

Short communication

Novel phlebovirus detected in *Haemaphysalis parva* ticks in a Greek island



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ABSTRACT

During the last decade the number of novel tick-borne phleboviruses has increased rapidly, especially after the identification of severe fever with thrombocytopenia syndrome and Heartland viruses which can cause severe disease in humans. A novel virus, Antigone virus was recently detected in ticks collected from the mainland of Greece. The aim of the present study was to investigate the presence of tick-borne phleboviruses in an island in Greece. During November 2015, 31 ticks were collected from sheep in Lesvos island. Phleboviral RNA was detected in 12/22 adult *Haemaphysalis parva* ticks. The virus was provisionally named Lesvos virus after the name of the island. Phylogenetic analysis of a 1108-bp L RNA fragment revealed that the Lesvos virus sequences cluster together with Dabieshan and Yongjia tick viruses detected in China in *H. longicornis* and *H. hystricis* ticks, respectively. Further studies are needed to investigate its exact distribution, epidemiology and virulence. It is expected that the research studies on tick biology and pathogen-tick-host interactions will allow a better understanding of the virus life cycle and the elucidation of the possible role of the novel tick-borne phleboviruses in public health.

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1. Introduction

Most viruses of the genus Phlebovirus (family *Bunyaviridae*) are transmitted by phlebotomine sandflies (Diptera: *Psychodidae*). However, some phleboviruses (like Rift Valley fever virus) are transmitted by mosquitoes (Diptera: *Culicidae*), while others (like Uukuniemi virus, UUKV) are transmitted by ticks (Acari: *Ixodidae* and *Argasidae*). Due to the use of modern molecular techniques, e.g. next generation sequencing, the number of described tick-borne phleboviruses is increasing rapidly. The scientific interest increased further following the characterization of two novel tick-borne phleboviruses associated with disease in humans: severe fever with thrombocytopenia syndrome virus (SFTSV) in China (Yu et al., 2011) and Heartland virus (HRTV) in the United States (McMullan et al., 2012). Both viruses cause to humans a disease characterized by fever, thrombocytopenia, leukocytopenia, and multiorgan dysfunction. Furthermore, a wide range of novel phleboviruses have been detected in ticks in several parts of the world (Matsuno et al., 2015; Tokarz et al., 2014; Wang et al., 2014; Li et al., 2015).

Phleboviruses are divided into 2 groups, the sandfly fever virus group (transmitted by sandflies and mosquitoes) and the Uukuniemi-like viruses transmitted by ticks (Elliott and Brennan, 2014). The sequences of SFTSV and related phleboviruses represent a new clade, roughly equidistant from the sandfly fever and Uukuniemi groups (Yu et al., 2011). Recently we identified a novel phlebovirus, Antigone virus (ANTV), detected in *Rhipicephalus sanguineus* sensu lato ticks collected from sheep in the mainland of Greece. It was shown that ANTV sequences form a distinct clade in the tick-borne phleboviruses, together with American dog tick phlebovirus, Bole tick virus, Changping tick virus and Lihan tick virus (Papa et al., 2016). Aim of the present study was to test ticks collected in a Greek island for probable phlebovirus infection.

2. Material and methods

During November 2015, ticks were collected from 16 sheep in 6 different farms located in 4 villages (Eresos, Chidira, Vatousa, Sigri) on the western part of Lesvos island, Greece. The identification was performed morphologically under a stereo-microscope using taxonomic keys (Estrada-Peña et al., 2004). Ticks were stored in -70°C until further testing.

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Table 1
Locations in Lesvos island, Greece, where tick-borne phleboviruses were detected.

Location (altitude, m)	Farms – Animals positive/tested		Ticks positive/tested			
	Farm	Sheep	<i>H. parva</i>	<i>D. marginatus</i>	<i>H. punctata</i>	Total
Eresos [73]	0/1	0/1	0/0	0/1	0/0	0/1
Chidira [308]	1/1	3/4	4/8	0/1	0/0	4/9
Vatousa [279]	3/3	6/8	8/14	0/4	0/0	8/18
Sigri [17]	0/1	0/3	0/0	0/0	0/3	0/3
Total	4/6	9/16	12/22	0/6	0/3	12/31

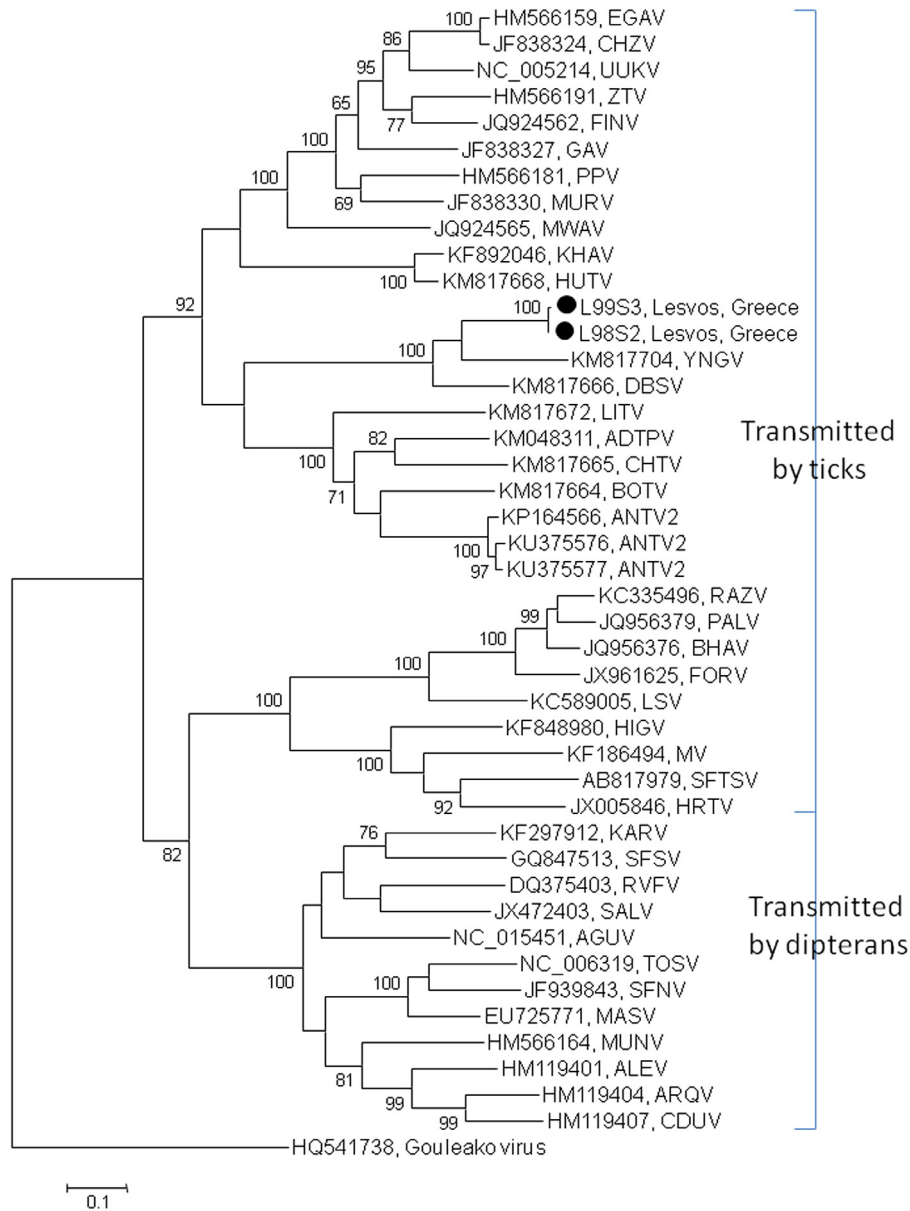


Fig. 1. Maximum Likelihood phylogenetic tree based on a 1108-bp fragment of phlebovirus L RNA segment. The numbers at the nodes indicate percentage bootstrap replicates of 1000; values below 60% are not shown. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The scale bar indicates 10% nucleotide sequence divergence. Sequences in the tree are indicated as GenBank accession number and strain name. Sequences of the present study are marked. ADTV (American dog tick virus); ANTV (Antigone virus); AGUV (Aguacate virus); ALEV (Alenquer virus); ARQV (Ariquemes virus); BHAV (Bhanja virus); BOTV (Bole Tick virus); CDUV (Chandiru virus); CHTV Changping Tick virus; CHZV (Chize virus); DBSV (Dabieshan virus); EGAV (EgAN 1825–61 virus); FINV (FinV707 virus); FORV (Forecariah virus); GAV (Grand Arbaud virus); HIGV (Hunter Island Group virus); HRTV (Heartland virus); HUTV (Huangpi Tick virus); KARV (Karimabad virus); KHAV (Khasan virus); LITV (Lihan Tick virus); LSV (Lone Star virus); MASV (Masillia virus); MUNV (Munguba virus); MURV (Murre virus); MV (Malsoor virus); MWAV (Manawa virus); PALV (Palma virus); PPV (Precarious point virus); RAZV (Razdan virus); RVFV (Valley fever virus); SALV (Salehabad virus); SFNV (Sandfly fever Naples virus); SFSV (Sandfly fever Sicilian virus); SFTSV (Severe fever with thrombocytopenia syndrome virus); TOSV (Toscana virus); UUKV (Uukuniemi virus); YNGV (Yongjia virus); ZTV (Zaliv Terpenia virus). Gouleako virus was used as outgroup.

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