

# Two clusters of Toscana virus meningo-encephalitis in Livorno Province and Elba Island, July-September 2018

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## Abstract

**Background.** Toscana virus (TOSV) is an arbovirus transmitted to humans by *Phlebotomus* spp sandflies. It causes aseptic meningitis and meningoencephalitis with marked seasonality. Here we describe the clinical, microbiological and epidemiological features of two clusters of cases occurred in Tuscany in 2018.

**Methods.** A confirmed case was defined as the detection of anti-TOSV IgM and IgG in serum sample, in presence of typical clinical manifestations. We consulted hospital records of hospitalized patients to collect clinical information and obtained epidemiological information from the local health authority investigation report. We telephonically interviewed patients using a standard questionnaire for a 6 months follow-up.

**Results.** A total of 12 cases of TOSV meningo-encephalitis with onset between 4<sup>th</sup> of July and 12<sup>th</sup> of September accessed health care services in the province of Livorno. Eight cases were males with median age 41,5 and four were not resident in the area. Serological investigations confirmed a recent TOSV infection. Eight cases reported visiting Elba Island and four had a possible occupational-related exposure.

**Conclusions.** This surge of infection emphasizes the need of information campaigns coupled with adequate surveillance and control interventions against TOSV that, among other arboviruses, is a growing issue of concern in Italy.

## Introduction

Toscana virus (TOSV) is an arbovirus belonging to the sandfly fever Naples complex of the *Phenuiviridae* family, first identified in 1971 from the sandfly

*Phlebotomus perniciosus* (1). The virus can be transmitted to humans through the bite of an infected female sandfly and was associated with human disease in early 1980s (2). Although infections are often asymptomatic, TOSV was reported as one

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of the most important etiological agents of viral meningitis in Italy (3, 4) and other Mediterranean countries (5, 6). The disease, prevalent among adults, has a marked seasonality with cases most often occurring during late summer (3, 4). The ecology of the virus is not well known, although antibodies against the virus have been detected in various vertebrates including horses, sheep and dogs (4, 7). Circulation of the virus in Tuscany and its islands is well described in the literature and seroprevalence surveys of the resident population reported up to 30% positivity to anti-TOSV antibodies (4, 8).

Besides, Tuscany is a world-famous holiday destination and cases in returning travelers have also been described over the years (9, 10) and also more recently (11). Yet, current surveillance activities in the region and at national level are suboptimal.

Here we describe two clusters of TOSV cases occurred in Tuscany during July-September 2018, in order to increase awareness about this arbovirolosis (12).

## Materials and methods

A confirmed case was defined as the detection of anti-TOSV IgM and IgG in serum sample, in presence of typical clinical manifestations.

All virological and serological analyses were performed on plasma samples by the Virology Unit of the Pisa University Hospital, Regional Reference Center for Arboviruses and the only center performing TOSV serology in the Toscana North-West Local Health Authority (LHA) to which Livorno and Elba island refer. The samples were collected from symptomatic patients by the Livorno Infectious Disease Unit and sent to the Pisa reference center. Positive cases were confirmed by the Italian National Institute of Health (Istituto Superiore di Sanità, ISS).

Search for TOSV RNA genome was performed using the Toscana Virus Real

Time Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) Kit (Liferiver, Shanghai, China). In brief, Viral RNA was extracted from 200  $\mu$ l of serum e/o liquor using Qiasymphony instrument (Qiagen) and a 5  $\mu$ l RNA sample was used for the Real-time reaction. Anti-TOSV antibodies were investigated using commercial enzyme-linked immunosorbent assay (ELISA) tests for TOSV IgG and IgM specific antibodies (Enzywell Toscana Virus – DIESSE Siena, Italy) and anti-West Nile antibody using SERION ELISA classic West Nile Virus IgG/IgM (Institut Virion\Serion GmbH, Wurzburg, Germany).

West-Nile and Chikungunya Virus RNA genome search was performed, respectively, with WNV ELITE MGB Kit (Elitech, Puteaux, France) and Chikungunya Virus Real Time RT-PCR Kit (Liferiver, Shanghai, China) and Usutu genome search with a home-made Real-time RT-PCR (13, 14). Confirmatory test was performed with ELISA Test (as above) and virus neutralization. Virus neutralization was performed on Vero cell monolayers by plaque reduction neutralization tests (PRNT). Neutralization antibody titres were calculated as the reciprocal of the serum dilution that gave an 80% reduction of the number of plaques (NT80), as compared to the virus control. Titres  $\geq 10$  were considered positive and all the 7 sera sent to the ISS had a titre PRNT80  $\geq 1:10$ .

All patients corresponding to the case definition during the period July-September 2018 in the database of the Virology Unit of Pisa were included in the study.

Hospital records of hospitalized patients in Livorno hospital (referral center for Infectious disease of Leghorn Province and Elba Island) were consulted to collect clinical information. In particular, data were collected in an anonymous form using a standard set of variables. The variables extracted were sex, age, clinical diagnosis, date of beginning of symptoms, use of

Emergency Departments (ED) services, use of intensive care unit, characteristics of symptoms, characteristics of liquor, date of hospitalization, number of days of hospitalization, health status at discharge, risk factors of the subject. Epidemiological information was obtained from the LHA investigation report. Telephonic interviews with patients were performed in February-March 2019 using a standard questionnaire to make a 6-month follow-up and collect additional information.

## Results

In September 2018 a cluster of seven cases of TOSV meningo-encephalitis, with onset between 30<sup>th</sup> of August and 12<sup>th</sup> of September, was reported to the LHA in Livorno. A retrospective analysis of laboratory records led to the identification of a previous cluster of five patients with onset between 4<sup>th</sup> and 12<sup>th</sup> July in the same area (Figure 1). The LHA launched an outbreak investigation in September 2018.

Eight of the twelve cases were males, aged 22-51 years (median 41.5); eight were resident in Tuscany (Table 1).

All patients scored positive for anti-TOSV IgM and IgG in serum samples. Five patients were tested for the presence of the viral genome and scored negative (Table 1). All patients scored negative for West Nile virus antibodies, one and two patients were also negative for Usutu virus and Chikungunya virus antibodies, respectively. Serum samples obtained from the seven patients belonging to the second cluster underwent confirmatory testing and scored positive both with ELISA and neutralization assays.

As for the interviews, one patient refused to participate, and one could not be contacted. Respondents reported having been, for work or leisure, in the south-eastern part of Elba Island or in rural areas of South Tuscany (Province of Livorno) (Figure 2). In particular, 8 of the 11 cases for whom the information was available had been on the Elba Island during the 15 days before symptoms onset. The most common reported

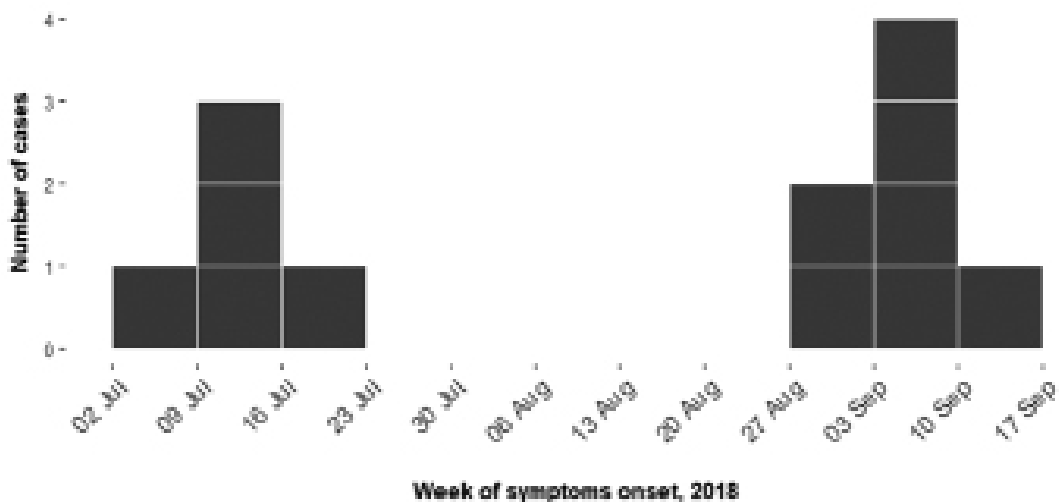


Figure 1 - Confirmed cases of TOSV meningo-encephalitis, Tuscany, Italy, 4 July -12 September 2018

Table 1 - Demographic, clinical, and epidemiological characteristics of cases

Case	Sex	Age (y)	Onset (Week)	Molecular diagnosis and serology(Blood)	Clinical presentation	Healthcare services	Risk factors
1	M	33	27	TOSV IgM+ TOSV IgG + TOSV genome –	Aseptic meningitis, asthenia, low fever, headache, nausea and vomiting, photophobia, arthralgia	2 ER accesses 7d Hospital stay	Resident in Livorno district, hiking in naturalistic area
2	F	43	28	TOSV IgM+ TOSV IgG + TOSV genome –	Aseptic meningitis, asthenia, high fever, headache, photophobia phonophobia, arthralgia, rigor nuclealis, vertigo	1 ER access 6d Hospital stay	Resident in Livorno, gardening, beach attendance
3	F	45	28	TOSV IgM+ TOSV IgG + TOSV genome –	Aseptic meningitis, asthenia, drowsiness, low fever, headache, photophobia arthralgia, rigor nuclealis, vertigo	1 ER access 5d Hospital stay	Resident on Elba Island, beach attendance, self-reported exposure to sandfly bites
4	M	46	28	TOSV IgM+ TOSV IgG +	Aseptic meningitis, asthenia, high fever, headache, nausea and vomiting, vertigo, otalgia	2 ER access 5d Hospital stay	Foreign visitor on Elba island
5	M	27	29	TOSV IgM+ TOSV IgG +	NA	1 ER access, Ambulatory care	NA
6	M	30	35	TOSV IgM+ TOSV IgG +	Aseptic meningitis, asthenia, drowsiness, low fever, photophobia, headache, rigor nuclealis	1 ER access, 5d Hospital stay	Boat storage worker, attendance of beach on Elba and Capraia islands
7	M	42	35	TOSV IgM+ TOSV IgG +	Aseptic meningitis, asthenia, low fever, photophobia, headache, rigor nuclealis	1 ER access, 5d Hospital stay	Boat storage worker, attendance of beach on Elba island 10d before onset
8	F	23	36	TOSV IgM+ TOSV IgG +	Aseptic meningitis, asthenia, low fever, headache, nausea and vomiting, photophobia, rigor nuclealis	2 ER access, 4d Hospital stay	Seasonal worker in an equestrian center on Elba island
9	M	51	36	TOSV IgM+ TOSV IgG +	Aseptic meningitis, asthenia, high fever, headache, nausea and vomiting, photophobia, arthralgia, vertigo, rigor nuclealis, delirium	1 ER access 7d Hospital stay	Resident in Livorno district, holiday on Elba island 15d before onset
10	F	41	36	TOSV IgM+ TOSV IgG +	Aseptic meningitis, asthenia, low fever, headache, nausea and vomiting, photophobia, arthralgia, vertigo	1 ER access, Ambulatory care	Resident in Livorno district, holiday on Elba island 7d before onset
11	M	25	36	TOSV IgM+ TOSV IgG + TOSV genome –	Aseptic meningitis, asthenia, high fever, headache, arthralgia, rigor nuclealis	1 ER access, 5d Hospital stay	Seasonal worker in an equestrian center on Elba island 2 w before onset
12	M	72	37	TOSV IgM+ TOSV IgG + TOSV genome –	Aseptic meningitis, asthenia, drowsiness, low fever, arthralgia	1 ER access, 10d hospital stay	Resident in Livorno district, gardening

activities were attendance of beaches and gardening. Possible occupational exposures were also reported, including horse riding training (2 cases) and boat storage (2 cases). One case was reported in a foreign traveler. All patients accessed ED with a clinical presentation compatible with aseptic meningitis. Three cases accessed ED twice. All but two patients were hospitalized, with a median stay of 5 days. Corticosteroid therapy and hydration were provided to all during hospitalization. A patient had an exacerbation of atrial fibrillation and was transferred to the cardiology unit. No fatalities were recorded and, at 6-month follow-up, 8 out of the 10 respondents had fully recovered, while two reported mild symptoms (asthenia, moderate visual impairment).

When asked, only two cases reported previous knowledge of TOSV. Increasing awareness and knowledge of the disease and of existing preventive measures not only among residents but also among clinicians was identified as important by all respondents.

## Discussion

Here we described the epidemiology and impact of two clusters of TOSV meningo-encephalitis occurred in Tuscany during summer 2018, involving both resident and visitors. Elba island and the South of Tuscany are important touristic destinations that attract seasonal workers and both national and international travelers, which may be at risk of contracting the infection. Despite the general increase of arbovirus-related burden of disease in Italy (13, 14) and the characteristics of TOSV epidemiology in Tuscany and central Italy at large (15), awareness is quite limited among the population. Targeted information campaigns for residents and visitors would be needed to inform about the disease and existing preventive measures to avoid sandfly - and other vectors- bites, such as repellants or long-sleeved tops and trousers (16). Similar information should be provided to travelers planning to stay in rural or seashore areas of Elba and south of Tuscany in summer season. As noted elsewhere(9, 10), TOSV

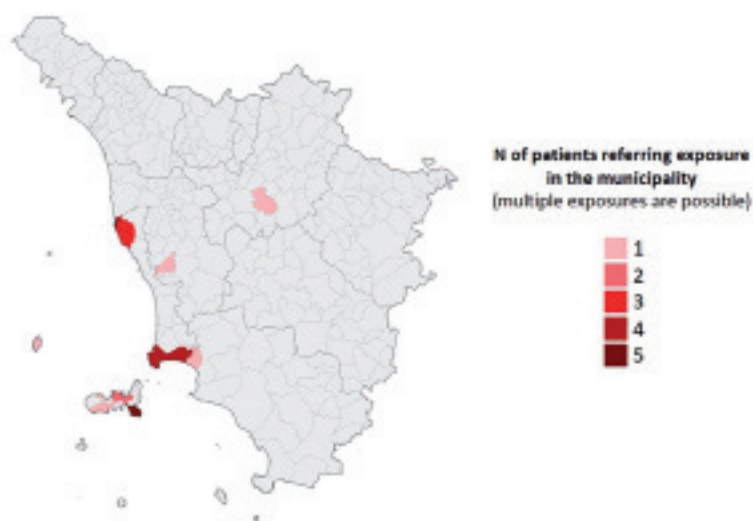


Figure 2 - TOSV outbreak: probable exposure locations

should be considered as a differential diagnosis for people presenting with aseptic meningitis symptoms, including returning travelers reporting a stay in Tuscany.

The two clusters of TOSV cases we described determined a significant use of healthcare resources for a preventable disease in terms of access to primary care service, ED, transfer to specialized care, and hospitalization days. Repeated access to ED may suggest that awareness of the disease among healthcare workers and/or referral system could be improved to increase system efficiency and quality of care.

Surveillance is a key element of the public health strategy to control vector-borne diseases. In 2016 the Italian Ministry of Health issued a circular letter on the “Surveillance of Arboviroses different from Chikungunya, Dengue, Zika and West Nile” (17), promoting the notification of human symptomatic cases, including TOSV. Yet, the identification of the cluster occurred in July was not timely and only subsequent to a retrospective case review prompted by the second cluster. Better knowledge of the importance of arboviruses surveillance among clinicians and public health officers is paramount, even more so when considering the growing relevance of vector-borne disease threats. Moreover, notification to LHA of positive results from microbiological laboratory could be an effective approach to report already collected data in order either to start or to facilitate the investigation of arbovirus outbreaks.

Due to the retrospective nature of our investigation, we could not perform vector surveillance to understand the entomological factors behind the clusters. However, presence of known TOSV vectors such as *Phlebotomus perniciosus*, *perfiliewi*, *papatasi* has already been reported in southern Tuscany (18). Vector control interventions (insecticide spraying) in highly frequented areas remains the most effective preventive option available, alongside with

personal protection (16). While we could not investigate the coverage and extension of local vector control activities during the 2018 summer season, disinfestation may need to be considered and possibly monitored at least in more touristic areas.

Impact of climate change on the distribution of sandflies species may lead to an increase of the population at risk of sandfly-borne phleboviral diseases (16). The growing rates of travel, transport, and international trade could further help the spread of imported viruses, vectors, and infected hosts (19).

Our study has some limitations. First of all, the nature of the disease and the large proportion of asymptomatic cases did not allow us to fully assess the extent of the two clusters. Due to the lag time between onset of diseases and patients' interviews, recall bias may have influenced reliability of responses. Secondly, the retrospective identification of the first cluster limited the possibility to perform advanced virological assays on a subset of samples or test samples. It was not possible to retest patients at the convalescent stage in order to confirm the diagnosis, based on demonstration of neutralising antibodies. Finally, molecular characterization of the pathogen was not feasible.

In conclusion, in the context of a surge in vectors-borne diseases reported in Italy in recent years (13, 14), TOSV infections and other phleboviruses are an issue of concern for public health, which may require additional investments in surveillance and control activities.

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## Riassunto

### *Due cluster di meningo-encefalite da virus Toscana in provincia di Livorno e all'isola d'Elba, Luglio-Settembre 2018*

**Introduzione.** Il virus Toscana (TOSV) è un arbovirus trasmesso all'uomo da flebotomi appartenenti al genere *Phlebotomus*. Provoca meningite asettica e meningo-encefalite con spiccata stagionalità. Descriviamo qui di seguito le caratteristiche cliniche, microbiologiche ed epidemiologiche di due cluster di casi avvenuti in Toscana nel 2018.

**Metodi.** Un caso confermato è stato definito come la rilevazione di IgM e IgG anti-TOSV nel siero, in presenza di manifestazioni cliniche tipiche. Abbiamo consultato le cartelle cliniche dei pazienti ricoverati per raccogliere informazioni cliniche e integrato le informazioni dell'indagine epidemiologica dell'autorità sanitaria locale. Abbiamo inoltre intervistato telefonicamente i pazienti utilizzando un questionario standard per un follow up a 6 mesi.

**Risultati.** Un totale di 12 casi di meningoencefalite TOSV con insorgenza tra il 4 luglio e il 12 settembre hanno avuto accesso ai servizi sanitari della provincia di Livorno. Otto casi erano maschi con età media 41,5 anni e quattro non erano residenti nell'area. Le indagini virologiche hanno confermato la recente infezione da TOSV. Otto casi hanno riferito di aver visitato l'Isola d'Elba e 4 hanno avuto una possibile esposizione professionale.

**Conclusioni.** Questo aumento dell'infezione sottolinea la necessità di campagne informative e di adeguati interventi di sorveglianza e controllo contro il TOSV che, insieme agli altri arbovirus, è un problema in crescita in Italia.

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