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Adler hantavirus, a new genetic variant of Tula virus identified in Major's pine voles (*Microtus majori*) sampled in southern European Russia

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ABSTRACT

Although at least 30 novel hantaviruses have been recently discovered in novel hosts such as shrews, moles and even bats, hantaviruses (family *Bunyaviridae*, genus Hantavirus) are primarily known as rodent-borne human pathogens. Here we report on identification of a novel hantavirus variant associated with a rodent host, Major's pine vole (*Microtus majori*).

Altogether 36 hantavirus PCR-positive Major's pine voles were identified in the Krasnodar region of southern European Russia within the years 2008–2011. Initial partial L-segment sequence analysis revealed novel hantavirus sequences. Moreover, we found a single common vole (*Microtus arvalis*) infected with Tula virus (TULV). Complete S- and M-segment coding sequences were determined from 11 Major's pine voles originating from 8 trapping sites and subjected to phylogenetic analyses.

The data obtained show that Major's pine vole is a newly recognized hantavirus reservoir host. The newfound virus, provisionally called Adler hantavirus (ADLV), is closely related to TULV. Based on amino acid differences to TULV (5.6–8.2% for nucleocapsid protein, 9.4–9.5% for glycoprotein precursor) we propose to consider ADLV as a genotype of TULV. Occurrence of ADLV and TULV in the same region suggests that ADLV is not only a geographical variant of TULV but a host-specific genotype. High intra-cluster nucleotide sequence variability (up to 18%) and geographic clustering indicate long-term presence of the virus in this region.

1. Introduction

Hantaviruses are known as rodent-borne viruses causing hemorrhagic fever with renal syndrome (HFRS) in Eurasia and hantavirus cardiopulmonary syndrome in the Americas (Krüger et al., 2011; Peters et al., 1999). Recently, knowledge of hantavirus host range has been significantly extended due to at least 30 novel hantaviruses have been discovered in "unexpected" hosts such as shrews (Arai et al., 2007; Klempa et al., 2007; Song et al., 2007a,b), moles (Arai et al., 2008; Kang et al., 2009a,b), and most recently even bats (Guo et al., 2013; Sumibcay et al., 2012; Weiss et al., 2012).

Despite the current "hunt" for hantaviruses in these newly recognized hosts, several new hantaviruses have also been identified in rodents including voles of the genus *Microtus*. Most recently, Tatenale virus (TATV) was identified in a single field vole (*Microtus agrestis*) in northwestern England and in fact represents the first hantavirus found in the United Kingdom (Pounder et al., 2013), thus disproving the long held belief that the British Isles are hantavirus-free. In addition, several hantaviruses associated with reed voles (*Microtus fortis*) and Maximowicz's voles (*Microtus maximowiczii*) were recently reported from China (Zou et al., 2008a,b).

Microtus-borne hantaviruses can be phylogenetically classified into the group of *Arvicolinae*-associated hantaviruses. Probably the most prominent members of this group are Puumala virus (PUUV), Tula virus (TULV), and Prospect Hill virus (PHV). PUUV,

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the causative agent of HFRS, has been detected widely in Europe and is associated with bank voles (*Myodes glareolus*) as carrier. In some countries, such as Finland, Germany, Sweden and the European part of Russia, thousands of HFRS cases caused by PUUV can occur in epidemic peak years (Krüger et al., 2013; Tkachenko et al., 1999, 2013; Vaheri et al., 2013).

There are several aspects which make the *Microtus*-borne hantaviruses particularly interesting. They are generally believed to not be pathogenic to humans. There is only limited evidence for few clinical cases associated with TULV infection (Klempa et al., 2003; Zelená et al., 2013). TULV also seems to be less host-specific than generally assumed for hantaviruses which are usually considered to be associated with a single host species (Schlegel et al., 2012b; Schmidt-Chanasit et al., 2010). In contrast to other rodent-borne hantaviruses, *Microtus*-borne hantaviruses are present in both the Old World and the New World. Besides PHV which is associated with *Microtus pennsylvanicus* voles (Lee et al., 1985) there is Isla Vista virus (ILV; associated with *Microtus californicus*, Song et al., 1995) and Prairie vole virus (PVV; associated with *Microtus ochrogaster* Acc. No. U19303; Song et al., unpublished data) all of which have been discovered in North America.

Here we report the identification of a new genetic variant of Tula virus associated with a rodent host, Major's pine vole, *Microtus majori* (*Terricola majori* according to the systematics of Russian mammals; Pavlinov and Lisovskyi, 2012) in the Black Sea coast area of European Russia, the region where the highly pathogenic Sochi genotype of Dobrava-Belgrade virus (DOBV) is known to circulate (Dzagurova et al., 2012; Klempa et al., 2008; Tkachenko et al., 2005).

2. Material and methods

2.1. Screening of tissue samples by reverse-transcription PCR (RT-PCR)

Small mammals were trapped in frame of the epizootiological study focused on hantavirus reservoir hosts in the Krasnodar region of southern European Russia from 2008–2011 (Russian Ministry of Public Health task, #88 order of March 17, 2008). Frozen lung tissue samples of voles collected within the study were first screened for presence of hantavirus antigens by ELISA using "HANTAGNOST" kit (Federal State Unitary Enterprise on Manufacture of Bacterial and Viral Preparations of Chumakov Institute of Poliomyelitis & Viral Encephalitides) according to the manufacturer's instructions (Ivanov et al., 1996). Presence of hantavirus specific antibodies was tested by IFA with slides containing combined antigens from Vero E6 cells infected with PUUV, DOBV, Hantaan virus, and Seoul virus. Anti-mouse FITC-conjugated IgM and IgG mixture (Imtek, Russia) was used as secondary antibody (Tkachenko et al., 2005). The positive samples were then screened for hantavirus RNA by RT-PCR. Briefly, total RNA was extracted from the homogenized tissue samples using TRIzol (Invitrogen). RNA was then reverse transcribed with Moloney murine leukemia virus reverse transcriptase with random hexamers used as primers. Hantavirus RNA was detected with the genus-specific PCR assay based on degenerated primers targeting a conserved region within the L-segment (Klempa et al., 2006).

2.2. Sequencing of S- and M-segments

To obtain complete S- and M-segment sequences, a broad variety of oligonucleotide primers has been applied in a series of RT-PCR assays (Table 1) in order to obtain overlapping PCR fragments. The obtained PCR products were then column-purified and either directly sequenced or cloned into pSCA vector using a StrataClone PCR cloning kit (Stratagene). At least three clones were sequenced in both directions.

2.3. Molecular host identification

To ensure correct classification of the collected voles, both the *cytochrome b* gene encoded by mitochondrial DNA as well as the mitochondrial DNA control region, D-loop, were sequenced for

Table 1
List of PCR primers used within the study.

Target	Primer name	Primer sequence (5′ → 3′)	References
S segment	S1n	CCAAGTGGRCARACWGCWGAYTGG	Sibold et al. (1995)
	SnMa2	TTAGATTTTTARYGGTTCCTG	Sibold et al. (1995)
	TULS1F	TAGTAGTAKRCTCCTTGAAAAGC	This study
	TULS27F	TACTRAARCCGCTGGKATGA	This study
	ADLS527R	TCATCYTTRAAYCKTATACGRGT	This study
	ADLS1201F	ATGATGGARTGGGGTGC	This study
	ADLS1243F	GGGGATGAYATGGAYCCWGA	This study
	TULS1760R	CGTGCATATATATAAGTGTACRGAGG	This study
M segment	TULM1F	TAGTAGTAGACTCCGCAAGAAGAAGC	This study
	TULM15F	GCAAGAAGAAGCAAAYACAGA	This study
	ADLM1691R	CCATWGTTTTCTGRTAYTCYTG	This study
	ADLM540F	TGGGYTTAGGRGATCAYCGG	This study
	ADLM1426R	ATAAATGCAYTGCCCRATYAC	This study
	ADLM1591F	ATGATMATAATCCGYATYCTT	This study
	ADLM2103R	GGCAAWGARAARTCTARCTCT	This study
	ADLM2640F	TGAAGARGGYGGGATGATATT	This study
	ADLM3179R	TGCCCTCCYTTACCYA	This study
	ADLM2000F	TGGGCWGCWAGTGCIGAIAC	This study
	TULM3681R	CGCARGAACAAAAGTCCAGG	This study
	TULM3694R	TAGTAGTAKICTCCGCARGAAC	This study
L segment	Han-L-F1	ATGTAYGTBAGTGCWGATGC	Klempa et al. (2006)
	Han-L-R1	AACCADTCWGTYCCRTCATC	Klempa et al. (2006)
	Han-L-F2	TGCWGATGCHACIAARTGGTC	Klempa et al. (2006)
	Han-L-R2	GCRTCRTCWGARTGRTGDGCAA	Klempa et al. (2006)
D-loop	CB1n	GGAGGMCARCCAGTWGAAYACCCATT	This study
	12S1n	TAATTATAAGGCCAGGACCAAACC T	This study
Cytochrome B	CytB Uni fw	TCATCMTGATGAAAYTTYGG	Schlegel et al. (2012a)
	CytB Uni rev	ACTGGYTGDCCBCCRATTCA	Schlegel et al. (2012a)

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