First detection of Toscana virus in Corsica, France

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Abstract

Toscana virus (TOSV) was detected for the first time from *Phlebotomus perniciosus* sandflies in Corsica, a French Mediterranean island. Genetic analysis showed that Corsican TOSV belongs to lineage A, together with Italian, Tunisian, Turkish and other French strains. The demonstration of TOSV in Corsica indicates that autochthonous and tourist populations are at risk of infection. Hence, physicians must consider TOSV as a possible cause of aseptic meningitis and unidentified febrile illness during the warm season.

Keywords: Emerging viruses, phlebotomine sandflies, phlebovirus, summer aseptic meningitis, summer febrile illness, Toscana virus
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Introduction

Toscana virus (TOSV) is a sandfly-borne phlebovirus transmitted mostly by *Phlebotomus perniciosus* and *Phlebotomus perfiliewi*. Initially discovered in central Italy in 1971 [1], first evidence for its human pathogenicity and neurotropism was reported 15 years later [2]. TOSV was progressively identified in other European countries (i.e. Portugal, Spain, France, Croatia and Turkey) [3,4]. In countries where it circulates, TOSV is now recognized as one of the main cause of aseptic meningitis during the warm season, i.e. the activity period of vectors [3]. Sandflies are widely distributed in all countries around the Mediterranean; their seasonal activity is affected by temperature and rainfall and it peaks during summertime, between May and October, mostly in July and August. Owing to their specific ecological conditions, the Mediterranean islands situation merits being analysed separately. The presence of TOSV has been proved indisputably in Elba, Cyprus [3], Sardinia [5] and Sicily [6]. In Corsica, a French island in the Mediterranean, almost 10% of the blood donors possessed anti-TOSV IgG, detected by ELISA test [7]. However, techniques such as ELISA and immunofluorescence assay are prone to cross-reactions, and positive results may be biased by the circulation of another antigenically related virus. To demonstrate the presence of TOSV in Corsica, we organized sandfly captures and subsequent virological investigations in Corsica.

The Study

A total of 951 sandflies (51.8% *P. perniciosus*, 46.3% of *Sergentomyia minuta*, 0.2% of *Phlebotomus mascitii*, 1.7% of *Phlebotomus* sp.) were collected during summer 2010 (between 12 and 20 July) in southwest Corsica (France), near Propriano (41°40′ N, 8°55′ E) (Fig. 1), by using CDC light traps (John W. Hock Company, Gainesville, FL, USA) [8]. Sandflies were organized in 112 pools (maximum ten specimens per pool) and processed as previously described [9] for (i) PCR detection of phlebovirus RNA using two systems of primers targeting two genes independently [10,11] and (ii) virus isolation onto Vero cells.

Of 112 pools processed, one pool of six male *P. perniciosus* was positive through the PCR system targeting the L-RNA segment. Sequencing the partial region of the L gene (201 nucleotides) confirmed the presence of TOSV, whose sequence was distinct from all other sequences manipulated in our laboratory and more generally available in the GenBank database (including the prototype Italian strain ISS.Phl3). Moreover, all mock samples were negative. To confirm the presence of TOSV RNA, the positive pool was also tested by real-time RT-PCR using two different systems [3], which both provided a positive result. Virus isolation was not obtained, probably because of a problem in sandfly preservation in the field.

The sequence of TOSV France-Corsica2010-A1 (GenBank accession no. KC700343) was aligned using CLUSTAL X [12] with homologous sequences of other TOSV strains and selected phleboviruses retrieved from the GenBank database.

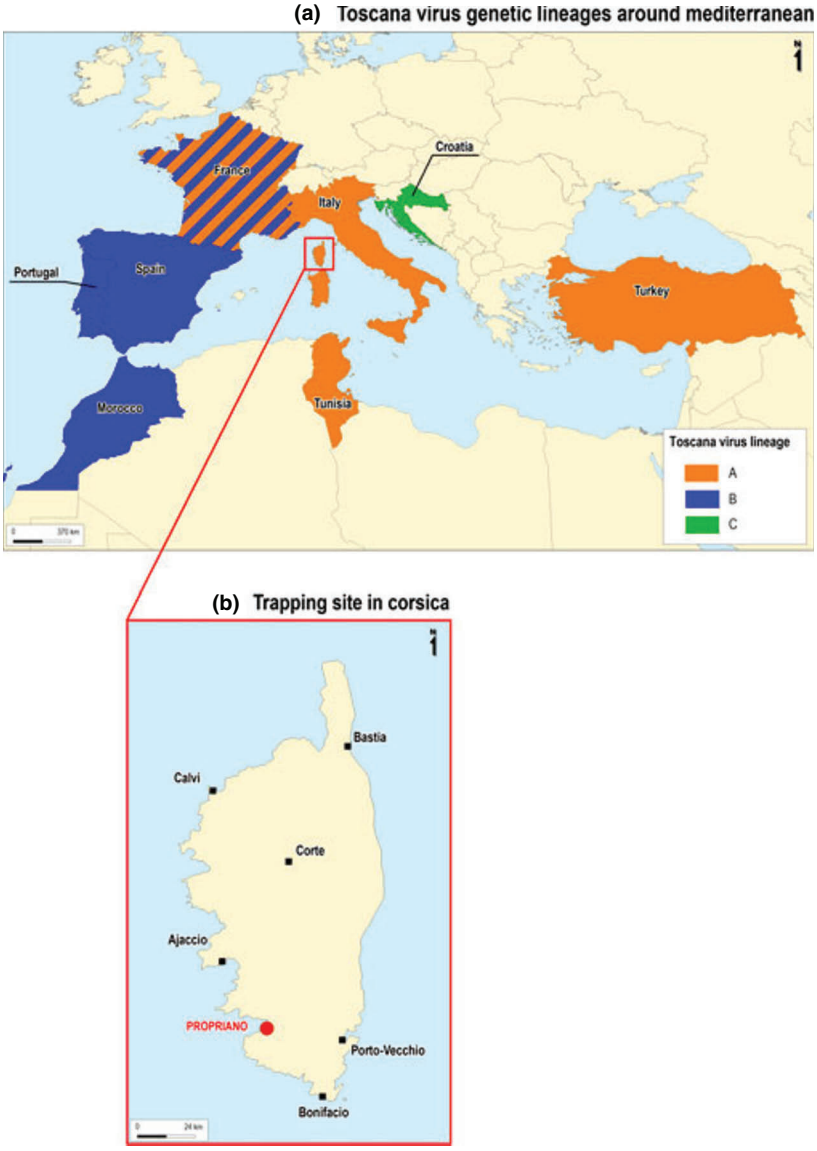

FIG. 1. (a) Geographic distribution of Toscana virus genetic lineages around the Mediterranean. (b) Geographic location of trapping sites in Corsica in July 2010.

Genetic distances were calculated at the amino acid and nucleotide levels by using the p-distance algorithm. Phenetic studies were performed using the neighbour-joining method in MEGA5 [13] (Fig. 2). The robustness of the nodes was tested by 1000 bootstrap replications.

Previous phylogenetic analyses of TOSV recognized two geographically associated lineages: (i) the lineage A that is endemic in Italy [1] (including Sardinia and Sicily, [5,6]) and lineage B, which is endemic in Spain [14]. Interestingly, in France both lineages were reported to circulate [11]. In Turkey and Tunisia, TOSV isolates were found to belong to lineage A [15,16]. In contrast, Moroccan and Portuguese TOSV belonged to lineage B [17]. Recently, a third lineage (lineage C) was described in Croatia, suggesting a genetic diversity that is higher than initially believed [4] (Fig. 1), which may hinder the performances of the diagnostic techniques based on RT-PCR. Genetic distance analyses and phylogram topologies indicate that TOSV France-Corsica2010-A1 belonged to lineage A, together with another French strain isolated from a patient living in Marseille, the largest city in southeastern continental France.

Of a total of 951 sandflies collected, one pool was positive for TOSV, yielding an infection rate of 0.10%. Lower infection rates were observed in Tunisia (0.03%) [16] and in Spain (0.05%) [14]. The infection rates in Italy (0.22%) and in metropolitan France (0.29%) are substantially higher [11,18]. The detection of Corsican TOSV from a pool of male sandflies suggests transovarial transmission in nature, as reported in Italy and Spain [14,18], resulting in maintenance of TOSV in sandfly populations for long periods, with persisting risk of infection in humans.

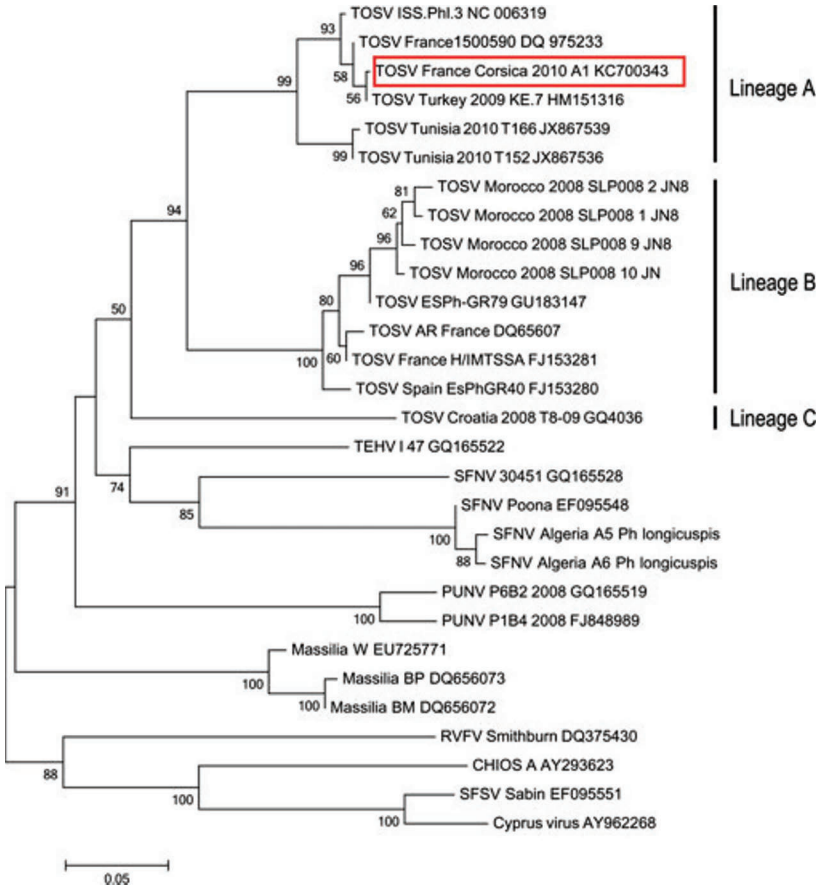

FIG. 2. Phenetic analysis of L segment of Toscana virus (TOSV) detected from pools of sandflies collected in Corsica (201 nucleotides) and homologous sequences of other selected phleboviruses. Sequences are identified by virus name or acronym, strain name, and GenBank accession number. Scale bars indicate nucleotide substitutions per site. TEHV, Tehran virus; SFNV, sandfly fever Naples virus; PUNV, Punique virus; RVFV, Rift Valley fever virus; CHIOS, phlebovirus Chios-A; SFSV, sandfly fever Sicilian virus.

In Corsica a seroprevalence study conducted with volunteer blood donors reported that 8.7% of the tested population possessed anti-TOSV IgG using ELISA tests [7]. However, seroprevalence data could not be considered as definitive evidence of TOSV circulation, because of the possible presence of closely related phleboviruses that could induce cross-reactions. This situation has been demonstrated with Massilia virus (MASV), another sandfly-borne phlebovirus, recently identified in southern France [19]. MASV was also detected in sandflies from Corsica (Bichaud L and Charrel RN, unpublished data). Both MASV and TOSV are members of the same antigenic complex (Sandfly fever Naples virus serocomplex), so techniques such as ELISA and immunofluorescence assay are not capable of discriminating between antibodies elicited by these viruses. For this reason, the fact that 8.7% of sera were reactive against TOSV antigens does not imply that TOSV is present in Corsica because the presence of MASV could lead to confounding results. Microneutralization assay, using the two viruses in a comparative manner, is the test of choice to analyse the specificity of antibodies and confirm definitively the identity of viruses that circulate in human populations.

To our knowledge, this is the first time that TOSV has been detected in Corsica. This first unambiguous detection from sandflies confirms that local population and visitors are at risk. In Corsica, TOSV must be considered by physicians as a possible cause in aseptic meningitis and unidentified febrile illness during the warm season, and should be tested for by virology laboratories. Phlebovirus investigation should also be part of differential diagnosis with other viral pathogens such as West Nile virus.

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Transparency Declaration

The authors declare no conflicts of interest.

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