Phleboviruses

Anna Papa
National Reference Centre for Arboviruses and Hemorrhagic Fever viruses
Aristotle University of Thessaloniki, Greece
Main vectors of phleboviruses: phlebotomine sandflies

Etymology *Phlebotomus*
From the Greek words: phlebo + tomi (cut a vein)
> 500 different species
- Widely distributed in the Med countries
- Abundant in peri-urban and rural environments, close to domestic animals and human populations.
- Ideal environment: a cool, shaded, slightly damp area.
- Active during night - silent flight - short flight range (100 m from their breeding sites).
- Active: May to October. The number increases after rainy season.

The sandflies become infected when biting an infected humans in the period between 48 h before the onset of fever and 24 h after the end of the fever, and remain infected for their lifetime.
TAXONOMY

Order **Bunyavirales**
Family **Phenuiviridae**
Genus **Phlebovirus** (the genus currently comprises 10 species + additional viruses as tentative species)

Species differentiation is based on a 4-fold difference in neutralization tests.

Over the past decade, a wide variety of novel phleboviruses were detected, particularly in the Mediterranean basin.

Classification is complicated by their reassorting ability, particularly in the M segment.
Phlebovirus transmission

- by **sandflies**, e.g. Toscana virus (TOSV)
- By **mosquitoes**, e.g. Rift Valley fever virus (RVFV)
- By **ticks**, e.g. Uukuniemi virus, Severe fever with thrombocytopenia syndrome virus (SFTSV)
**VIRION**

Enveloped, spherical. Diameter 80-120 nm. Glycoproteins serve as neutralizing and hemagglutinin-inhibiting antibody targets and are exposed to selective pressure.

**GENOME**

Segmented negative-stranded RNA genome. Encodes for 6 proteins.

S : N protein and a NSs. Uses an ambisense coding strategy
M : precursor of the viral glycoproteins Gn and Gc, and NSm.
L : viral RNA polymerase.
Sandfly fever viruses: Historical perspectives

1886: Pick. In the endemic Balkans

1908: Vector: *P. papatasi*. Austrian military commission (Doerr *et al*) working on the Dalmatian coast (Herzegovina) investigated the etiology and transmission of sandfly fever.

outbreaks mostly among soldiers who had recently arrived in endemic regions - most of the literature in military journals or reports

During World War II: 8,500 cases among US soldiers in the Sicily campaign (summer 1943), Naples outbreak (summer 1944).

1943-44: Isolation of Sicilian and Naples viruses from patients in Egypt and Palermo, Sicily and Naples (Sabin 1951).

August 1944: First use of DDT for the control of malaria vectors, decrease of SF cases.

1948: the largest outbreak occurred in Serbia (>1 million cases).

1971: Isolation of Toscana virus from *P. perniciosus* in Tuscany, Italy.

1983: Detection of TOSV in CSF of a patient with meningitis
Phleboviruses are of great military importance since large number of invading non-immune persons may be incapacitated at the time when they are mostly needed.

**Chart 1.** Incidence of sandfly fever and fever of undetermined origin in the North African-Mediterranean Theater of Operations, U.S. Army, 1943–45

[Preliminary data based on summaries of statistical health reports]
[Rate expressed as number of cases per annum per 1,000 average strength]

---

*Sabin et al.* JAMA 1944. Phlebotomus (Papatassi or sandfly) fever, a disease of military importance. Summary of existing knowledge and preliminary report of original investigations.
<table>
<thead>
<tr>
<th>Species or tentative species (ts)</th>
<th>Virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sandfly fever Naples</td>
<td>Sandfly fever Naples virus (SFNV)</td>
</tr>
<tr>
<td></td>
<td>Toscana virus (TOSV)</td>
</tr>
<tr>
<td></td>
<td>Massilia virus (MASV)</td>
</tr>
<tr>
<td></td>
<td>Tehran virus (TEHV)</td>
</tr>
<tr>
<td></td>
<td>Granada virus (GRV)</td>
</tr>
<tr>
<td></td>
<td>Punique virus (PUNV)</td>
</tr>
<tr>
<td></td>
<td>Fermo virus</td>
</tr>
<tr>
<td></td>
<td>Saddaguia virus (SADV) [md/ni]</td>
</tr>
<tr>
<td></td>
<td>Arrabida virus</td>
</tr>
<tr>
<td></td>
<td>Zerdali virus (ZERV)</td>
</tr>
<tr>
<td>Salehabad</td>
<td>Salehabad virus (SALV)</td>
</tr>
<tr>
<td></td>
<td>Arbia virus (ARBV)</td>
</tr>
<tr>
<td></td>
<td>Adria virus (ADRV) [md/ni]</td>
</tr>
<tr>
<td></td>
<td>Alcube virus</td>
</tr>
<tr>
<td></td>
<td>Edirne virus [md/ni]</td>
</tr>
<tr>
<td></td>
<td>Adana virus (ADAV)</td>
</tr>
<tr>
<td></td>
<td>Medjerda Valley virus (MVV)</td>
</tr>
<tr>
<td>Sandfly fever Sicilian (ts)</td>
<td>Sandfly fever Sicilian virus (SFSV)</td>
</tr>
<tr>
<td></td>
<td>Cyprus virus (SFCV)</td>
</tr>
<tr>
<td></td>
<td>Turkey virus (SFTV)</td>
</tr>
<tr>
<td></td>
<td>Utique virus [md/ni]</td>
</tr>
<tr>
<td>Corfou (ts)</td>
<td>Corfou virus (CFUV)</td>
</tr>
<tr>
<td></td>
<td>Toros virus (TORV)</td>
</tr>
<tr>
<td></td>
<td>Sicilian-like virus</td>
</tr>
<tr>
<td></td>
<td>Girne 1 virus [md/ni]</td>
</tr>
<tr>
<td></td>
<td>Girne 2 virus [md/ni]</td>
</tr>
<tr>
<td></td>
<td>Olbia virus [md/ni]</td>
</tr>
<tr>
<td></td>
<td>Provencia virus [md/ni]</td>
</tr>
<tr>
<td>Karimabad (ts)</td>
<td>Karimabad virus (KARV)</td>
</tr>
</tbody>
</table>
Distribution of sandfly-transmitted phleboviruses in the Med region
Phleboviral clinical syndromes

- Asymptomatic infections
- Mild disease, flu-like symptoms
- CNS infections (meningitis, encephalitis)

- Sandfly fever Sicilian virus
- Sandfly fever Naples virus
- Toscana virus (neurotropic)

Central Italy: 80% of acute CNS viral infections in children and 50% of cases in adults during summer.
Greece: 7-17% of CNS infections
Central Anatolia, Turkey: 15.7% of aseptic meningitis.
Portugal: 5.6% of meningitis cases.
Sandfly fever

Phlebotomus fever, papataci fever, 3-day fever

Incubation time: 2-5 d
Abrupt onset of the disease with fever, chills, frontal headache, malaise, photophobia, muscle and joint pain, and retro-orbital pain.

After 2 days the fever begins to subside and the temperature returns to normal.

Fatigue, depression, bradycardia and subnormal blood pressure may persist from few days to several weeks.

Recovery is usually complete.

Similar symptoms to dengue fever, but without rash.
Toscana virus infections

- Asymptomatic
- Influenza-like syndrome
- Meningitis or meningoencephalitis

Incubation period: 3–7 days

Initially as mild febrile illness without CNS involvement

Neuroinvasive infections usually begin with headache, fever, nausea, vomiting, and myalgia. Physical examination may show neck rigidity, Kernig sign, and in some cases unconsciousness, tremors, and paresis.

Outcome: usually favorable without sequelae.

CSF: 5–10 cells/mL, normal levels of glucose and proteins. Leucopenia or leucocytosis can be observed.
Severe Encephalitis Caused by Toscana Virus, Greece

30.6.2012. Female, 49 y.
PCR in CSF: Toscana virus lineage C.
Laboratory diagnosis of phleboviral infections

**Virus isolation and RT-PCR** in blood or cerebrospinal fluid is only possible in early stages of infection i.e. the first 1-3 days after symptom onset and before the IgM detection.

**Serology** (ELISA, IFA) of acute and early convalescent sera.

Serological **cross reactions** exist within the antigenic complex.

**Neutralization assays** using early convalescent sera are the reference method to identify the viruses or to assess the antibody response specificity.

The patient has lifelong immunity to the infecting phlebovirus **but not** to heterologous serotypes.
SF outbreak in Cyprus, 2002

Time: May to September 2002
Place: Cyprus, Athalassa National Forest Park, 5 km SE of Nicosia
Patients: 256/581 (44%) soldiers of the Greek Army forces, 17-22 y.
Signs and Symptoms: high fever (39-41°C), headache, rigors, arthralgia, myalgia, lower back pain.
Duration of symptoms: 60-72 h. Post infectious asthenia syndrome (2 weeks)
Laboratory findings: leucopenia, monocytosis, 2X elevated transaminases (mainly ALAT)
Laboratory diagnosis:
  - Genetic detection using pan-phlebo PCR
  - Sequencing: Sicilian-like virus
  - Isolation in Vero E6 cells (Cyprus virus)
  - Serological cross-reactivity for phleboviruses
Cyprus virus (isolation from patient’s blood, 1st day of illness)

\[ \delta = 100 \text{ nm} \]

Cyprus – Sicilian:
6.7% (nt)
1.5% (aa)
Summer of 2007 and 2008 Turkey in Kozan and Izmir in Med Turkey, and Ankara in central Turkey.
A SFSV-like strain was isolated (SFTV) highly homologous to Cyprus virus.
Massilia Virus, A Novel Phlebovirus (Bunyaviridae) Isolated from Sandflies in the Mediterranean

Rémi N. Charrel, Grégory Moureau, Sarah Temmam, Arezki Izri, Pierre Marty, Philippe Parola, Amelia Travassos da Rosa, Robert B. Tesh, and Xavier de Lamballerie

It is most closely related to viruses in the SFNV complex.

Granada Virus: a Natural Phlebovirus Reassortant of the Sandfly Fever Naples Serocomplex with Low Seroprevalence in Humans

Ximena Collao, Gustavo Palacios, Fernando de Ory, Sara Sanbonmatsu, Mercedes Pérez-Ruiz, José María Navarro, Ricardo Molina, Stephen K. Huchison, W. Ian Lipkin, Antonio Tenorio, and María Paz Sánchez-Secco

Department of Virology, National Center of Microbiology, Institute of Health “Carlos III,” Madrid, Spain; Virology, Medicine School, Universidad de Valparaíso, Valparaíso, Chile; Center for Infection and Immunity, Mailman School of Public Health, Columbia University, New York, New York; Service of Microbiology, University Hospital “Virgen de las Nieves,” Service of Parasitology, National Center of Microbiology, Institute of Health “Carlos III,” Madrid, Spain; 454 Life Sciences, Branford, Connecticut.
Isolation of three novel reassortant phleboviruses, Ponticelli I, II, III, and of Toscana virus from field-collected sand flies in Italy
Punique virus, a novel phlebovirus, related to sandfly fever Naples virus, isolated from sandflies collected in Tunisia

Elyes Zhioua,† Grégory Moureau,²† Ilhem Chelbi,¹ Laetitia Ninove,²,³ Laurence Bichaud,² Mohamed Derbal,¹ Mylène Champs,² Saïfeddine Chreni,¹ Nicolas Salez,² Shelley Cook,⁴ Xavier de Lassale,²,³ and Remi N. Charrel²,³

Sandfly Fever Sicilian Virus, Algeria

Arezki Izri,⁎ Sarah Temmam,† Grégory Moureau,† Boussad Hamrioui,† Xavier de Lassale,† and Remi N. Charrel†

Molecular and Serological Evidence for the Presence of Novel Phleboviruses in Sandflies from Northern Algeria

Grégory Moureau¹, Laurence Bichaud¹, Nicolas Salez¹, Laetitia Ninove¹, Boussad Hamrioui², Smail Belazzoug³, Xavier de Lassale¹, Arezki Izri⁴ and Rémi N. Charrel¹

Sandflies: Two sequences closer to Poona virus of SFNV & one closer to Cyprus virus
Location: Lezhe and Kruje districts
Time: May - September 2005
Sites: peridomestic sites, and inside chicken coops, cow barns and pigpens
Method: CDC miniature light traps
Sandflies pooled by sex, trapping area and date of collection
438 sandflies, 69.9% female.
2/12 pools phlebovirus positive.
Collection dates: 27 June, 5 September 2005

A novel phlebovirus in Albanian sandflies

A. Papa¹, E. Velo² and S. Bino²
1) 1st Department of Microbiology, Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece and 2) Control of Infectious Diseases Department, National Institute of Public Health, Tirana, Albania

Adria virus

ADRV vs ARBV: 22.9% and 6.7% at nt and aa level
History of phleboviruses in Greece

July-August 1941. 20% of German troops in Athens and islands had sandfly fever.
1945. SF cases among British troops in Athens.
1948. SF cases among Germans in Athens.
1946. Application of DDT. Sandfly fever very rare.
1981. Rodhain et al., Isolation of Corfou virus (SFSV-like) from *Phlebotomus major* in Corfu island.

Seroprevalence:
*Tesh and Papaevangelou, 1977*
Among residents of Athens >30 years: SFNV 36%, SFSV 13%.
< 30 years: SFNV 4%, SFSV 0%.

*Antoniadis et al, 1990*
Among healthy farmers SFNV: 16.7%, SFSV 2%, Corfu virus 4%.

*Papa et al, 2010*
Against TOSV: 51.7% in Corfu, 39% in Cephalonia islands
Severe Encephalitis Caused by Toscana Virus, Greece

30.6.2012. Female, 49 y.
PCR in CSF: **Toscana virus lineage C.**
2.5-y boy admitted to a hospital in Thessaloniki, Greece, because of a first episode of febrile seizures. No underlying disease.
Fever (38.2°C) and vomit while in the nursery school. Sudden adherence of eye gaze, peroral cyanosis, masseter muscle spasm, tonic convulsions of the body and extremities and involuntary loss of urine. The episode lasted 3 minutes. He presented irritability and felt sleepy. By the time he entered the hospital, he was apyretic.
Clinical examination normal.
leukocytosis (22.600/μl) - 85.7% neutrophils.
Full recover, discharge 2 days later.
One pool of female sandflies collected on 7 July 2009: **positive** for phleboviruses.

32% different from SALV

Inoculation on Vero E6 cells: CPE 5 days post inoculation
Phleboviruses in Greece

- Adria virus
- TOSV
- Cyprus virus
- Corfu virus
- Kerkira virus
- Lefkas virus
Since 2007 many cases of a life-threatening disease with sudden fever, gastrointestinal symptoms, thrombocytopenia, and leukopenia were reported in China. After that the virus was detected in additional provinces. Fatality 30%.

Patients reported of tick bites.
Novel tick-borne phleboviruses in Europe

Detected in ticks
Unknown pathogenicity

- Greece 2016, 2017
- Portugal 2017
- France 2017
- Germany, Belgium 2017
 Prevention

✔ Prevention of sandfly bites (insect repellents)

✔ Control of sandflies and their breeding grounds with insecticides

Ordinary mosquito nets and screening are not sufficient to prevent sandfly bites: unfed female flies can pass through.