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Research Letter

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Nine autochthonous cases of Toscana virus infection in France, 2022–2024: a clinical and virological overview

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Toscana virus (TOSV) is an RNA virus (species *Phlebovirus toscanaense*, family *Phenuiviridae*) circulates seasonally in the Mediterranean basin, in association with its vectors' activity.[1] TOSV possesses three segmented genome composed of segment small (S), medium (M) and large (L).[1] The primary vectors responsible for TOSV transmission are *Phlebotomus (Ph.) perniciosus* and *Ph. perfiliewi*. TOSV transmission to humans occurs via sand fly bite. Although the virus is capable of transovarial and/or venerial transmission within its insect vectors, the vertebrate reservoir (if any) for TOSV remains unidentified.[1]

High seroprevalence rates in humans of TOSV in the Mediterranean countries such as Italy, France, Algeria, Tunisia, Greece and Turkiye confirm high circulation of the virus (range between 17% and 41%).[1] While TOSV infections are often asymptomatic, symptomatic cases can include a central nervous system (CNS) disorder typically accompanied by fever.[1] During the acute phase of infection, detection of viral genome in cerebrospinal fluid (CSF) and serum/plasma by RT-qPCR is the preferred diagnostic approach along with the serological techniques to detect TOSV-specific antibodies.[2] A confirmed case of TOSV infection with direct diagnostic metods include either isolation of the virus or detection of viral RNA in blood or CSF samples. Although the majority of TOSV cases have historically been reported in Italy, the surveillance efforts has identified authochthonous a number of human cases in France.[3–5] This study describes nine laboratory-confirmed autochthonous TOSV cases detected in France between 2022 and 2024, providing a comprehensive analysis of clinical, epidemiological, and virological characteristics, including phylogenetic data linking these cases to known viral strains. Between 2022 and 2024, patients with neurological symptoms were tested for TOSV infection at the Laboratory of Virology of AP-HM University Hospitals, and at the French National Reference Center (NRC) for Arboviruses. The NRC receives approximately 500 samples annually for suspected

TOSV infection, primarily from patients presenting with CNS diseases symptoms between May and November. These samples are typically referred to the NRC after initial screening for the common bacterial and viral pathogens responsible for CNS diseases, such as enteroviruses, parechoviruses, meningococcus and herpesviruses in the admitted Central University Hospitals. Since 2006, the annual number of laboratory-confirmed TOSV cases in France has ranged between 1 and 11, with the year 2019 representing a peak incidence with 11 confirmed cases. At AP-HM, serum, plasma and CSF were tested using an in-house RT-quantitative reverse transcription polymerase chain reaction (qPCR) assay[6] on LightCycler 480 (Roche Diagnostics), after extraction on EZ2 (QIAGEN) using the EZ1 virus mini kit (QIAGEN); a cycle threshold (Ct) value < 40 was considered positive, a $Ct >= 40$ and < 45 equivocal, and > 45 negative. Immunofluorescent test Sandfly fever virus mosaic 1 (EUROIMMUN) was used to detect anti-TOSV IgM on IF Sprinter (EUROIMMUN), providing qualitative results (positive/negative).

At NRC, RT-qPCR was performed with the Panther Fusion (HOLOGIC) using the same in-house assay.[7] The results of RT-qPCR were interpreted as described above. For serology testing, an in-house Enzyme-Linked Immuno Sorbent Assay (ELISA) was used to detect anti-TOSV IgM and IgG, and samples with > 3 ratio were considered as positive.

Viral isolation was attempted on Vero E6 cells for five RT-qPCR positive samples. Cell cultures were monitored daily for cytopathic effects, and 200 μl of supernatant collected on post-infection day 5 was tested using a specific TOSV RT-qPCR assay.[7] Viral genome sequencing was performed on nucleic acids extracted from either cell culture supernatant or from clinical samples. Sequencing was performed using the Illumina Klenow next-generation sequencing platform. The L segment sequences were aligned using ClustalW alongside homologous sequences of selected phleboviruses obtained from GenBank.

Seven men and two women with a median age of 58 years (range 42–74) were identified with TOSV infection between May 2022 and August 2024. Most cases originated from southern France: Bouches-du-Rhône (5), Vaucluse (1), Var (1) and Gard (1) (Supplementary Fig. 1). One case (Case 3) was identified in Haute-Savoie, in eastern France—a region not previously associated with TOSV presence. All patients presented neurological manifestations during the acute phase, with headache (8/9) and fever (5/9) being the most common clinical features (Table 1). Comorbidities were reported in three patients. Hospitalization was required for all, however, full recovery was observed in all cases without any reported long-term sequelae. All but one case were confirmed by RT-qPCR that yielded positive results on CSF (8/9) collected during the acute phase, with Ct values ranging from 27 to 38. Patient 2 had an equivocal RT-qPCR result with Ct value at 43 although both the shape of the curve and the RFU (relative fluorescence units) were convincing. Anti-TOSV IgM antibodies were detected in all tested CSF samples (8/8), as well as in the serum or plasma of five cases (Table 1). All acute-phase CSF and serum/plasma samples were negative for IgG except Case 1 plasma collected 42 days post symptom onset (PSO).

Leukocyte counts (7/9) in the CSF ranged from 63 to 290 cells/mm^3, with lymphocytic predominance (69% to 99%). CSF protein levels showed a range between 0.98 and 1.19 g/L (6/9) and glucose concentrations were between 2.27 and 3.90 mmol/L. TOSV was successfully isolated from CSF samples of cases 4, 5 and 6, with full genome sequencing (GenBank numbers PV340479, PV344504, PV359168, PV340480, PV344505, PV359169, PV340481 PV359167, PV359170); partial genome sequence was obtained from CSF of Case 1 (GenBank a number: PV359171). Phylogenetic analysis realized on the L segment of the three complete genomes showed that all strains belong to TOSV lineage B, closely related to TOSV strains were detected previously from patients in France[8] (Fig. 1).

In this study, we describe nine laboratory-confirmed TOSV cases that occurred in France between 2022 and 2024. The data highlight the ongoing circulation of TOSV lineage B in southern France and suggest stable local transmission over the past two decades. The unexpected case from Haute-Savoie suggests northward expansion of the virus's range, possibly driven by vector spread linked to climate change. Though sand fly presence in Haute-Savoie is not well-documented, species capable of TOSV transmission, such as *P. perniciosus*, have been reported in northeastern France.[9] We identified TOSV in seven men and two women, which is concordant with recent estimates that men are significantly more likely to develop symptoms of TOSV infection compared to women.[1]

All patients presented symptoms typical of neurotropic arbovirus infections, such as photophobia, neck stiffness and confusion (Table 1). None of the patients developed complications or long-term effects, consistent with the generally favourable prognosis in immunocompetent individuals.

All patients were laboratory confirmed through the detection of viral RNA in CSF, with high viral loads for most cases (low Ct values). This allowed for the successful isolation of only three strains. Additionally, anti-TOSV IgM antibodies were detected between 1 and 42 days PSO, a timeframe consistent with the expected humoral response in neuroinvasive TOSV infections.[10]

Laboratory analyses revealed elevated leukocyte counts in the CSF with a predominance of lymphocytes, indicative of lymphocytic pleocytosis characteristic of viral meningitis. In patients for whom data were available, CSF protein levels were mildly elevated, while glucose concentrations remained within normal ranges.

France is among the countries where both TOSV lineages A and B co-circulate.[6] Phylogenetic analysis reveals that the virus circulating between 2022 and 2024 is closely related to lineage B TOSV strains from the past two decades in France, indicating long-term maintenance of the virus in the country. All these elements highlight the importance of ongoing monitoring to detect any emerging changes that could impact its epidemiology or public health significance.

Travellers to TOSV endemic regions advised to take preventive measures against sand fly bites, including the use of insect repellents and wearing protective clothing, and sleeping in insect-proof accommodations. Public awareness campaigns could also help reduce exposure during peak sand fly activity.

Our article confirms the recent circulation of TOSV in France and emphasizes the importance of testing TOSV as a cause of disease in patients hospitalized for neuro-invasive manifestations

Table 1 Characteristics and laboratory findings of nine autochthonous TOSV cases identified in France, 2022–2024

Case	Residence and travel information	Period of exposure	Age/Sex	Clinical acute symptoms	Laboratory findings	Sample type	Days PSO	RT-qPCR (Ct)	Anti-TOSV IgM (ratio)	Anti-TOSV IgG (ratio)
1	France, Bouches-du-Rhône, Les Pennes Mirabeau	August 2024	44/M	Frontal and bitemporal headache, photophobia, moderate neck stiffness, Brudzinski sign	CSF: 184 leukocytes/mm^3 including including 95% lymphocytes, protein: 0,98 g/l, glucose: 3,35 mmol/l	CSF	2	28	5.4	0,9
						Serum	4	NT	7.7	1
						Plasma	42	NT	4.5	3,4
2	France, Vaucluse travel to Ardèche	August 2024	69/M	Fever, headache, arthralgia, myalgia, asthenia, confusion	CSF: 170 leukocytes/mm^3 including including 74% lymphocytes, protein: 1.00 g/L, glucose: 2,27 mmol/l	CSF	5	43	8	0.9
						Serum	5	neg	14.5	1,1
3	France, Haute-Savoie, Annecy	July 2024	68/M	Fever, headache, vomiting, retro-orbital pain, neck stiffness	CSF: 145 leukocytes/mm^3 including 98% lymphocytes, protein 1.16 g/L, glucose: 3.9 mmol/Ll	CSF	2	37	3.1	1
						CSF	5	38	8.5	1.1
						Plasma	6	neg	7	1.1
						Whole Blood	6	neg	NT	NT
4	France, Bouches-du-Rhône, Auriol	July 2023	58/M	Severe headaches and nausea, unresponsive to acetaminophen	CSF: 264 leukocytes/mm^3 including 88% lymphocytes, protein: 1.10 g/L, glucose: 3.10 mmol/L	CSF	1	33	NT	NT
5	France, Bouches-du-Rhône, La Ciotat,	October 2022	49/F	Severe headache with febrile meningeal symptoms	CSF: 290 leukocytes/mm^3 including 99% lymphocytes, protein 1,06 g/l glucose: 3,00 mmol/l	CSF	1	31	4.4	1
6	France, Bouches-du-Rhône, Eguilles travel to Var	September 2022	43/F	Severe headaches with nausea, vomiting, photophobia, neck stiffness	CSF: 106 leukocytes/mm^3 including 69% lymphocytes.	CSF	1	27	5.9	1
7	France, Bouches-du-Rhône, La Ciotat	June 2023	42/M	Severe headache with retro orbital radiating, phonophobie, photophobie and vertiginous	CSF: 63 leukocytes/mm^3 including 99% lymphocytes, protein 1,19 g/l, glucose: 3,40 mmol/l	CSF	2	30	5.3	0,9
8	France, Var	October 2022	74/M	Fever, headaches, meningitis, myalgia, digestive problems	NA	CSF	6	28	3.6	1
						Serum	6	neg	6.2	1,1
9	France, Gard	July 2022	69/M	Fever, headaches, meningitis, myalgia	NA	CSF	6	28	9.5	1,4
						Plasma	7	neg	11.1	2

PSO: post symptoms onset; Neg: negative; NT: not tested; CFS: cerebrospinal fluid, Ct: Cycle Threshold, NA: not available

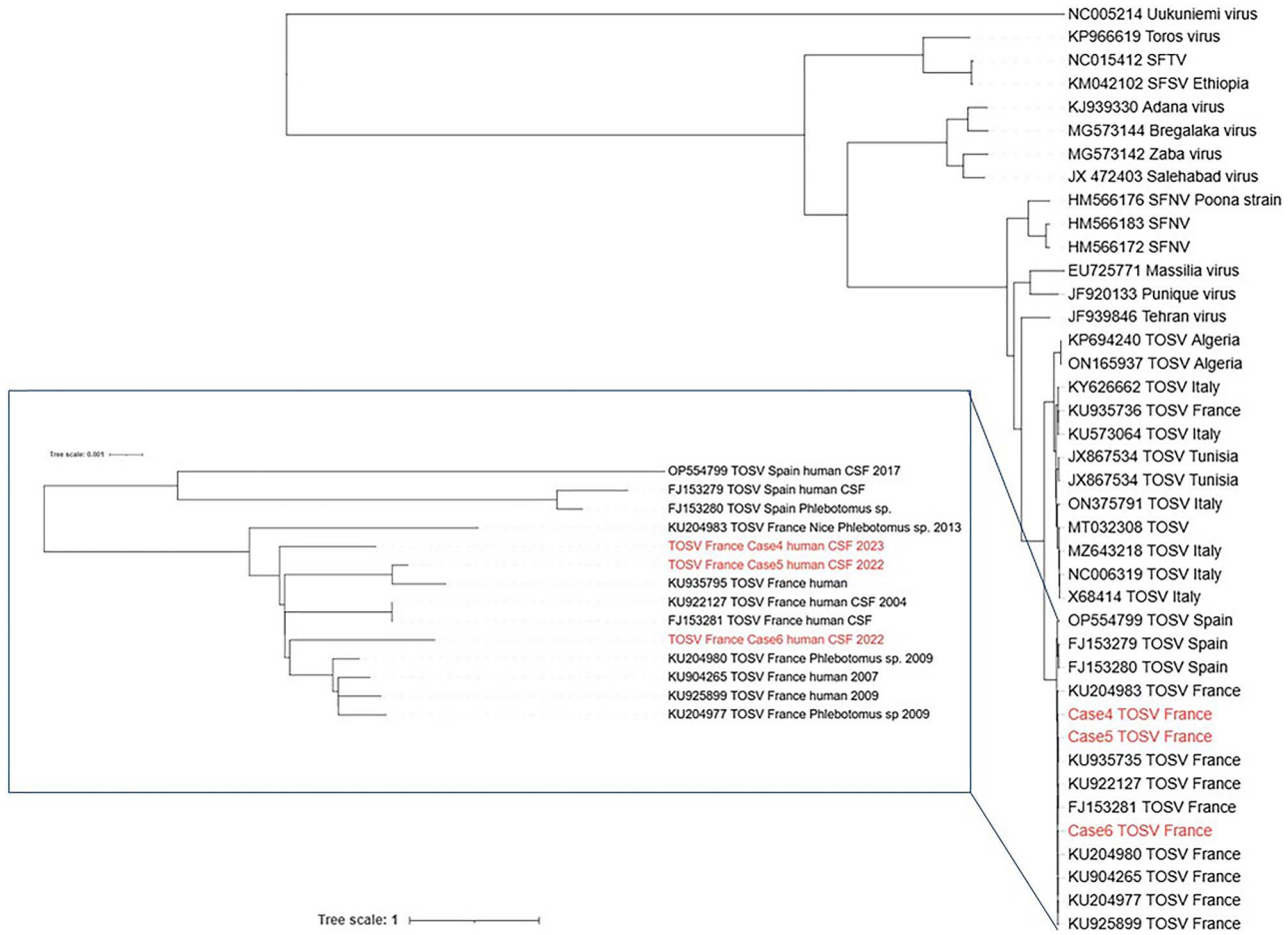

Figure 1 Maximum likelihood phylogenetic tree based on L segment RNA dependent RNA polymerase gene sequences. Sequences from three cases identified in France in 2022–2024 for which a complete genome was obtained are highlighted. Phylogenetic inference was performed using IQTREE2 under the best substitution model identified by ModelFinder with ultrafast bootstrap approximation (1000 replicates). The tree was midpoint rooted.

living or having visited regions where the vector species are present.

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Authors Contributions

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Data availability

The data underlying this article are available in GenBank Nucleotide Databas and can be accessed with GenBank accession numbers PV340479, PV344504, PV359168, PV340480, PV344505, PV359169, PV340481 PV359167, PV359170 and PV359171.

Ethical statement

This study was part of the national public health surveillance program of the National Reference Centre (NRC) for Arboviruses supervised by the National Public Health Agency (Santé Publique France, SPF). Therefore, as an epidemiological record, consultation with an ethics committee was not required. Samples transferred to the NRC are submitted with a form that includes the patient's consent (non-opposition clause).

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