Toscana Virus Infection Clinical Characterization

Nazli Ayhan[1,2*], Carole Eldin[1,3], Remi Charrel[1,3,4]

[1]Unité des Virus Émergents (UVE: Aix-Marseille Univ, Università di Corsica, IRD 190, Inserm 1207, IRBA), France.

[2]Centre National de Référence des Arbovirus, Inserm-IRBA, Marseille, France.

[3] Laboratoire des Infections Virales Aigues et Tropicales, Pole des Maladies Infectieuses, AP-HM Hopitaux Universitaires de Marseille, France

[4] Le Service de Prévention du Risque Infectieux (LESPRI), CLIN AP-HM Hôpitaux Universitaires de Marseille, France

*Corresponding Author : Unité des Virus Émergents (UVE) Méditerranée Infection, 19-21, Bd. Jean Moulin 13005 Marseille, Tel: +33(0)4 13 73 21 51, nazli.ayhan@univ-amu.fr

Abstract

Background: Toscana virus (TOSV) is a sandfly-borne phlebovirus causing central nervous system (CNS) infection in Mediterranean countries, during summer season. However, clinical aspects of the disease caused by this virus are poorly known by clinicians, so that its prevalence is probably underestimated due to a lack of diagnosis.

Study design: We gathered data from all available case series and retrospective studies identifying TOSV as the causative viral agent. The informations of age, sex, clinical characteristics, laboratory findings, imaging results and clinical outcomes of TOSV infection were recorded and analyzed.

Results: In our review a total of 95 articles including TOSV infections resulting in a total of 1,381 cases were analyzed. Our findings indicate, TOSV affects individuals across various age groups, with a median age of 44.45 years. A notable disparity in infection rates between genders, with men being significantly more likely to present symptoms due to TOSV than women, with a sex ratio of 2.0 (p<0.001). The

clinical presentation of TOSV infection encompasses a range of symptoms, including fever, headache, retro-orbital pain, neurological and muscular manifestations with less common reports of cutaneous and gastrointestinal symptoms. To date, six fatalities have been attributed to TOSV infections, with a median age of 76 years.

Diagnostic evaluation of TOSV infections often involves the analysis of cerebrospinal fluid, where findings may include an elevated white blood cell count.

Conclusions: These findings underscore the diverse clinical manifestations of TOSV infections and highlight the importance of considering this pathogen in the differential diagnosis of patients presenting with acute febrile illness, especially in endemic regions. TOSV represents an emerging infectious threat that warrants inclusion in the diagnostic protocols for patients presenting with CNS, particularly within the Mediterranean basin or for those with recent travel history to endemic regions during warmer months when sandflies are actively circulating.

Introduction

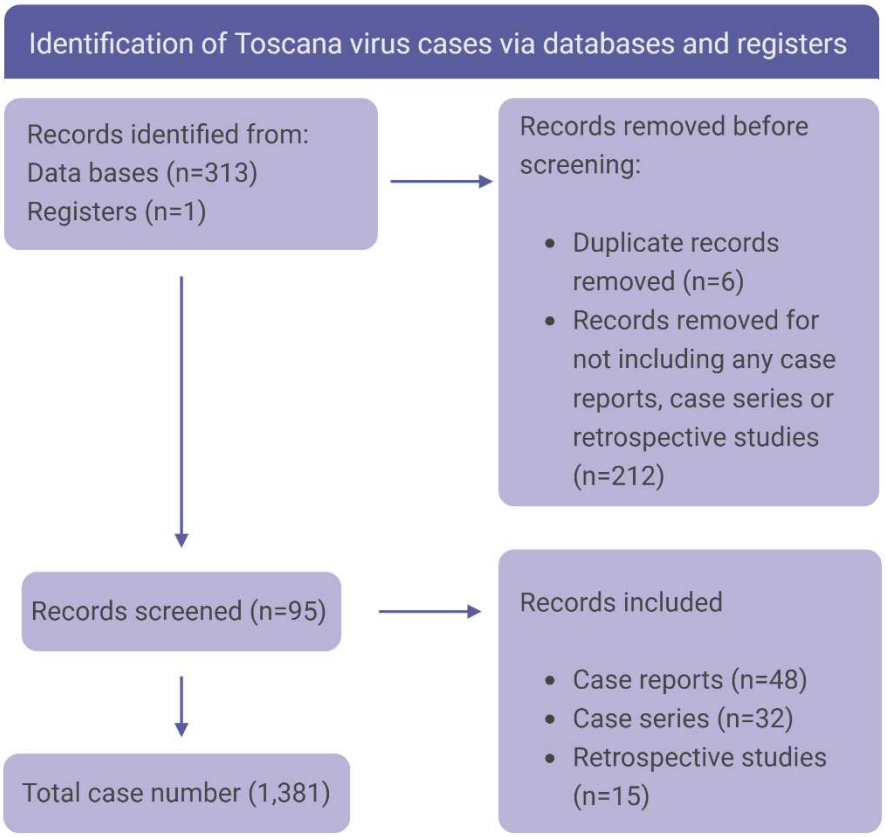
Toscana virus (TOSV) is classified within the *Phlebovirus toscanaense* species of the *Phenuiviridae* family within the *Bunyavirales* order. TOSV is recognized as a significant human pathogen prevalent in the Mediterranean region (1). Recent studies, including Medlock et al. (2) have documented a concerning increase in the population density of blood-feeding insects, along with their dissemination into territories previously considered free of sand flies. This expansion of the sand fly habitats substantially increases the risk of TOSV exposure among human population. Cases of TOSV infection have been reported in various Mediterranean countries such as; Italy, Spain, Portugal, France, Türkiye, Croatia, Greece, Algeria, Tunisia (3). Seroprevalence studies in both human and non-human vertebrates have revealed significant infection rates, with high prevalence reported in Italy (19.8%) (4), Türkiye (17.8%) (5), Greece (21%) (6), North Africa (22%-41%) (7); and the Balkans (37.5%) (8) indicating the widespread presence of TOSV across the Mediterranean basin. A recent retrospective study in Germany by Dersch et al. (2021) identified cases of TOSV-neuroinvasive disease in patients with meningoencephalitis with no recent travel history to endemic areas, suggesting a broader geographical spread than previously thought (9). TOSV is listed in the first three viral agents causing neurological infection at least in Italy, Spain and France together with enteroviruses and herpesviruses during warm season (10). However, the rare inclusion of TOSV in the diagnostic algorithm of nervous system infections (CNS) infections results in an important underestimation of the incidence (3).

The objective of our study was to review the available data about clinical characteristics of TOSV infections to raise the awareness of physicians about this emerging pathogen.

63 # Material and Method

64 ## Study design

65 Relevant entries in global Web-based resources that comprise Scopus (http://www.scopus.com/),
66 Web of Science (https://isiknowledge.com), and PubMed (https://pubmed.ncbi.nlm.nih.gov), Google
67 scholar (https://scholar.google.com.tr/) were searched. Database investigations were performed
68 using the keywords “bunyavirus”, “phlebovirus,”, “Toscana virus”, “TOSV”, “Toscana virus case
69 report”, ”nervous system infection”. Reports unrelated to TOSV infection were omitted, as well as
70 conference reports with recurring data in publications. The references cited in each report were
71 examined for further publications, which were included in the analysis (Figure1).



72

73 **Figure2.** Toscana virus local and imported case distribution map.

Statistical Analysis

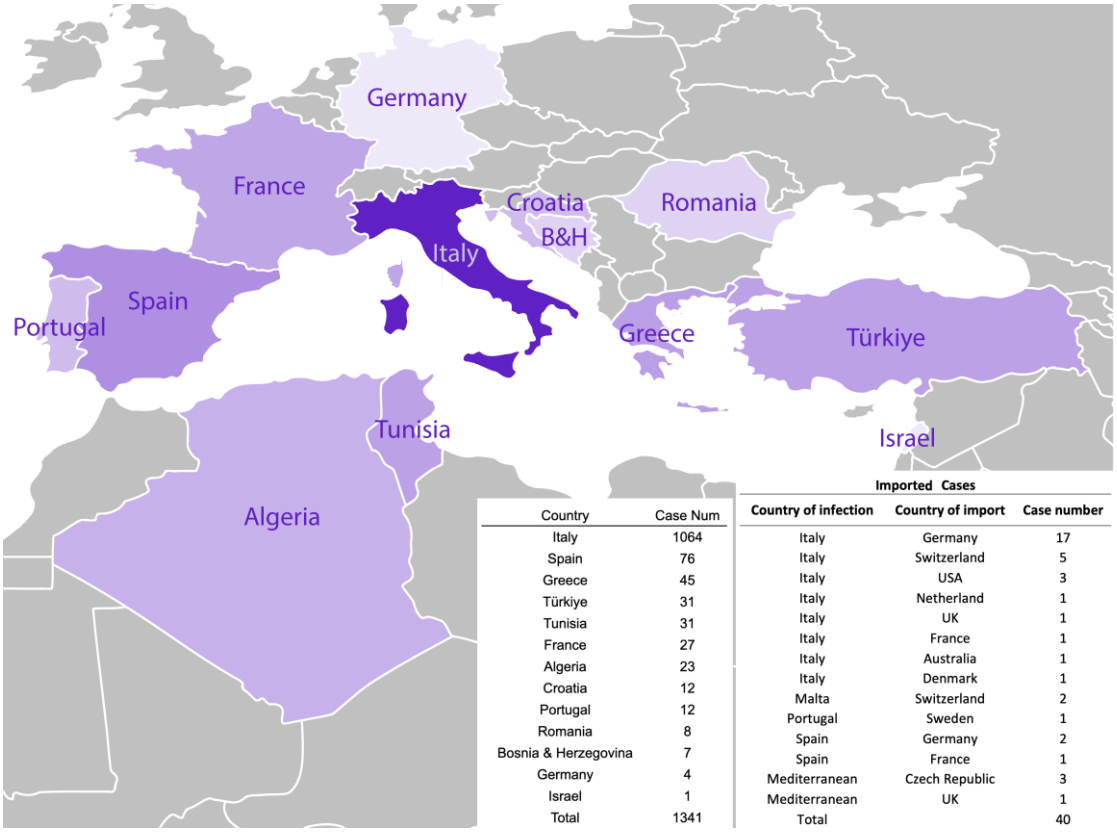
All variables (described in Table 1) were screened using a logistic regression univariate analysis to check for statistically significant associations of demographic characters with TOSV in SPSS version 24.

Results

Demographic and geographic characteristics

We conducted an inventory of 95 articles including 48 case reports, 32 case series and 15 retrospective studies, documenting a total of 1,381 cases of TOSV infection cases between 1985 and 2023. Age and sex demographics were obtained for 762 and 1,115 patients respectively (Table 1). Significantly more male patients were reported with TOSV infection compared to female patients (744 males to 371 females; $p<0.001$).

TOSV infections were recorded in 12 countries: with the majority of cases originating from Italy (n=1064), followed by Spain (n=76), Greece (n=45), Türkiye and Tunisia each with (n=31), France (n=27), Algeria (n=23), and lesser numbers from Croatia (n=12), Portugal (n=12), Romania (n=8), Bosnia & Herzegovina (n=7), Germany (n=4) and Israel (n=1). A total of 40 travelers returning from Mediterranean countries were reported, of which 30 had visited Italy, including both mainland and islands (Figure2).



Country	Case Num
Italy	1064
Spain	76
Greece	45
Türkiye	31
Tunisia	31
France	27
Algeria	23
Croatia	12
Portugal	12
Romania	8
Bosnia & Herzegovina	7
Germany	4
Israel	1
Total	1341

Imported Cases

Country of infection	Country of import	Case number
Italy	Germany	17
Italy	Switzerland	5
Italy	USA	3
Italy	Netherland	1
Italy	UK	1
Italy	France	1
Italy	Australia	1
Italy	Denmark	1
Malta	Switzerland	2
Portugal	Sweden	1
Spain	Germany	2
Spain	France	1
Mediterranean	Czech Republic	3
Mediterranean	UK	1
Total		40

Figure2. Toscana virus local and imported case distribution map.

TOSV infections were reported across a wide range of age groups, with the youngest patient being between 0-5 year old (11) and the oldest between 90-95 year old (12); the median age of patients was 44.4 years. The disease manifested in 82 pediatric patients under 15 years of age, as documented in nine studies (11–19). Notably, the prevalence of TOSV infection in children was significantly lower, at 5.9%.

Clinical characteristics

Typical manifestations

Retro-orbital pain was the most common symptom (156/163, 95.7%). Then headache was observed in 93.2% of cases (566/607) sometimes reported as the “worst headache of their life”. Fever was described in 91.9% (678/738) of cases with an abrupt onset and temperature ranging from 38°C to 39.5°C. Neck rigidity was reported in 88.2% (473/536). Other non-specific signs such as

nausea/vomiting, muscle weakness, asthenia and fatigue were described in almost 80% of cases (Table 1).

Table 1. Demographic characteristics and non-specific clinical signs.

	Number of cases	Total	%
Age, median [range]	44.45	762	
Sex (male/female)	744/371	1115	
Signs			
Fever	678	738	91.9
Headache	566	607	93.2
Nausea/Vomiting	340	431	78.9
Neck rigidity	473	536	88.2
Muscle weakness / Asthenia / Fatigue	34	43	79.1
Retro-orbital pain	156	163	95.7

Neurological manifestations

Of the 644 documented cases, 519 patients (representing 80.6%) presented with meningitis or aseptic meningitis, while 111 patients presented with either pure encephalitis or meningo-encephalitis (Table 2); myelitis was very rarely reported. Until recently, a total of 49 cases were recorded as meningitis/meningo-encephalitis. More recently, Mellace et al. (2022) identified 331 cases featuring neurological symptoms; however, the distinction between meningitis and encephalitis/meningoencephalitis was not clearly defined.. As a consequence, although central manifestations are not unusual, pure meningitis is the most prominent neurological manifestation of TOSV infections.

116 **Table 2. TOSV neurological manifestations.**

	Number of cases	Total analysed cases	%
Clinical Presentations			
Meningitis /Aseptic Meningitis	519	644	80.6
Encephalitis	77	241	32.0
Meningoencephalitis	34	221	15.4
Meningitis/Aseptic Meningitis or Meningoencephalitis	49		
Myelitis	2		
Total	684+331* = 1015	1382	73.44

117 * However recent article include 331 patients with meningitis, meningoencephalitis, encephalitis and polineuropatia didn't

118 included the number count due to lack of information for each clinical presentation but included in total count (19).

119 Three cases of hydrocephalus were documented in young patients (range between 15-25 year-old); in

120 all cases, hydrocephalus was observed as a complication following viral meningoencephalitis (20,21).

121 Specific neurological manifestations were categorized based on their involvement of either the central

122 or peripheral nervous system, as detailed in Table 3.

123 **Table 3. TOSV neurological signs and symptoms.**

Neurological Manifestations:
signs and symptoms denoting a central nervous system involvement
abnormal behavior, agitation, anhedonia irritability, aphasia, ataxia, blurred vision, cerebral atrophy, confusion, depression, double vision, dysmetria, epilepsy, facial paresis, focal neurological deficit, hallucinations, hemiparesis/paresis, hydrocephalus, hypotonia, impaired consciousness, insomnia, nystagmus, obtundation, occipital/ischemic stroke, paresis, paresthesia, phonophobia, photophobia, kernig's sign, seizure, sleepiness, stumbling and dysmetria, tetra paresis, tonic-clonic seizure, tremor

signs and symptoms denoting a peripheral nervous system involvement
brachial plexitis, Guillain Barré like syndrome, hyperaesthesia, limited atrophy, motor ans sensory neuropathy, peripheral polyneuropathy,

Out of 712 patient, 569 exhibited at least one neurological signs or symptoms listed in Table3. Among central neurological manifestations, Kernig's sign was the most prevalent, observed in 135 cases, followed by decreased consciousness and photophobia (n=41), facial or leg hemiparesis /paresis (n=17) and confusion (n=16). Speech impairmentwas reported in eight patients(22–28) while six patients experienced hearing impairment, characterized by bilateral deafness that persisted beyond the acute phase and 6 patients reported hearing impairment (29–32). Additionally, changes in personality, sexual and social disinhibition, aggressiveness, and other abnormal behaviors were also documented (33,34) (Table2). The presence of symptoms signing a peripheral neuropathy was observed in 14 cases of which 10 presented with Guillain-Barre like syndrome (35–37).

Ocular manifestations

Retro-orbital pain or pressure were very frequently observed, documented in 156 out of 163 cases. In contrast, conjunctivitis was reported only in a single case (38) (Table4).

Gastro-intestinal manifestations

Gastro-intestinal manifestations consisting of gastroenteritis, abdominal pain, dysphagia or diarrhea were reported rarely (16/43). (20,37,39–41) (Table4).

Cutaneous manifestations

Dermatological symptoms including petechiae, rash, exanthema, and febrile erythema were reported in 15 out of 38 cases. Rash was the most frequently observed symptom, noted in 11 cases (20,29,39,41–45) (46) (Table4).

Muscular manifestations

The musculoskeletal symptoms observed included cramps, myalgia/arthralgia, myositis/fasciitis, and muscle stiffness. Among the 51 patients who exhibited these symptoms, all presented with myalgia and/or arthralgia (12,18,22,35,38,44,45,47–53) (Table4).

Table 4. Number of TOSV cases with muscular, ocular, gastro-intestinal, cutaneous and neurological manifestations.

	Number of cases	Total	%
Muscular Manifestations	51	211	24,2
Myalgia / Arthralgia	51	211	24,2
Ocular Manifestations	157	164	95,7
Retroorbital pain or pressure	156	163	95,7
Gastro-intestinal Manifestations	16	43	37,2
Gastroenteritis	7	16	43,8
Cutaneous Manifestations	15	38	39,5
Rash	11	32	34,4
Neurological Manifestations	569	712	79.9
Central Nervous System Manifestations	560	683	82
Peripheral Nervous System Manifestations	14	26	53,8

Testicular manifestations

Testicular manifestations, including epididymo-orchitis, testicular pain, and swelling, were reported in five patients (20,39,41,54,55) (Table 5). These symptoms were significantly more frequent (p-value <0.001) among younger patients, with a median age of 27 years (range 16-44) (Table 5). Recently, TOSV RNA was detected in the seminal fluid of a patient in the age range of 20-25 year old until day 59 after infection without any testicular manifestations (28).

Table 5. TOSV infection cases with testicular manifestations.

Characteristic / References	Age range / Age median [range] (n)	Fever	Headache	Neck rigidity / pain	Nausea / vomiting
Echevarria et al. 2003	25-29	NR*	NR*	NR*	NR*
Baldelli et al. 2004	15-19	1	1	1	1
Zanelli et al. 2013	25-29	1	1	-	-
Tschumi et al. 2019	20-24	1	1	-	-
Mascitti et al. 2020	40-44	1	1	-	-
	27 [16-44] (5)	4	4	1	1

*, NR, not reported

Severe cases and Mortality

Although TOSV infections are frequently associated with severe clinical manifestations, the vast majority of patients recover completely. Nonetheless, six fatal cases have been reported across two studies: the first fatality occurred in Italy (31); and five subsequent fatalities were reported in Romania (31,49). The overall the mortality rate is 0.43% with deceased patients having a median age of 76 years-old (range 70-91). Age was found to have a statistically significant impact on mortality (*p*-value <0.001). In addition, five out of six fatal cases had comorbidities such as hypertension and diabetes (Table6).

There were nine cases of severe TOSV infections leading to coma (20,31,49,56). Seven of these were elder patients with median age of 79, six of whom had comorbidities such as diabetes and hypertension and two patients were two siblings with the age range between 15 to 20, previously mentioned as having developed hydrocephalus following infection.

Table6. TOSV infection lethal cases.

Reference	Nb of case	Sex	Age Range	Country	Comorbidities	Period	In-hospital length of stay (days)
Bartels et al. 2012	1	male	70-74	Italy	-	-	14
Popescu et al. 2021	1	male	90-94	Romania	Hypertension, congestive heart failure, ischemic heart disease, stroke sequelae	June	10
	1	female	65-69	Romania	Hypertension, diabetes mellitus, obesity, NonHodgkin lymphoma	August	4
	1	male	75-79	Romania	Diabetes mellitus type II, Ischemic heart disease	August	19
	1	male	70-74	Romania	Diabetes mellitus type II, Ischemic heart disease, Atrial fibrillation, Congestive heart failure, Diabetic polyneuropathy and arteriopathy	August	4
	1	female	85-89	Romania	Hypertension, Stroke sequelae, Chronic renal failure	August	6

175

176

177 *Laboratory characteristics*

178 Cerebrospinal fluid (CSF) analysis showed elevated white blood cell (WBC) levels in 22 cases.

179 Lymphocytic predominance ratio was high in 82% of the patients (47 out of 57) (>50%). CSF samples

showed high protein levels in 102 patients. Mildly elevated glucose levels have been showed in 179 patients (Table 7).

Table 7. TOSV laboratory characteristics.

Laboratory Findings	Mean (n) [range]	Normal range/percentage
CSF protein level (mg/dL)	56.34 (77) [11-757]	15-45
CSF WBC (cells/mm3)	432.50 (22) [6-3500]	0-5
CSF Lymphocytes ratio (%)	73.40% (57) [18%-100%]	50%
CSF Glucose (mg/dL)	63.21 (60) [29-132]	>40

Electro-physiology analysis

The electroencephalogram (EEG) showed abnormal results in 43 patients (11,23,31,56–62) with non-specific abnormalities, occasional spikes and slow waves (58).

Imaging and Radiological findings and electrocardiogram tests

When available, magnetic resonance imaging (MRI) results showed diffuse encephalopathy, diffuse atrophy abnormalities, diffuse bilateral asymmetric myositis or scattered punctuate T2-hyperintense non-enhancing white matter lesions in 11 patients (21,27,45,53,63). Cerebral atrophy and/or occipital stroke sequelae were detected in 2 patients with computerized tomography (CT) scan (49).

Few patients were examined by with electrocardiogram (ECG) and chest X-ray. Chest X-ray demonstrated an opacity in the right middle lobe of the lung of one patient (29). No abnormalities were recorded with ECG after TOSV infection (Table8).

Table8. TOSV electro-physiology analysis and radiology findings.

Electro-physiology analysis and radiology findings	Number of abnormal cases	Total
EEG	43	82
MRI	11	30
CT	3	30
Chest X-ray	1	8
ECG	0	3

Discussion

Infections caused by TOSV exhibit a high diversity of clinical manifestations, none of them being neither pathognomonic nor highly specific. It is crucial to provide a comprehensive analysis of the observed signs in order to improve the clinical orientation for physicians seeing both residents and visitors in the Mediterranean regions during the warm season when TOSV can be transmitted by sand flies (Figure1). Unlike other neuroinvasive viruses such as herpes viruses and enteroviruses, TOSV infections occur exclusively during the period of activity of phlebotomine sand flies, typically from March to November in the Mediterranean, with slight variations depending on local climate conditions (64).

From 1985 to2023, a total of 1,381 cases were reported from 12 different countries of which the majority (1,064 cases, 77%) occurred in Italy. The annual incidence rate of cases fluctuates considerably in relation with complex biology of vectors, hosts and environmental factors. TOSV infections have been documented across a broad age spectrum (median age 44.45 years, ranging from 0 to 95 years old); however, age itself is not indicative of etiology, though most cases occur between 20 and 60 years old. Using data collected in travelers returning to their homeland, the median incubation period was

calculated at 12 days (10-14 days) for the neuroinvasive forms (65). Whether the same incubation period applies for milder cases remains unexplored.

TOSV symptomatic infections are observed twice as frequently in males as in females. The reasons for this gender disparity in symptomatology could be related to differences in immune response, viral susceptibility, or possibly due to variations in exposure factors and the prevalence of high-risk behaviors. However, similar seroprevalence rates between males and females suggest that behavioral factors alone may not explain this difference (66). Interestingly, the same tendency is also observed for WNV infections (67).

Both length of hospitalization and symptoms duration vary significantly based on the severity of the case. The longest hospitalization was 60 days (68) and the longest symptoms duration was 28 days (52,69). The letality rate of TOSV infections is 0.43% which is lower than other neuroinvasive arboviruses such as WNV, Japanese encephalitis virus (JEV) or tick-borne encephalitis virus (TBEV). All the fatal cases were older than 60 year-old; five of the six fatal cases had comorbidities such as hypertension and/or diabetes mellitus.

Severe neuroinvasive forms, characterized by encephalitis or meningo-encephalitis, are reported in 15.8 to 32.0% of neurological cases, the latter representing almost 80% of 712 studied cases. Since TOSV cases reported in the literature are the most severe ones, neuroinvasive forms are certainly overrepresented. Common forms are frequent although they are likely to remain either undetected or unpublished (70); those forms are associated with unspecific clinical manifestations such as fever, headache, nausea, vomiting, retro-orbital pain and muscle weakness.

Severe long-lasting or permanent neurological sequelae such as consciousness impairment, hydrocephalus, ischemic stroke and aphasia (20,49,68,71,72) were described. In contrast with other arboviruses, severe neurological forms are not more frequent in the elderly (19). However, the lower incidence rate of TOSV infections in the pediatric group (5.9%) can either be explained by a lower exposure and/or by a reporting bias, or by biological factors such as immune response and/or viral susceptibility.

Among system specific manifestations, CNS signs are the most frequently observed with 79.9% of cases followed by ocular and muscular manifestations. Peripheral neurological manifestations, gastro-intestinal and cutaneous manifestations were reported rarely. Additionally, a unique case involved a patient with gastrointestinal manifestations who was also co-infected with West Nile virus (37). Although testicular manifestations are noteworthy, they have been documented in only five patients. To date, aside from the Zika virus (ZIKV), no other arbovirus has been identified as a causative agent for testicular manifestations. Recently, the presence and persistence of TOSV RNA in seminal fluid has been demonstrated without evidence for sexual transmission (28).

In most of cases presenting with clinical picture justifying CSF collection, CSF was clear and colorless. The CSF formula showed elevated WBC levels in 22 patients and lymphocytic meningitis in 58 patients. Mildly elevated protein and glucose levels were described in 102 patients and 179 patients respectively. Hypoglycorachia was never reported. CSF parameters were available only for a subset of the reviewed cases.

Electro-physiology analysis and radiology findings showed abnormalities in EEG or in MRI for 56 cases (4%). CT scan and chest X-ray were performed occasionally and abnormalities were rarely recorded. No abnormalities were recorded for ECG which was performed only for three patients (58,73,74)

In almost all cases, the recovery is complete without persisting functional sequelae. However, there are a few documented cases where individuals have experienced complicated forms of meningitis and/or encephalitis with lingering effects attributed to TOSV. These effects include impaired cognitive functions and altered social and sexual behaviors (60,68) Other neuroinvasive arboviruses, such as WNV, JEV or TBEV, cause long-lasting or permanent sequelae (75).

Finally, most TOSV infections remain undiagnosed, as TOSV is not included in the list of pathogens to be screened in patients presenting with febrile illness and/or neurological manifestations in areas where the virus is endemic. The only exception is Italy where it is recommended to include TOSV in the

267 panel of viruses to be tested during summertime for suspect cases. This is likely why most cases are
268 reported in Italy besides the fact that TOSV has been discovered in Italy and that physicians are much
269 more aware of its existence than in other at risk countries. This oversight is highlighted by numerous
270 retrospective studies, which suggest that many cases of TOSV infection are classified as "infections due
271 to unknown pathogenic agents" due to the absence of specific laboratory screening for TOSV
272 (35,51,76,77). Nevertheless, most TOSV cases exhibit nonspecific signs or symptoms with short
273 duration in non-severe cases.
274 Together, the combination of lack of awareness of physician with absence of recommendations to
275 screen suspect cases using specific laboratory tests has and continue to greatly contribute to the
276 underestimation of TOSV cases despite its public health importance.
277
278

279 ## Acknowledgement
280

281 This study was funded by European Commission grant 101057690 and UKRI grants 10038150 and
282 10039289, and is catalogued by the CLIMOS Scientific Committee as CLIMOS number XXXX* (http://
283 www.climos-project.eu). The contents of this publication are the sole responsibility of the authors and
284 do not necessarily reflect the views of the European Commission, the Health, and Digital Executive
285 Agency, or UKRI. Neither the European Union nor granting authority nor UKRI can be held responsible.
286 The funders had no role in study design, data collection and analysis, decision to publish, or preparation
287 of the manuscript. For the purposes of Open Access, the authors have applied a CC BY [option: CC BY-
288 ND] public copyright license to any Author Accepted Manuscript version arising from this submission.
289 CLIMOS forms part of the Climate Change and Health Cluster comprising six Horizon Europe projects:
290 BlueAdapt, CATALYSE, CLIMOS, HIGH Horizons, IDAlert, and TRIGGER.
291 *The number will be added after publication.
292

References

1. Charrel RN, Bichaud L, de Lamballerie X. Emergence of Toscana virus in the mediterranean area. World J Virol . 2012;1(5):135–41. DOI: https://doi.org/10.5501/wjv.v1.i5.135

2. Medlock JM, Hansford KM, Van Bortel W, Zeller H, Alten B. A summary of the evidence for the change in European distribution of phlebotomine sand flies (Diptera: Psychodidae) of public health importance. J Vector Ecol. 2014:72–7. DOI: https://doi.org/10.1111/j.1948-7134.2014.12072.x

3. Ayhan N, Charrel RN. An update on Toscana virus distribution, genetics, medical and diagnostic aspects. Clin Microbiol Infect. 2020;26(8). DOI: https://doi.org/10.1016/j.cmi.2019.12.015

4. Terrosi C, Olivieri R, Bianco C, Cellesi C, Cusi MG. Age-dependent seroprevalence of Toscana virus in central Italy and correlation with the clinical profile. Clin Vaccine Immunol. 2009;16(8):1251–2. DOI: https://doi.org/ 10.1128/CVI.00376-08

5. Ergunay K, Aydogan S, Ilhami Ozcebe O, Cilek EE, Hacioglu S, Karakaya J, et al. Toscana Virus (TOSV) exposure is confirmed in blood donors from Central, North and South/Southeast Anatolia, Turkey. Zoonoses Public Health . 2012;59(2):148–54. DOI: https://doi.org/10.1111/j.1863-2378.2011.01436.x

6. Anagnostou V, Papa A. Seroprevalence of Toscana virus among residents of Aegean Sea islands, Greece. Travel Med Infect Dis . 2013;11(2):98–102. DOI: https://doi.org/10.1016/j.tmaid.2012.11.006

7. Alkan C, Allal-Ikhlef AB, Alwassouf S, Baklouti A, Piorkowski G, de Lamballerie X, et al. Virus isolation, genetic characterization and seroprevalence of Toscana virus in Algeria. Clin Microbiol Infect . 2015;21(11):1040.e1-1040.e1. DOI: https://doi.org/10.1016/j.cmi.2015.07.012

8. Punda-Polic V, Jeroncic A, Mohar B, Kraljevic KS. Prevalence of Toscana virus antibodies in residents of Croatia. Clin Microbiol Infect. 2012;18(6):200–3. DOI:

https://doi.org/10.1111/j.1469-0691.2012.03840.x

9. Dersch R, Sophocleous A, Cadar D, Emmerich P, Schmidt-Chanasit J, Rauer S. Toscana virus encephalitis in Southwest Germany: a retrospective study. BMC Neurol . 2021;21(1):1–5. DOI: https://doi.org/10.1186/s12883-021-02528-7

10. Gori Savellini G, Gandolfo C, Cusi MG. Epidemiology of Toscana virus in South Tuscany over the years 2011-2019. J Clin Virol. 2020;128:104452. DOI: https://doi.org/10.1016/j.jcv.2020.104452

11. Braito A, Ciufolini MG, Pippi L, Corbisiero R, Fiorentini C, Gistri A, et al. Phlebotomus-transmitted toscana virus infections of the central nervous system: a seven-year experience in Tuscany. Scand J Infect Dis . 1998;30(5):505–8. DOI: https://doi.org/10.1080/00365549850161539

12. Papa A, Kontana A, Tsergouli K. Phlebovirus infections in Greece. J Med Virol . 2015;87(7):1072–6. DOI: https://doi.org/10.1002/jmv.24163

13. Mendoza-Montero J, Gamez-Rueda M-I, Navarro-Mari J-M, de la Rosa-Fraile M, Oyonarte-Gomez S. Infections Due to Sandfly Fever Virus Serotype Toscana in Spain. Clin Infect Dis . 1998;27(3):434–6. DOI: https://doi.org/10.1086/514684

14. Braito A, Corbisiero R, Corradini S, Fiorentini C, Ciufolini MG. Toscana virus infections of the central nervous system in children: A report of 14 cases. J Pediatr . 1998;132(1):144–8. DOI: https://doi.org/10.1016/S0022-3476(98)70500-1

15. Santos L, Simões J, Costa R, Martins S, Lecour H. Toscana virus meningitis in Portugal, 2002-2005. Euro Surveill . 2007;12(6):E3-4. DOI: https://doi.org/10.2807/esm.12.06.00715-en

16. Anagnostou V, Pardalos G, Athanasiou-Metaxa M, Papa A. Novel phlebovirus in febrile child, Greece. Emerg Infect Dis . 2011;17(5):940–1. DOI: https://doi.org/ 10.3201/eid1705.101958

17. Punda-Polić V, Mohar B, Duh D, Bradarić N, Korva M, Fajs L, et al. Evidence of an autochthonous Toscana virus strain in Croatia. J Clin Virol . 2012;55(1):4–7. DOI: https://doi.org/10.1016/j.jcv.2012.06.006

18. Dupouey J, Bichaud L, Ninove L, Zandotti C, Thirion-Perrier L, de Lamballerie X, et al. Toscana virus infections: A case series from France. J Infect . 2014;68(3):290–5. DOI: https://doi.org/10.1016/j.jinf.2013.11.006

19. Mellace F, Del Manso M, Oradini-Alacreu A, Ceccarelli E, Mateo-Urdiales A, Petrone D, et al. Meningiti, meningo-encefaliti ed encefaliti da virus Toscana in Italia, 2016-2021. Punta dell'iceberg di una arbovirosi endemica poco conosciuta. Boll Epidemiol Naz . 2024;3(2):10–9.

20. Baldelli F, Grazia Ciufolini M, Francisci D, Marchi A, Venturi G, Fiorentini C, et al. Unusual Presentation of Life-Threatening Toscana Virus Meningoencephalitis. Clin Infect Dis . 2004;38(4):515–20. DOI: https://doi.org/10.1086/381201

21. Oechtering J, Petzold GC. Acute hydrocephalus due to impaired CSF resorption in Toscana virus meningoencephalitis. Neurology . 2012;79(8):829–31. DOI: https://doi.org/10.1212/WNL.0b013e3182661f1

22. Calisher CH, Weinberg AN, Muth DJ, Lazuick JS. Toscana virus infection in United States citizen returning from Italy. Lancet (London, England) . 1987;1(8525):165–6. DOI:

23. Epelboin L, Hausfater P, Schuffenecker I, Riou B, Zeller H, Bricaire F, et al. Meningoencephalitis Due to Toscana Virus in a French Traveler Returning From Central Italy. J Travel Med . 2008;15(5):361–3. DOI: https://doi.org/10.1111/j.1708-8305.2008.00221.x

24. 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(11):1701–7. DOI: https://doi.org/ 10.3201/eid1111.050851

25. Kay MK, Gibney KB, Riedo FX, Kosoy OL, Lanciotti RS, Lambert AJ. Toscana virus infection in American traveler returning from Sicily, 2009. Emerg Infect Dis . 2010;16(9):1498–500. DOI: https://doi.org/ 10.3201/eid1609.100505

26. Greco F, Mauro MV, Tenuta R, Apuzzo G, Giraldi C. A new case of meningitis due to Toscana virus . Vol. 35, NEW MICROBIOLOGICA. 2012; 1:35(1):99.

27. Suardi LR, Di Lauria N, Pozzi M, Rogasi PG, Barilaro A, Azzolini F, et al. Acute cerebellar ataxia:

a rare Toscana Virus (TOSV) meningoencephalitis complication. 2019;130(3):276–8. DOI: https://doi.org/10.1080/00207454.2019.1673748

28. Matusali G, D'Abramo A, Terrosi C, Carletti F, Colavita F, Vairo F, et al. Infectious Toscana Virus in Seminal Fluid of Young Man Returning from Elba Island, Italy. Emerg Infect Dis . 2022;28(4):865. DOI: https://doi.org/ 10.3201/eid2804.211920

29. Howell BA, Azar MM, Landry ML, Shaw AC. Toscana virus encephalitis in a traveler returning to the United States. J Clin Microbiol . 2015;53(4):1445–7. DOI: https://doi.org/ 10.1128/JCM.03498-14

30. Ocal M, Orsten S, Inkaya AC, Yetim E, Acar NP, Alp S, et al. Ongoing Activity of Toscana Virus Genotype A and West Nile Virus Lineage 1 Strains in Turkey: A Clinical and Field Survey. Zoonoses Public Health . 2014;61(7):480–91. DOI: https://doi.org/10.1111/zph.12096

31. Bartels S, Boni L, Kretzschmar HA, Heckmann JG. Lethal encephalitis caused by the Toscana virus in an elderly patient. J Neurol . 2012;259(1):175–7. DOI: https://doi.org/10.1007/s00415-011-6121-y

32. Martínez-García FA, Moreno-Docón A, Segovia-Hernández M, Fernández-Barreiro A. [Deafness as a sequela of Toscana virus meningitis]. Med Clin (Barc) . 2008;130(16):639. DOI: https://doi.org/10.1157/13120347

33. Osborne JC, Khatamzas E, Misbahuddin A, Hart R, Sivaramakrishnan A, Breen DP. Toscana virus encephalitis following a holiday in Sicily. Pract Neurol . 2016;16(2):139–41. DOI: https://doi.org/10.1136/practneurol-2015-001265

34. Serata D, Rapinesi C, Del Casale A, Simonetti A, Mazzarini L, Ambrosi E, et al. Personality Changes After Toscana Virus (TOSV) Encephalitis in a 49-Year-Old Man: A Case Report. Int J Neurosci . 2011;121(3):165–9. DOI: https://doi.org/10.3109/00207454.2010.537412

35. Ocal M, Orsten S, Inkaya AC, Yetim E, Acar NP, Alp S, et al. Ongoing Activity of Toscana Virus Genotype A and West Nile Virus Lineage 1 Strains in Turkey: A Clinical and Field Survey. Zoonoses Public Health . 2014;61(7):480–91. DOI: https://doi.org/10.1111/zph.12096

36. Rota E, Morelli N, Immovilli P, De Mitri P, Guidetti D. Guillain-Barré-like axonal polyneuropathy associated with Toscana virus infection: A case report. Medicine (Baltimore) . 2017;96(38):e8081. DOI: https://doi.org/10.1097/MD.0000000000008081

37. Okar SV, Bekircan-Kurt CE, Hacıoğlu S, Erdem-Özdamar S, Özkul A, Ergünay K. Toscana virus associated with Guillain–Barré syndrome: a case–control study. Acta Neurol Belgica 2020 1213 . 2020;121(3):661–8. DOI: https://doi.org/10.1007/s13760-020-01279-5

38. Tappe D, Schmidt-Chanasit J, Günther S, Ries A, Ziegler U, Müller A, et al. Acute Toscana virus infection mimicked by Yersinia-induced reactive arthritis syndrome after journey to Spain. J Clin Virol. 2010;47(1):104–5.

39. Echevarría J-M, de Ory F, Guisasola M-E, Sánchez-Seco M-P, Tenorio A, Lozano Á, et al. Acute meningitis due to Toscana virus infection among patients from both the Spanish Mediterranean region and the region of Madrid. J Clin Virol . 2003;26(1):79–84. DOI: https://doi.org/10.1016/S1386-6532(02)00041-0

40. Anagnostou V, Pardalos G, Athanasiou-Metaxa M, Papa A. Novel phlebovirus in febrile child, Greece. Emerg Infect Dis . 2011;17(5):940–1. DOI: https://doi.org/ 10.3201/eid1705.101958

41. Zanelli G, Bianco C, Cusi MG. Testicular involvement during Toscana virus infection: an unusual manifestation? Infection . 2013;41(3):735–6. DOI: https://doi.org/10.1007/s15010-012-0368-9

42. Schultze D, Korte W, Rafeiner P, Niedrig M. First report of sandfly fever virus infection imported from Malta into Switzerland, October 2011. Euro Surveill . 201;17(27). DOI: https://doi.org/10.2807/ese.17.27.20209-en

43. Arden KE, Heney C, Shaban B, Nimmo GR, Nissen MD, Sloots TP, et al. Detection of Toscana virus from an adult traveler returning to Australia with encephalitis. J Med Virol . 2017;89(10):1861–4. DOI: https://doi.org/10.1002/jmv.24839

44. Dominati A, Sap L, Vora S. [Fever in a returning traveler from Tuscany]. Rev Med Suisse . 2018;14(592):294–6.

423 45. Vilibic-Cavlek T, Zidovec-Lepej S, Ledina D, Knezevic S, Savic V, Tabain I, et al. Clinical,
424 Virological, and Immunological Findings in Patients with Toscana Neuroinvasive Disease in
425 Croatia: Report of Three Cases. Trop Med Infect Dis 2020, Vol 5, Page 144 . 2020;5(3):144.
426 DOI: https://doi.org/10.3390/tropicalmed5030144
427 46. Gámbaro F, Pérez AB, Prot M, Agöera E, Baidaliuk A, Sánchez-Seco MP, et al. Untargeted
428 metagenomic sequencing identifies Toscana virus in patients with idiopathic meningitis,
429 southern Spain, 2015 to 2019. Eurosurveillance. 2023;28(45):2200913. DOI:
430 https://doi.org/10.2807/1560-7917.ES.2023.28.45.2200913
431 47. Nicoletti L, Renzi A, Caciolli S, Nicoletti L, Bartolozzi D, Balducci M, et al. Central Nervous
432 System Involvement during Infection by Phlebovirus Toscana of Residents in Natural Foci in
433 Central Italy (1977–1988). Am J Trop Med Hyg . 1991;45(4):429–34. DOI:
434 https://doi.org/10.4269/ajtmh.1991.45.429
435 48. Pauli C, Schwarz TF, Meyer CG, Jager G. [Neurological symptoms after an infection by the
436 sandfly fever virus]. Dtsch Med Wochenschr . 1995;120(43):1468–72. DOI:
437 https://doi.org/10.1055/s-2008-1055501
438 49. Mosnier E, Charrel R, Vidal B, Ninove L, Schleinitz N, Harlé JR, et al. Toscana virus myositis and
439 fasciitis. Med Mal Infect . 2013;43(5):208–10. DOI:
440 https://doi.org/10.1016/j.medmal.2013.04.002
441 50. Erdem H, Ergunay K, Yilmaz A, Naz H, Akata F, Inan AS, et al. Emergence and co-infections of
442 West Nile virus and Toscana virus in Eastern Thrace, Turkey. Clin Microbiol Infect .
443 2014;20(4):319–25. DOI: https://doi.org/10.1111/1469-0691.12310
444 51. Schirmer L, Wölfel S, Georgi E, Ploner M, Bauer B, Hemmer B. Extensive Recruitment of
445 Plasma Blasts to the Cerebrospinal Fluid in Toscana Virus Encephalitis. Open Forum Infect Dis .
446 2015;2(3):ofv124. DOI: https://doi.org/10.1093/ofid/ofv124
447 52. Popescu CP, Cotar AI, Dinu S, Zaharia M, Tardei G, Ceausu E, et al. Emergence of Toscana
448 Virus, Romania, 2017–2018. Emerg Infect Dis . 2021;27(5):1482. DOI: https://doi.org/

10.3201/eid2705.204598

53. Quattrone F, Mazzetti P, Aquino F, Sani S, Carneglia L, Pistello M, et al. Two clusters of Toscana virus meningo-encephalitis in Livorno Province and Elba Island, July-September 2018. Ann Ig . 2020;32(6):674–81. DOI: https://doi.org/10.7416/ai.2020.2387

54. Tschumi F, Schmutz S, Kufner V, Heider M, Pigny F, Schreiner B, et al. Meningitis and epididymitis caused by Toscana virus infection imported to Switzerland diagnosed by metagenomic sequencing: A case report. BMC Infect Dis. 2019;19(1). DOI: https://doi.org/10.1186/s12879-019-4231-9

55. Mascitti H, Calin R, Dinh A, Makhloufi S, Davido B. Testicular pain associated with clear fluid meningitis: How many cases of Toscana virus are we missing? Int J Infect Dis. 2020;1:93:198–200. DOI: https://doi.org/10.1016/j.ijid.2020.02.008

56. Kuhn J, Bewermeyer H, Hartmann-Klosterkoetter U, Emmerich P, Schilling S, Valassina M. Toscana virus causing severe meningoencephalitis in an elderly traveller. J Neurol Neurosurg Psychiatry . 2005;76(11):1605–6. DOI: https://doi.org/10.1136/jnnp.2004.060863

57. Braito A, Corbisiero R, Corradini S, Marchi B, Sancasciani N, Fiorentini C, et al. Evidence of Toscana virus infections without central nervous system involvement: A serological study. Eur J Epidemiol . 1997;13(7):761–4. DOI: https://doi.org/10.1023/A:1007422103992

58. Dionisio D, Valassina M, Ciufolini MG, Vivarelli A, Esperti F, Cusi MG, et al. Encephalitis without Meningitis Due to Sandfly Fever Virus Serotype Toscana. Clin Infect Dis . 2001;32(8):1241–3. DOI: https://doi.org/10.1086/319759

59. Gabriel M, Resch C, Günther S, Schmidt-Chanasit J. Toscana virus infection imported from Elba into Switzerland. Emerg Infect Dis . 2010;16(6):1034–6. DOI: https://doi.org/ 10.3201/eid1606.091763

60. Serata D, Rapinesi C, Del Casale A, Simonetti A, Mazzarini L, Ambrosi E, et al. Personality Changes After Toscana Virus (TOSV) Encephalitis in a 49-Year-Old Man: A Case Report. Int J Neurosci . 2011;121(3):165–9. DOI: https://doi.org/10.3109/00207454.2010.537412

61. Nicuolo G Di, Pagliano P, Battisti S, Starace M, Mininni V, Attanasio V, et al. Toscana Virus Central Nervous System Infections in Southern Italy. J Clin Microbiol . 2005;43(12):6186–8. DOI: https://doi.org/10.1128/JCM.43.12.6186-6188.2005

62. Doudier B, Ninove L, Million M, de Lamballerie X, Charrel RN, Brouqui P. Unusual Toscana virus encephalitis in southern France. Med Mal Infect . 2011;1(41):50–1. DOI: https://doi.org/10.1016/j.medmal.2010.09.006

63. Marlinge M, Crespy L, Zandotti C, Piorkowski G, Kaphan E, Charrel RN, et al. A febrile meningoencephalitis with transient central facial paralysis due to Toscana virus infection, south-eastern France, 2014. Eurosurveillance. 2014; 4;19(48):20974. DOI: https://doi.org/10.2807/1560-7917.ES2014.19.48.20974

64. Jaijakul S, Arias CA, Hossain M, Arduino RC, Wootton SH, Hasbun R. Toscana meningoencephalitis: A comparison to other viral central nervous system infections. J Clin Virol . 2012;55(3):204–8. DOI: https://doi.org/10.1016/j.jcv.2012.07.007

65. Laroche L, Jourdain F, Ayhan N, Bañuls AL, Charrel R, Prudhomme J. Incubation Period for Neuroinvasive Toscana Virus Infections. Emerg Infect Dis . 2021;27(12):3147. DOI: https://doi.org/10.3201/eid2712. 203172

66. Masse S, Ayhan N, Capai L, Bosseur F, de Lamballerie X, Charrel R, et al. Circulation of Toscana Virus in a Sample Population of Corsica, France. Viruses. 2019;11(9):817. DOI: https://doi.org/10.3390/v11090817

67. McDonald E, Mathis S, Martin SW, Erin Staples J, Fischer M, Lindsey NP. Surveillance for West Nile Virus Disease — United States, 2009–2018. MMWR Surveill Summ. 2021;70(1):1–15. DOI: http://dx.doi.org/10.15585/mmwr.ss7001a1

68. Sanbonmatsu-Gámez S, Pérez-Ruiz M, Palop-Borrás B, Navarro-Marí JM. Unusual manifestation of toscana virus infection, Spain. Emerg Infect Dis . 2009;15(2):347–8. DOI: https://doi.org/10.3201/eid1502.081001

69. Schwarz TF, Jager G, Gilch S, Pauli C. Serosurvey and laboratory diagnosis of imported sandfly

fever virus, serotype Toscana, infection in Germany. Epidemiol Infect . 1995;114:501–10. DOI: https://doi.org/10.1017/ S0950268800052213

70. Hemmersbach-Miller M, Parola P, Charrel RN, Paul Durand J, Brouqui P. Sandfly fever due to Toscana virus: an emerging infection in southern France. Eur J Intern Med. 2004 1;15(5):316–7. DOI: https://doi.org/10.1016/j.ejim.2004.05.006

71. Kuhn J, Bewermeyer H, Hartmann-Klosterkoetter U, Emmerich P, Schilling S, Valassina M. Toscana virus causing severe meningoencephalitis in an elderly traveller. J Neurol Neurosurg Psychiatry . 2005;76(11):1605–6. DOI: https://doi.org/10.1136/jnnp. 2004.060863

72. Oechtering J, Petzold GC. Acute hydrocephalus due to impaired CSF resorption in Toscana virus meningoencephalitis. Neurology . 2012;79(8):829–31. DOI: https://doi.org/10.1212/WNL. 0b013e3182661f1a

73. Epelboin L, Hausfater P, Schuffenecker I, Riou B, Zeller H, Bricaire F, et al. Meningoencephalitis Due to Toscana Virus in a French Traveler Returning From Central Italy. J Travel Med . 2008;15(5):361–3. DOI: https://doi.org/10.1111/j.1708-8305.2008. 00221.x

74. Zanelli G, Bianco C, Cusi MG. Testicular involvement during Toscana virus infection: an unusual manifestation? Infection . 2013;41(3):735–6. DOI: https://doi.org/10.1007/s15010-012- 0368-9

75. Kvam KA, Stahl JP, Chow FC, Soldatos A, Tattevin P, Sejvar J, et al. Outcome and Sequelae of Infectious Encephalitis. J Clin Neurol . 2024;20(1):23. DOI: https://doi.org/10.3988/jcn. 2023.0240

76. Francisci D, Papili R, Camanni G, Morosi S, Ferracchiato N, Valente M, et al. Evidence of Toscana virus circulation in Umbria: First report. Eur J Epidemiol. 2003;18(5):457–9. DOI: https://doi.org/10.1023/A: 1024295710118

77. Vocale C, Bartoletti M, Rossini G, Macini P, Pascucci MG, Mori F, et al. Toscana Virus Infections in Northern Italy: Laboratory and Clinical Evaluation. Vector-Borne Zoonotic Dis . 2012;12(6):526–9. DOI: https://doi.org/10.1089/vbz.2011.0781

527