

Of phlebotomines (sandflies) and viruses: a comprehensive perspective on a complex situation

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Old World sandfly-borne phleboviruses are classified into three serological complexes: Sandfly fever Sicilian, Sandfly fever Naples and Salehabad. Human pathogens (febrile illness ['sandfly fever'], neuroinvasive infections) belong to the two first complexes. The increasing number of newly discovered sandfly-borne phleboviruses raises concerns about their medical and veterinary importance. They occupy a wide geographic area from Mediterranean basin to North Africa and the Middle East to the central Asia. At least nine species of sandflies can transmit these viruses. Recent results suggest that sandfly vectors are not as specific for viruses as initially believed. Recent seroprevalence studies demonstrate that humans and domestic animals are heavily exposed. Specific molecular diagnostic methods must be developed and implemented in clinical microbiology laboratories.

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Sandflies: the vectors

Phlebotomine sandflies (Diptera: Psychodidae, Phlebotominae) show worldwide distribution including southern Europe, Asia, Africa, Australia, Central and South America [1]. They are small (1.5–3 mm), hairy, mostly nocturnal insects, with weak flight capacity. Subfamily Phlebotominae contains three genera: *Phlebotomus* spp. and *Sergentomyia* spp., which are present in the Old World, and *Lutzomyia* spp., which is present only in the New World [2]. Sandfly activity shows seasonal correlation and peaks during summer. Despite sandflies have limited dispersal ability, recent climate changes resulted in the expansion of geographic areas where sandflies circulate

[3,4*]. For instance, *Phlebotomus perniciosus* and *P. mascittii* are now reported in south-western Germany and southern Austria, respectively [5,6]. Faunistic studies which map the distribution and the abundance of sandflies provide crucial information for vector control.

Phleboviruses

Phleboviruses are negative-sense, enveloped, three-segmented (L, M and S segments) RNA viruses. Old World sandfly-borne phleboviruses can be classified using their antigenic properties into three serological complexes, namely Sandfly fever Sicilian complex (grouping SFSV and CFUV), Sandfly fever Naples complex and Salehabad complex.

According to the International Classification for Taxonomy of Viruses (ICTV), the genus *Phlebovirus* contains two Old World sandfly-borne viral species: the *Sandfly fever Naples* species and the *Salehabad* species. The *Sandfly fever Naples* species includes the following viruses: Sandfly fever Naples [SFNV], Toscana sandfly virus [TOSV], Tehran virus [THEV] and Massilia sandfly virus [MASV]. The *Salehabad species* virus includes Salehabad virus [SALV] and Arbia virus [ARBV].

In addition to these two recognized species complex, ICTV has listed two eponymous tentative species corresponding to the Sandfly fever Sicilian virus [SFSV] and Corfou virus [CFUV] [7].

During the last decade, new phleboviruses have been discovered, but are still unclassified (Box 1). For clarity, species (recognized and tentative) are italicized and acronyms do apply only to viruses.

Finally, it was recently demonstrated that Karimabad virus [KARV] does not belong to the *Sandfly fever Naples* species [8*].

Sandflies are generalist vectors

Phlebotomine sandflies are vectors of parasites (*Leishmania*), bacteria (*Bartonella*) and viruses (*Phlebovirus*) [9,10*]. The majority of viruses within the *Phlebovirus* genus have been associated with sandflies. Specifically, viruses belonging to the *Sandfly fever Naples* species were detected and isolated from *P. perfiliewi*, *P. perniciosus*, *P. longicuspis*, *P. papatasi*, *P. sergenti* and *Sergentomyia minuta* [11–18,19*,20–23]. Viruses belonging to the tentative *Sandfly fever Sicilian* species and *Corfu* species were detected and isolated from *P. ariasi*, *P. papatasi*, *P. neglectus*, *P. perniciosus*

Box 1 Schematic overview of sandfly-borne phlebovirus groups depending on the antigenic relationships.

SANDFLY-BORNE PHLEBOVIRUSES		
SANDFLY FEVER NAPLES SPECIES		
ICTV RECOGNISED	NEW (ISOLATION + SEQUENCE)	SEQUENCE ONLY
SANDFLY FEVER NAPLES VIRUS (SFNV)(Italy) TEHRAN VIRUS (THEV)(Iran) MASSILIA VIRUS (MASV)(France) TOSCANA VIRUS (TOSV) ^a	TOSCANAVIRUS(LIN-B) ^b ZERDALIVIRUS(Turkey) ARRABIDAVIRUS(Portugal) GRANADAVIRUS(Spain) PUNIQUEVIRUS(Tunisia)	TOSCANA VIRUS (LIN-C)(Croatia, Greece) FERMO VIRUS(Italy) BALKAN VIRUS (the Balkans) GIRNE1 VIRUS (Cyprus) PROVENCIA VIRUS(France)
SALEHABAD SPECIES		
ICTV RECOGNISED	NEW (ISOLATION + SEQUENCE)	SEQUENCE ONLY
SALEHABAD VIRUS (SALV)(Iran) ARBIA VIRUS (ARBV)(Italy)	ADANAVIRUS(Turkey) ALCUBEVIRUS(Portugal) MEDJERDAVALLEYVIRUS(Tunisia)	ADRIA VIRUS (Greece, Albania) EDIRNE VIRUS (Turkey) OLBIA VIRUS (France)
SANDFLY FEVER SICILIAN VIRUS		
ICTV TENTATIVE	NEW (ISOLATION + SEQUENCE)	SEQUENCE ONLY
SANDFLY FEVER SICILIAN VIRUS (SFSV)(Italy)	SANDFLYFEVER SICILIAN CYPRUS VIRUS(Cyprus) SANDFLYFEVER SICILIAN TURKEY VIRUS(Turkey) DASHLIVIRUS(Iran)	KABYLIA VIRUS(Algeria) TUN 166 (Tunisia)
CORFOU VIRUS		
ICTV TENTATIVE	NEW (ISOLATION + SEQUENCE)	SEQUENCE ONLY
CORFOU VIRUS (CFUV)(Greece)	TOROSVIRUS(Turkey)	UTIQUE VIRUS (Tunisia) GIRNE2 VIRUS (Cyprus) CHIOS VIRUS (Greece)
<p>a, Italy, Tunisia, Algeria, France, Turkey</p> <p>b, Portugal, Spain, France, Morocco, Turkey</p>		

and *P. longicuspis* [21,22,24–27]. Viruses belonging to the *Salehabad* species were detected and isolated from *P. perniciosus* and *P. perfiliewi* [18,28–30].

Phleboviruses were identified both in male and female sandflies at equal rates [18,21,31,32^{*}], suggesting the

existence of both transovarial (vertical) and venereal (horizontal) transmission during mating [2,33–36].

The number of known phleboviruses has dramatically increased over last decade owing to the flourishing of entomological and virological studies and to investigations

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