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Short communication

Uukuniemi virus, Czech Republic

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ABSTRACT

Following the identification of severe fever with thrombocytopenia syndrome and Heartland viruses, the interest on tick-borne phleboviruses has increased rapidly. Uukuniemi virus has been proposed as a model for tick-borne phleboviruses. However, the number of available sequences is limited. In the current study we performed whole-genome sequencing on two Uukuniemi viral strains isolated in 2000 and 2004 from *Ixodes ricinus* ticks in the Czech Republic. Both strains cluster together with Potepli63 strain isolated in the country in 1963. Although the Czech strains were isolated many years apart, a high identity was seen at the nucleotide and amino acid levels, suggesting that UUKV has a relatively stable genome.

1. Introduction

According to the latest (10th) report of the International Committee on Taxonomy of Viruses, Uukuniemi phlebovirus (UUKV) is one of the 10 species of the genus *Phlebovirus* in the family *Phenuiviridae*, in the order *Bunyavirales* (International Committee on Taxonomy of Viruses (ICTV), 2016). UUKV has been associated with disease in humans and it has been proposed as a model for tick-borne viruses, especially in studying virus replication and early virus-mammalian receptor/cell interactions (Mazelier et al., 2016; Rezelj et al., 2015). Phleboviruses are negative-sense RNA viruses with a tri-segmented genome: the large (L), coding for the viral RNA-dependent RNA polymerase, the medium (M), coding the polyprotein precursor (which after cleavage gives rise to the envelope glycoproteins Gn and Gc), and the small (S), coding for the nucleoprotein N and the non-structural protein NSs (Walter and Barr, 2011). While M and L segments code their proteins in a negative-sense manner, the S segment of phleboviruses follows an ambisense coding strategy (Simons et al., 1990).

Most phleboviruses are transmitted by sandflies (e.g. Toscana virus), while few are transmitted by mosquitoes (e.g. Rift Valley fever virus) and ticks (e.g. UUKV). Tick-borne phleboviruses differ from the other viruses of the genus in that they do not express the non-structural protein NSm. The interest on tick-borne phleboviruses increased rapidly after the discovery of severe fever with thrombocytopenia syndrome and Heartland viruses (SFTSV and HRTV) in China and the United States, respectively, which cause severe and often fatal disease in humans, which share high sequence homology with UUKV (McMullan et al., 2012; Yu et al., 2011). As a result, several novel tick-borne phleboviruses have been detected or isolated in various countries. Phylogenetically they are divided into at least 4 groups: Uukuniemi, Bhanja, SFTS, and Kaisodi (Matsuno et al., 2015). Besides UUKV, the Uukuniemi group includes several other species (such as Murre, Grand Arbaud, and Precarious Point). A recently identified tick-borne phlebovirus in Japan, Kabuto Mountain virus, branches between the Uukuniemi and Kaisodi group viruses (Ejiri et al., 2017).

The prototype UUKV S23 strain was originally isolated in 1960 from a pool of *Ixodes ricinus* ticks collected in southern Finland (Saikku and Brummer-Korvenkontio, 1973). Three years later (1963), several UUKV strains were isolated in the Czech Republic, in a region which was known endemic for the European type of Tick-borne encephalitis virus (TBEV), a flavivirus transmitted also by *I. ricinus* ticks (Kolman et al., 1966). One of the Czech UUKV strains, Potepli-63, was studied in detail regarding its biological, physical and chemical properties (Kolman, 1970a,b). A study in *I. ricinus* ticks in the Potepli region, in central Bohemia, showed that their minimum infection rate with UUKV is double than that with TBEV (3.95 versus 1.91) (Kolman and Husova, 1971) suggesting that UUKV is widespread in the country. Up to now, the available number of whole-genome UUKV sequences is very limited including that of Potepli-63 strain. Aim of the present study was to gain an insight into the genetic diversity and evolution of UUKV, by identifying the whole-genome sequence of two UUKV strains isolated in the Czech Republic.

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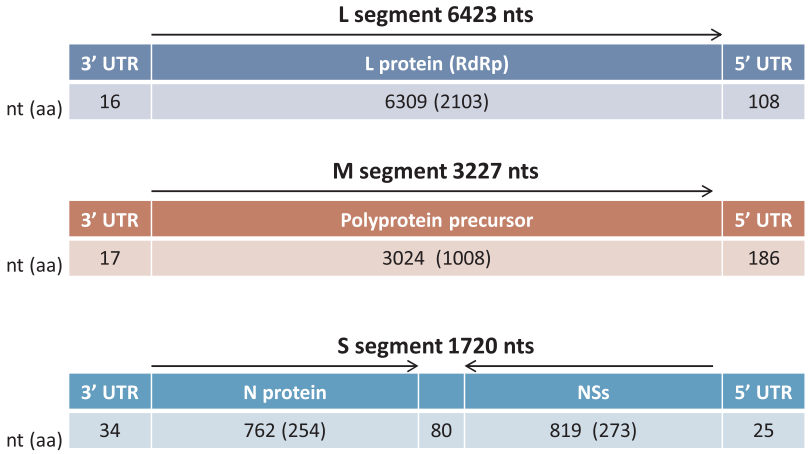

Fig. 1. Schematic representation of the three RNA segments, L (large), M (medium) and S (small) of the two Czech Uukuniemi virus strains and the proteins encoded.

Table 1
Mean genetic differences at nucleotide (nt) and amino acid (aa) level. A: among Czech UUKV strains; B: between Czech UUKV strains and the prototype UUKV strain S3.

Group	L protein		M polyprotein		N protein		NSs protein	
	6309 nt	2103 aa	3024 nt	1008 aa	762 nt	254 aa	819 nt	273 aa
A	1.36	0.04	1.79	0.32	0.52	0	1.13	0.74
B	1.33	0.24	7.48	1.64	3.30	0.76	3.60	2.60

2. Material and methods

2.1. Virus isolates

Two UUKV strains, Losov/Czech Republic/2000 (in short Losov) and Lichnov/Czech Republic/2004 (in short Lichnov), isolated from questing *I. ricinus* ticks collected from the vegetation by flagging were used in this study. Losov strain was isolated from a pool of 40 *I. ricinus* nymphs collected on October 5, 2000, in Losov village in the Czech Republic (49.6169147N, 17.3724894E); Lichnov strain was isolated from a pool of 40 *I. ricinus* nymphs collected on September 24, 2004, in Lichnov village in the Czech Republic (50.0221992N, 17.6511833E). Both strains were isolated by intracerebral inoculation in suckling mice.

The isolation procedure from the ticks was as follows: the pool of ticks was homogenized with cell culture medium (D-MEM), and a 20% suspension was prepared. Following centrifugation at 2000*g* for 30 min, 0.01–0.02 ml of the supernatant was inoculated intracerebrally into 1–3 days old suckling mice (specific-pathogen-free CD1 mice provided by the BioTest s.r.o. company, Konárovice, Czech Republic). The animals were observed daily for 14 days. In case of presence of neurological symptoms a second passage was performed: the mice were decapitated and a 20% brain tissue suspension with D-MEM was prepared. Further procedure was identical to the first passage. In case of development of neurological symptoms, the experiment was terminated and the result was considered as positive. The moribund mice were decapitated and the brain tissues were processed for virus identification by serology or PCR. Animal experiments were approved by the Central National Animal Committee (approval numbers are 1020/396/A00 and 21209/2003-1020). Institutional and national guidelines for the care and use of laboratory animals were followed.

Following the same isolation procedure, four additional pools consisting of 67 nymphs and 11 adult *I. ricinus* ticks (6 males and 5 females) collected on the same day in the same region as Losov strain were negative. Similarly, three additional pools consisting of 100 nymphs and 1 adult *I. ricinus* tick (male) collected on the same day in the same region as Lichnov strain were negative.

2.2. RNA extraction and next-generation sequencing

RNA was extracted from the second passage of the viruses using the viral RNA mini kit (Qiagen, Hilden, Germany).

Whole-genome sequencing was achieved from culture supernatant on a genetic analyser Ion Torrent PGM (Thermo Fisher Scientific) using a 316 chip. Following cDNA synthesis using the Superscript III reverse transcriptase kit (Thermo Fisher Scientific) and DNA fragmentation, a library was prepared and amplified using the Ion Xpress Plus Fragment Library Kit (Thermo Fisher Scientific). After sequencing, *de novo* assembly was performed with SPADES (http://bioinf.spbau.ru/spades), and mapping was done with BWA (https://www.msi.umn.edu/sw/bwa) using sequences from UUKV Potepli-63 strain (GenBank accession numbers KM114246, KM114247, KM114248) as reference.

2.3. Phylogenetic analysis

Whole-genome sequences from viral strains of the Uukuniemi group were aligned using CLUSTAL W and phylogenetic analysis was performed in MEGA 7 (Kumar et al., 2016). Phylogenetic trees based on complete genome sequences of the three RNA segments were constructed using the maximum likelihood method. As outgroup was used Kabuto Mountain virus, which branches between the Uukuniemi and the Kaisodi group viruses (Ejiri et al., 2017).

3. Results and discussion

A total of 321,667 and 270,815 reads were taken from UUKV Lichnov and Losov strains, respectively. The lengths of the coding and non-coding regions are identical among the Czech UUKV strains (Losov, Lichnov, Potepli-63), with L, M and S genomic segments comprising 6423, 3227 and 1720 nucleotides, respectively. Sequences were submitted to GenBank DataBase under the accession numbers MG969381-MG969386.

The schematic representation of the three segments and the encoded proteins are shown in Fig. 1. In all three segments the eight nucleotides of the 3′ and 5′ terminal sequences were conserved and complementary