Nested reverse transcription–PCR using degenerate primers was applied to amplify a 222-bp fragment of the large RNA segment of phleboviruses (9). The retrieved sequence was identical to sequences detected in sandflies collected in 2005 in the Adriatic coastal region of Albania; that strain was provisionally named Adria virus (10). Adria virus is distinct from other recognized members of the genus Phlebovirus and clusters with phleboviruses of the Salehabad serocomplex, such as Salehabad virus and Arbia virus, differing by 21.6% and 29.6% with Salehabad virus and Arbia virus at the nucleotide level and by 3% and 17.7% at the amino acid level, respectively (Table).

Detection of the Adria virus sequence in the patient’s blood suggests that this virus is pathogenic to humans. As expected, serologic testing of the sample taken at the time of admission produced negative results for phleboviruses; a convalescent-phase blood sample was not available. Although the course of the disease in the child was mild, further studies will show the role of this strain in public health.

Because the duration of viremia in persons with phlebovirus infections is short, use of molecular methods for the laboratory diagnosis of phleboviral infections is limited; and even when a phleboviral infection is confirmed by serologic testing, the exact strain is difficult to determine. Physicians in Greece, as in other Mediterranean countries, should be aware of the circulation of phleboviruses and potential risk for phlebovirus-associated infections during the summer. Such infections, especially with neurologic signs, should be included in the differential diagnosis of summer febrile syndromes.

Acknowledgments

We thank the 2 anonymous referees for valuable comments on the initial submission.

The work was supported by the Hellenic Centre for Diseases Control and Prevention.

Vassiliki Anagnostou, Grigorios Pardalos, Miranda Athanasiou-Metaxa, and Anna Papa

Author affiliations: Aristotle University of Thessaloniki Thessaloniki, Greece (V. Anagnostou, A. Papa); and Hippokration Hospital, Thessaloniki (G. Pardalos, M. Athanasiou-Metaxa)

DOI: 10.3201/eid1705.101958

References

1. Nichol ST, Beaty BJ, Elliott RM, Goldbach R, Plyusnin A, Schmaljohn CS, et al. Genus Phlebovirus. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA, editors. Virus taxonomy: eighth report of the International Committee on Taxonomy of Viruses. San Diego (CA): Elsevier Academic Press; 2005. p. 709–11.

2. Charrel RN, Gallian P, Navarro-Mari JM, Nicoletti L, Papa A, Sanchez-Seco MP, et al. Emergence of Toscana virus in Europe. Emerg Infect Dis. 2005;11:1657–63.

3. 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.

4. Anagnostou V, Sdouga M, Volakli H, Violaki A, Papa A. Phlebovirus meningoencephalitis complicated by Pseudomonas aeruginosa pneumonia: a case report. Vector Borne Zoonotic Dis. Epub Jun 24, 2010.

5. Papa A, Andriotis V, Tzilianos M. Prevalence of Toscana virus antibodies in residents of two Ionian islands, Greece. Travel Med Infect Dis. 2010;8:302–4. doi:10.1016/j.tmaid.2010.09.002

6. Rodhain F, Madulo-Leblond G, Hannoun C, Tesh R. Virus Corfu: a new phlebovirus isolated from phlebotomes in Greece [in French]. Ann Inst Pasteur/Virol. 1985;136E:161–6.

7. Dobler G, Treib J, Haass A, Frosner G, Woessner R, Schimrigk K. Toscana virus infection in German travelers returning from the Mediterranean. Infection. 1997;25:325. doi:10.1007/BF01720413

8. Papa A, Konstantinou G, Pavlidou V, Antoniadis A. Sandfly fever virus outbreak in Cyprus. Clin Microbiol Infect. 2006;12:192–4. doi:10.1111/j.1469-0691.2005.01330.x

9. Sanzheze-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;71:140–9. doi:10.1002/jmv.10465

10. Papa A, Velo E, Bino S. A novel phlebovirus in Albanian sandflies. Clin Microbiol Infect. In press 2011.

Address for correspondence: Anna Papa, Department of Microbiology, Medical School Aristotle University of Thessaloniki, 54124, Thessaloniki, Greece; email: annap@med.auth.gr
analyzed according to their address of residence in each territorial division. Information related to these donors is reported in the Table.

Presence of immunoglobulin (Ig) G and IgM against TOSV was investigated by using a commercial enzyme immunoassay kit (EIA Enzywell Toscana virus IgG and IgM; DIESSE Diagnostica Senese S.p.A., Siena, Italy) developed by using the recombinant nucleocapsid (N) protein of TOSV. This serologic test was validated in a previous study that revealed high specificity and sensitivity (3).

Our results showed that 84 (11.5%) of 729 plasma samples were positive for IgG against TOSV N protein. Twenty-four (3.3%) plasma samples were positive for IgM, and 5 (0.7%) were positive for IgG and IgM (Table).

To confirm the ELISA results, IgG-positive samples were further subjected to Western blot (WB) analysis by using TOSV (isolate H/IMTSSA [2])-infected cell lysate (4). In 233 (32%) of samples, we detected a protein of molecular mass compatible with that of the N protein. A previously reported antibody-positive control was used to validate the WB assay (5). Our WB analysis showed a reduced sensitivity when compared with results of ELISA. After chemical/heat treatment of the protein samples, WB will only detect the linear epitopes on the N protein, while ELISA detects both linear and conformational epitopes. Furthermore, a less recent exposure of the blood donor population to the virus would have resulted in weaker N protein detection by WB as a consequence of a lower antibody titer. However, we cannot exclude some aspecific cross-reactivity as a consequence of well-conserved N protein sequence among the genus.

Finally, to detect TOSV RNA, we processed IgM-positive plasma samples by reverse transcription–PCR (6). The finding of IgM is an indication of a recent exposure to the virus and hence a possible presence in blood. Our PCR did not detect any viral RNA in the samples. Such negative results could indicate either cleared viremia or a low viral load, below the sensitivity limit of the test.

Sero logic information obtained in our study confirms the circulation of TOSV in southeastern France. Factors such as commercial exchange and movement of humans, animals, and arthropods between France and Italy may explain the highest prevalence observed (18.8%) in the Alpes Maritimes territorial district, which borders Italy. Our results regarding this area appear of the same order of magnitude as those reported in the general Italian population (>20%) (7).

Geographic and climatic conditions (e.g., temperature, humidity), factors that affect vector distribution and abundance (7), could explain the lower prevalence found in the mountainous districts (collectively ≈400–2,000 meters in elevation). The lower temperatures in these districts may also affect the ability of vectors to efficiently transmit the virus in the field (8).

TOSV prevalence in Corsica, an island in the Mediterranean Sea, was unexpectedly high. In this region, ≈8.7% (10 donors of 115) of the population sampled showed an IgG- or IgM-positive response. In the other districts, the IgM seroprevalence did not exceed 4.4%. The vector that transmits TOSV is known to be present in this area (7), and TOSV infections have been reported on nearby Sardinia (9). The elevated IgM titer in the population in Corsica could indicate 1) recent virus contacts; 2) recent infections with a new TOSV strain circulating in Corsica; or 3) presence of related phleboviruses that are inducing cross-reactivity in the N protein–based IgM ELISA.

Our results demonstrate that 14.1% (IgG and IgM) of the healthy population (blood donors) in France living on the Mediterranean border

| Demographic characteristic | No. donors | % IgG-positive samples | % IgM-positive samples |
|-----------------------------|------------|-----------------------|-----------------------|
| Age, y                      |            |                       |                       |
| <30                         | 211        | 11.8                  | 3.8                   |
| 30–39                       | 133        | 11.3                  | 3                     |
| 40–49                       | 156        | 11.5                  | 2.6                   |
| 50–60                       | 158        | 10.8                  | 4.4                   |
| >60                         | 71         | 12.7                  | 1.4                   |
| Sex                         |            |                       |                       |
| F                           | 353        | 13.9                  | 2.5                   |
| M                           | 376        | 9.3                   | 4                     |
| French territorial division†|            |                       |                       |
| Alpes de Haute Provence     | 29         | 10.3                  | 3.4                   |
| Hautes Alpes                | 64         | 6.25                  | 1.6                   |
| Alpes Maritime              | 111        | 19                    | 0                     |
| Bouches du Rhône            | 143        | 12.6                  | 2.8                   |
| Corsica                     | 115        | 8.7                   | 8.7                   |
| Var                         | 154        | 8.4                   | 1.9                   |
| Vaucluse                    | 113        | 13.3                  | 4.4                   |
| Total                       | 729        | 11.5                  | 3.3                   |

*Plasma samples were determined as positive by using an ELISA to detect immunoglobulin (Ig) G against Toscana virus (absorbance cutoff value [optical density at 450 nm (OD450)>0.47]) and IgM (absorbance cutoff value of OD450>0.15). Mean age of seropositive blood donors were the following: women, 40 y (SD 13.73 y); men, 41.8 y (SD 13.71 y).
†French territorial division elevations: Alpes de Haute Provence, 280–3,412 m; Hautes Alpes, 430–4,101 m; Alpes Maritimes, 0–3,143 m; Bouches du Rhône, –2–1,042 m; Corsica, 0–2,706 m; Var, 0–1,714 m; Vaucluse, 12–1,909 m.
have been in contact with TOSV and show asymptomatic or mild, unidentified symptoms, as it is the case for many other arbovirus infections (10). Such findings raise concerns about the risks of virus transmission to virus-naive persons by blood transfusions and organ transplants.

Further investigation is needed to better assess how widespread TOSV is in populations. For example, a donor–recipient investigation might confirm virus transmission by blood transfusion, and studies related to the behavior of sandfly vectors, virus biology, and mammalian reservoir hosts could help define populations at higher risk for exposure.

Acknowledgments
We thank Isabelle Leparc-Goffart, Marc Grandadam, and Hugues Tolou for providing an aliquot of Toscana virus isolate H/IMTSSA (FJ153286).

Nadège Brisbarre, Houssam Attoui, Pierre Gallian, Paola Di Bonito, Colomba Giorgi, Jean-Francois Cantaloube, Philippe Biagini, Mhammed Touinssi, Francois Jordier, and Philippe de Micco

Author affiliations: Université de la Méditerranée, Marseille, France (N. Brisbarre, P. Gallian, J.-F. Cantaloube, P. Biagini, M. Touinssi, F. Jordier, P. de Micco); Institute for Animal Health, Woking, UK (H. Attoui); and Instituto Superiore di Sanità, Rome, Italy (P. Di Bonito, C. Giorgi)

DOI: 10.3201/eid1705.101052

References
1. Cusi MG, Savellini GG, Zanelli G. Toscana virus epidemiology: from Italy to beyond. Open Virol J. 2010;4:22–8.
2. Peyrefitte CN, Devetakov I, Pastorino B, Villeneuve L, Bessaud M, Stolidi P, et al. Toscana virus and acute menigitis, France. Emerg Infect Dis. 2005;11:778–80.
3. Soldateschi D, dal Maso GM, Valassina M, Santini L, Bianchi S, Cusi MG. Laboratory diagnosis of Toscana virus infection by enzyme immunoassay with recombinant viral nucleoprotein. J Clin Microbiol. 1999;37:649–52.
4. Attoui H, Billoir F, Bruyer JM, de Micco P, de Lamballerie X. Serologic and molecular diagnosis of Colorado tick fever viral infections. Am J Trop Med Hyg. 1998;59:763–8.
5. Di Bonito P, Nicoletti L, Mochi S, Accardi L, Marchi A, Giorgi C. Immunological characterization of Toscana virus proteins. Arch Virol. 1999;144:1947–60. doi:10.1007/s007050050717
6. Sánchez-Seco MP, Echevarría JM, Hernández 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;71:140–9. doi:10.1002/jmv.10465
7. Chamaillé L, Tran A, Meunier A, Bourdoiseau G, Ready P, Dedet JP. Environmental risk mapping of canine leishmaniasis in France. Parasit Vectors. 2010;3:31. doi:10.1186/1756-3305-3-31
8. Barbazan P, Guiserix M, Boonyuan W, Tuntaprasart W, Pontier D, Gonzalez JP. Modelling the effect of temperature on transmission of dengue. Med Vet Entomol. 2010;24:66–73. doi:10.1111/j.1365-2915.2009.00848.x
9. Venturi G, Makeddu G, Rezza G, Ciccozzi M, Pettinato ML, Cilliano M, et al. Detection of Toscana virus central nervous system infections in Sardinia Island, Italy. J Clin Virol. 2007;40:90–1. doi:10.1016/j.jcv.2007.06.005
10. Schmaljohn CS, Nichol ST. Bunyaviridae. In Fields BN, Knipe DM, Howley PM, editors. Fields virology, 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2010.

Quinine-Resistant Malaria in Traveler Returning from French Guiana, 2010

To the Editor: Resistance of Plasmodium falciparum to antimalarial drugs is one of the most worrying problems in tropical medicine. For P. falciparum malaria acquired in French Guiana, the combination of quinine and doxyxycycline is one of the first-line recommended treatments (1). Since 1996, only 2 treatment failures with quinine have been reported from that country (2). An elevated 50% inhibitory concentration (IC50), classified as in vitro quinine resistance, was reported for 17% of 32 P. falciparum isolates obtained during 1983–1987 in French Guiana (3). Throughout 1994–2005, isolates were susceptible to quinine, with a mean IC50 <200 nmol/L (4).

We report quinine treatment failure in a 35-year-old man who was infected during a 3-month stay in Saül, a rural area of French Guiana. The patient did not use antivectorial or antimalarial prophylaxis. The patient sought treatment with fever 4 days after returning to France on June 22, 2010 (day 0), and a diagnosis of P. falciparum malaria was made on the basis of results of a rapid diagnostic test performed by a private medical laboratory. The man, who weighed 58 kg, was treated as an outpatient with 500 mg of quinine to be taken orally 3×/d for 7 days; he did not receive doxyxycycline. He was admitted to the Laveran Military Teaching Hospital in Marseille on July 15 (day 24 and first day of recrudescence) for uncomplicated malaria with a P. falciparum parasitemia level of 4%. He was given artemether, 80 mg/d, by intramuscular injection for 3 days. Blood samples taken on day 27 (third day of recrudescence) and day