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Short communication

Molecular identification of Puumala orthohantavirus in Bulgaria

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ABSTRACT

In Bulgaria, only Dobrava orthohantavirus has been detected in patients and in rodents. In order to elucidate possible Puumala orthohantavirus (PUUV) circulation, 131 bank voles (*Myodes glareolus*) were captured. PUUV RNA was detected in 14 (10.7%). Partial L segment sequences were recovered from six *M. glareolus*. Phylogenetic analysis showed that all PUUV sequences from this study clustered together among the Alpe-Adrian lineage. It is the first genetic evidence of the virus circulation in a Balkan country outside north-western Balkans. The findings in this study extended the known edge of virus distribution towards Southeastern Europe.

Orthohantaviruses cause two human diseases – haemorrhagic fever with renal syndrome (HFRS) and hantavirus cardiopulmonary syndrome (HCPS). Until recently, it was believed that HFRS is only spread in Europe and Asia, while HCPS is restricted to America. Recent investigations, however, have revised this concept showing HFRS in the New World and HCPS in the Old World (Clement et al., 2019) as well as considerable clinical overlap between HFRS and HCPS (Clement et al., 2014).

Orthohantaviruses are enveloped viruses with tri-segmented single-stranded RNA genome of negative polarity, members of the genus *Orthohantavirus* of the *Hantaviridae* family (order *Bunyavirales*) (Adams et al., 2017).

Puumala orthohantavirus (PUUV) carried by the bank vole (*Myodes glareolus*) causes a milder form of haemorrhagic fever with renal syndrome (HFRS) also known as nephropathia epidemica with case fatality rate < 1% in Northern and Central-Western Europe and western Russia (Vaheri et al., 2013).

Dobrava-Belgrade orthohantavirus (DOBV) carried by the yellow-necked field mouse (*Apodemus flavicollis*) causes a severe form of HFRS with case fatality rate 5–15% in South-eastern Europe: the Balkan countries and the Alpe-Adrian region (Klempa et al., 2013).

PUUV and DOBV co-circulate in the countries of West Balkan Peninsula (Slovenia, Croatia, Bosnia & Herzegovina). In Slovenia, 73.8% of HFRS were due to PUUV infection (Avšič-Županc et al., 2014). In Croatia, two-thirds of HFRS patients were infected with PUUV (Markotić et al., 2002).

So far only DOBV has been detected in the central, eastern and southern Balkan Peninsula (Serbia, Bulgaria, Romania, Greece) (Avšič-Županc et al., 2014).

In Bulgaria, DOBV infections have been confirmed by RT-PCR in patients and in rodents (Papa and Christova, 2011; Christova et al., 2015). Correspondingly, HFRS cases in the country are characterized by severe course, pronounced toxic syndrome and kidney involvement (Christova et al., 2017).

Recently, we detected PUUV-specific antibodies in patients even with severe HFRS (Christova et al., 2017). However, direct genetic detection of PUUV was never been successful neither in patients nor in rodents in Bulgaria.

In order to elucidate possible PUUV circulation in the country, we collected a total of 131 *M. glareolus* (bank voles) in three regions (Smolyan, Batak and Velingrad) close to dams and lakes, where HFRS cases were reported. Sherman live capture traps were placed in foothills and mountainous deciduous and coniferous forests at altitude above 800 m during 2015–2017. Rodents were processed according to the national guidelines for use of animals.

Viral RNA was extracted from bank vole homogenized spleen samples by QIAamp Viral RNA Kit (QIAGEN, Germany). One step real-time RT-PCR for detection of PUUV RNA was performed using degenerated primers set and probe targeting highly conserved region within the S-segment as described (Kramski et al., 2007). All samples with positive real-time RT-PCR result were amplified by RT-nested PCR targeting regions in L-segment (Klempa et al., 2006) and amplicons were sequenced on GenomeLab™ GeXP Genetic Analysis System (Beckman Coulter, USA).

Phylogenetic analysis of partial L segments was conducted by BioEdit sequence alignment editor. Phylogenetic trees were generated by MEGA 6 software (http://megasoftware.net) based on one thousand bootstrap replicates of the original nucleotide sequence alignments.

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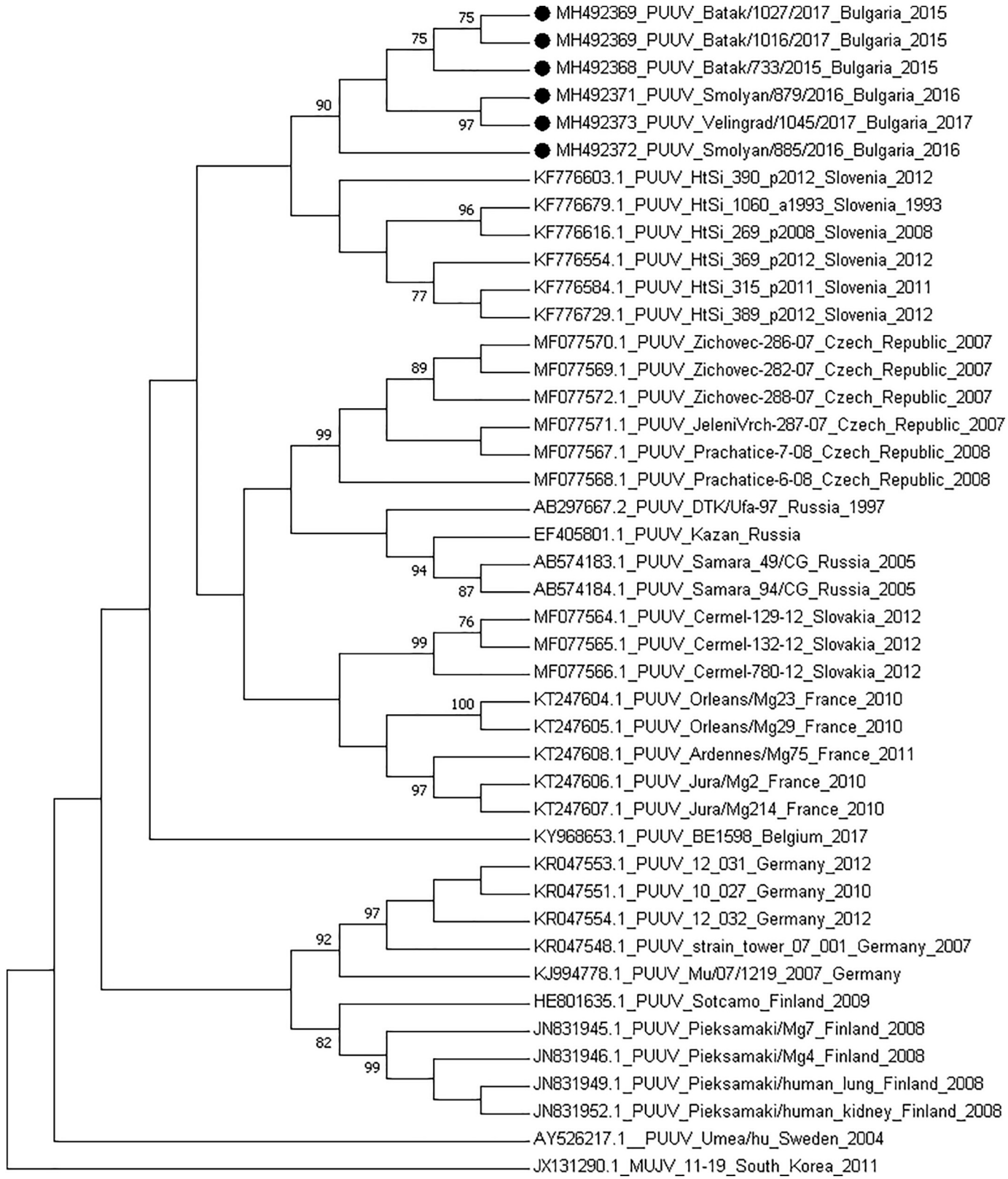

Fig. 1. Phylogenetic tree of Puumala orthohantaviruses calculated for the partial L segment sequences (nt 2988 to 3258). The Muju virus DQ138133 was used as outgroup. Bootstrap support values > 80% are shown at the nodes. Orthohantavirus sequences used for comparison were recovered from the GenBank. Scale bar indicates genetic distance. Bulgarian PUUV from this study are marked by dots.

Evolutionary history was inferred using the Neighbor-Joining algorithm. Related PUUV sequences used for comparison were recovered from GenBank.

PUUV RNA was detected in 14 (10.7%) bank voles by real-time RT-PCR and in 8 of them (6.1%) by RT-nested PCR. Partial L segments of 6 samples were sequenced and analyzed. Sequences were deposited in GenBank under accession numbers: MH492368-MH492373 for isolates: Batak/733/2015, Batak/1016/2017, Batak/1027/2017, Smolyan/879/2016, Smolyan/885/2016, and Velingrad/1045/2017 respectively.

Phylogenetic analysis showed that all recovered PUUV sequences from this study clustered together among the Alpe-Adrian lineage (bootstrap support value of 92%) (Fig. 1). The genome sequences showed similarity with Slovenian PUUV_HtSi strains. The mean genetic differences of Bulgarian PUUV sequences in the L RNA segment (270 nts) was 2.6%.

PUUV circulation among bank voles, confirmed in our study, showed that PUUV is present in Bulgaria and most probably, due to similar environment and distribution of the hosts, circulate as well in the neighbor countries in central, eastern and southern Balkan Peninsula, where PUUV have not been recovered.

It should be noted that unlike PUUV infections in the rest of Europe, PUUV in the Balkans can cause severe HFRS with clinical manifestations similar to those caused by DOBV, as shown by Slovenian and Croatian authors (Avšič-Županc et al., 1999; Markotić et al., 2002).

In conclusion, PUUV detected in bank voles in Bulgaria extended the known edge of virus distribution towards Southeastern Europe. Further studies on PUUV from this region and on specific clinical manifestations of PUUV and DOBV infections are needed.

Declarations of interest

None.

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