

## Serological Survey of Toscana Virus Infections in a High-Risk Population in Italy

Marcello Valassina,<sup>1\*</sup> Melissa Valentini,<sup>1</sup> Agostino Pugliese,<sup>2</sup>  
Pier Egisto Valensin,<sup>1</sup> and Maria Grazia Cusi<sup>1</sup>

Microbiology Section, Department of Molecular Biology, University of Siena, Siena,<sup>1</sup> and Department of Medical and Surgical Sciences, Clinical Microbiology Section, University of Turin, Turin,<sup>2</sup> Italy

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**Toscana virus is the most important agent responsible for meningitis in central Italy. We report a serosurveillance study, using an immunoenzymatic assay, of 360 serum samples harvested from a high-risk population occupationally exposed to Toscana virus in two regions of Italy, Tuscany and Piedmont. The results indicates a seroprevalence of Toscana virus of 77.2% in the forestry workers, particularly in the Tuscany region. This fact is strictly correlated with the ecological niches specific for the survival of Toscana virus arthropod vector.**

Sandfly fever viruses cause human illness and are transmitted by sand fly vectors (*Phlebotomus* spp.); three serotypes that circulate in the Mediterranean area have been identified: sandfly Naples, sandfly Sicilian, and Toscana (TOS). Serotype Naples and Sicilian viruses are the etiologic agents of a usually mild, self-limited disease characterized by fever, myalgia, and headache (1, 7). TOS virus shows major neurovirulence (14), and the infection involves the central nervous system, causing meningitis, meningoencephalitis, or encephalitis, particularly in the male young-adult seronegative population (2, 13; C. H. Calisher, A. N. Weinberg, D. J. Muth, and J. S. Lazuick, Letter, *Lancet* **i**:165, 1987).

TOS virus has been recognized as one of the most important etiologic agents of meningitis and meningoencephalitis in countries of endemicity (Spain, Portugal, Italy, and Cyprus). This neuropathogenic infection is more frequent during the summer, with a peak in August because of the life cycle of the insect vector and because of increased tourism in areas of endemicity (3, 5, 8; Schwarz, T. F., S. Glich, and G. Jäger, Letter, *Lancet* **ii**:803, 1993). The direct diagnostic procedures for the TOS virus infection are the isolation method and, more recently, the detection of viral RNA directly in the cerebrospinal fluid (3–5, 11–13; Calisher et al., letter; Schwarz et al., letter). The detection of the specific antibodies is evaluated by serological techniques, such as indirect immunofluorescence assay, plaque reduction neutralization test, immunoblotting, and enzyme immunoassay (EIA). Recently, the EIA test has been performed using the recombinant viral nucleoprotein (rN) as antigen (5, 6, 9, 10, 12). We used this method to evaluate the circulation and the pathogenicity of TOS virus in the area of endemicity of central and southern Tuscany, including in this study a selected population of forestry workers occupationally exposed to the habitat of *Phlebotomus* spp.

A total of 360 subjects (351 male and 9 female; ages, 25 to 62 years) occupationally exposed to arbovirus infection were enrolled in this study. All subjects were healthy and each under-

went an anamnestic evaluation that gave a negative result for a history of neurologic symptomatology. The serum samples were collected beginning in July and August 2000. The 360 high-risk subjects came from three geographical areas of middle and central-southern Tuscany: Florence (area 1; 128 subjects), Siena (area 2; 145 subjects), and Arezzo (area 3; 87 subjects). As a random control, we analyzed serum samples that had been collected in our laboratory from 290 patients (ages, 23 to 74 years) living in the urban area of Siena. A similar serological survey was developed in the Piedmont area, a region of northwestern Italy, where the incidence of TOS virus meningitis is very low (P. G. Pistono, F. Piro, P. Pauri, M. Burgia, M. Pecorari, P. Pietrosemoli, M. C. Medici, M. C. Arcangeleti, and M. Valassina, 31st AMCLI Meet. Natl. Soc. Italian Clin. Microbiol. 2002, abstr. G07, p. 247, 2002). Serum samples were collected from 50 subjects that were usually present in forested areas (males [32 to 68 years old]; hunters and wild boar breeders). As a negative control, 40 serum samples harvested from residents of the urban area of the city of Turin were analyzed. All sera were analyzed for the presence of immunoglobulin G (IgG)- and IgM-specific anti-TOS virus antibodies using the EIA with rN (IgG/IgM TOS virus detection kit; DIESSE, Siena, Italy), following the procedures described in the kit instructions. The optical density at 450 nm (OD<sub>450</sub>) was measured in a microplate reader (Labsystem). In IgG testing, samples giving an OD<sub>450</sub> of >0.360 were considered positive. For IgM detection, the procedure provides a microcapture method; samples giving an OD<sub>450</sub> of >0.400 were considered positive. A total of 278 out of 360 (77.2%) of the serum samples collected in Tuscany were TOS virus positive by IgG detection. For the three different areas, seropositivity was detected in 95 of 128 (74.2%) cases in area 1, 115 of 145 (79.3%) cases in area 2, and 68 of 87 (78.1%) cases in area 3. The analysis of the data collected, referring to the distribution in the three different areas, does not show a significant statistical difference ( $\chi^2 = 1.06$ ;  $P = 0.58$ ). On the contrary, the presence of specific anti-TOS virus IgG was detected in 66 of 290 (22.7%) subjects in the random control group, which is a significantly different seropositivity profile from that of the aforementioned high-risk group ( $P < 0.0001$ ). The serum sam-

\* Corresponding author. Mailing address: Department of Molecular Biology, Microbiology Section, University of Siena, Via Laterina 8, 53100 Siena, Italy. Phone: 39 577-233850. Fax: 39 577-233870. E-mail: valassina@unisi.it.

TABLE 1. Prevalence of TOS virus antibodies in occupationally exposed and unexposed residents of two Italian regions

Population	No. (%) with TOS virus antibody result		
	Positive	Negative	Total
Tuscany			
Forestry workers	278 (77.2)	82 (22.8)	360
Control	66 (22.7)	224 (77.3)	290
Piedmont			
Forestry workers	3 <sup>a</sup> (6)	47 (94)	50
Control	1 (2.5)	39 (97.5)	40

<sup>a</sup> Two sera with borderline results.

ples collected in Piedmont showed the presence of specific anti-TOS virus IgG in 3 of 50 cases, two of which had a OD<sub>450</sub> very close to the cutoff value in the immunoenzymatic test. The borderline value was therefore confirmed by repeated EIA and additional assays. The sera harvested from 40 residents of Turin showed negative results for IgG detection, except for one. Results for specific anti-TOS virus IgM (Table 1) were negative in all the subjects and in the control population. These data confirmed the absence of an acute TOS virus infection at the time of sampling. It is interesting that the anamnestic inquiry related to the high-risk population gave negative results for neurological symptoms such as meningitis or meningoencephalitis in all the subjects in the areas of Tuscany and Piedmont. This result is in agreement with the observation that the infection is often asymptomatic or the clinical picture is not relevant (1). For comparison with another arthropod-borne virus, the same serum samples were analyzed for serological detection of tick-borne encephalitis (TBE) virus infection by a commercially available EIA (FSME/Tick-Borne-Encephalitis Virus, IgG, and IgM ELISA; ICN Diagnostic Division, Costa Mesa, Calif.). All the serum samples collected in Tuscany were negative for IgG- and IgM-specific anti-TBE virus, except one; 5 of 50 cases (10%) of the serum samples collected from the high-risk population in Piedmont resulted positive for TBE virus IgG (data not shown). These data confirm the spread of TBE virus and TOS virus in different ecological niches specific for the survival of their arthropod vectors. The presence of

TOS virus and its vector in central-southern Tuscany is demonstrated by the high prevalence of the specific antibodies in the population with an elevated risk of contact. The TOS virus appears to be the most common viral agent transmitted by arthropods in this geographic area. This is the first observation of the seroprevalence of TOS virus in a selected population of forest workers.

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