole samples, with a single nucleotide polymorphism at position 145 (adenine, 2 individuals; guanine, 1 individual). Whilst the recovered sequences were close to Tatenale virus, there was significant divergence from this (respectively 86.0-86.3% and 95.9-96.7% identity at the nucleotide and amino acid level) (Technical appendix). Phylogenetic analysis (Figure, panel B) of the L segment demonstrated this level of divergence was comparable to the divergence amongst many western European lineages of PUUV.

Taken together with the original record these data are sufficient to suggest that Tatenale-like hantavirus lineages are widespread and locally common in northern England. Furthermore, the considerable sequence divergence between samples in Cheshire and Northumberland is consistent with long-standing endemicity in northern England. Given that PUUV has never been recorded in the U.K.(*2, 8*), the possibility should be considered that a Tatenale-like virus could be responsible for some U.K. HFRS cases. More studies are now needed to confirm the status of other common U.K. rodents as hosts for this virus, and to further characterize its phyletic relationships and zoonotic potential. Importantly, the cross-reactivity of Tatenale-like virus infected sera to antigens from other relevant hantaviruses should be determined to inform future serological surveys and the diagnosis of human HFRS cases.

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Figure (main article) legend

Figure. (A) Map of mainland U.K. showing localities for Tatenale virus-like hantavirus lineages; original site at Tattenhall, Cheshire (*1*) and additional sites at Kielder, Northumberland (GRD: 55° 11' 1.61" N, -2° 35' 3.05" W; CHE: 55°13' 8.39" N, -2° 32' 26.50" W; SCP: 55° 15' 44.18" N, -2° 32' 41.05" W). (B) Bayesian phylogenetic tree for the hantavirus genomic L segment (318 bp fragment, with no missing data), showing relationships amongst Tatenale virus-like lineages and other relevant lineages. Phylogenetic analysis was conducted using a GTR G+I model within *MrBayes* (*9*) software using Markov chain Monte Carlo chain lengths of 1 million and a strict clock. Substitution models were estimated using *MrModelTestV2* (*10*). The tree is drawn to scale with branch lengths measuring the number of substitutions per site, and node values representing the posterior probabilities. Scale bar represents 0.1 nucleotide substitutions per site. Sequences are represented by the taxonomic names, strain (if more than one is included) and GenBank ascension numbers. **Kielder_Hantavirus** represents the 145>A sequence found in this study (the phylogeny is unchanged if the other sequence is substituted). ADLV, Adler Virus; CATV, Catacamas Virus; HOKV, Hokkaido virus; KBRV, Khabarovsk virus; LNV, Laguna

Negra virus; PHV, Prospect Hill virus; PUUV, Puumala virus; TULV, Tula virus; YN05-YN06, unnamed hantaviruses.