

A novel phlebovirus in Albanian sandflies

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Abstract

Sandflies were collected in two districts of Albania and tested for a probable phlebovirus infection. A novel phlebovirus, provisionally named Adria virus, was detected in 2/12 pools, both trapped in a region close to the Adriatic Sea. The new virus is genetically closer (similarity 77.1% at nucleotide level) to Arbia virus, which belongs to the Salehabad serocomplex. Its distribution and probable pathogenicity to humans remain to be elucidated.

Keywords: Albania, Arbia virus, PCR, phlebovirus, sandfly

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Sandfly fever viruses (genus *Phlebovirus*, *Bunyaviridae* family) cause in humans a variety of syndromes, from a mild febrile illness to severe central nervous system infections. They are transmitted to humans by phlebotominae sandflies (*Diptera: Psychodidae*), which remain infected for life. The virus is most efficiently replicated in sandflies and transmitted when temperatures exceed 25°C; thus, sandfly fever cases are observed mainly in summer.

Three phleboviruses are known to circulate in the Mediterranean countries: Toscana virus (TOSV), Sandfly fever Naples virus (SFNV) and Sandfly fever Sicilian virus (SFSV). SFSV and SFNV cause a transient febrile illness (sandfly fever,

phlebotomus fever or pappataci fever), whereas TOSV is neurovirulent, leading to aseptic meningitis and meningoencephalitis [1–3]. A number of additional phlebovirus strains have been detected recently in Cyprus, France, Spain, Algeria and Tunisia [4–8].

Albania is a Balkan country with a coast on the Adriatic Sea to the west, where the climate is typically Mediterranean (mild, wet winters and warm, sunny and dry summers). Former studies on the phlebotomine fauna of Albania showed that eight species are present: *P. neglectus*, *P. papatasi*, *P. perfiliewi*, *P. tobbi*, *P. similis*, *P. simici*, *Sergentomya dentata* and *S. minuta*, with *P. neglectus* being the most abundant species, widespread in almost all Albania [9,10]. Activity of adult sandflies in Albania lasts from May to early November. Up to date, the entomological research in Albania has been carried out only on sandflies' fauna composition, their feeding habits and the vector competence in *Leishmania* transmission. Concerning phleboviral infections, the available data are very limited, as sandfly fever is seldom recognized because of its mild presentation, while in endemic populations it is mixed with other febrile illnesses of childhood [11]. In addition, viral central nervous system infections have a wide spectrum of causes and clinical presentations, and their incidence is not well defined, as the aetiology of many cases remains unknown [12]. In order to gain a first insight into the epidemiology of phleboviruses in Albania, sandflies trapped in two Albanian districts were tested for a probable phlebovirus infection.

The entomological survey was conducted in Kruje and Lezhe districts (Fig. 1); they represent endemic regions for leishmaniasis, which is also transmitted by sandflies [13]. Three collecting sites were selected, one in Kruje (altitude 658 m) and two in Lezhe (altitude 43 and 58 m). Collection of sandflies was carried out from May to September 2005 using CDC miniature light traps, in peridomestic sites, and inside cow barns, chicken coops and pigpens. Sandflies were transported alive to the laboratory, anaesthetized with low temperature, pooled by gender, trapping area and date of collection and stored at –20°C for further analysis.

RNA was extracted from homogenized sandfly pools using the RNeasy kit (Qiagen, Hilden, Germany). An RT-nested PCR was applied using degenerated primers amplifying a fragment of the L gene of phleboviruses [14]. PCR products were sequenced in an ABI 3130 genetic analyser (Applied Biosystems, Foster City, CA, USA). Sequences were aligned with respective ones retrieved from GenBank using CLUSTAL W, while phylogenetic analysis was performed using the PHYLIP software and the neighbour-joining method with 100 multiple data sets.

A total of 438 sandflies were collected: 132 males (30.1%) and 306 females (69.9%), 47.4% of them blood fed (Table 1);



FIG. 1. Map of Albania, where the locations of Lezhe and Kruje districts are seen.

TABLE 1. Number of sandflies trapped in three stations in Kruje and Lezhe districts in Albania

Month	Locality	Station	No. of males	No. of females (blood fed)	Total
May	Kruje	I	1	23 (16)	24
June	Kruje	I	28	56 (26)	84
Subtotal	Kruje		29	79 (42)	108
May	Lezhe	II	49	37 (37)	86
May	Lezhe	III	0	20 (4)	20
June	Lezhe	II	21	58 (38)	79
June	Lezhe	III	17	53 (24)	70
August	Lezhe	II	2	14 (0)	16
September	Lezhe	II	9	27 (0)	36
September	Lezhe	III	5	18 (0)	23
Subtotal	Lezhe		103	227 (103)	330
Total			132	306 (145)	438

they were divided into 12 pools. Phlebovirus RNA was detected in 2/12 pools: one consisted of six sandflies (one male, five females, four blood fed) collected on 27 June 2005, in station II in Lezhe (41.78°N, 19.63°E, altitude 43 m), and the second consisted of 23 sandflies (five males, 18 females) collected on 15 September 2005, in station III in Lezhe (41.78°N, 19.63°E, altitude 58 m). Sequencing and phylogenetic analysis showed that the two strains were very closely related (they differ by 0.08% and 0.01% at nucleotide and aminoacid level, respectively), differing greatly from all known phleboviruses (Fig. 2). The strain was provisionally named Adria virus. Sequences were submitted in GenBank under the accession numbers HM043725 and HM043726. The new virus clusters together with Arbia virus (ARBV, DQ862467); however, the two viruses differ by 22.9% and

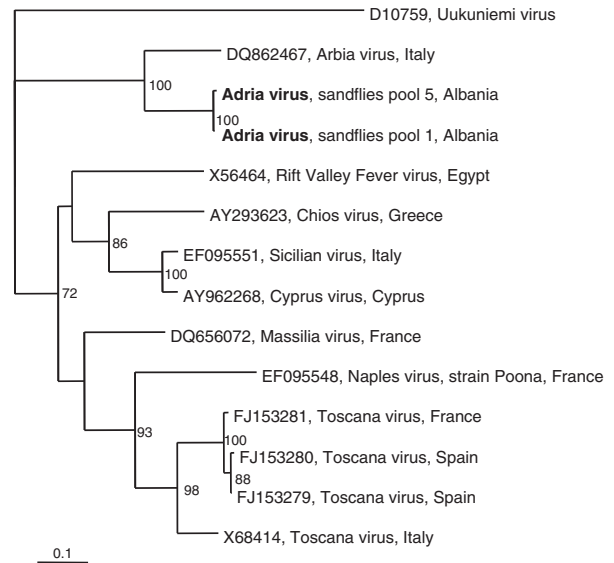


FIG. 2. Phylogenetic tree based on a 234-bp fragment of the L RNA segment of phleboviruses. The numbers at the nodes indicate percentage bootstrap replicates of 100; values below 60% are not shown. Horizontal distances are proportional to the nucleotide differences. The scale bar indicates 10% nucleotide sequence divergence. Sequences are indicated as: accession number, strain, country.

6.7% at nucleotide and aminoacid level, respectively. ARBV, belongs in the Salehabad serocomplex, and was isolated from sandflies in the Tuscany region in Italy, where it has been reported to circulate in populations of *P. perniciosus* (*P. tobbi*) and *P. perfiliewi*, at rates greater than TOSV (56% vs. 44%) [15]. So far there are no reports of human disease caused by ARBV [16]. The Salehabad serocomplex also contains the Salehabad virus (SALV), originally isolated in 1959 from Iranian sandflies [17]. The Adria virus differs by >60% from TOSV, SFSV and SFNV, and represents a distinct clade among phleboviruses. Although more than 60 phleboviruses are known, classified into nine serocomplexes [17], the number of available phlebovirus sequences, especially of the L segment, is very limited, making the genetic comparison unattainable.

In conclusion, a novel phlebovirus was detected in sandflies collected in the coastal region of Albania. Application of molecular methods enables the detection of viruses in human samples; however, in the case of phleboviruses this does not happen often, as the duration of viraemia in humans is very short (24–36 h). For this reason, most novel phleboviruses have been detected in sandflies, and their pathogenicity has not yet been elucidated. Further studies are needed to determine the distribution of the new virus in Albania and other Mediterranean countries, while testing patients' samples taken during the first days of illness will probably provide evidence of its pathogenic potential.

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Transparency Declaration

No conflict of interest.

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