

Toscana Virus Central Nervous System Infections in Southern Italy

Giuseppe Di Nicuolo,^{1*} Pasquale Pagliano,² Sonia Battisti,¹ Maria Starace,¹ Vera Mininni,¹
Vittorio Attanasio,² and Francesco Saverio Faella²

Virology Laboratory¹ and Department of Neurological Infectious Diseases,² D. Cotugno Hospital, Naples, Italy

Received 30 May 2005/Returned for modification 19 July 2005/Accepted 16 September 2005

Toscana virus was detected by reverse transcription-nested PCR in 5.6% of cerebrospinal fluid (CSF) samples from patients with meningitis and encephalitis during the summer in southern Italy. The central nervous system infections were associated with young adults and with a substantially benign clinical course. Presenting features and CSF findings are also discussed in the present report.

Toscana virus (TOSV) is an arthropod-borne virus (*Phlebovirus* genus, *Bunyaviridae* family) transmitted by sand flies of *Phlebotomus* species (*Phlebotomus perniciosus* and *Phlebotomus perfiliewi*) residing in humid areas around the Mediterranean (11, 14). TOSV has been associated with acute meningitis and encephalitis occurring during the summer in individuals living in central Italy (1, 7, 12), Spain (3, 6), and France (5), as well as in travelers returning from Mediterranean countries (2, 4, 10). TOSV infection in southern Italy has not been reported previously. Here, we report the incidence rates, seasonal occurrence, clinical presentations, and cerebrospinal fluid (CSF) findings of central nervous system (CNS) infections due to TOSV remarked from 2001 to 2004 among the people of the southern Italian Province of Naples in a prospective study.

The Cotugno Hospital in Naples is the referral center for patients affected by CNS infections who live in the communities of the Naples Province (population, 3 million). From 2001 to 2004, 336 patients with acute aseptic meningitis or encephalitis have been hospitalized in the Cotugno Hospital. At admission, CSF specimens for cyto-biochemical and viral investigation were collected by lumbar puncture. All patients were subjected to electroencephalography (EEG), computerized tomography scanning, and/or magnetic resonance imaging within 24 h after the patient's hospitalization. Inclusion criteria in the study for TOSV infection of the CNS were clinical evidence of acute neurological disease, fever, clear CSF, and presentation during the outbreak period from May to September. Patients with AIDS were not included in the study. The study comprised 126 patients with aseptic meningitis and/or encephalitis (28 in the year 2001, 53 in 2002, 25 in 2003, and 20 in 2004). It included 54 females who were 3 to 79 years of age (median, 31 years) and 72 males who were 2 to 81 years of age (median, 28 years).

CSF samples from all patients were tested for herpesviruses, enteroviruses, TOSV, and West Nile virus. TOSV was detected by a reverse transcription (RT)-nested PCR method. The primers were designed on the basis of a published sequence of the TOSV S segment-encoding N protein (GenBank accession number X53794), as previously described (9, 12, 13). The

nested PCR products were labeled with digoxigenin and detected colorimetrically in microwell plates coated with a specific biotinylated capture probe by using a PCR-enzyme-linked immunosorbent assay digoxigenin labeling kit and a PCR-enzyme-linked immunosorbent assay digoxigenin detection kit (Roche Diagnostics, Mannheim, Germany). The probe sequence was 5'-biotinyl-CGT GTC CTG TCA GAA TCC CT-3' (GenBank accession number X53794; positions 1440 to 1459). An RNA preparation of TOSV-infected cells was used as a positive control.

A viral agent was detected from the CSF of 35 patients (27.8% of cases); enteroviruses were found in 15 patients (11.9%), herpes simplex virus (HSV) type 1 in 10 patients (7.9%), TOSV in 7 patients (5.6%), varicella-zoster virus in 1 patient (0.8%), and human herpes virus 6 in 2 patients (1.6%). In no case was West Nile virus detected. The seven cases of TOSV infection of the CNS included three females and four males, 16 to 44 years of age (median, 23 years). One case occurred in the year 2001 (3.6% of cases), three in 2002 (5.7% of cases), two in 2003 (8.0% of cases), and one in 2004 (5.0% of cases). The CNS infection occurred in six residents and in one American tourist. Five of the residents were living in suburbs of the province, and one was living in Naples City. All patients had a prodromal illness of 2 to 7 days' length with fever (37.5 to 38.5°C), malaise, and severe headache; two of them also reported vomiting and myalgia. A fleeting erythematous rash, involving the trunk and legs, occurred with two patients. Presenting characteristics of CNS involvement are listed in Table 1. The signs and symptoms of meningeal inflammation (fever, headache, and stiff neck) were present in all cases. The firmness of a stiff neck was very slight in three cases. Two patients showed at presentation lethargy resolving within 48 h after hospitalization. Nevertheless, all patients appeared to be alert without any sign of neurological deficit. EEG abnormalities were detected in four patients (57.1% of cases), including a generalized slowing of waves, which was more prominent in the left temporal region in one case. Computerized tomography scan findings were normal in all patients. Magnetic resonance imaging analyses performed on the patients presenting with EEG abnormalities did not show parenchymal changes. The CSF pressure was normal in five cases or slightly increased in two. The CSF samples from all patients presented high white blood cell (WBC) counts with lymphocytic predominance, high protein levels, and normal CSF/se-

* Corresponding author. Mailing address: Laboratory of Virology, D. Cotugno Hospital, Via G. Quagliariello 54, Naples 80131, Italy. Phone: 39 081 590 8403. Fax 39 081 590 8303. E-mail: giuseppe.dinicuolo@ospedalecotugno.it.

TABLE 1. Epidemiological, clinical, and CSF characteristics of seven patients with Toscana virus infection of the CNS

Characteristic(s)	Patients						
	1	2	3	4	5	6	7
Epidemiological data:							
Month of infection	July	July	May	September	September	September	September
Age (years)	16	19	23	23	27	37	44
Sex (M/F)	F	F	M	M	M	F	M
Clinical features:							
Fever max. (°C)	38.5	37.8	37.6	37.5	38.2	38.5	38
Headache	Severe	Severe	Severe	Severe	Severe	Severe	Severe
Stiff neck	Strong	Strong	Weak	Strong	Weak	Weak	Strong
Vomiting	Yes	No	No	Yes	No	Yes	Yes
Neurological deficits	No	No	No	No	No	No	No
Neuroimaging-change	No	No	No	No	No	No	No
EEG-change	Yes	Yes	No	Yes	No	No	Yes
Length of illness (days)	16	10	22	13	13	14	18
Sequelae	No	No	No	No	No	No	No
CSF findings:							
WBC count (×10 ⁶ / liter)	200	200	300	900	40	350	30
Lymphocytes (%)	90	60	85	77	88	85	86
Protein (mg/liter)	668	1430	1840	1420	1450	1830	1100
Glucose (ratio)	0.69	0.65	0.72	0.61	0.66	0.67	0.66

rum glucose ratios. All cases received a course of supportive treatment with dexamethasone (0.4 mg/kg of body weight/day) during the first 4 days from hospitalization. The meningeal signs disappeared within 2 to 4 days after hospitalization. EEG and CSF changes returned to normal within 28 to 60 days after the clinical onset of disease. The median duration of hospitalization was 14 days (range, 10 to 22 days). All patients were discharged without neurological impairment.

The main demographic characteristics and presenting features for patients with HSV, enterovirus, and TOSV infection of the CNS are reported in Table 2. No finding was closely related to TOSV infection only. On the other hand, a Glasgow coma score of ≤12 and neuroimaging abnormalities were present only in patients with HSV or enterovirus infections.

The present study is the first in which CNS infections by TOSV have been analyzed in the population of southern Italy. The CNS infections by TOSV occur in the Province of Naples from the late spring to early autumn among young-adult people who are residents of the coast and neighboring areas. Several cases of human visceral leishmaniasis, transmitted by *P. perniciosus*, occur every year among the population of the Province of Naples (8). Both the geographic area and the seasonal occurrence of the two infections overlap. It is likely that the same *Phlebotomus* vector is also responsible for the spreading of TOSV among the population of this region. TOSV has been reported to be the most frequent etiologic agent of viral meningitis during the summer in central Italy (1, 7, 12). CNS infections among the population of Naples Province showed an estimated incidence rate of 0.03 to 0.1/100,000 persons/year. Our findings suggest that TOSV infection is endemic in southern Italy but that CNS involvement is not as common as in central Italy. The presenting features of CNS infection were of aseptic meningitis with brain involvement in more than half of cases. Although self-resolving in almost cases, TOSV infection of the CNS may be severe in some

patients who need intensive care and who experience a long period of inactivity.

Moreover, during our study, most cases of meningitis and encephalitis that occurred during the warm months were undiagnosed. Infections by other viruses of the sandfly fever

TABLE 2. Demographic characteristics and presenting features for 32 patients according to etiologic agent

Characteristics	No. (%) of cases		
	HSV (n = 10)	Enteroviruses (n = 15)	TOSV (n = 7)
Age (years):			
<5	1 (10)	3 (20)	0
5–65	7 (70)	10 (66.6)	7 (100)
>65	2 (20)	2 (13.3)	0
Fever (>37.5°C)	9 (90)	13 (86.5)	7 (100)
Glasgow coma score:			
15	2 (20)	3 (20)	5 (71.4)
12–14	5 (50)	4 (26.6)	2 (28.5)
8–11	1 (10)	6 (40)	0
<8	2 (20)	2 (13.3)	0
Neuroimaging-change	9 (90)	10 (66.6)	0
EEG-change	10 (100)	13 (86.6)	4 (57.1)
CSF WBC count (×10⁶/liter):			
<5	0	0	0
5–500	9 (90)	15 (100)	6 (85.7)
>500	1 (10)	0	1 (14.2)
CSF protein level (mg/liter):			
<450	1 (10)	2 (13.3)	0
450–1000	2 (20)	3 (20)	1 (14.2)
>1000	7 (70)	10 (66.6)	6 (85.7)

group, including TOSV variants, or by other mosquito-borne and tick-borne arboviruses circulating in the Mediterranean areas must be investigated. Viral cultures and broad-range RT-PCR assays followed by sequencing analysis are necessary to detect these viruses. Specific antibody tests for arboviral infection might help the diagnosis if RT-PCR assays are not available. Finally, some obligate intracellular bacteria (i.e., rickettsiae) spreading in the region may be involved in CNS infections and need to be investigated.

We thank Donato Greco (Istituto Superiore Sanità, Rome, Italy) for technical assistance.

This study was supported in part by the Public Health Authority (Assessorato Ricerca Scientifica) of the region of Campania, Italy.

REFERENCES

1. Braito, A., M. Ciufolini, L. Pippi, R. Corbisiero, C. Fiorentini, A. Gistri, and L. Toscano. 1999. Phlebotomus-transmitted Toscana virus infection of the central nervous system: a seven-year experience in Tuscany. *Scand. J. Infect. Dis.* **30**:505–508.
2. Dobler, G., J. Treib, A. Haass, G. Frosner, R. Woesner, and K. Schimrigk. 1997. Toscana virus infection in German travellers returning from the Mediterranean. *Infection* **25**:325.
3. Echevarría, J. M., F. de Ory, M. E. Guisasola, M. P. Sanchez-Seco, A. Tenorio, A. Lozano, J. Cordoba, and M. Gobernado. 2003. Acute meningitis due to Toscana virus infection among patients from both the Spanish Mediterranean region and the region of Madrid. *J. Clin. Virol.* **26**:79–84.
4. Eitrem, R., B. Niklasson, and O. Weiland. 1991. Sandfly fever among Swedish tourists. *Scand. J. Infect. Dis.* **23**:451–457.
5. Hemmersbach-Miller, M., P. Parola, R. N. Charrel, J. P. Durand, and P. Brouqui. 2004. Sandfly fever due to Toscana virus: an emerging infection in southern France. *Eur. J. Intern. Med.* **15**:316–317.
6. Mendoza-Montero, J., M. I. Gamez-Rueda, J. M. Navarro-Mari, M. de la Rosa-Fraile, and S. Oyonarte-Gomez. 1998. Infections due to sandfly fever serotype Toscana in Spain. *Clin. Infect. Dis.* **27**:434–436.
7. Nicoletti, L., P. Verani, S. Caciolli, M. G. Ciufolini, A. Renzi, D. Bartolozzi, P. Paci, F. Leoncini, P. Padovani, E. Traini, M. Baldareschi, and M. Balducci. 1991. Central nervous system involvement during infection by phlebotomus Toscana of resident in natural foci in central Italy (1997–1998). *Am. J. Trop. Med. Hyg.* **45**:429–434.
8. Pagliano, P., M. Rossi, C. Rescigno, S. Altieri, M. G. Coppola, M. Gramiccia, A. Scalone, L. Gradoni, and F. Faella. 2003. Mediterranean visceral leishmaniasis in HIV-negative adults: a retrospective analysis of 64 consecutive cases (1995–2001). *J. Antimicrob. Chemother.* **52**:264–268.
9. Schwarz, T. F., G. Jäger, S. Gilch, and H. Nitschko. 1995. Nested RT-PCR for detection of sandfly fever virus, serotype Toscana, in clinical specimens, with confirmation by nucleotide sequence analysis. *Res. Virol.* **146**:355–362.
10. Schwarz, T. F., S. Gilch, G. Jager, and C. Pauli. 1993. Travel-related Toscana virus infection. *Lancet* **342**:803–804. (Letter.)
11. Tesh, R. B., J. Lubroth, and H. Guzman. 1992. Simulation of arbovirus overwintering: survival of Toscana virus (Bunyaviridae: Phlebotomus) in its natural sandfly vector *Phlebotomus perniciosus*. *Am. J. Trop. Med. Hyg.* **47**:574–581.
12. Valassina, M., F. Meacci, P. E. Valensin, and M. G. Cusi. 2000. Detection of neurotropic viruses circulating in Tuscany: the incisive role of Toscana virus. *J. Med. Virol.* **60**:86–90.
13. Valassina, M., M. G. Cusi, and P. E. Valensin. 1996. Rapid identification of Toscana virus by nested PCR during an outbreak in the Siena area of Italy. *J. Clin. Microbiol.* **34**:2500–2502.
14. Verani, P., M. G. Ciufolini, S. Caciolli, A. Renzi, G. Nicoletti, G. Sabatinelli, D. Bartolozzi, G. Volpi, L. Amaducci, M. Coluzzi, P. Paci, and M. Balducci. 1988. Ecology of viruses isolated from sand flies in Italy and characterization of a new Phlebotomus (Arabia virus). *Am. J. Trop. Med. Hyg.* **38**:433–439.