Sandfly fever virus activity in central/northern Anatolia, Turkey: first report of Toscana virus infections

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Abstract

Sandfly fever viruses (SFVs) cause febrile diseases as well as aseptic meningitis/encephalitis and include serotypes sandfly fever Sicilian virus (SFSV), sandfly fever Naples virus (SFNV) and Toscana virus (TOSV). Infections are endemic in the Mediterranean basin and data on SFV activity in Turkey are limited. In this study, sera from 1533 blood donors from the Ankara, Konya, Eskisehir and Zonguldak provinces of Turkey were evaluated for SFV exposure by indirect immunofluorescence test (IIFT) and confirmed by virus neutralization test (VNT). One hundred and two patients with central nervous system (CNS) infections of unknown aetiology were also tested via IIFT and real-time reverse-transcription PCR for SFV/TOSV. Rate of overall IgG reactivity in IIFT was 32.9% (505/1533) among blood donors. TOSV exposure was confirmed by VNT in all study regions. Exposure to the recently-identified serotype sandfly fever Turkish virus, as evaluated by VNT, was revealed in Konya and Ankara. SFNV exposure was identified in Konya and SFSV was observed to be present in all regions except Zonguldak. TOSV RNA was detected in 15.7% (16/102) and was accompanied by TOSV IgM in 25% (4/16) of the patients. Partial L and S sequences suggested that TOSV circulating in Turkey can be grouped into TOSV genotype A strains. Exposure to TOSV and other SFV serotypes was revealed in blood donors and CNS infections by TOSV were identified for the first time in Turkey. Infections are observed to be endemic in central Anatolia and should be considered as aetiologic agents in cases/outbreaks of fever and meningoencephalitis.

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Introduction

Sandfly fever viruses (SFV) are arthropod-borne viruses transmitted to humans by the bite of phlebotomine sandflies of the *Psychodidae* family [1]. They are single stranded RNA viruses, classified in the Phlebovirus genus of the *Bunyaviridae* and comprise three major serotypes; sandfly fever Sicilian virus (SFSV), sandfly fever Naples virus (SFNV) and Toscana virus (TOSV) [1,2]. SFNV and SFSV serotypes cause a self-limited febrile condition known as phlebotomus, papatacci or sandfly fever [1,2]. Sandfly fever Cyprus virus (SFCV), a variant of the SFSV serotype, is also responsible for acute febrile diseases [3]. TOSV displays a distinct neurotropism and causes aseptic meningoencephalitis in infected individuals [4–6]. In endemic regions, such as countries in the Mediterranean basin, sandfly fever is common, as revealed by serosurveys, and TOSV infections account for a high proportion of human aseptic meningitis during the summer months [7–12]. Information about SFV activity in Turkey, as a country in the endemic region, is limited [13,14]. In sera collected in 1955, seroprevalence rates of 22% and 62% for SFSV and SFNV, respectively, were reported in 50 adults from the Mediterranean province of Antalya [13]. In the Aegean region, seroprevalence rates of 0.84% and 13.9% were observed [14]. Ozbel *et al.* also identified SFNV and TOSV activity in two towns (Akbuk and Olukbasi) in the same region (3rd Balkan Conference of Microbiology Proceedings; 2003, pp. 152–155). A recent report indicates that outbreaks of febrile diseases without central nervous system (CNS) symptoms associated with sandfly bites in Izmir (Aegean coast), Adana (Mediterranean coast) and Ankara (Central Anatolia) provinces were attributable to SFSV and a

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novel variant of SFSV, provisionally named sandfly fever Turkish virus (SFTV), was identified [15]. Nevertheless, clinical cases of TOSV have not yet been described from Turkey and the seroepidemiology in the regions affected by the outbreaks is not known. Therefore, we aimed to assess SFV exposure in healthy blood donors from central/northern Anatolia and the role of TOSV and other SFVs in CNS infections of unidentified aetiology from central Anatolia.

Materials and Methods

Samples from blood donors

A total of 1533 sera, collected after informed consent between January and April 2009 from volunteer blood donors at four major branches of the Turkish Red Crescent Middle Anatolia Regional Blood Center (in Ankara, Konya, Eskişehir and Zonguldak, cities of Turkey), were included. The distribution of sera according to the sampling locations was: Ankara, 884; Konya, 388; Eskisehir, 143; and Zonguldak, 118; this had been determined according to the population in each area (Fig. 1). Ankara is the capital and second most densely populated city in Turkey. Mean age of the study group was 40.7 (range, 23–64; SD, 13.16), with a female percentage of 12.1%. All participants filled out a questionnaire to reveal the presence of risk factors for vector-borne infections. All sera were transported on dry ice and stored in aliquots at −20°C.

Samples from patients

Sera obtained from 102 adult patients with the preliminary diagnosis of aseptic meningoencephalitis, collected between April and October 2009, were evaluated retrospectively. The majority of the patients (98/102; 96%) resided in central Anatolia. The samples, obtained within 2–4 days after the onset of symptoms, were stored in aliquots at −20 and −80°C for future analysis at the Hacettepe University Hospital Central Laboratory. Cultures for *Mycobacterium tuberculosis* and other causes of bacterial/fungal meningitis, as well as polymerase chain reaction (PCR) assays for herpes viruses, were negative. Clinical history and laboratory data of the patients were retrieved from medical records.

The study was performed after approval from Hacettepe University Medical Ethics Committee and according to the blood donation official guidelines of the Turkish Red Crescent Society as approved by the Turkish Ministry of Health.

Detection and confirmation of SFV exposure in blood donors

Sera from blood donors were evaluated for SFV IgG by a commercial indirect immunofluorescence test (IIFT) (sandfly fever virus IgG Mosaic I; EuroImmun, Luebeck, Germany) that allows simultaneous detection of four viral serotypes (SFSV, SFNV, TOSV and SFCV), performed as indicated by the manufacturer. The virus neutralization tests (VNTs) were performed on Vero B4 cells, using standard methods [16,17], and described virus strains (SFSV Oct-85 Sabin, SFNV Oct-85 Sabin and TOSV ISS.Phl.3) [18] and the novel SFTV isolate [15]. Briefly, the assays were carried out with serial serum dilutions of 1/10–1/320 and virus strains were used at a final concentration of 100 $TCID_{50}$. Readout was performed after 72 h of incubation at 37°C and positivity was determined by the lack of cytopathogenic effect above a serum dilution of 1/20.

Evaluation of patient samples

Sera from patients were tested for SFV IgM and IgG by IIFT (sandfly fever virus IgG and IgM Mosaic I) as explained above and for viral RNA by PCR. RNA was extracted using the Qiamp viral RNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions and subjected to quantitative real-time RT-PCR targeting the S segment of SFSV, SFNV and TOSV as previously described [18,19]. For the recently-identified SFTV, forward (5′-TCTGAGAACT GAGCTACAAGTGTTTATTAT-3′) and reverse (5′-TTCCC ATCTCTCTTCTGAAGAGTG-3′) primers and TaqMan probe (5′-FAMAGGTCATAGACAGTATCATGAGAATTGC TAGGTG-TAMRA-3′) were employed for the real-time RT-PCR with the temperature profile: RT at 63°C 3 min, activation at 95°C 30 s, and 45 cycles of two-step PCR at 95°C 5 s, 60°C 15 s [15]. IgM and/or RNA positive sera were inoculated onto monolayers of Vero B4 cells for virus isolation. S segment PCR positive samples and inoculated culture supernatants at day 7 were further subjected to amplification via generic phlebovirus L segment primers (NPhlebo1+, NPhlebo1−, NPhlebo2+ and ATos2−) as described previously [20].

Results

SFV exposure in blood donors

A total of 505 of 1533 samples (32.9%) were reactive in the IgG IIFT (Table 1). Samples reactive against a single serotype in the IIFT (46 TOSV, 21 SFSV, 12 SFNV, 7 SFCV positives; total, 86) and dual positives that included a reactivity against TOSV (total, 17) were selected for VNT. The most common dual reactivities observed in the IIFT were for SFSV + SFCV and TOSC + SFNV (data not shown). Eight sera were excluded from VNTs due to insufficient amounts. A total of 95 and 74 samples could be evaluated via VNTs for SFSV, SFNV, TOSV and SFTV (Table 1). In the VNTs, exposure to more than one serotype (SFSV + SFTV and/or

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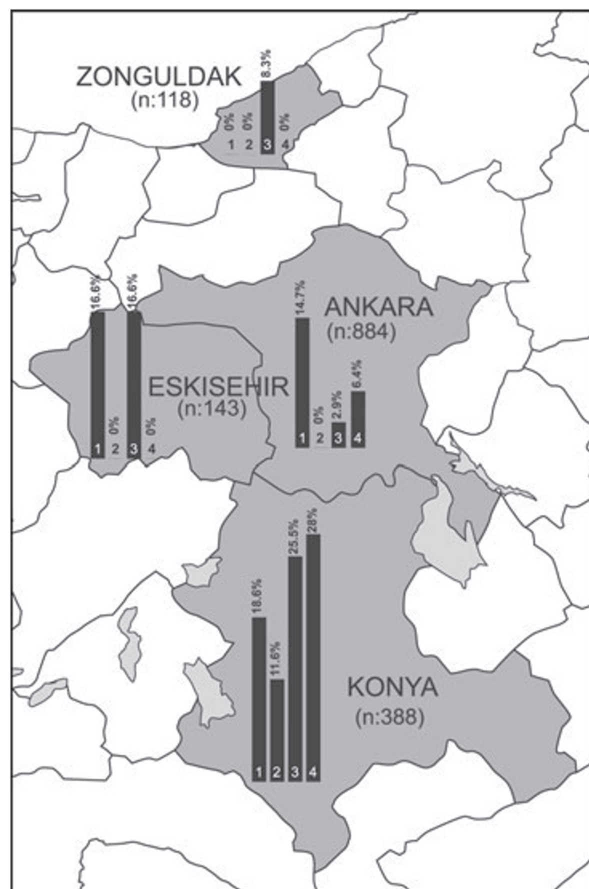

FIG. 1. Map showing the blood donor sampling locations and confirmed SFV exposure rates according to the region. The total number of blood donors evaluated by IIFT between January and April 2009 in each region is indicated in parentheses. The bars represent: 1, sandfly fever Sicilian virus (SFSV); 2, sandfly fever Naples virus (SFNV); 3, Toscana virus (TOSV); 4, sandfly fever Turkish virus (SFTV). The rates were calculated according to the total number of samples evaluated by virus neutralization for each area (see Table 1 and Materials and methods for details).

SFSV + SFTV + SFNV) was confirmed in eight samples from Konya and in five samples from Ankara (total, 13/74; 17.6%). Distribution of IIFT and VNT results are summarized in Table 1 and Fig. 1. The highest VNT titers observed were 1:40 for TOSV, 1:160 for SFNV and 1:320 for SFSV and SFTV.

TOSV in aseptic meningitis/encephalitis cases

TOSV RNA was detected in 16 (16/102, 15.7%) sera and was accompanied by TOSV IgM in four cases (4/16, 25%) (Table 2). TOSV IgM was observed in seven (7/102, 6.9%) sera without detectable RNA. Real-time RT-PCR for SFV serotypes other than TOSV was negative in all samples. Mean and median TOSV loads determined in the cases were 4.39×10^6 and 2.59×10^7 copies/mL, respectively (range, 48 794–2.31×10^8, Table 2). Attempts at viral isolation from PCR and/or IgM reactive cases were not successful.

Patient history and clinical progress could be reviewed in 11 out of 16 RT-PCR positive individuals. All persons had

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TABLE 1. Distribution of IIFT and VNT results according to the study region

	SFV IgG IIFT negative (%)	SFV IgG IIFT positive				VNT confirmed[b] (positive/total, %)			
		Single	Dual[a]	Triple + all positive[a]	Total (%)	SFSV	SFNV	TOSV	SFTV
Ankara (*n* = 884)	701 (79.3)	28	99	56	183 (20.7)	5/34 (14.7)	0/34 (0)	1/34 (2.9)	2/31 (6.4)
Konya (*n* = 388)	132 (34.1)	36	137	83	256 (65.9)	8/43 (18.6)	5/43 (11.6)	11/43 (25.5)	7/25 (28)
Eskisehir (*n* = 143)	112 (78.4)	8	13	10	31 (21.6)	1/6 (16.6)	0/6 (0)	1/6 (16.6)	0/6 (0)
Zonguldak (*n* = 118)	83 (70.4)	14	14	7	35 (29.6)	0/12 (0)	0/12 (0)	1/12 (8.3)	0/12 (0)
Total (*n* = 1533)	1028 (67.1)	86	263	156	505 (32.9)	14/95 (14.7)	5/95 (5.2)	14/95 (14.7)	9/74 (12.1)

SFV, sandfly fever virus; IIFT, indirect immunofluorescence test; VNT, virus neutralization test; SFSV, sandfly fever Sicilian virus; SFNV, sandfly fever Naples virus; TOSV, Toscana virus; SFTV, sandfly fever Turkish virus.
[a]Includes any combination of the four SFV serotypes, SFSV, SFNV, TOSV and SFCV (see text for details).
[b]Ninety-five samples were evaluated for SFSV, SFNV and TOSV; 74 samples were evaluated for SFTV.

TABLE 2. Clinical symptoms and laboratory data of the TOSV real-time RT-PCR positive cases

No.	Age/gender/place of residence	CSF evaluation	Meningitic symptoms[a]	Encephalitic symptoms[b]	Gastrointestinal symptoms[c]	TOSV load[d] (copies/mL)	TOSV IgM[c]
1	35, Male, Ankara	Normal	+	–	–	1.69×10^5	–
2	51, Male, Konya	Pleocytosis, increased protein	+	+	+	8.26×10^6	–
3	19, Female, Ankara	Normal	+	–	–	6.99×10^6	–
4	43, Female, Ankara	n.i.	n.i.	n.i.	n.i.	1.80×10^7	–
5	32, Female, Ankara	Pleocytosis	+	–	–	2.31×10^8	–
6	35, Female, Ankara	Normal	+	–	+	2.35×10^5	–
7	34, Female, Ankara	Increased protein	+	–	–	6.24×10^7	–
8	18, Male, Konya	n.i.	n.i.	n.i.	n.i.	6.58×10^5	–
9	26, Male, Ankara	Pleocytosis, increased protein and glucose	+	–	–	8.84×10^6	+
10	29, Female, Ankara	Pleocytosis	–	+	–	1.94×10^6	+
11	29, Male, Ankara	Normal	+	–	–	3.47×10^6	–
12	26, Female, Ankara	Normal	+	–	–	7.94×10^5	+
13	30, Male, Eskisehir	Increased protein	+	–	–	5.42×10^4	–
14	31, Female, Ankara	n.i.	n.i.	n.i.	n.i.	5.04×10^6	+
15	32, Male, Ankara	n.i.	n.i.	n.i.	n.i.	6.73×10^7	–
16	20, Female, Ankara	n.i.	n.i.	n.i.	n.i.	4.88×10^4	–

CSF, cerebrospinal fluid; TOSV, Toscana virus; n.i., no information.
[a]Includes neck rigidity and/or Kernig signs.
[b]Includes muscle paresis, alterations of consciousness, tremor and/or cranial nerve involvement.
[c]Includes nausea/vomiting (skin rash, lymphadenopathy, hepatic or renal involvement were not observed in any of the cases).
[d]As evaluated in serum.

fever (>37.5°C) and headache, but no symptoms or test results indicating systemic involvement could be demonstrated and all had been discharged without sequelae (Table 2).

The S-segment real-time RT-PCR amplicon of patient 4 and a partial L-segment PCR amplicon of patient 7 were ligated into pCRII plasmid (Invitrogen, Breda, Netherlands) and successfully sequenced. Phylogenetic analyses of these sequences were carried out using the MEGA 3 program by using the Kimura model for nucleotide substitutions and multiple sequence alignment was performed by CLUSTALW2 (Fig. 2a,b) [21].

FIG. 2. Phylogenetic trees reconstructed by the neighbour-joining method from (a) partial S-segment sequence (TGCAGgGACACCAT CACTCTGTCAAGGGTGTCAGCCGCATTTGTTCCGTGGACTGTTCAGGCACTACGTGTCCTGTCAGAATCCCTGCCCGTGTCTGGGACC ACTATGGATGCCATTGCTGGTGTAACCTACCCAAGAGCCATGATGCACCCA; 153 nt) and (b) L segment sequence (TTGTCAAAAA TCCCAAAAGTGCTCAGGTCTGAGCTACAAGTATTTCTAACCCACAGGCTATTCAGCACAATGCAGCGAATATCAG; 85 nt). For each sequence, serotype, strain name and country of isolation are given. Bootstrap values are given in percentages. GenBank accession numbers of the S segment sequences are HM051104 (Turkish isolate), EF570141.1, GU270841.1, EF570140.1, DQ904354, EU327772.1, DQ975231.1, EF201830.1, EF201831.1, EF201828.1, EF201832.1, EF201831.1, DQ656078.1, DQ656077.1, EU725773.1, EF201821.1, EF201816.1, EF201817.1, EF201818.1, AY705943.1, EF120631.2, AY705940.1, AY705938.1, EF120632.1, AY766034, EF201833, EF120630 and DQ656075. GenBank accession numbers of the L segment sequences are HM151316 (Turkish isolate), X68414, NC_006319, DQ975233, FJ356705, DQ656070, FJ153281, DQ656071, FJ153279, FJ153280, EF095548, EU725771, EF095551, EU240882, NC_005214.

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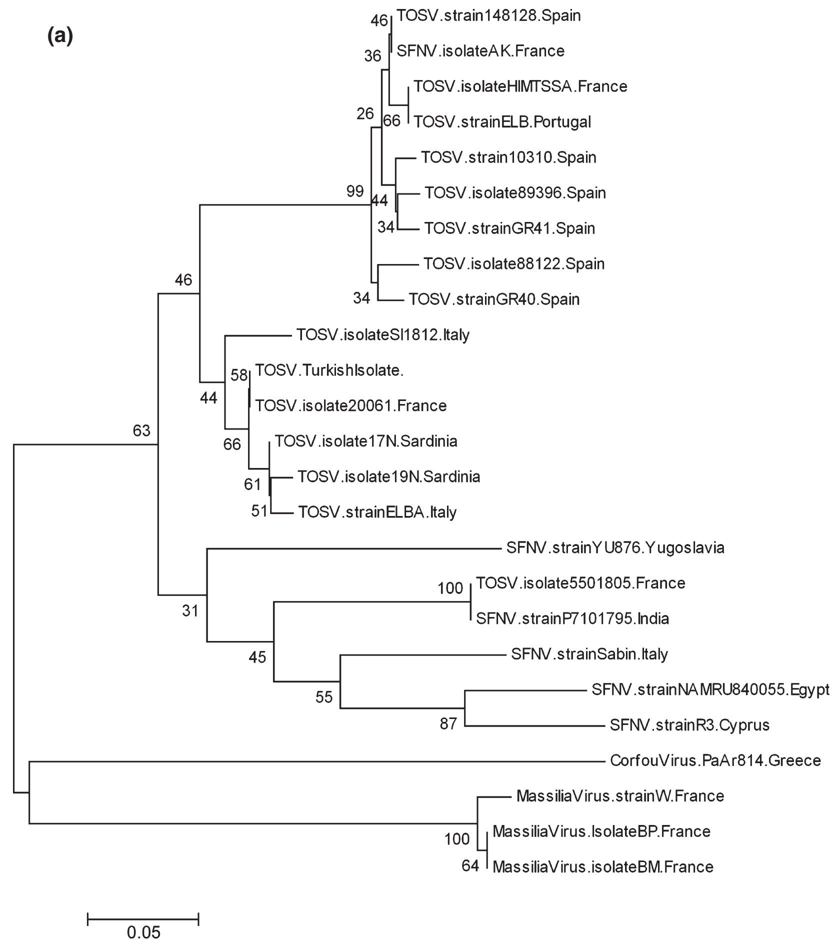

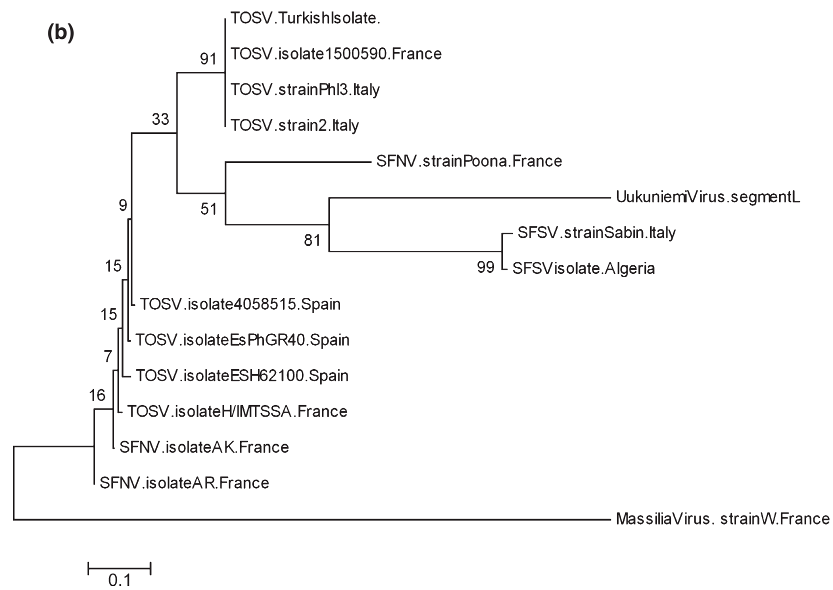

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Discussion

It is known that in endemic regions, the seroprevalences of SFVs may be as high as 60%, with frequent multiple infections [12]. Previous studies on SFV activity in Turkey had identified human exposures to SFSV, SFNF and TOSV in Aegean and Mediterranean coastal regions [13,14], but no data regarding central Anatolia were available. To our knowledge, this is the largest serosurveillance study from Turkey and the first to identify human SFV exposure in central/northern Anatolia. In this study, focusing on the central Anatolian provinces of Ankara, Konya and Eskisehir and the Black Sea coastal province of Zonguldak, frequent exposure to TOSV and other SFV serotypes in blood donors was revealed. The IgG reactivities in IIFT were above 20% for all study regions, with an overall rate of 32.9% and as high as 65.9% in Konya (Table 1). Human TOSV exposure was identified and confirmed by VNT in all regions, with the highest seroprevalence observed in Konya (25.5%) followed by Eskisehir, Ankara and Zonguldak (Table 1). Exposure to the new SFV serotype SFTV, as identified by VNT, was observed in Konya and Ankara and comprised the most frequent serotype in Konya (28%). SFNV exposure was identified only in Konya and SFSV was observed to be present in all regions except Zonguldak (Table 1). Exposure to multiple SFV serotypes was detected in Ankara and Konya. These results show that SFVs are endemic in central Anatolia and must be considered as the etiologic agent in cases/outbreaks of febrile diseases with unknown etiology. Also of importance is the detection of TOSV activity in all regions including the northern Anatolian province of Zonguldak, which indicates TOSV as a probable etiologic agent for aseptic meningoencephalitis cases observed in central/northern Anatolia.

There is only one laboratory-confirmed SFV case from Turkey, which involves a travel-related SFSV infection presenting as meningitis in a 15-year-old girl, after a vacation in Turkey [22]. Recently, SFSV and SFTV have been identified in outbreaks of febrile diseases with nausea/vomiting in 2007–2008 from the Izmir (Aegean), Adana (Mediterranean) and Ankara (central Anatolia) regions [15]. In this study, we have identified, for the first time, acute TOSV CNS infection by viral RNA detection in 15.7% (16/102) of the aseptic meningitis cases of unidentified etiology in central Anatolia, Turkey. The clinical picture observed in the affected individuals is consistent with TOSV infections from various endemic Mediterranean countries, where symptoms of mild meningitis, usually without encephalitic symptoms and with frequent recovery without sequelae, are common (Table 2) [5,9–12]. We included only RNA-positive individuals as confirmed TOSV infections because cross-reactions between SFV serotypes cannot be ruled out in serologic tests and asymptomatic infections and/or IgM persistence may result in false-positive interpretations in endemic regions [1]. Because the study was performed with previously stored samples, convalescent serum samples were not available for further serological confirmation. SFV serotypes other than TOSV were not detected in patients.

It has been suggested that genetically divergent TOSV strains may co-circulate in endemic regions and at least two geographically-distinct TOSV populations are present in the Mediterranean countries [23–25]. The existence of two lineages of TOSV were proposed for Spain and France [24,25]. Phylogenetic analyses of the determined partial S-segment sequence (153 nt) and partial L-segment sequence (85 nt) showed highest homologies to TOSV sequences from Italy (S segment, X53794, NC_006318; L segment, NC006319, X68414) and France (S segment, DQ975232; L segment, DQ975233), clearly placing them into group A of TOSV sequences [23]. Our results also suggest that previously designed real-time RT-PCR primers targeting the S segment are effective in identifying acute TOSV infections in Turkey and can be used in diagnostics [18].

The activity of various species of Phlebotomine sandflies has been observed in Mediterranean, Aegean and central Anatolian regions in Turkey, including *P. papatasi* and *P. perfiliewi*, well-known vector species for SFVs, but virus characterization has never been accomplished [26,27]. Detailed entomologic studies are required for displaying viral genomic heterogeneity in Anatolia.

In conclusion, we have displayed frequent human exposure to SFVs and the role of TOSV in CNS infections in central/northern Anatolia. SFVs must be considered as etiologic agents in cases of febrile diseases and aseptic meningitis in local residents as well as travellers.

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Preliminary IIFT results of some of the blood donors from the Ankara region were presented as a poster at the 12th Annual Meeting of the European Society for Clinical Virology in Istanbul, Turkey, between 27 and 30 September 2009.

Author Contributions

Koray Ergünay: project planning, organization, IIFT assays, overall evaluation of results, phylogenetic analyses and manuscript preparation. Mehmet B. Saygan: project planning, sam-

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ple collection, processing and evaluation (blood donors). Sibel Aydoğan: sample processing (blood donors and patients) and IIFT assays. Modou Moustapha Lo: neutralization assays. Manfred Weidmann: PCR assays and manuscript editing. Meik Dilcher: DNA sequencing. Burçin Şener: sample collection, processing and evaluation (patients). Gülşen Hasçelik: sample collection, processing and evaluation (patients). Ahmet Pınar: sample processing (blood donors and patients) and IIFT assays. Dürdal Us: project planning, organization and IIFT assays.

Transparency Declaration

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References

1. Mertz GJ. Bunyaviridae: Bunyaviruses, Phleboviruses, Nairoviruses and Hantaviruses. In: Richman DD, Whitley RJ, Hayden FG, eds. *Clinical Virology*. New York: Churchill-Livingstone, 1997; 943–971.
2. Dionisio D, Esperti F, Vivarelli A, Valassina M. Epidemiological, clinical and laboratory aspects of Sandfly Fever. *Curr Opin Infect Dis* 2003; 16: 383–388.
3. Papa A, Konstantinou G, Pavlidou V, Antoniadis A. Sandfly fever virus outbreak in Cyprus. *Clin Microbiol Infect* 2006; 12: 192–194.
4. Verani P, Nicoletti L, Ciufolini MG. Antigenic and biological characterization of Toscana virus, a new Phlebotomus fever group virus isolated in Italy. *Acta Virol* 1984; 28: 39–47.
5. Nicoletti L, Verani P, Caciolli S *et al.* Central nervous system involvement during infection by Phlebovirus Toscana of residents in natural foci in central Italy (1977–1988). *Am J Trop Med Hyg* 1991; 45: 429–434.
6. Ehrnst A, Peters CJ, Niklasson B, Svedmyr A, Holmgren B. Neurovirulent Toscana virus (a sandfly fever virus) in Swedish man after visit to Portugal. *Lancet* 1985; 25: 1212–1213.
7. Nicoletti L, Ciufolini MG, Verani P. Sandfly fever viruses in Italy. *Arch Virol* 1996; 11: 41–47.
8. 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: 685–691.
9. Mendoza-Montero J, Gamez-Rueda MI, Navarro-Mari JM, de la Rosa-Fraile M, Oyonarte-Gomez S. Infections due to sandfly fever virus serotype Toscana in Spain. *Clin Infect Dis* 1998; 27: 434–436.
10. Valassina M, Meacci F, Valensin PE, Cusi MG. Detection of neurotropic viruses circulating in Tuscany: the incisive role of Toscana virus. *J Med Virol* 2000; 60: 86–90.
11. Hemmersbach-Miller M, Parola P, Charrel RN, Durand JP, Brouqui P. Sandfly fever due to Toscana virus: an emerging infection in southern France. *Eur J Intern Med* 2004; 15: 316–317.
12. Peyrefitte CN, Devetakov I, Pastorino B *et al.* Toscana virus and acute meningitis, France. *Emerg Infect Dis* 2005; 11: 778–780.
13. 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: 663–674.
14. Serter D. Present status of arbovirus sero-epidemiology in the Aegean region of Turkey. *Zentralbl Bakteriol* 1980; 9: 155–161.
15. Çarhan A, Uyar Y, Özkaya E*et al.* Characterization of a new new phlebovirus related to Sandfly fever Sicilian virus isolated during a Sandfly fever epidemic in Turkey. *J Clin Virol* 2010; 48: 264–269.
16. Davies FG, Jacobsen P, Sylla D. Laboratory manual on Rift Valley fever: isolation and identification technics. In: Report of FAO/WHO group on emergency preparedness for Rift Valley Fever control in West Africa (WHO-VPH/88.77), 1988: 1–134.
17. Schwarz TF, Gilch S, Pauli C, Jager G. Immunoblot detection of antibodies to Toscana virus. *J Med Virol* 1996; 49: 83–86.
18. Weidmann M, Sanchez-Seco MP, Sall AA *et al.* Rapid detection of important human pathogenic Phleboviruses. *J Clin Virol* 2008; 41: 138–142.
19. Weidmann M, Muhlberger E, Hufert FT. Rapid detection protocol for filoviruses. *J Clin Virol* 2004; 30: 94–99.
20. Sanchez-Seco MP, Echevarria JM, Hernandez L, Estevez D, Navarro-Mari JM, Tenorio A. Detection and identification of Toscana and other phleboviruses by RT-nested-PCR assays with degenerated primers. *J Med Virol* 2003; 71: 140–149.
21. Kumar K, Tamura K, Nei M. MEGA 3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* 2004; 5: 150–163.
22. Becker M, Zielen S, Schwartz TF, Linde R, HofmanN D. Pappataci fever. *Klin Pediatr* 1997; 209: 377–379.
23. Sanbonmatsu-Gamez S, Perez-Ruiz M, Collao X *et al.* Toscana virus in Spain. *Emerg Infect Dis* 2005; 11: 1701–1707.
24. Charrel RN, Izri A, Temmam S *et al.* Cocirculation of 2 genotypes of Toscana virus, southeastern France. *Emerg Infect Dis* 2007; 13: 465–468.
25. Collao X, Palacios G, Sanbonmatsu-Gamez S *et al.* Genetic diversity of Toscana virus. *Emerg Infect Dis* 2009; 15: 574–577.
26. Simsek FM, Alten B, Caglar SS *et al.* Distribution and altitudinal structuring of phlebotomine sand flies (Diptera: Psychodidae) in southern Anatolia, Turkey: their relation to human cutaneous leishmaniasis. *J Vector Ecol* 2007; 32: 269–279.
27. Yaman M, Dik B. An inventory of the phlebotomine sandflies (Diptera: Psychodidae) found in the Turkish province of Konya. *Ann Trop Med Parasitol* 2006; 100: 265–275.

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