On July 25, 2012, SBV infection was identified in a cow in Jura Canton in the northwestern, French-speaking region of Switzerland (Romandie) (*7*). A serologic study conducted in the United Kingdom showed that several cattle and sheep seroconverted for SBV in 2012 (*8*). However, our data show that SBV survived the winter, when midge numbers decreased. The precise mechanisms of SBV overwintering are not known and need to be explored.

The consequences of SBV recirculation should be investigated, particularly in pregnant cows, ewes, and goats. The 2 SBV-positive farms described in this report are located in a previously SBV-free area (Finistère-Brittany) or an area in which the infection rate was low (Pyrénées-Atlantiques) in the winter of 2011–2012, during which seroprevalence for most herds was probably weak (C. Sailleau et al., unpub. data). Therefore, reemergence of cases of congenital forms of SBV infection in France and others areas of Europe can be expected.

Acknowledgments

We thank the veterinary services in France and Nicolas Fradin for contributing to this study, the veterinary practitioners for collecting blood samples, and the farmers for permitting their animals to be included in this study.

Corinne Sailleau, Emmanuel Bréard, Cyril Viarouge, Alexandra Desprat, Virginie Doceul, Estelle Lara, Jérôme Languille, Damien Vitour, Houssam Attoui, and Stéphan Zientara

Author affiliations: Agence Nationale de Sécurité Sanitaire, Maisons-Alfort, France (C. Sailleau, E. Bréard, C. Viarouge, A. Desprat, V. Doceul, E. Lara, D. Vitour, S, Zientara); Direction Générale de l'Alimentation, Paris, France (J. Languille); and Institute for Animal Health, Pirbright, UK (H. Attoui)

DOI: http://dx.doi.org/10.3201/eid1902.121281

References

1. Toussaint JF, Vandenbussche F, Mast J, De Meester L, Goris N, Van Dessel W, et al. Bluetongue in northern Europe. Vet Rec. 2006;159:327. http://dx.doi.org/10.1136/vr.159.10.327-a
2. Hoffmann B, Scheuch M, Hoper D, Jungblut R, Holsteg M, Schirrmeier H, et al. Novel orthobunyavirus in cattle, Europe, 2011. Emerg Infect Dis. 2012;18:469–72. http://dx.doi.org/10.3201/eid1803.111905
3. van den Brom R, Luttikholt SJ, Lievaart-Peterson K, Peperkamp NH, Mars MH, van der Poel WH, et al. Epizootic of ovine congenital malformations associated with Schmallenberg virus infection. Tijdschr Diergeneeskd. 2012;137:106–11.
4. European Food Safety Authority. Schmallenberg virus: analysis of the epidemiological data and assessment of impact. European Food Safety Authority. 2012;10: 2768–857. http://www.efsa.europa.eu/en/efsajournal/doc/2768.pdf
5. Dominguez M, Hendrikx P, Zientara S, Calavas D, Jaÿ M, Touratier A, et al. Preliminary estimate of Schmallenberg virus infection impact in sheep flocks, France. Vet Rec. 2012;171:426. http://dx.doi.org/10.1136/vr.100883
6. Bilk S, Schulze C, Fischer M, Beer M, Hlinak A, Hoffmann B. Organ distribution of Schmallenberg virus RNA in malformed newborns. Vet Microbiol. 2012;159:236–8. http://dx.doi.org/10.1016/j.vetmic.2012.03.035
7. Schmallenberg virus - Europe (48), 1st case 2012. ProMed 2012 [cited 2012 Jul 22]. www.promedmail.org, archive no. 20120721.1210343
8. Anonymous. Schmallenberg virus 'still circulating' in the UK. Vet Rec. 2012;171:140. http://dx.doi.org/10.1136/vr.e5373

Address for correspondence: Corinne Sailleau, Unité Mixte de Recherche Virologie 1161, Agence Nationale de Sécurité Sanitaire, 23 Ave du Générale de Gaulle, Maisons-Alfort 94076, France; email: corinne.sailleau@anses.fr

Toscana Virus Isolated from Sandflies, Tunisia

To the Editor: Toscana virus (TOSV; genus *Phlebovirus*, family *Bunyaviridae*) is transmitted by sandflies, mostly the species *Phlebotomus perniciosus* and *P. perfiliewi* (*1*). Initially discovered in central Italy, TOSV was recently identified in other European countries (i.e., Portugal, Spain, France, Croatia, and Turkey) (*2*). TOSV is a primary cause of aseptic meningitis during warm months (*2*). A seroprevalence study suggested that TOSV is present in Tunisia and may cause neuroinvasive infections, but definitive evidence of TOSV circulation has not been possible because it is difficult to distinguish from the antigenically related phlebovirus Punique virus (*3*,*4*); both viruses are members of the species *Sandfly fever Naples virus*. We investigated the prevalence of TOSV among sandflies in northern Tunisia.

A total of 5,288 sandflies (3,547 females, 1,740 males) were collected during June–October 2010 by using CDC light traps (John W. Hock Company, Gainesville, FL, USA) at Utique (37°08′N, 7°74′E), a focus for visceral leishmaniasis in northern Tunisia. Sandflies were separated by sex and trapping nights and pooled with ≥30 specimens by pool. Pools were processed as described (*4*) and subjected to PCR detection of phlebovirus RNA targeting 2 genes independently (*4*–*6*) and virus isolation onto Vero cells. Of 249 pools processed, 8 strains of phleboviruses were isolated: 2 TOSV, 3 Punique virus, and 3 other phleboviruses currently being characterized.

TOSV strains were obtained from 2 pools of sandflies trapped in September 2010, T152 and T166, consisting of 30 males and 30 females, respectively. These pools were positive for TOSV RNA by sequencing of

2 PCR products (201-nt and 280-nt sections in the large [L] and small [S] gene segments, respectively). Supernatant of the third passage was prepared for electron microscopy, which showed spherical and pleomorphic structures, 80–120 nm in diameter, compatible with viruses of the family *Bunyaviridae*. Complete genome sequencing was then done by using the Ion PGM Sequencer (Life Technologies SAS, Saint Aubin, France) (*7*); a total of 165,307 reads were obtained, of which 135,700 matched with the sequence of TOSV Iss.PhL3 used as reference. The viral genome of TOSV Tunisia-2010-T152 (GenBank accession nos. JX867534–JX867536) was composed of 12,488 nt; the complete sequence consisted of 1,869 nt, 4,215 nt, and 6,404 nt for the S, medium [M], and L RNA segments, respectively. The partial S sequence of the TOSV Tunisia-2010-T166 strain (GenBank accession nos. JX867537–JX867539) was identical to that of T152, but 1 synonymous mutation was observed in the partial L sequence and 1 nonsynonymous mutation in the partial M sequence (I906V).

The TOSV Tunisia-2010-T152 strain was aligned with homologous sequences retrieved from the GenBank database. Genetic distances were calculated at the amino acid and nucleotide levels by using the p-distance algorithm (online Technical Appendix Tables 1–3, wwwnc.cdc.gov/EID/article/12-1463-Techapp1/.pdf). Phylogenetic studies were performed by using the neighbor-joining method in MEGA5 (*8*) (Figure). The robustness of the nodes was tested by 1,000 bootstrap replications. We found that TOSV Tunisia-2010-T152 was most closely related to the prototype strain from Italy, Iss.PhL3, with nucleotide/amino acid distances of 0.031/0.052, 0.032/0.073, and 0.039/0.012 for the S, M, and L RNA sequences, respectively. Together, these genetic distances and phylogram topologies indicate that TOSV Tunisia-2010-T152 is most closely related to strains within the Italian lineage, although it may represent a distinct sublineage, more distantly related to strains belonging to the Spanish lineage (*9*).

Concomitantly with virus isolation, the phenology of sandfly species was studied during May–November 2010. Sandflies were identified, and the density was calculated as described (*10*). Most of the sandflies belonged to the subgenus *Larroussius* (98.3%). *P. perniciosus* sandflies were the most abundant species (71.74%), followed by *P. longicuspis* (17.47%) and *P. perfiliewi* (8.82%). Other sandfly species, such as *Phlebotomus* (*Phlebotomus*) *papatasi*, *Phlebotomus* (*Paraphlebotomus*) *sergenti*, *Sergentomyia minuta parotti*, *S. christophersi*, and *S. antennata* were found, but these were much less abundant. The phenology of 3 main sandfly species showed 2 main peaks: 1 small peak in June and a second, larger peak during September–October (online Technical Appendix Figure).

In summary, of a total of 5,288 sandflies collected, 2 pools were positive for TOSV, yielding an infection rate of 0.03%. A similar infection rate was observed in Spain (0.05%) (*9*); however, the infection rates in Italy (0.22%) and in France (0.29%) are substantially higher (*1*,*6*). The isolation of TOSV from male and female sandflies suggests transovarial transmission in nature, as reported

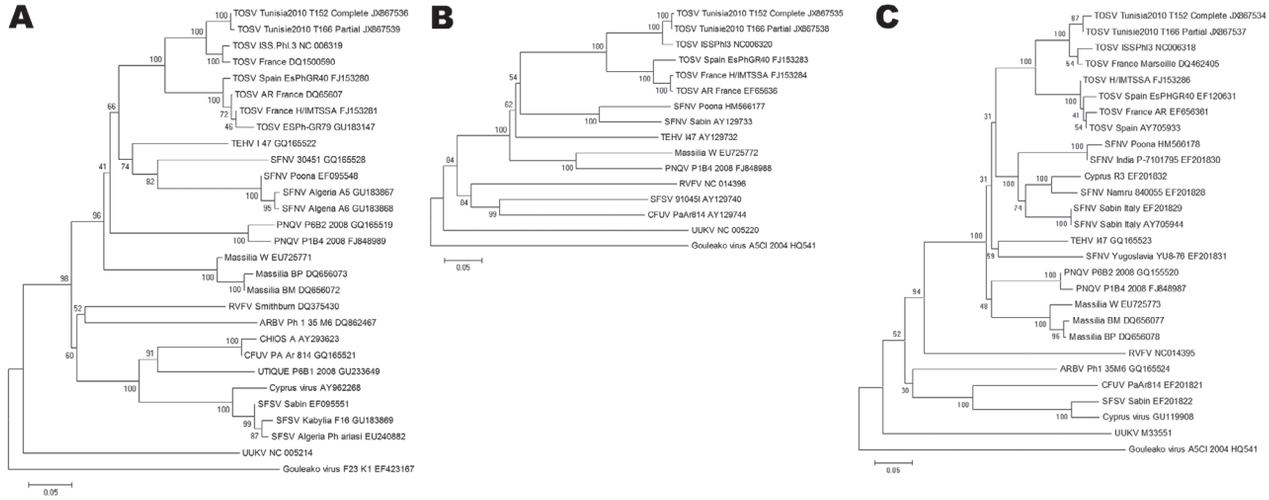

Figure. Phylogenetic analysis of 3 segments of Toscana virus (TOSV) isolates from pools of sandflies collected in Tunisia and homologous sequences of other selected phleboviruses. A) Large segments; B) medium segments; C) small segments. Sequences are identified by virus name or acronym, strain name, and GenBank accession number. Scale bars indicate nucleotide substitutions per site. TEHV, Tehran virus; SFNV, sandfly fever Naples virus; PNQV, Punique virus; RVFV, Rift Valley fever virus; ARBV, Arbia virus; CHIOS, phlebovirus Chios-A; CFUV, Corfou virus; SFSV, sandfly fever Sicilian virus; UUKV, Uukuniemi virus.

in Italy and Spain (*1*,*9*). In southern European countries, TOSV is mostly transmitted by *P. perniciosus* and *P. perfiliewi* sandflies (*1,6,9*), whereas *P. perniciosus*, *P. longicuspis*, and *P. perfiliewi* are the most abundant sandfly species in northern Tunisia. It is therefore probable that TOSV is transmitted by sandfly species of the subgenus *Larroussius*.

We found that 2 phleboviruses belonging to the *Sandfly fever Naples virus* species, TOSV and Punique virus, are cocirculating in northern Tunisia. This finding calls for further investigation of these viruses' potential effect on human health in this area.

This study was funded in part by the French "Agence Nationale de la Recherche" and "Agence inter-établissements de recherche pour le développement" through the MIE Phlebo-MED project, by European Virus Archive within the European FP7 CAPACITIES Project GA no. 228292, and by the Institut Pasteur, Tunis, Tunisia.

Laurence Bichaud,[1] Khalil Dachraoui,[1] Géraldine Piorkowski, Ifhem Chelbi, Gregory Moureau, Saifedine Cherni, Xavier De Lamballerie, Sonia Sakhria, Rémi N. Charrel,[2] and Elyes Zhioua[2]

Author affiliations: Aix-Marseille University (AMU, IRD, EHESP), Marseille, France (L. Bichaud, G. Piorkowski, G. Moureau, X. De Lamballerie, R.N. Charrel); IHU Méditerranée Infection, Marseille (L. Bichaud, X. De Lamballerie, R.N. Charrel); and Institut Pasteur, Tunis, Tunisia (K. Dachraoui, I. Chelbi, S. Cherni, S. Sakhria, E. Zhioua)

DOI: http://dx.doi.org/10.3201/eid1902.121463

[1]These authors contributed equally to this manuscript.

[2]These authors contributed equally to this manuscript.

References

1. Verani P, Ciulfolini MG, Caciolli S, Renzi A, Nicoletti L, Sabatinelli G, et al. Ecology of viruses isolated from sand flies on Italy and characterization of a new phlebovirus (Arbia virus). Am J Trop Med Hyg. 1988;38:433–9.
2. Charrel RN, Gallian P, Navarro-Mari JM, Nicoletti L, Papa A, Sánchez-Seco MP, et al. Emergence of Toscana virus in Europe. Emerg Infect Dis. 2005;11:1657–63. http://dx.doi.org/10.3201/eid1111.050869
3. Bahri O, Fazaa O, Ben Alaya-Bouafif N, Bouloy M, Triki H, Bouattour A. Role of Toscana virus in meningo-encephalitis in Tunisia [in French]. Pathol Biol (Paris). 2011;59:e125–7. Epub 2010 Apr 7.
4. Zhioua E, Moureau G, Chelbi I, Ninove L, Bichaud L, Derbali M, 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. http://dx.doi.org/10.1099/vir.0.019240-0
5. Sánchez-Seco MP, Echevarría JM, Hernández L, Estévez D, Navarro-Marí 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. http://dx.doi.org/10.1002/jmv.10465
6. Charrel RN, Izri A, Temmam S, Delaunay P, Toga I, Dumon H, et al. Cocirculation of 2 genotypes of Toscana virus, southeastern France. Emerg Infect Dis. 2007;13:465–8. http://dx.doi.org/10.3201/eid1303.061086
7. Rothberg JM, Hinz W, Rearick TM, Schultz J, Mileski W, Davey M, et al. An integrated semiconductor device enabling non-optical genome sequencing. Nature. 2011;475:348–52 http://dx.doi.org/10.1038/nature10242.
8. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: Molecular Evolutionary Genetics Analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol. 2011;28:2731–9. Epub 2011 May 4. http://dx.doi.org/10.1093/molbev/msr121
9. Sanbonmatsu-Gámez S, Pérez-Ruiz M, Collao X, Sánchez-Seco MP, Morillas-Márquez F, de la Rosa-Fraile M, et al. Toscana virus in Spain. Emerg Infect Dis. 2005;11:1701–7. http://dx.doi.org/10.3201/eid1111.050851
10. Chelbi I, Derbali M, Al-Ahmadi Z, Zaafouri B, El Fahem A, Zhioua E. Phenology of *Phlebotomus papatasi* (Diptera: Psychodidae) relative to the seasonal prevalence of zoonotic cutaneous leishmaniasis in central Tunisia. J Med Entomol. 2007;44:385–8. http://dx.doi.org/10.1603/0022-2585(2007)44[385:POPPDP]2.0.CO;2

Address for correspondence: Rémi N. Charrel, Unite des Virus Emergents, UMR190 "Emergence des Pathologies Virales," Faculte de Medecine, 27 Blvd Jean Moulin, 13005 Marseille, France; email: remi.charrel@univ-amu.fr

Seroprevalence of Dengue in American Samoa, 2010

To the Editor: Since the 1970s, regular dengue epidemics have caused considerable illness in the Pacific region (*1*). In 2009, an epidemic year, the incidence of reported clinical dengue cases in American Samoa reached 644 cases/100,000 population; in 2010, incidence decreased to 77 cases/100,000 population (*2*). Dengue surveillance in American Samoa is being developed, but the effects of this disease are unknown.

In 2010, blood samples were collected in American Samoa primarily for a leptospirosis seroprevalence study. Samples were also tested for IgG antibodies against dengue virus, and a seroprevalence of 95.6% was observed. We report this finding and advocate improved surveillance and integrated control programs to limit dengue transmission in American Samoa.

A cross-sectional seroprevalence study was conducted during May–July 2010 with the primary aims of identifying risk factors for human leptospirosis and providing an evidence base to direct public health interventions in American Samoa (*3*,*4*). During the study, investigators encountered community concern about dengue and were asked by health authorities to use the remaining collected serum for a dengue seroprevalence study. Amendments to the original human research ethics applications submitted to the American Samoa Institutional Review Board and the University of Queensland