Chapter 11

Phlebotomus Fever—Sandfly Fever

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CASE PRESENTATION

During mid-August, a 27-year-old male was admitted to the emergency ward with high fever, chills, severe headache, joint pain, watery diarrhea, nausea and, vomiting, which had started the day before. The initial complete physical examination demonstrated a fever of 38.9°C, generalized muscle tenderness, and multiple skin lesions suggesting insect bites on the upper left limb. Neurological examination was normal without meningeal signs. Medical history revealed no previous disease of significance but an exposure to mosquitoes during his stay at his cousin's cottage 5 days ago. He had vomited three times during the last 24 hours. No apparent risk for infectious gastroenteritis could be identified. Laboratory evaluation demonstrated decreased leukocyte count ($3.8 \times 10^3/\mu L$) with relative lymphocytosis, decreased platelet count ($1.32 \times 10^5/\mu L$), elevated alanine aminotransferase (ALT, 101 U/L), aspartate aminotransferase (AST, 128 U/L), gamma glutamyl transpeptidase (GGT, 107 U/L), creatinine phosphokinase (CPK, 428 U/L), and lactate dehydrogenase (LDH, 354 U/L) levels. Hemoglobulin, C-reactive protein, total protein, blood urea nitrogen (BUN), albumin, creatinine, prothrombin time (PT), activated partial thromboplastin time (aPTT), international normalized ratio (INR), and the chest X-ray were within normal limits. The patient was transferred to the infectious diseases department with the preliminary diagnosis of undifferentiated viral febrile condition, and blood and stool samples were submitted for microbiological analyses. Symptomatic treatment with intravenous rehydration and anti-pyretics was initiated.

The patient responded favorably to the treatment and fever subsided within 48 hours. Platelet and leukocyte counts returned to normal limits in 36–48 hours while hepatic enzymes remained over the threshold for another

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24–48 hours. No leukocyte, erythrocyte, or parasite eggs were detected in stool microscopy. Blood and stool cultures were evaluated as negative for pathogenic bacteria. Serological assays ruled out acute hepatitis by hepatitis A–E viruses. Due to the history and observation of insect bites, West Nile virus and phlebovirus assays were also performed in blood samples obtained during admission. While West Nile virus as well as Toscana and sandfly fever Sicilian virus immunoglobulins could not be detected, a pan-phlebovirus polymerase chain reaction demonstrated reactive results. Sequencing of the amplicons revealed complete homology to the sandfly fever Sicilian virus variant, sandfly fever Turkey virus, and diagnosis of sandfly fever was established. A serum sample collected on the fifth day was reactive for sandfly fever Sicilian virus IgM antibodies via commercial assays. The patient was discharged with recovery on the sixth day of admission with normalized biochemical and hematologic parameters. During the follow-up visit on the fourth week after discharge, the patient complained about weakness, fatigue, and lack of concentration, which had lasted about 10 days. A convalescent serum obtained during the visit demonstrated an IgG seroconversion.

1. WHY THIS CASE WAS SIGNIFICANTLY IMPORTANT AS AN EMERGING INFECTION

Sandfly fever, also known as phlebotomus, pappataci, or 3-day fever, is an arthropod-borne febrile disease transmitted by phlebotomine sandflies. Sandfly fever is prevalent in many regions including the countries in the Mediterranean Basin, northern Africa, the Middle East, and parts of Central and Southern Asia where it constitutes a significant health problem affecting non-immune persons.[1] Although the disease is not frequently associated with mortality and residual sequelae, it is a highly incapacitating and debilitating condition that can significantly affect the individual and the population.[2] In endemic regions, epidemics, especially occurring during summer months, have been noted. Sandfly fever is also of concern as a travel-related infection.[3–6] Historically, the disease affected a large number of Allied and Axis troops serving in the Mediterranean region during the Second World War.[7] Several cases of imported sandfly fever have been reported in persons visiting endemic countries.[8–10]

2. WHAT IS THE CAUSATIVE AGENT?

Sandfly fever is caused by viruses classified in the *Phlebovirus* genus of the *Bunyaviridae* family. The *Phlebovirus* genus consists of over 50 distinct virus serotypes, some of which have not been fully characterized. According to the 9th Report of the International Committee for Taxonomy of Viruses (ICTV), phleboviruses consist of nine viral species or serotypes (Sandfly fever Naples, Salehabad, Rift Valley fever, Uukuniemi, Bujaru, Candiru,

Chilibre, Frijoles, and Punta Toro) and several tentative species (including Sandfly fever Sicilian and Corfu).[11]

Phleboviruses possess an enveloped spherical virion approximately 100 nm in diameter; a tripartite single-stranded RNA genome having a negative and ambisense coding strategy. The structural proteins of phleboviruses comprise the surface glycoproteins (Gn and Gc) encoded by the M (medium-sized) genome segment, the nucleocapsid protein encoded by the S (small) segment, and the viral RNA-dependent RNA polymerase, encoded by the L (large) genome segment. The replication site is the cytoplasm of the infected cell and virion maturation occurs in the Golgi complex.[12,13]

Within the *Phlebovirus* genus, well-known agents responsible for febrile diseases occuring in the Old World are Sandfly fever Sicilian virus (SFSV) and Sandfly fever Naples virus (SFNV)[2]. In addition to SFSV and SFNV, other phlebovirus strains are associated with sandfly fever as well. These include Sandfly fever Cyprus virus (SFCV), which was identified in Greek troops in Cyprus, and Sandfly fever Turkey virus (SFTV), which was characterized during outbreaks in central and Aegean/Mediterranean coastal provinces in Turkey.[3,14] SFCV and SFTV are considered as SFSV variants due to significant genomic sequence homology and antigenic cross-reactions.[3] Another frequently observed clinically significant phlebovirus is Toscana virus (TOSV). Due to its distinct neurotropism, TOSV has been identified as an important agent of sporadic seasonal meningitis/meningoencephalitis in endemic countries.[15] Febrile disease without central nervous system involvement due to TOSV has also been reported.[16,17] Moreover, a surprisingly vast diversity of phleboviruses has been revealed in vector sandflies in the endemic regions. Phleboviruses characterized in sandflies include Salehabad, Karimabad, and Tehran viruses in Iran, Corfu virus in Greece, Arbia virus in Italy, Massilia virus in France, Granada virus in Spain, Punique virus in Tunisia, Adria virus in Albania, as well as several putative isolates.[18–27] Although serologic data indicate human exposure to some of these strains, their pathogenicity and association with clinical disease have not yet been fully elucidated.[20–22,24,27,28] Nevertheless, recent evidence suggests Granada virus to be responsible for mild febrile diseases.[29] In the New World, Alenquer, Chagres, Candiru, and Punta Toro phleboviruses have been detected in individuals with sporadic febrile diseases in Panama, Brasil, and in northern parts of South America. The diseases in tropical America are probably forest associated and rodents living in forests presumably act as amplifying hosts.[30–32]

3. WHAT IS THE FREQUENCY OF THE DISEASE?

The risk for infection with sandfly-transmitted phleboviruses affects large areas of the Old World including southern Europe, Africa, the Middle East, and Central and Western Asia, in association with the presence of sandfly

vectors.[28,33] Recent investigations have demonstrated that phlebovirus diversity in the Mediterranean basin is higher than initially suspected, and that populations living south and east of the Mediterranean Sea have a high risk for virus exposure during their lifetime.[15,28,33,34]

Historical evidence of sandfly fever in the Mediterranean region dates back to the Napoleonic Wars and SFSV-SFNV isolation was performed following the epidemics around Italy during the Second World War.[7,35] Allied forces stationed in the Mediterranean and Middle East reported tens of thousands of cases and attack rates of 3−10% (locally up to 80%). Outbreaks of sandfly fever occurred repeatedly in the former Soviet Union in the period from 1945 to 1950, predominantly in Crimea, Romania, Moldavia, and the Central Asian republics. Epidemics related to the activity of *Phlebotomus papatasi* were reported in northern Africa, southern Europe, the Middle East, and Central Asia.[36,37] Human disease in the Americas is reported from Panama, Brazil, and northern regions of South America, although phleboviruses have been isolated as far as Rio Grande Valley in Texas and southern Brasil.[30−32]

Seroepidemiological studies have shown that the prevalence of antibodies to SFSV and SFNV in indigenous populations follows the distribution of *P. papatasi* around the Mediterranean region, the Middle East, North Africa and Western Asia.[2,18,28,38,39] Evidence for SFSV activity is present in Bangladesh, India, Greece, Cyprus, Iraq, Morocco, Saudi Arabia, Somalia, Sudan, Tunisia, Turkey, Pakistan, Croatia, Iran, the southern European and Central Asian republics of Turkmenia, Tajikistan, Uzbekistan, Azerbaijan and Moldavia, former Yugoslavia, France, and Portugal.[4,18,28,39−42] SFNV follows a similar distribution and has been detected in Bangladesh, Ethiopia, Greece, Cyprus, Iraq, Morocco, Saudi Arabia, Sudan, Djibouti, Turkey, and former Yugoslavia, Turkmenia, Tajikistan, Uzbekistan, Azerbaijan and Moldavia.[28,33,39−41,43] Serosurveillance and clinical data from areas surrounding the Mediterranean suggest that SFNV infections have significantly decreased during the last decades.[33,44] Since 2006, sandfly fever epidemics were reported from Cyprus, Iraq, and Turkey.[3,4,14,45] Infections in non-immune individuals, frequently acquired as travel-related infections, are well known.[4,5,8,9,46] Figure 11.1 shows countries with documented sandfly fever phlebovirus activity in the Old World.

4. HOW IS THE VIRUS TRANSMITTED?

The only known transmission route for viruses causing sandfly fever is via blood sucking of female phlebotomine sandflies of the *Psychodidae* family.[1,33,36] Direct human-to-human or parenteral transmission has not been demonstrated to date. Many phleboviruses have been characterized in Africa, Europe, and Central Asia mainly from *Phlebotomus* and also from *Sergentomyia* species, and in the Americas from sandflies belonging to the

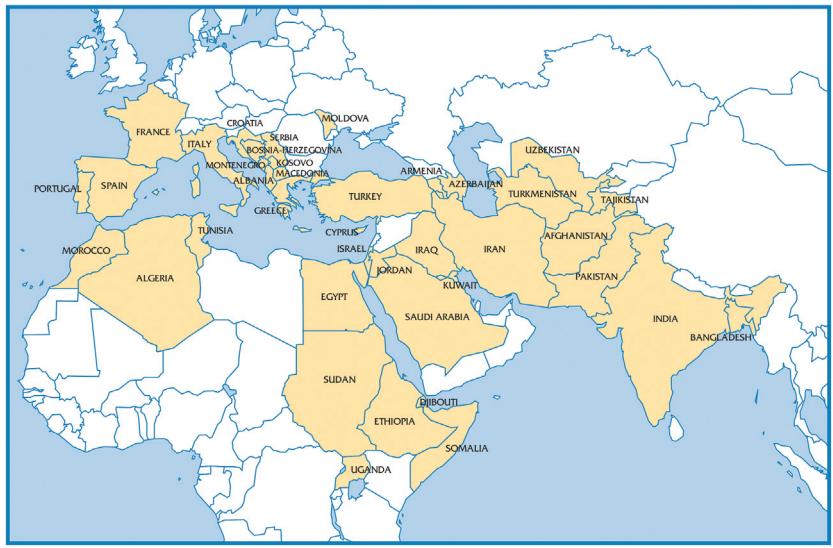

FIGURE 11.1 Countries with documented sandfly fever phlebovirus activity in the Old World.

genera *Lutzomyia sensu lato*[1,33]. *P. papatasi* has been widely acknowledged as the vector for SFSV and SFNV.[37,47,48] Furthermore, virus sequences have been detected in *P. major*, *P. ariasi*, *P. perniciosus*, *P. longicuspis*, *P. perfiliewi*, *P. neglectus*, and *Sergentomyia minuta*, implicating virus replication in a variety of sandfly species. Novel virus strains with unexplored clinical significance have also been characterized in sandflies.[18–27,49,50]

The sandfly acquires the virus as a result of biting an infected person any time from 48 hours before until 24 hours after the onset of fever. Following transmission, the virus requires an incubation period of 7–10 days, after which the sandfly remains infected for life. Many of the phleboviruses are maintained in their arthropod vectors by vertical (transovarial) transmission and that vertebrate hosts play little or no role in the basic virus maintenance cycle. This allows phleboviruses to persist during periods when adult vectors are absent or when susceptible vertebrate hosts are unavailable. Although different vertebrate species have been implicated in the SFSV life cycle, no confirmed reservoirs have been identified so far.[1,2,33,47,50,51]

Phlebotomine sandflies are present in the warm zones of Asia, Africa, Australia, southern Europe, and the Americas.[33,52] They are typically found in the moist subtropical countries of the Eastern Hemisphere between latitudes 20–45° North, particularly around the Mediterranean Sea, in the Middle East, and in parts of India. Their northward distribution reaches around the latitude of 50° North in southwest Canada and in northern France and Mongolia.[53,54] Although the southernmost distribution of sandflies

extends to the latitude of 40° South, they are absent from New Zealand and the Pacific islands. The altitude range of sandfly breeding extends from 0 to 3300 meters.[55]

The epidemiological pattern of sandfly fever directly reflects the life cycle of Phlebotomine vectors. Cases usually begin to appear in April and gradually build to a peak in September. The disease may break out in epidemic form during the summer season following sandfly breeding. The factor currently known to limit the spread of sandfly fever is the distribution areas of potential vectors. The disease can emerge and/or reemerge in any geographical region, given that the sandfly vectors are active.[1,33]

5. WHICH FACTORS ARE INVOLVED IN DISEASE PATHOGENESIS? WHAT ARE THE PATHOGENIC MECHANISMS?

Since phleboviruses causing sandfly fever are not associated with human death or severe morbidity, no autopsy reports are available and pathological or histological changes in the affected individuals are not known. An adequate animal model is also lacking to study pathogenesis of the Old World sandfly fever phleboviruses, with the exception of TOSV.[56] Clinical or field isolates demonstrate poor infectivity for various laboratory animals including hamsters, mice, rats, rabbits, and guinea pigs.[7–36] No evidence of disease has been obtained with SFSV after inoculating various non-human primates, and febrile disease can only be induced in rhesus monkeys after intracerebral inoculation.[57] Adult female Syrian golden hamsters subcutaneously inoculated with Punta Toro virus develop a fulminant fatal illness characterized by hepatic and splenic necrosis and interstitial pneumonitis.[58]

The seroprevalence data from endemic countries indicate frequent virus exposure and inapparent or subclinical infections in indigenous populations. Viral replication is successfully controlled by the immune response, and specific IgM antibodies become detectable in blood during the first week after disease onset. The immunity to sandfly fever phleboviruses is type specific. Neutralizing antibodies produced during previous exposure are sufficient to suppress the occurrence of symptoms upon rechallenge with the homologous agent but do not confer cross-immunity, thus the person remains susceptible to infections with other phlebovirus serotypes.[2,34,59]

6. WHAT ARE THE CLINICAL MANIFESTATIONS?

The clinical presentation of sandfly fever due to infections with SFSV and SFNV are identical. After an incubation period of 3–6 days, the disease onset is characterized by the fever (38.8–40.3°C; 102–104.5°F), headache, retroorbital pain, photophobia, generalized aching, malaise, and chills. The face can be suffused, with injection of the conjunctivas and scleras. Photophobia may

be present and can be accompanied by intense ocular pain on movement of the eyes. A faint pink erythema may be observed over the shoulders and thorax. Sandfly bites may also be recognized on the skin. Although lymphadenopathy and hepatosplenomegaly are uncommon, the spleen may be palpable in a small percentage of patients. Abdominal pain, discomfort, diarrhea, or constipation may also occur. The duration of fever is 2–4 days in 85% of cases, but may extend to 11 days. During the first day of fever, the pulse may be accelerated. Rarely, a second febrile episode can be observed. Leukopoenia is present in most cases at admission to the hospital and the lowest counts are recorded in the immediate post-febrile period. Following the febrile stage, there is fatigue and weakness, accompanied by slow pulse and frequently subnormal blood pressure. Convalescence may require a few days to several weeks and can be severely incapacitating. The prognosis is favorable without any complications or sequelae and no mortality that can be directly attributed to sandfly fever has ever been recorded.[34,59]

In experimentally-induced SFSV infections in volunteer young adults, the incubation period ranged from 50 hours to 9 days, with an average of 42–44 hours after intravenous inoculation. The onset was sudden with fever, chills, frontal headache, pain in the eyes, photophobia, backache, arthralgia, nuchal stiffness, loss of appetite, vomiting, alteration of taste, sore throat, and epistaxis. Not all patients had all symptoms. Some had giddiness and weakness, especially during recovery. Fever lasted 2–4 days. Bradycardia was noted toward the end of the febrile period, and into convalescence. Rash did not occur, although erythema of the face and conjunctival injection was common. Leukopenia was noted on the second day of fever with relative lymphopenia and neutropenia. The lymphocytes recovered more rapidly than neutrophils. The urine, liver function tests, and cerebrospinal fluid were normal. Five percent had recurrence of fever 5–7 days after initial recovery.[7,34,59,60]

In addition to the symptoms of sandfly fever, SFTV infections frequently present with aggravated gastrointestinal symptoms, elevation of hepatic enzymes, creatine kinase, and thrombocytopenia. Increased levels of alkaline phosphatase and gamma-glutamyl transpeptidase may also be noted.[3,61] Similar clinical and laboratory findings have also been documented for SFCV.[14] The post-infectious asthenia syndrome is well characterized in patients recovering from SFTV infections.[61]

In contrast to TOSV, SFSV and SFNV infections have generally not been associated with neurological manifestations and symptoms involving the central nervous system are rare or absent in the majority of the sandfly fever infections. However, cases of meningitis/meningoencephalitis due to SFSV and/or related viruses and probable central nervous system invasion of certain strains have been recognized.[1,8,27,62] Mechanisms, underlying conditions, or risk factors for central nervous system infections with phleboviruses other than TOSV require further investigation.

7. HOW DO YOU DIAGNOSE?

The diagnosis of sandfly fever is frequently based on clinical symptoms in endemic regions and laboratory confirmation is usually sought in specific incidents, travel-related/imported infections, or outbreaks. Routine laboratory examinations may reveal leukopenia, lymphopenia, neutropenia, or thrombocytopenia, elevation of hepatic enzymes, creatine kinase, alkaline phosphatase, and/or gamma-glutamyl transpeptidase, which are transient and may be pronounced, mild, or absent depending on the phase of the disease.

Serum or plasma is usually sufficient for definitive diagnostic testing for sandfly fever unless there are signs/symptoms of central nervous system involvement, when cerebrospinal fluid samples must also be submitted. Specific diagnosis can be achieved via direct viral detection or via the demonstration of virus-specific immunoglobulins.[2] Direct viral diagnosis can be performed via virus isolation on cell cultures or via viral RNA detection. Although SFSV and SFNV can be propagated in Vero, BHK-21, and LLC-MK2 cells, virus isolation is rarely attempted for diagnosis, has lower sensitivity compared to RNA detection, and is performed only in specialized laboratories.[2,63] Reverse transcription polymerase chain reaction (RT-PCR) techniques are commonly employed for viral RNA detection in clinical samples. RT-PCR applications involving various primer/probe sequences and detection methods for SFSV, TOSV, and other phleboviruses have been described.[64–67] However, since viremia is low and transient in the majority of the cases, direct methods for virus detection can only be effective during the early stages of infection (1–3 days after onset) and prior to seroconversion.[2,34,59,60]

Diagnosis via the demonstration of specific immunoglobulins is usually performed in sandfly fever. Complement fixation and hemagglutination inhibition methods have been historically used in detecting seroconversion.[36,59,60,68] Solid phase immunoassays such as immunofluorescence assay (IFA), enzyme-linked immunosorbent assay (ELISA), and immunoblot assays have been developed, which enable the detection of IgM or IgG class antibodies.[2,69,70] However, commercial tests for TOSV diagnosis are more common, and assays readily available for sandfly fever phleboviruses are currently few. Commercial or in-house solid phase immunoassays demonstrate variations of sensitivity and specificity due to the detection method and viral antigens employed in the test.[2,70] Cross-reactivity in serological assays is well known among phleboviruses, particularly among antigenically similar isolates belonging to the same genus or serocomplex such as SFNV or SFSV.[3,62,70,71] Viral neutralization test (VNT) remains as the reference method to assess the specificity of the antibody response. Thus, when precise serological characterization of a particular phlebovirus is required, reactive results in ELISA or IFA must be confirmed via VNT incorporating viruses of the same and distinct serocomplexes. Although labor intensive and only established in specialized laboratories, VNT is regarded as the gold standard assay for specificity and for calculating the antibody titer.[2,28,71,72]

8. HOW DO YOU DIFFERENTIATE THIS DISEASE FROM SIMILAR ENTITIES?

Sandfly fever presents with signs and symptoms of an undifferentiated febrile illness and a similar clinical picture can be observed in many infectious and non-infectious diseases. Since sandfly fever is non-fatal and frequently self-limited with or without supportive treatment, not all affected individuals seek medical assistance and a significant portion of symptomatic infections may be undocumented. Epidemiological data are of particular significance in the diagnosis due to the seasonality and geographical distribution of vector activity. In suspected cases, a thorough medical history should be obtained, including recent travels to the endemic areas and insect bites. Travel-related sandfly fever is usually reported during or after visits to the Mediterranean area and during summer months when the sandflies are active.[3,5,8–10,45] In the physical examination, sandfly, mosquito, or tick bites should be sought, suggesting exposure to arthropod vectors. The differential diagnosis should include other vector-borne diseases depending on the travel history, viral and parasitic infections, mild forms of hepatitis, and hematologic diseases. Although rarely observed in sandfly fever, symptoms suggesting central nervous system involvement should be recognized to differentiate and diagnose TOSV infections with similar epidemiological features and other viral encephalitides for timely intervention and proper management.

9. WHAT IS THE THERAPEUTIC APPROACH?

The treatment of phlebovirus infections is mainly symptomatic and hepatotoxic medications as well as aspirin and other non-steroidal anti-inflammatory drugs such as ibuprofen and ketoprofen are not recommended.[1] Animal models have demonstrated that viruses in the *Bunyaviridae* family are inhibited with interferon and interferon inducers. Moreover, bunyaviruses are generally considered sensitive to ribavirin, as evident in currently suggested therapies for Crimean-Congo hemorhaggic fever.[73] Ribavirin administered as an oral dose of 400 mg every 8 hours, started 1 day before experimental infection for 8 days, has been demonstrated to protect volunteers against SFSV challenge.[74] Ribavirin combined with human recombinant interferon-α was proposed as a treatment for SFSV infection, based on *in vitro* efficacy.[75] In addition to ribavirin, SFSV replication could be suppressed *in vitro* by 6-azauridine, interferon-α, glycyrrhizin, suramin sodium, dextran sulfate, and pentosan polysulphate.[75] Phleboviruses, as well as other members of the *Bunyaviridae* family, were observed to be inhibited by interferon-induced MxA protein *in vitro*.[76] Selenazole, a nucleoside carboxamide, was also effective in suppressing the replication of SFSV in cell cultures.[77] Favipiravir, a pyrazine derivative, has been found to be effective against SFNV *in vitro* and demonstrated higher therapeutic indexes compared to

ribavirin in hamsters with Punta Toro virus infections, suggesting these compounds as promising antivirals.[78,79]

Although the disease course in sandfly fever is frequently benign and an evidence-based drug therapy is not possible due to the lack of randomized human trials, interferon−ribavirin combination might be considered as an emergency therapy in unique circumstances, such as a worsening disease in critically ill patients.[2]

10. WHAT ARE THE PREVENTIVE AND INFECTION CONTROL MEASURES?

Currently, no vaccines against sandfly-borne phleboviruses are available. Since the immunity is serotype specific and do not confer cross-protection with other virus serotypes, an effective vaccine must be able to mount an immune response against all relevant phlebovirus strains capable of producing clinical disease.[59] The prevention of sandfly fever relies on the control of vector proliferation in areas where people are likely to be exposed and use of individual protective measures[1]. General personal protection measures against sandfly bites include wearing of long sleeves and pants, usage of impregnated bednets, avoiding outdoor activities in the evening, and using insect repellents. Sandflies breed in vegetation within a few hundred feet of human habitations. Since these breeding sites are hard to discover, larvicidal control is impractical and control measures should be aimed at interrupting contact between humans and adult female sandflies.[80] The bloodsucking females feed only from sunset to sunrise and generally remain close to the ground. Ordinary mosquito netting and screening are of limited use, because unfed female sandflies can pass through 18-mesh squares.[33,80] Insecticide-impregnated or non-impregnated bednets with small mesh are effective in preventing sandfly bites.[81,82] Travelers to endemic countries should be encouraged to employ personal protection, especially during seasons when sandflies are active.

REFERENCES

1. Depaquit J, Grandadam M, Fouque F, et al. Arthropod-borne viruses transmitted by Phlebotomine sandflies in Europe: a review. *Euro Surveill* 2010;**11**(15):19507.
2. Dionisio D, Esperti F, Vivarelli A, Valassina M. Epidemiological, clinical and laboratory aspects of Sandfly Fever. *Curr Opin Infect Dis* 2003;**16**:383−8.
3. Carhan A, Uyar Y, Ozkaya E, et al. Characterization of a new phlebovirus related to sandfly fever sicilian virus isolated during a sandfly fever epidemic in Turkey. *J Clin Virol* 2010;**48**:264−9.
4. Ellis SB, Appenzeller G, Lee H, et al. Outbreak of sandfly fever in central Iraq, September 2007. *Mil Med* 2008;**173**:949−53.
5. Niklasson B, Eitrem R. Sandfly fever among Swedish UN troops in Cyprus. *Lancet* 1985;**1**:1212.

6. Eitrem R, Vene S, Niklasson B. Incidence of sandfly fever among Swedish United Nations soldiers on Cyprus during 1985. *Am J Trop Med Hyg* 1990;**43**:207–11.
7. Sabin AB. Experimental studies on Phlebotomus (pappataci, sandfly) fever during World War II. *Arch Gesamte Virusforsch* 1951;**4**:367–410.
8. Becker M, Zielen S, Schwartz TF, et al. Pappataci fever. *Klin Padiatr* 1997;**209**:377–9.
9. Schultze D, Korte W, Rafeiner P, Niedrig M. First report of sandfly fever virus infection imported from Malta into Switzerland, October 2011. *Euro Surveill* 2012;**17**:20209.
10. Schwarz TF, Jager G, Gilch S, Pauli C. Serosurvey and laboratory diagnosis of imported sandfly fever virus, serotype Toscana, infection in Germany. *Epidemiol Infect* 1995;**114**:501–10.
11. Plyusnin A, Beaty BJ, Elliott RM, Goldbach R, Kormelink R, Lundkvist A, et al. Virus Taxonomy: Classification and Nomenclature of Viruses. In: King AMQ, Lefkowitz E, Adams MJ, Carstens EB, editors. *Ninth Report of the International Committee on Taxonomy of Viruses*. San Diego: Elsevier; 2011. p. 693–709.
12. Elliott RM, Schmaljohn CS, Collett MS. Bunyaviridae genome structure and replication. *Curr Top Microbiol Immunol* 1991;**169**:91–141.
13. Suzich JA, Kakach LT, Collett MS. Expression strategy of a phlebovirus: biogenesis of proteins from the Rift Valley fever virus M segment. *J Virol* 1990;**64**:1549–55.
14. Papa A, Konstantinou GV, Pavlidou V, Antoniadis A. Sandfly fever virus outbreak in Cyprus. *Clin Microbiol Infect* 2006;**12**:192–4.
15. Charel RN, Gallian P, Navarro-Mari JM, Nicoletti L, et al. Emergence of Toscana virus in Europe. *Emerg Infect Dis* 2005;**11**:1657–63.
16. Hemmersbach-Miller M, Parola P, Charrel RN, Paul Durand J, Brouqui P. Sandfly fever due to Toscana virus: an emerging infection in southern France. *Eur J Intern Med* 2004;**15**:316–7.
17. Portolani M, Sabbatini AM, Beretti F, Gennari W, Tamassia MG, Pecorari M. Symptomatic infections by toscana virus in the Modena province in the triennium 1999–2001. *New Microbiol* 2002;**25**:485–8.
18. Tesh R, Saidi S, Javadian E, Nadim A. Studies on the epidemiology of sandfly fever in Iran. I. Virus isolates obtained from Phlebotomus. *Am J Trop Med Hyg* 1977;**26**:282–7.
19. Rodhain F, Madululeblond G, Hannoun C, Tesh RB. Corfou virus—a new phlebovirus isolated from phlebotomine sandflies in Greece. *Ann Inst Pasteur Virol* 1985;**136**:161–6.
20. Charel RN, Moureau G, Temmam S, et al. Massilia virus, a novel phlebovirus (*Bunyaviridae*) isolated from sandflies in the Mediterranean. *Vector Borne Zoonotic Dis* 2009;**9**:519–30.
21. Collao X, Palacios G, de Ory F, et al. Granada virus: a natural phlebovirus reassortant of the sandfly fever Naples serocomplex with low prevalence in humans. *Am J Trop Med Hyg* 2010;**83**:760–5.
22. Zhioua E, Moureau G, Chelbi I, et al. Punique virus, a novel phlebovirus, related to sandfly fever Naples virus, isolated from sandflies collected in Tunisia. *J Gen Virol* 2010;**91**:1275–83.
23. Papa A, Velo E, Bino S. A novel phlebovirus in Albanian sandflies. *Clin Microbiol Infect* 2011;**17**:585–7.
24. Moureau G, Bichaud L, Salez N, et al. Molecular and serological evidence for the presence of novel phleboviruses in sandflies from northern Algeria. *Open Virol J* 2010;**4**:15–21.
25. Peyrefitte CN, Grandadam M, Bessaud M, et al. Diversity of Phlebotomus perniciosus in Provence, Southeastern France: Detection of two putative new phlebovirus sequences. *Vector Borne Zoonotic Dis* 2013;**13**:630–6.

26. Verani P, Ciufolini MG, et al. Ecology of viruses isolated from sand flies in Italy and characterized of a new Phlebovirus (Arabia virus). *Am J Trop Med Hyg* 1988;**38**:433–9.
27. Anagnostou V, Pardalos G, Athanasiou-Metaxa M, Papa A. Novel phlebovirus in febrile child, Greece. *Emerg Infect Dis* 2011;**17**:940–1.
28. Tesh RB, Saidi S, Gajdamovic SJ, Rodhain F, Vesenjak-Hirjan J. Serological studies on the epidemiology of sandfly fever in the Old World. *Bull World Health Organ* 1976;**54**:663–74.
29. Navarro-Mari JM, Gomez-Camarasa C, Perez-Ruiz M, Sanbonmatsu-Gamez S, Pedrosa-Corral I, Jimenez-Valera M. Clinicoepidemiologic study of human infection by Granada virus, a new Phlebovirus within the Sandfly fever Naples serocomplex. *Am J Trop Med Hyg* 2013;**88**:1003–6.
30. Calisher CH, McLean RG, Smith GC, Szmyd DM, Muth DJ, Lazuick JS. Rio Grande—a new phlebotomus fever group virus from south Texas. *Am J Trop Med Hyg* 1977;**26**:997–1002.
31. Tesh RB, Chaniotis BN, Peralta PH, Johnson KM. Ecology of viruses isolated from Panamanian phlebotomine sandflies. *Am J Trop Med Hyg* 1974;**23**:258–69.
32. Trapp EE, Andrade AH, Shope RE. Itaporanga a newly recognized arbovirus from Sao Paulo state, Brazil. *Proc Soc Exp Biol Med* 1965;**118**:421–2.
33. Maroli M, Feliciangeli MD, Bichaud L, Charrel RN, Gradoni L. Phlebotomine sandflies and the spreading of leishmaniases and other diseases of public health concern. *Med Vet Entomol* 2013;**27**:123–47.
34. Ergunay K, Whitehouse C, Ozkul A. Current status of human arboviral infections in Turkey. *Vector Borne Zoonotic Dis* 2011;**11**:731–41.
35. Sabin AB, Philip CB, Paul JR. Phlebotomus (pappataci or sandfly) fever. A disease of military importance: summary of existing knowledge and preliminary report of original investigations. *JAMA* 1944;**125**:603–6.
36. Sabin AB. Recent advances in our knowledge of dengue and sandfly fever. *Am J Trop Med Hyg* 1955;**4**:198–207.
37. Hertig M, Sabin AB. Sandfly fever (pappataci, phlebotomus, three-day fever). In: Hoff EC, editor. *Preventive Medicine in World War II*, Vol. 7. Washington DC: Communicable Diseases Office of the Surgeon General, U.S. Department of the Army; 1964. p. 109–74.
38. Javadian E, Tesh R, Saidi S, Nadim A. Studies on the epidemiology of sandfly fever in Iran. Host-feeding patterns of Phlebotomus papatasi in an endemic area of the disease. *Am J Trop Med Hyg* 1977;**26**:294–8.
39. Gaidamovich SI, Obukhova VR, Sveshnikova NA, Cherednichenko IN, Kostiukov MA. Natural foci of viruses borne by Phlebotomus papatasi in the U.S.S.R. according to a serologic study of the population. *Vopr Virusol* 1978;**5**:556–60.
40. Eitrem R, Stylianou M, Niklasson B. High prevalence rates of antibody to three sandfly fever viruses (Sicilian, Naples, and Toscana) among Cypriots. *Epidemiol Infect* 1991;**107**:685–91.
41. Goverdhan MK, Dhanda V, Modi GB, et al. Isolation of Phlebotomus (sandfly) fever virus from sandflies and humans during the same season in Aurangabad district, Maharashtra state, India. *Indian J Med Res* 1976;**64**:57–63.
42. Filipe AR. Serological survey for antibodies to arboviruses in the human population of Portugal. *Trans R Soc Trop Med Hyg* 1974;**68**:311–4.
43. Gaidamovich SY, Kurakhmedova SA, Melnikova EE. Aetiology of Phlebotomus fever in ashkhabad studied in retrospect. *Acta Virol* 1974;**18**:508–11.
44. Tesh RB, Papaevangelou G. Effect of insecticide spraying for malaria control on the incidence of sandfly fever in Athens, Greece. *Am J Trop Med Hyg* 1977;**261**:163–6.

45. Guler S, Guler E, Caglayik DY, et al. A sandfly fever virus outbreak in the East Mediterranean region of Turkey. *Int J Infect Dis* 2012;**16**:e244–6.
46. Eitrem R, Niklasson B, Weiland O. Sandfly fever among Swedish tourists. *Scand J Infect Dis* 1991;**23**:451–7.
47. Tesh RB, Modi GB. Studies on the biology of phleboviruses in sand flies (Diptera: Psychodidae). Experimental infection of the vector. *Am J Trop Med Hyg* 1984;**33**:1007–16.
48. Verani P, Lopes MC, Nicoletti L, Balducci M. Studies on Phlebotomus-transmitted viruses in Italy: I. Isolation and characterization of a Sandfly fever Naples-like virus. Arboviruses in the Mediterranean Countries. *Zentralbl Bakteriol* 1980;(Suppl 9):195–201.
49. Izri A, Temmam S, Moureau G, Hamrioui B, de Lamballerie X, et al. Sandfly fever Sicilian virus, Algeria. *Emerg Infect Dis* 2008;**14**:795–7.
50. Ergunay K, Erisoz Kasap O, Kocak Tufan Z, Turan MH, Ozkul A, Alten B. Molecular evidence indicates that Phlebotomus major sensu lato (Diptera: Psychodidae) is the vector species of the recently-identified sandfly fever Sicilian virus variant: sandfly fever turkey virus. *Vector Borne Zoonotic Dis* 2012;**12**:690–8.
51. Tesh RB, Modi GB. Maintenance of Toscana virus in *Phlebotomus perniciosus* by vertical transmission. *Am J Trop Med Hyg* 1987;**36**:189–93.
52. Killick-Kendrick R. The biology and control of phlebotomine sandflies. *Clin Dermatol* 1999;**17**:279–89.
53. Young DG, Perkins PV. Phlebotomine sandflies of North America (Diptera: Psychodidae). *J Am Mosq Control Assoc* 1984;**44**:263–304.
54. Lewis DJ. A taxonomic review of the genus Phlebotomus (Diptera: Psychodidae). *Bull Brit Mus (Nat Hist) Entomology Series* 1982;**45**:121–203.
55. Lane RP. Sandflies (Phlebotominae). In: Lane RP, Crosskey RM, editors. *Medical Insects and Arachnids*. London: Chapman & Hall; 1993. p. 78–119.
56. Cusi MG, Gori Savellini G, Terrosi C. Development of a mouse model for the study of Toscana virus pathogenesis. *Virology* 2005;**333**:66–73.
57. McClain DJ, Summers PL, Pratt WD, Davis KJ, Jennings GB. Experimental infection of nonhuman primates with sandfly fever virus. *Am J Trop Med Hyg* 1997;**56**:554–60.
58. Fisher AF, Tesh RB, Tonry J, Guzman H, Liu D, Xiao SY. Induction of severe disease in hamsters by two sandfly fever group viruses, Punta toro and Gabek Forest (Phlebovirus, Bunyaviridae), similar to that caused by Rift Valley fever virus. *Am J Trop Med Hyg* 2003;**69**:269–76.
59. Bartelloni PJ, Tesh RB. Clinical and serologic responses of volunteers infected with phlebotomus fever virus (Sicilian type). *Am J Trop Med Hyg* 1976;**25**:456–62.
60. Beisel WR, Herman YF, Sauberlich HE, Herman RH, Bartelloni PJ, Canham JE. Experimentally induced sandfly fever and vitamin metabolism in man. *Am J Clin Nutr* 1972;**25**:1165–73.
61. Kocak Tufan Z, Weidmann M, Bulut C, et al. Clinical and laboratory findings of a sandfly fever Turkey Virus outbreak in Ankara. *J Infect* 2011;**63**:375–81.
62. Ergunay K, Ismayilova V, Colpak IA, Kansu T, Us D. A case of central nervous system infection due to a novel Sandfly Fever Virus (SFV) variant: Sandfly Fever Turkey Virus (SFTV). *J Clin Virol* 2012;**54**:79–82.
63. Karabatos N, editor. *International Catalogue of Arboviruses including Certain other Viruses of Vertebrates*. 3rd ed. San Antonio, Texas, USA: American Society of Tropical Medicine and Hygiene; 1985.
64. Sanchez-Seco MP, Echevarria JM, Hernandez L, et al. Detection and identification of Toscana and other phleboviruses by RT-nested-PCR assays with degenerated primers. *J Med Virol* 2003;**71**:140–9.

65. Weidmann M, Sanchez-Seco MP, Sall AA, et al. Rapid detection of important human pathogenic Phleboviruses. *J Clin Virol* 2008;**41**:138–42.
66. Hasib L, Dilcher M, Hufert F, Meyer-König U, Weidmann M. Development of a flow-through microarray based reverse transcriptase multiplex ligation-dependent probe amplification assay for the detection of European Bunyaviruses. *Mol Biotechnol* 2011;**49**:176–86.
67. Ibrahim SM, Aitichou M, Hardick J, Blow J, O'Guinn ML, Schmaljohn C. Detection of Crimean-Congo hemorrhagic fever, Hanta, and sandfly fever viruses by real-time RT-PCR. *Methods Mol Biol* 2011;**665**:357–68.
68. Clarke DH, Cassals J. Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses. *Am J Trop Med Hyg* 1958;**7**:561–73.
69. Eitrem R, Vene S, Niklasson B. ELISA for detection of IgM and IgG antibodies to sandfly fever Sicilian virus. *Res Virol* 1991;**142**:387–94.
70. Ergünay K, Litzba N, Lo MM, et al. Performance of various commercial assays for the detection of Toscana virus antibodies. *Vector Borne Zoonotic Dis* 2011;**11**:781–7.
71. Tesh RB, Peralta PH, Shope RE, Chaniotis BN, Johnson KM. Antigenic relationships among phlebotomus fever group arboviruses and their implication for the epidemiology of sandfly fever. *Am J Trop Med Hyg* 1975;**24**:135–44.
72. Calisher CH, Beaty BJ, Chandler LJ. Arboviruses. Revised and Expanded In: Lenette EH, Smith TF, editors. *Laboratory Diagnosis of Viral Infections*. 3rd ed. New York: Marcel Dekker; 1999. p. 305–32.
73. Sidwell RW, Smee DF. Viruses of the Bunya- and Togaviridae families: potential as bioterrorism agents and means of control. *Antiviral Res* 2003;**57**:101–11.
74. Huggins JW. Prospects for treatment of viral hemorrhagic fevers with ribavirin, a broad-spectrum antiviral drug. *Rev Infect Dis* 1989;**11**(Suppl 4):S750–61.
75. Crance JM, Gratier D, Guimet J, Jouan A. Inhibition of sandfly fever Sicilian virus (Phlebovirus) replication in vitro by antiviral compounds. *Res Virol* 1997;**148**:353–65.
76. Frese M, Kochs G, Feldmann H, et al. Inhibition of bunyaviruses, phleboviruses, and hantaviruses by human MxA protein. *J Virol* 1996;**70**:915–23.
77. Kirsi JJ, North JA, McKernan PA, et al. Broad-spectrum antiviral activity of 2-beta-D-ribofuranosylselenazole-4-carboxamide, a new antiviral agent. *Antimicrob Agents Chemother* 1983;**24**:353–61.
78. Gowen BB, Wong MH, Jung KH, et al. In vitro and in vivo activities of T-705 against arenavirus and bunyavirus infections. *Antimicrob Agents Chemother* 2007;**51**:3168–76.
79. Gowen BB, Wong MH, Jung KH, Smee DF, Morrey JD, Furuta Y. Efficacy of favipiravir (T-705) and T-1106 pyrazine derivatives in phlebovirus disease models. *Antiviral Res* 2010;**86**:121–7.
80. Faiman R, Cuno R, Warburg A. Control of phlebotomine sand flies with vertical fine-mesh nets. *J Med Entomol* 2009;**46**:820–31.
81. Jalouk L, Al Ahmed M, Gradoni L, Maroli M. Insecticide-treated bednets to prevent anthroponotic cutaneous leishmaniasis in Aleppo Governate, Syria: results from two trials. *Trans R Soc Trop Med Hyg* 2007;**101**:360–7.
82. Alten B, Caglar SS, Simsek FM, Kaynas S. Evaluation of protective efficacy of K-OTAB impregnated bednets for cutaneous leishmaniasis control in Southeast AnatoliaTurkey. *J Vector Ecol* 2003;**28**:97–107.